Journal of Environmental Chemical Engineering 13 (2025) 117049

Contents lists available at ScienceDirect

Journal of Environmental Chemical Engineering

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

Innovative strategies for identifying and grouping chemicals, nanomaterials and materials to improve their safety of use

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ARTICLE INFO

Keywords:
Chemicals
Materials
Nanomaterials
Safety
Strategy
SSbD
in-silico

ABSTRACT

To enhance the safety of chemicals, nanomaterials, and materials, innovative identification and grouping strategies are urgently needed. There are various methods which can improve hazard assessment, reduce testing burdens, and support regulatory decisions. This study explores cutting-edge approaches to enable more efficient and accurate safety evaluations, ensuring sustainable and safer use, as part of an European-wide project. Considering the Chemicals Sustainability Strategy (CSS) and the challenges it addresses regarding the production and use of chemicals to meet societal needs and protect people and ecosystems, two key research and innovation actions are being undertaken, namely 1) Strategic Research and Innovation Agenda and 2) implementation of Safe and Sustainable by Design concept. To address these needs, the CheMatSustain project aims to develop new research methods or improve existing ones to enhance the safety and sustainability assessments of chemicals and materials. The project uses photoelectron spectra to identify chemicals, nanomaterials and materials (CNMs), and alternatively, transcriptome and proteome profiles of EA.hy926 cells exposed to contact with the tested CNMs. In the latter alternative case, the cells serve as selective biosensors that repeatedly and specifically recognize the stress factor resulting from contact with artificial surfaces. Identifying similarities and differences in photoelectron spectra and transcriptome and proteome profiles are crucial. All tested samples of CNMs are also used in *in vitro* biological studies to assess cytotoxicity and genotoxicity, the impact on the processes of free radicals' formation, apoptosis, repair of damaged DNA, and to assess ecological effects *in vivo* in relation to aquatic organisms. The collected data are stored in a database and utilized to develop computational QSAR models for predicting CNMs' activity in various toxicological and ecotoxicological endpoints (in silico risk assessment). Data obtained within the CheMatSustain project also will allow the combined use of CNMs risk assessment and life cycle assessment to estimate the environmental impacts and human health risks at each stage of the life cycle of the CNMs studied.

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https://doi.org/10.1016/j.jece.2025.117049
Received 20 February 2025; Received in revised form 7 May 2025; Accepted 10 May 2025
Available online 14 May 2025

1. Introduction

In the manufacturing industry, chemicals are fundamental to processes such as fabrication, surface treatment, and product formulation, as they are used in creating polymers, paints, adhesives, and dyes, contributing to improved product quality and performance [1]. For instance, specialty chemicals enhance the durability of coatings and enable the production of lightweight composites that are increasingly important in automotive and aerospace applications [2]. These chemicals ensure that materials can withstand extreme conditions, making products technically safer and more efficient [3]. In addition, nanomaterials, defined as materials with structures at the nanoscale (1–100 nm), have also transformed manufacturing. Their unique surface area-to-volume ratio and quantum properties allow for novel applications [4]. In electronics, nanomaterials are essential for developing smaller, faster, and more efficient components, such as transistors and sensors. The semiconductor industry utilizes nanotechnology to push the limits of miniaturization, facilitating the continued advancement of consumer electronics [4–6]. In healthcare, the role of chemicals and nanomaterials is equally significant. Pharmaceuticals often rely on chemical synthesis to create active ingredients in medicines, while nanomedicine explores the use of nanoparticles for drug delivery systems [7]. These systems enhance the bioavailability of drugs, allowing for targeted therapy that minimizes side effects and improves treatment efficacy [8]. Moreover, nanomaterials are employed in imaging techniques, enabling earlier and more accurate disease detection through advanced imaging agents [9]. The environmental industry benefits from chemicals and nanomaterials as well. Chemical processes are vital for waste treatment, pollutant removal, and environmental remediation [10]. Nanotechnology also assists in developing more effective catalysts and filtration systems that can clean water and air more efficiently [11,12]. For example, nanomaterials in solar panels can increase energy conversion efficiency, promoting cleaner energy alternatives [13]. Furthermore, the agriculture sector utilizes chemicals for fertilizers, pesticides, and herbicides to enhance crop yields. Nanotechnology in agriculture is emerging with nanofertilizers that improve nutrient delivery to plants and reduce environmental impact [14]. Overall, the integration of chemicals and nanomaterials as well as some materials (e.g. cellulose) across various industries not only enhances product performance and efficiency but also fosters innovation in addressing modern challenges. As technology advances, the synergistic application of chemicals, nanomaterials and materials (CNMs) promises to lead to further breakthroughs, ultimately contributing to a more sustainable and efficient industrial landscape [3,15].

In order to mark some future steps towards safety and sustainability of CNMs, it is needed to understand the processes and assess their behavior in contact with the environment and humans. Therefore, the aim of this paper is to present to the scientific community in the form of a report the objectives and scope of the CheMatSustain project and methodologies to be used to improve identification and grouping of chemicals, nanomaterials and materials used in different industrial sectors, with the final aim to contribute their safe and sustainable production and usage. The report also explains how the objectives of the project will be achieved, as well as the potential social and environmental benefits of the project findings, without delaying the time required to complete the project.

