 doi:10.20944/preprints202205.0229.v1

Review

Nanosafety: an evolving concept to bring the safest possible nanomaterials to society and environment

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Abstract: The use of nanomaterials has been increasing in recent times, and they are widely used in industries such as cosmetics, drug, food, water treatment and agriculture. The rapid development of new nanomaterials demands a set of approaches to evaluate the potential toxicity and risks related to them. In this regard, nanosafety has been using and adapting already existing methods (toxicological approach), but the unique characteristics of nanomaterials demand new approaches (nanotoxicology) to fully understand the potential toxicity, immunotoxicity and (epi)genotoxicity. Also, new technologies, such as organ-on-chip and sophisticated sensors, are under development and/or adaptation. All the information generated is used to develop new *in silico* approaches trying to predict the potential effects of newly developed materials. The overall evaluation of how from the production to final disposition chain of nanomaterials is evaluated under Life Cycle Assessment (LCA), which is becoming an important element of nanosafety considering sustainability and environmental impact. In this review we give an overview of all these elements of nanosafety.

Keywords: Nanomaterials; Nanotoxicology; Immunotoxicity; Genotoxicity; Epigenetics; Advanced *in vitro* models; *In silico*; Life Cycle Assessment.

1. Introduction

The concepts of nanomaterials and nanotechnology were coined in the 20th century, and from the birth of quantum theory it was evident that the physicochemical properties of matter in the nano-size range (i.e., nanomaterials) would be challenging to handle and understand [1,2]. Despite examples of nanomaterials can be found in ancient products [3], it was not until recently that the knowledge and understanding of these materials came to light. Nanotechnology was first suggested by Richard Feynman in the late 1950's [4] and in 1974, Norio Taniguchi coined the term Nanotechnology [5]. With the discovery of fullerenes in 1985 the revolution of nanomaterials took momentum and up to date we talk about carbon nanotubes (CNT), graphene, quantum dots, nanorods, nanowires, nanofibers and all sort of composites that include different types and other materials [6].

Nanosized structures have been under commercial use for several years already. Cosmetics, drug delivery, food, water treatment and agriculture are among the industries that use a large variety of these types of structures, including liposomes, magnetic nanoparticles, and titanium dioxide (TiO_2), among a large variety [7]. We also must consider that accidental and intentional release of nanomaterials into the environment is a growing concern [8]. For these reasons, exposure to nanomaterials can be related to multiple routes, and the differences in type (chemical composition, physical properties, shape, and size)

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and sources give a very complex dimension to the task of assessing their safety and potential risks [9]. Just as an example, recently it has been reported that plastics with submicron size were found in the bloodstream of healthy donors [10]. This finding is not surprising considering that the translocation of particles in that size range was reported in experimental studies in 2002 [11], and since then many other studies have shown that particulate matter can translocate to the circulation [12] and can reach different tissues including the brain [13], even in a fetal stage [14]. The difference between the plastics in blood study and previous reports, is that the plastics report comes in a time when the discussion related to microplastics in the oceans [15] has reached the mass media [16], therefore giving big mediatic attention to this report [17].

The production and use of nanomaterials can be found in a wide variety of products, from components for electronic devices, to cosmetics and food [18]. The potential impact on the environment is also a problem to consider, due to the fact that the final disposal can lead to contamination of soil, water, and air [19]. But what are the routes of exposure to nanomaterials? In the following image we try to compile the main and most common routes of exposure (Figure 1).