2. Innovative strategy for identifying materials

Identification and characterization of chemical substances and materials, including nanomaterials (CNMs), is the basis for preparing dossiers for their registration in accordance with the Registration, Evaluation, Authorization, and Restriction of Chemicals (REACH) regulation issued by the European Commision, which is essential for their safe production, use, processing, and disposal. The use of standard experimental analytical methods is very effective in relation to chemicals and macroscopic forms of materials but are ineffective when involving nanomaterials. Unlike macroscopic forms of matter, the properties of nanomaterials are mainly determined by the geometric factor (the ratio of the surface area to the linear dimensions of the nanoparticle) and the directly related quantum effects, similarly to the case of chemical molecules. It is generally accepted that the properties of chemical substances depend on the atomic composition and the type of bonds present there, as well as the number of atoms that make up the molecule. It is no surprise that extending the hydrocarbon chain by another carbon atom causes a change in the physicochemical properties of the modified molecule in relation to the initial molecule. Quantum chemistry successfully explains these changes [16,17]. However, there is no such approach in relation to nanoparticles, the size of which is also dependent on the number and type of atoms that build these particles, and physicochemical properties and biological effects are determined by quantum effects, as in the case of chemical molecules. Therefore, the persistent use of analytical methods, which are excellent for identifying and characterizing macroscopic forms of matter, but are not necessarily effective on a nanometric scale, is surprising [18,19]. In connection with the above, we decided to redefine the needs of experimental analysis of nanomaterials and base them on quantum effects. We have experimental methods at our disposal, in the form of imaging molecular orbitals using scanning tunneling microscopy (STM) [20], as well as photoelectron spectroscopy (PS) [21], which provides information on the energy states of electrons in the objects being studied. Thus, in practice, we can use solutions of the Schrödinger equation, which are a pair of the eigenwave function and the eigenstates of electrons, for analysis. This analysis can be supported by purely theoretical calculations using density functional theory (DFT) [22]. Although it is not easy for many to accept, the functioning of very complex biological objects, such as cells, is based on the specific recognition of biochemical molecules, their binding, processing and release in the chain of interactions creating metabolic pathways. Both molecular recognition and the resulting binding, processing and disintegration of the resulting complexes are controlled by quantum effects, exactly as in the case of chemical molecules [23]. Both living and inanimate matter are composed of the same atoms, and their mutual relations are controlled by the same laws of quantum physics [24]. We propose that living cells can act as sensors, specifically and consistently detecting stress factors triggered by exposure to chemicals, nanoparticles, or macroscopic material surfaces (CNMs). The idea presented here became the basis for a patent specifying the possibility of using molecular biology methods to identify products of material engineering and nanotechnology [25], and the potential usefulness of this solution is presented in the monograph [26], which extensively develops the idea of an innovative approach to substance identification considering quantum effects common on the nanometric scale. The research methods mentioned above are usually used for in-depth analysis of the structure of the studied object (photoelectron spectrometry) and the processes of the cell's metabolic response to stress (transcriptome and proteome). In our approach, however, we are less interested in such in-depth procedures, and we are more focused on the analysis of similarities and differences between the obtained photoelectron energy spectra and transcriptome and proteome profiles.

3. Selection, preparation, and physicochemical characterization of chemical substances and nanomaterials for the purposes of project implementation

The selection of CNMs was performed within organic and inorganic substances and, within those two main groups, the most important representatives of chemicals, nanomaterials, and materials were identified (CNMs). The selection process included CNMs necessary from the different point of views: i) CNMs important from the scientific point of view enabling the identification of relationships between materials (those so-called “model materials” with precisely defined and repeatable physicochemical parameters) and their biological impacts; ii) CNMs widely used in industry; iii) CNMs with potential applications. The selected set of CNMs consists of: i) metallic nanomaterials; ii) polymeric nanomaterials, iii) micro/nano-materials as a pair of the same materials in micro and nanoscale range size; iv) chemical substances often used for nanomaterials synthesis/stabilization.

The main selection criterion for metallic nanoparticles (mNPs) was to test the scientific postulates of the CheMatSustain connected with the recognition of the nanomaterials by physical and biological response. Nanoparticles (NPs) have distinct physical and chemical properties and cause distinct biological effects compared to their macro-counterparts. NPs differ from each other in terms of material composition, size, shape, and dimension, the material core type and surface modifier present on the surface. NPs size plays a key role in their long circulation, biodistribution, and clearance [27]. The properties of nanoparticles (material composition, size, shape, surface charge, and porosity) are intimately connected with their functionality and their effects on health and the environment [28]. The size of individual particles can impact the properties and performance of a material or product. For these reasons, it was decided to study nanoparticles made of different materials, sizes, shapes and functional groups on the surface.

As representatives of polymeric nanostructures polystyrene spheres and dendrimers were selected. The polystyrene polymer nanoparticles are composed of linear polystyrene molecules with homogeneous shape and mono-modal size. *In vivo* and *in vitro* studies suggest that polystyrene nanoparticles (PS-NPs) may penetrate organisms through several routes i.e. respiratory and digestive tracts. They can be deposited in living organisms and accumulate further along the food chain [29]. Dendrimers are nano-sized, radially symmetric molecules with well-defined, homogeneous, and monodisperse structure that has a typically symmetric core, an inner shell, and an outer shell [30]. Dendrimers are constituted by repetitive units (so-called "generations") that are chemically bound to each other by an arborescent process around a multifunctional central core. Although the literature [31] reports indicate the potentially high toxicity of all dendrimers, those nanostructures are recognized as potential pharmaceutical nanocarriers.

As the representatives of micro/nano-materials the two pairs of materials were selected: i) titanium dioxide and ii) cellulose, with the particle size in micro- and nanoscale range. Titanium dioxide (TiO_2) was selected due to its wide range of applications [32] and presence in the environment. Titanium dioxide exists in three common crystalline phases: brookite, anatase, and rutile. However, in industry, the anatase and rutile are the mainly used crystalline phases of TiO_2, hence for this subgroup of the CNMs anatase-TiO_2 and rutile-TiO_2 in the nano- and micro-forms were selected. Cellulose is Earth's most abundant natural polymer, synthetized and primarily found in plant cell walls and produced by certain bacteria. It is a linear polysaccharide with β-(1→4) linked D-glucose units. The properties of cellulose—such as biodegradability, renewability, and robust mechanical strength make it an ideal candidate for extensive research for both scientific and application purposes. Microcrystalline cellulose (MCC) is a refined form of cellulose that is widely used across various industries due to its properties and functionalities [33]. Nanocellulose is a material based on natural fibers of nanometric size, exhibiting unique properties due to its high surface area-to-volume ratio and nanoscale dimensions.

The last group of the materials investigated are chemical substances eg. stabilizers (sodium citrate, poly (ethylene glycol) methyl ether thiol, polyvinylpyrrolidone) and bioactive compounds (tormentic acid and triterpenic acids obtained from RS (Red Sentinel) callus extract).

All selected CNMs are currently subjected to thorough physicochemical analysis using high-resolution microscopic and spectroscopic techniques.

4. Synergies between EU policies and industries relevant for the selected CNMs

Although CNMs marked a paradigm shift across multiple industries at the close of 19th century [34], their usage in healthcare, electronics, aerospace, water treatment and textiles are expected to drive market growth in the next years, with projection estimating USD 10.34 billion in 2020 to USD 38.17 billion in 2029 [35]. For their physicochemical properties, CNMs have dominated the biomedicine industry: while gold (Au) was widely utilized for medical treatments in the 1990s, Au nanorods have become prominent for electrochemical reduction applications [36]. Dendrimers are used as anticancer drugs, in pain management, and in drug delivery [37]. Driven by optical properties, silver is one of the most supplied CNM by sensors producers [38], especially for medical devices and electric and electronic equipment, like refrigerators and washing machines [39]. Likewise, silver is applied as an antimicrobial, where medical and consumer applications are the main application areas. Metal oxides like TiO_2 exhibit a huge potential in the wastewater treatment for their photocatalytic degradation capabilities [40]. Concurrently, TiO_2 is also highly demanded by the construction industry for its advantage to increase mechanical and heating properties of roads [41]. In the textile sector, nanocoatings, nanofibers, and nanocomposites have revolutionized the development of functional textiles, enabling the manufacturing of flame-retardant, UV-protective, antimicrobial, and water- or oil-repellent fabrics [42]. Carbon nanotubes and composited with organic matrixes are continuously being utilized in aerospace engineering to innovate the design of spacecraft and aircraft [41]. Significant advancements are also registered in the food industry with regards to food nanosensing, oriented to detect pathogens, nanostructured food ingredients, aimed at facilitating digestion and metabolism [43] and lastly, nutraceuticals for their contribution to improve nutritional value [44].

Despite nanotechnology's substantial market impact driven by breakthroughs in cosmetics, energy, water treatment, electronic devices, and medical products, regulatory and policy developments have not kept pace. This has led to emerging concerns regarding the potential risks that nanomaterials cause to environmental and human health [45]. At the same time, the different momentum of nanotechnology development and the increasing globalization of industries have created substantial barriers to achieving harmonization, standardization, and global regulation of nanomaterials due to differences in regulatory landscapes worldwide [46].

Europe and the United States have taken a leading role in addressing these challenges by developing regulatory frameworks. These efforts have been supported by collaborative initiatives involving government agencies, public and private organizations, and the development of guidelines and proposals aimed at fostering effective governance of nanotechnologies. A critical objective is to reach a balance between regulatory flexibility and control, ensuring the responsible use of nanotechnology while enabling economic benefits, particularly in the global trade of nanotechnology-based products. Simultaneously, these regulations aim to safeguard human health and the environment [45]. In this context, it has been two decades since the European Commission published its "preliminary risk analysis", which laid the foundation for the "incremental approach" to regulating nanomaterials. This approach prioritizes the use of existing legislative frameworks, revisiting and amending them as necessary to address the specificities of nanomaterials [47]. Consequently, numerous European Union legislations and directives have been revised to incorporate nano-specific considerations. In 2022, the European Commission introduced a significant update to the definition of nanomaterials, representing a milestone in their regulatory efforts [48]. However, challenges persist, particularly in the application and implementation of nano-specific requirements across diverse industrial activities, which often needs case-specific analysis and tailored regulatory responses.

The European Commission has implemented a series of initiatives to address the regulatory challenges associated with nanomaterials, with the European Chemicals Agency (ECHA) playing an important role. Since 2020, explicit legal requirements have been in place under the REACH (Registration, Evaluation, Authorization, and Restriction of

Chemicals) and CLP (Classification, Labelling and Packaging) regulations for companies manufacturing or importing "nanoforms" [49]. These regulations aim to ensure that no new chemical substances will be placed on the market without robust safety data, adhering to the principle of "no data, no market" [45]. In addition, the European Union Observatory for Nanomaterials (EUON) was established in 2017 to provide a platform for information regarding the use of nanomaterials within the EU market. These measures are part of a broader strategy to enhance the management and governance of nanomaterials, aiming for more effective oversight and safety assurance [50]. However, despite these advancements, the regulatory frameworks have not yet evolved into proper laws. The regulatory gaps and the insufficient data on the risks associated with nanomaterials, continue to allow environmental and health concerns to remain unresolved [45].

5. Safe and Sustainable by Design (SSbD) framework in industry

The environmental and health concerns related to CNMs are growing. In particular, the increasing attention paid to the toxic effects on human health associated with CNMs exposure to and absorption by the human body has led to deepened efforts for better investigating the environmental risks [34]. Additionally, driven by the need to address the dispute among the scientific community regarding proper safety and sustainability assessment [51], EU Commission introduced the Safe and Sustainable by Design (SSbD) Framework in 2022 as voluntary approach to guide such assessments [52,53]. This approach emphasizes the integration of sustainability principles at the design stage, ensuring that products are not only safe for workers and consumers but also environmentally friendly throughout their lifecycle. Recent research highlights the importance of adopting the EU SSbD Framework to address the challenges posed by traditional practices, which often neglect the environmental and health impacts of products. The integration of chemical risk assessment and life cycle assessment (LCA) in the field of nanotechnology has emerged as a critical area of research[54]. Nanotechnology has significantly influenced various industries, including those producing the most commonly used products such as textiles, cosmetics and paper, by enhancing product performance and functionality. However, the introduction of CNMs raises concerns due to their potential safety and environmental implications. Vance et al. highlight the importance of developing inventories of nanomaterial consumer products to prioritize which products require thorough evaluation for human exposure and toxicity studies, as well as LCA [55]. This proactive approach is essential in identifying potential risks early in the product development process. The most frequent knowledge gap according to a survey among European projects focused on SSbD is data for LCA [56]. For instance, inventory data on production of NMs as well as on potential releases of NMs during products' life cycle [56]. This is a challenge that has been addressed earlier by a number of researchers [57–60] but the challenge remains.

CNMs have been used in textiles for antimicrobial, self-cleaning, UV-protection and several other functionalities [61–63]. Some studies have been conducted on both health risks and environmental impact throughout the life cycle [62–64]. Although most of the studies are performed before the emergence of the EU SSbD Framework, some are used prospectively, whereas others seem not to follow the principles of SSbD at all. In the textile sector, SSbD is gaining traction through innovative practices aimed at reducing waste and enhancing recyclability. The concept of Design for Disassembly (DfD) is particularly relevant, as it allows an easy separation of materials at the end of a product's life cycle, facilitating recycling and reducing landfill contributions [65]. Moreover, the shift towards circular textile practices, such as textile recycling and upcycling, is being recognized as a critical strategy for achieving sustainability goals [66].

The application of SSbD principles is particularly relevant in the development of nanomaterials in the cosmetics sector. For instance, Furxhi et al. discuss the need for a holistic SSbD approach that encompasses product safety, low environmental impact, material and energy efficiency, and process safety. The study specifically examines cosmetic sunscreens containing titanium dioxide nanoparticles as an alternative to conventional chemical UV absorbers, which are often associated with adverse health effects and environmental concerns [56]. This aligns with the broader goal of the ASINA project, focusing on establishment of a data-driven methodology for assessing the safety and sustainability of nano-enabled products throughout their lifecycle [67].

In the paper industry, one significant aspect of the SSbD approach is the emphasis on sustainable sourcing of raw materials. The paper industry has seen a shift towards using recycled paper as a primary raw material [68]. This not only reduces the demand for virgin fibers but also mitigates the environmental impact associated with deforestation and resource depletion. Cleaner production technologies are also adopted e.g. the implementation of low-chlorine or even chlorine-free bleaching processes has been a notable advancement in the paper industry, particularly in Scandinavian countries [69].

The potential for nanotechnology to contribute to sustainable practices is underscored by the need for a holistic approach to risk governance. The debate surrounding the environmental consequences of development and use of CNMs has intensified, as noted by Guinée et al., who emphasize the necessity of employing both risk assessment and LCA to quantify these impacts [70]. The integration of these methodologies allows for a comprehensive evaluation of the environmental performance of nanotechnology-based products, ensuring that sustainability is a core consideration in their development [71]. For instance, LCA has been recognized as a vital tool for assessing the environmental impacts of nanomaterials used in various applications, including nanocellulose-reinforced composites in the paper industry [72]. Subramanian et al. advocate for integrating risk management and sustainability principles, which can guide the responsible development of nano-enabled products [73]. This is particularly important in the cosmetics sector, where consumer safety and environmental sustainability are essential.

In conclusion, the current research on SSbD underscores the importance of integrating safety and sustainability principles at the design stage as exemplified here in textiles, cosmetics and paper.

6. Methods for testing metabolic response following exposure to selected chemicals and nanomaterials

Even the best performed identification of CNMs, although very important from the point of view of their safe use, is not sufficient to determine biological and environmental effects. For this purpose, it is necessary to conduct appropriate studies providing an image of the potential cytotoxicity, genotoxicity and ecotoxicity of the identified substances and materials. Both approaches are complementary to study the effects on cells and on organisms. Unifying parameters are the survival/death of the cells or animals and, besides others, oxidative stress parameters on the cellular basis. Combining the results of *in vitro* and *in vivo* observations with *in silico* modeling can provide a tool capable of predicting the biological and ecological effects of the presence of the identified substances and materials. For this purpose, the CheMatSustain project performs comprehensive studies of the above-selected CNMs.

6.1. In vitro studies

The EA.hy926 cell line, which is a hybrid of human umbilical cord vascular endothelial cells and lung cancer clone A549 cells, was selected for this project and is available in the collection offered by the American Type Culture Collection (ATCC). These cells exhibit the morphology of endothelial cells and are often used as model endothelial cells. They are very easy and stable to culture and allow for good experimental reproducibility, which is not always possible with primary endothelial cells. On the other hand, vascular endothelium is involved in direct contact with inhaled, ingested and skin-derived substances. This contact is due to the presence of blood vessels in the barrier structures of the epithelium and skin. Therefore, these cells are very flexible to the metabolic response resulting from contact with these substances.

In our studies, nanoparticles are stabilized with three types of stabilizers, i.e. sodium citrate, polyethylene glycol (PEG) and polyvinylpyrrolidone (PVP). Stabilizing substances are used in minimal concentrations that allow maintaining dispersed nanoparticles in suspension, but do not have a significant effect on cell functions.

The cytotoxicity test was selected as the basic test, which allows estimating the range of concentrations of the tested factors that may have a toxic effect on cells cultured *in vitro*. Cells are seeded at 10^4 per well and cultured for 24 hours under standard conditions. After this time, the test samples are added, and culture is continued for another 24 hours. Negative controls are cultures free of test samples, and positive controls are obtained by adding Dimethyl sulfoxide (DMSO - 6 %). The cytotoxicity effect of the tested nano- and micro-particles is assessed in the concentration range 0.5–20 μg/mL. Based on the determined EC10, EC25 and EC50 values, further *in vitro* tests are conducted at the indicated concentrations. The experiments are repeated at least three times and in the statistical analysis of the results we assume the significance level of $p < 0.05$. Using EA.hy926 cells in a classic two-dimensional culture, the following *in vitro* experiments are currently being conducted regarding the biological effects of selected CNMs:Fig. 1.

- assessment of cytotoxicity using the MTT [74] or XTT [75] assays (enzymatic-colorimetric tests),
- assessment of genotoxicity using the micronucleus (microscopic) [76] or comet (electrophoretic) [77] assays,
- assessment of the level of reactive oxygen species formation (cytofluorimetric test) [78],
- assessment of the level of apoptosis and activity of DNA damage repair (cytofluorimetric assays) [79],
- assessment of the transcriptome profile (using the NGS system) [80],
- assessment of the proteome profile (using the Shotgun system) [81].

6.2. *In vivo and ex vivo studies*

Nanoparticles are increasingly entering aquatic and marine habitats. Due to their small size, they have high mobility and reactivity, which favors their distribution in these ecosystems [82]. For instance, in water, they can agglomerate and precipitate or bind to organic and inorganic substances and finally, they can attach to or accumulate in aquatic organisms [82] (Fig. 2). Therefore, assessment of their interaction with and effects on biota is crucial to estimate their threat to living organisms, populations, and, finally, ecosystems. Zooplankton and phytoplankton are the foundations of aquatic food webs. Due to their small size, they are particularly susceptible to the harmful effects of NPs [83–86]. Bioassays will be conducted considering various levels of biological organization, ranging from cellular and molecular changes to acute organismic and chronic trans-generational effects. Growth inhibition tests are carried out with the microalgae *Phaeodactylum tricornutum* according to ISO standard 10253:2024. Acute toxicity tests are conducted with the rotifer *Brachionus plicatilis* [86]. The bioassays conform to ISO Standards 19827 and 14380, respectively. Water flea, *Daphnia* magna, completes this set of study organisms [87]. Depending on the tested species, limit tests are conducted at concentrations of 50–100 $mg \cdot L^{-1}$ and for 48–72 hours to identify toxic effects. Positive (toxic) results will be tested using defined dilution series for their effect on concentration and LC values.

The major route through which particles may enter the body is ingestion. Therefore, we document the ingestion/incorporation of the test materials in rotifers and daphnia.

Fig. 2: Species selection for eco-toxicological studies within the CheMatSustain-project. a) the diatom *Phaedactylumtricornutum (*https://www.algatech.com, b) the rotifer *Brachionusplicatilis*©AWI, c) the water flea *Daphnia magna* ©AWI. Will be exchanged.

In the second step, follow-up studies are carried out to investigate observed effects on organisms in more detail on the cellular and molecular level. Previous studies in our lab showed that ingested microparticles do alter the enzymatic features in the digestive tract of marine invertebrates. Therefore, we analyze a set of specific enzyme markers, such as acid and alkaline phosphatases, esterases, aminopetidases, as well as oxidative stress markers. Superoxide dismutase (SOD) is a key enzyme in the antioxidant defense system and catalyzes the conversion of superoxide radicals into hydrogen peroxide (Fig. 3). Increased SOD activity indicates oxidative stress, probably after activation of intracellular NADPH-oxidase upon incorporation of nanoparticles [88–90].

Besides enzymatic assays, NMR spectroscopy of metabolites offers several advantages in ecotoxicology research. As a non-invasive and high-resolution method, it enables the simultaneous identification and quantification of many metabolites in biological samples [91,92]. This is particularly useful for detecting early biochemical changes caused by toxic substances before visible physiological effects occur. It allows us to examine substances at low concentrations and identify changes in the metabolite composition that are difficult to detect with traditional toxicological methods.

7. Database: structure and functionality

Consequently, there is a need for a database be constructed and developed for the collection and exploitation of in-project research big data outputs (also from existing websites & databases), to properly collect results obtained. Output from the database will be used to develop i*n silico* (QSAR) models to extrapolate the knowledge gathered in the project to other materials to ensure the safety of the material (to humans and the environment) in the design phase. This will enable the

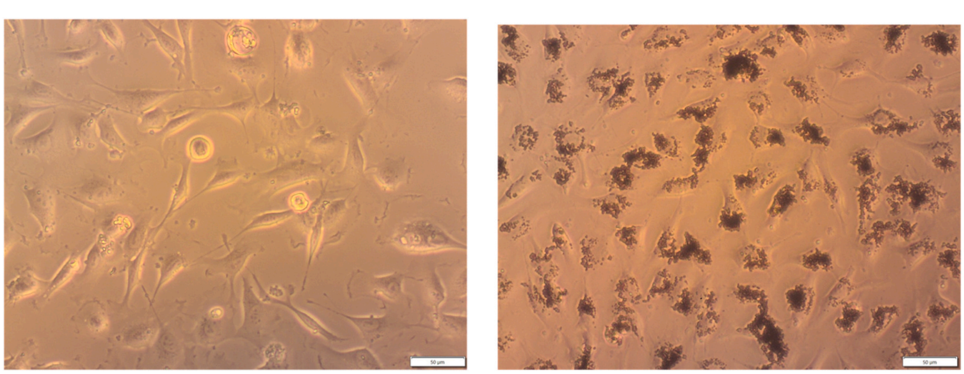

Fig. 1. EA.hy926 cells in control culture (left side) and in the presence of rutile TiO_2 nanoparticles. The culture was carried out under standard conditions (37C, 5 % CO_2, humid atmosphere) for 24 h and the concentration of TiO_2 nanoparticles was 75 μg/mL. Titanium dioxide nanoparticles were stabilized with PVP. The white bar corresponds to 50 μm. The right photo clearly shows large agglomerates of nanoparticles accumulated by the cells.

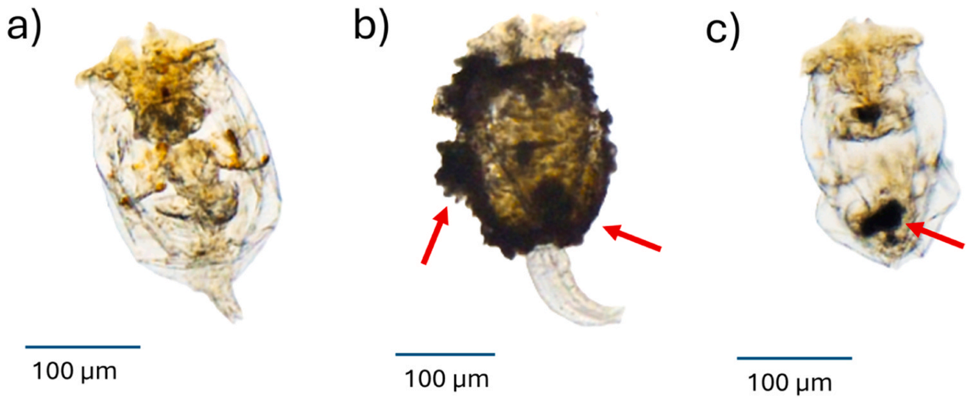

Fig. 2. Rotifers (*Brachionus plicatilis*) exposed to a) sole seawater (control), b) nano-TiO_2, rutile, and c) micro-TiO_2, rutile. The red arrows indicate the TiO_2 adhered on the body (b) or within the digestive system (c).

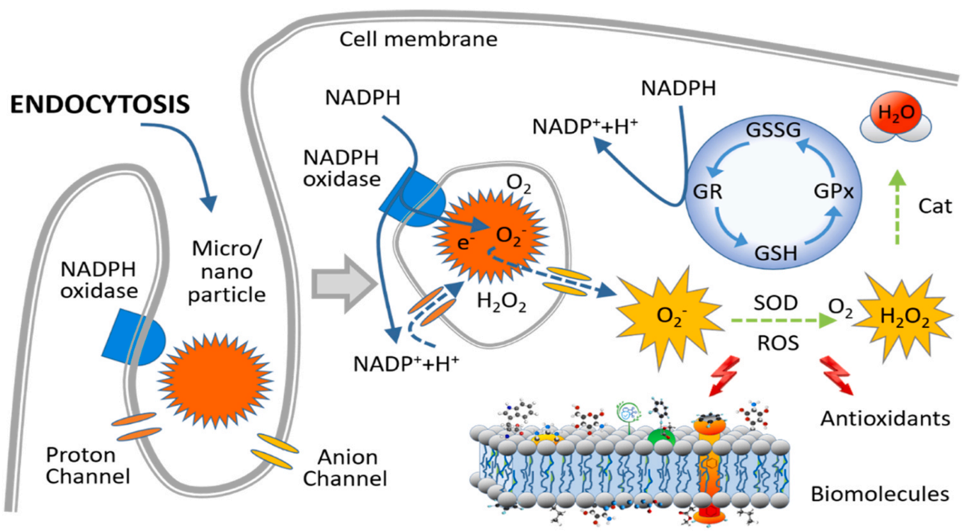

Fig. 3. Draft of the intra-cellular formation of reactive oxygen species (ROS) through a respiratory burst following micro/nanoparticle uptake by phagocytosis and actors of the subsequent antioxidant responses. NADPH = Nicotinamide adenine dinucleotide phosphate reduced form, SOD = Superoxide dismutase, Cat = Catalase, GPx = Glutathione peroxidase, GSSG = Glutathione oxidized, GR = Glutathione reductase, GSH = Glutathione reduced.

project to classify and group CNMs which will be used to qualify the assessment of risks in Life Cycle Assessment (LCA), develop Footprint Scorecard (FS) and dissemination of reliable information via CheMat Facility (a platform with specific content prepared for three main target groups of the project: policy makers, industry and citizens.

The research data are and will be collected in a way that aligns with the FAIR (findable, accessible, interoperable, and reusable) principles to maximise its overall impact with no compromise on integrity, the database is also designed to be user-friendly, secure, and exchangeable with seamless dissemination. CheMatSustain will ensure guidelines from EU funding body are met and research data is authentic, accurate, secure and in totality. In cases where personal data is collected, acknowledgement of General Data Protection Regulation (GDPR) and local regulations will be included.

Data collection from external websites (ECHA, EUON etc.) and databases (CBit etc.) is done by web scrapping using Python having API. The external interface (API) has been developed to facilitate and optimise data consumption and exchange from the database. Python is used as the scripting language and as a bridge to SQL queries and other data-serving operations. Internal research data is and will be collected in excel, CSV or JASON format.

The chosen model for the CheMatSustain Database is a relational database model, PostgreSQL or MySQL. A relational database is a database that stores and provides access to data points that have relation to one another. This system is based on the relational model, an intuitive, straightforward way of representing data in tables. Each table consists of rows and columns. Each row in a table represents a unique instance of data, often called a record, while each column represents a field or attribute of that data. The relational database uses Structured Query Language (SQL) for database management and operations, allowing for complex queries and data manipulation.

The database has been meticulously designed to be FAIR compliant, ensuring that the data is findable, accessible, interoperable, and reusable.

Findable: Each dataset within the database is assigned a unique identifier, allowing for precise retrieval and reference. Comprehensive metadata is provided for each entry, making it easy to locate and understand the data's context and relevance.

Accessible: The database is structured to facilitate easy access through standard protocols. Authentication and authorisation mechanisms ensure that the data is available to authorised users while protecting sensitive information. Clear documentation is provided, detailing how users can access and use the data efficiently.

Interoperable: Data formats and protocols used in the database are based on widely accepted standards, promoting seamless integration with other systems and databases. This ensures that data can be easily shared and combined with other datasets, fostering collaboration, and extending the utility of the data beyond its original purpose.

Reusable: To maximise reusability, the database includes rich metadata describing the data's origin, structure, and usage conditions. Data is stored in formats that support long-term preservation and are suitable for a variety of applications.

8. Analysis of similarities and differences of photoelectron spectra and -omics profiles

In accordance with the above-mentioned innovative strategy for identifying CNMs, experiments are currently being conducted to provide, for the selected and described above CNMs samples, both photoelectron spectra as well as transcriptome and proteome profiles of EA.hy926 cells exposed to these samples. Comparative analysis of these spectra and profiles will be used to evaluate existing similarities and differences to differentiate and identify the tested samples. In addition, it is used to create a database of spectra and profiles enabling fast and efficient identification of CNMs selected and studied within the CheMatSustain project and in the future their application in other projects for identification of other nanomaterials. For the identification of CNMs, the raw spectra and profiles treated as unique fingerprints will be primarily used. We assume here a certain analogy with the fingerprint method used to identify chemical compounds in mass spectrometry, where obtained MS spectrum is used to identify the compound of interest by comparing it with spectra collected in databases. Of course, there are many possible ways to implement this analysis, and its most effective way will be developed using the spectra and profiles obtained by us. However, we are confident that even a relatively simple standard correlation analysis can be effectively used for this purpose [26]. As an illustration we present here a comparative analysis of the transcriptome obtained for EA.hy926 cells treated with four different, coded CMNs samples. Shortly, EA.hy926 cells were incubated for 24 h under standard conditions (37°C in a 5 % CO_2 in humid air atmosphere, 24 h), in the presence of four different encoded material samples. After incubation, the cells were lysed, and RNA was isolated and used for a microarray experiment to assess the gene expression profile [93]. The obtained gene expression profiles of the treated cells were compared with those of the control cells and the FC (fold change) parameter characterizing the changes in gene expression was calculated. Then, the correlation analysis of the FC parameter values obtained for the tested coded samples was performed and results are presented in Fig. 4 and Table 1. This analysis clearly indicates a high similarity of two of the four samples, and after decoding it turned out that they were titanium alloy samples prepared by different techniques. In the statistical analysis of the results, we assume the significance level of $p < 0.05$ and the

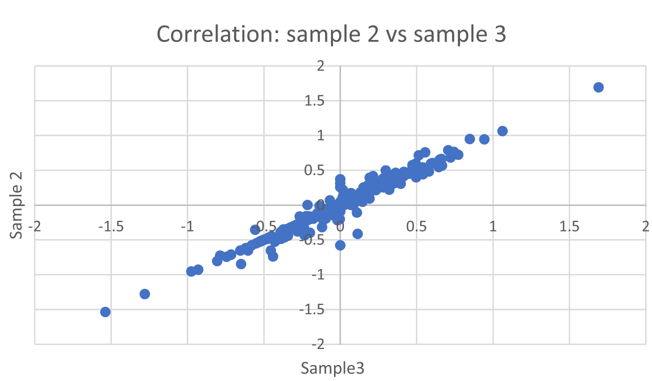

Fig. 4. Example of comparative analysis of similarities and differences in transcriptome profiles of EA.hy926 cells treated with four CNMs samples. A convenient measure of profile similarity is the correlation coefficient R. For the tested set of samples R = 0.9078 for samples 2 and 3, which indicates their very high similarity.

Table 1
Results of correlation analysis of gene expression scores (fold changes) of four coded CNMs samples. The experiments were repeated three times, and the standard deviation (SD) value did not exceed 1 % of the mean values. R = 0.9758 for samples 2 and 3, which indicates their very high similarity.

Correlation coefficient R	Sample 1	Sample 2	Sample 3	Sample 4
Sample 1	1.0000			
Sample 2	0.0052	1.0000		
Sample 3	−0.0016	0.9758	1.0000	
Sample 4	−0.1245	0.0197	0.0021	1.0000

correlation coefficient R> 0.9 is considered significant.

Regardless, the obtained experimental data in the form of photoelectron spectra and transcriptome and proteome profiles can and certainly will be used for an in-depth analysis of the CNM structure and the metabolic processes initiated in the cell by these CNMs. This in-depth analysis will find its place in the *in-silico* modeling described below.

9. Chemoinformatic prediction models and its usage in risk assessment

Computational (*in silico*) approaches are commonly used in several fields (such as pharmacy, cosmetics, agrochemistry) to predict relevant properties of chemicals. For example, they are broadly used in drug-discovery to predict the potential therapeutic activity and toxicity of drug candidates. These approaches are faster and cheaper than experimental assays at the traditional laboratories: once a computational model has been developed and validated, it can be rapidly used to screen hundreds or thousands of molecular structures (even not synthesized or theoretical ones). Furthermore, these *in silico* approaches allow to decrease or even suppress the number of animals sacrificed, thus contributing to the general effort to reduce, refine and replace them for ethical reasons (3 Rs) [94].

Amongst the computational methodologies, the development and application of Quantitative Structure-Activity Relationships models (QSARs) is one of the most used, due to its robustness and easy applicability. Those models are based on finding quantitative relationships between the structure of a chemical and its physicochemical or biological properties. QSAR models for organic chemicals are widely accepted and even promoted by regulatory bodies such as the European Chemicals Agency [94]. However, the use of QSAR on materials and nanomaterials (NMs) (nanoQSAR) is a quite novel and active field of research, due to the intrinsic difficulty of their structural characterization [95,96]. The number of relevant nanoQSARs is growing significantly in the last years, due to the definition of new descriptors adapted to the materials structure and the inorganic nature of most NMs, as well as the progressive generation of more experimental information on NMs.

As part of the CheMatSustain project, we have reviewed the *in silico* preexisting models to predict several toxicity parameters of NMs. We have found that most published models are devoted to toxicity in humans, modeled using *in vitro* data from targets such as bacteria and mammal cells. The most common endpoint studied is cytotoxicity, followed by genotoxicity, but we have also identified predictive models focused on the inflammatory and oxidative potentials [97,98]. Regarding environmental endpoints, very few studies have been published until now, for example about toxicity in *Daphnia magna* and in zebrafish. Regarding the composition of NMs, most of the models are based on metal oxides, both solely and as a part of a wider dataset [99]. Other core components found in publications are SiO_2, Cd-based quantum dots (QDs) and carbon-based inorganic materials such as fullerenes, carbon nanotubes and graphene flakes [100].

We have also searched for QSAR models available as computational tools (online servers and local installed). As expected, in the case of NMs only very few tools are currently available. We have compiled a list of predictive models for NMs which are available online on different platforms, such as OCHEM [101], Enalos Cloud [102] or NanoSolveIT [103]. Recently, the CheMatSustain partner ProtoQSAR has launched ProtoNANO [104], a NMs-focused tool proposing some nanoQSAR models related with different endpoints such as cytotoxicity (in bacteria, human cells and tumoral cells), zeta potential and partition coefficient.

From this review work, we have concluded that one of the biggest difficulties to develop nanoQSAR models is the scarcity of relevant data relating the chemical structure of NMs to their biological impact or toxicological behavior. The parameters with more information (such as cytotoxicity or genotoxicity) are apparently those requested for regulatory purposes by international entities such as ECHA or EFSA. On the other hand, there is not a systematic approach for structural characterization (e.g. not all sources in nano- and microparticles include the information on size), and different assays that are not always comparable can be used for this characterization. Similarly, there is a significant variance among the cell lines used for testing, and the lack of consistency makes it impossible to compile homogeneous single-cell databases as it would be the ideal situation for QSAR development purposes [95,96]. Those limitations are general to the nanoQSAR models with respect to the "classical" QSARs based on small organic molecules (e.g. drugs), which usually are developed from bigger data sets (in some cases with hundreds or thousands of chemicals and biological data), and which structures can be more easily characterized, for example by the use of simple topological descriptors based on the graph theory [105]

Taking into account all these considerations, priority is given to the development of QSARs for chemicals and NMs with high structural diversity and not focused strictly on particular chemical families. We will work with the more consistent and available data for the direct toxicity parameters, such as cytotoxicity, genotoxicity, algae growth inhibition and invertebrate acute toxicity. We compile a primary database of very consistent toxicological data for diverse families of chemicals and materials, which is a key requirement for better modeling. In all cases, we ensure that the external data are consistent with the experimental results provided by the partners in the CheMatSustain consortium. To increase data availability and potentially provide more general models, the effect of including external data related to alternative target cells/species in the modelling database is also evaluated. Expert partners will also take into consideration this analysis to guide and assess the selected techniques, as well as provide guidance in the pros and cons of the different cell-lines and assays and the main characteristics required to assess the reliability and usability of data for modeling. As a preliminary work on this, we have developed a series of models on toxicity of quantum dots [106]. A classification model for cytotoxicity in tumoral cells provided an accuracy of 0.83 and 0.72 respectively for the training set (533 compounds) and the validation set (80 compounds) previously selected by a random procedure. Following the same approach, a classification model for cytotoxicity in human primary cells has been developed, and the accuracy was of 0.90 and 0.85 for the training set and validation set (97 and 34 compounds respectively). These models and the future models developed in the context of the project follow strictly the requirements from the OECD regarding the validity of QSARs [94], especially regarding the clear definition of endpoints and algorithms, the definition of a defined domain of applicability for each model (since (Q)SARs are limited in terms of the types of chemical structures, physicochemical properties and mechanisms of action for which the models can generate reliable predictions), the use of appropriate measures of goodness-of-fit, robustness and predictivity, as well as the use of training and validation sets (including external datasets when available). Finally, a mechanistic interpretation will be also provided when possible. Furthermore, the findings from molecular-based models could be used to discuss aspects such as the influence of the cell type and assay conditions in the models, contributing to guide the selection and curation of data for the subsequent models on materials.

10. Conclusions

This paper reports on a study aimed at identifying innovative strategies for identifying and grouping chemicals, nanomaterials and materials to improve their safety of use.

Ensuring the safe use of chemicals, nanomaterials, and materials requires efficient identification and grouping methods. Traditional approaches often struggle with diverse substances, leading to inefficiencies in risk assessment.

Considering the need to improve and optimize safety and sustainability of chemicals, nanomaterials and materials, which is currently seen, the CheMatSustain project is taking a series of steps to move forward and provide solutions to the current situation. Together with many other projects dealing with similar issues, but focused on other CNMs and other strategies, a significant impact in this field is expected. The combination of different techniques, expertise, experience, and approaches of the project implementers has been carefully selected to reach the desired results which will be published as soon as available from the project.

Considering that it is based on an on-going European project, this paper has some limitations. Firstly, existing methodologies may not fully capture the complexities of nanomaterials, which can exhibit unique properties that differ significantly from their bulk counterparts. Current classification systems may lack the granularity required to address these differences, leading to potential safety oversights. Secondly, the rapid advancement in nanotechnology often outpaces regulatory frameworks, resulting in gaps in safety assessments and risk management. This can hinder the timely identification of potential hazards associated with new materials. Finally, the reliance on standardized testing protocols, which may not be universally applicable to all types of chemicals or nanomaterials. Variability in production processes, environmental interactions, and exposure scenarios complicate the grouping and identification efforts.

However, despite these limitations, the paper provides a welcome addition to the literature since it shows the variability in production processes, environmental interactions, and exposure scenarios. It also shows the need for integrative approaches for successful communication and collaboration among researchers and regulatory experts to enhance safety measures for chemicals and nanomaterials. The findings aim to support policymakers and researchers in adopting advanced methodologies, ultimately fostering a more sustainable and safety-focused approach to chemical management. This research is important for evolving regulatory frameworks and protecting human and environmental health.

For this reason, in order not to waste time on project completion, the authors decided to make public the goals, scope, and methods chosen for the implementation of the CheMatSustain project and to enable their use by interested research teams.

CRediT authorship contribution statement

Piotr Komorowski: Writing – original draft, Methodology, Formal analysis. **Walter Leal Filho:** Writing – review & editing, Funding acquisition, Conceptualization. **Kamal Khandelwal:** Writing – original draft. **Jarosław Grobelny:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Reinhard Saborowski:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Emma Stromberg:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation. **Bogdan Walkowiak:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Jelena Barbir:** Writing – review & editing, Writing – original draft, Visualization, Funding acquisition, Conceptualization. **Elena Bernalte:** Writing – original draft, Investigation. **Eleonora Foschi:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Olsen StigIrving:** Writing – original draft, Investigation. **Rafael Gozalbes:** Writing – review &

editing, Writing – original draft, Data curation.

Funding

This paper was funded by the Horizon Europe programme, the European Health and Digital Executive Agency (HADEA), grant number 101137990 – Project CheMatSustain.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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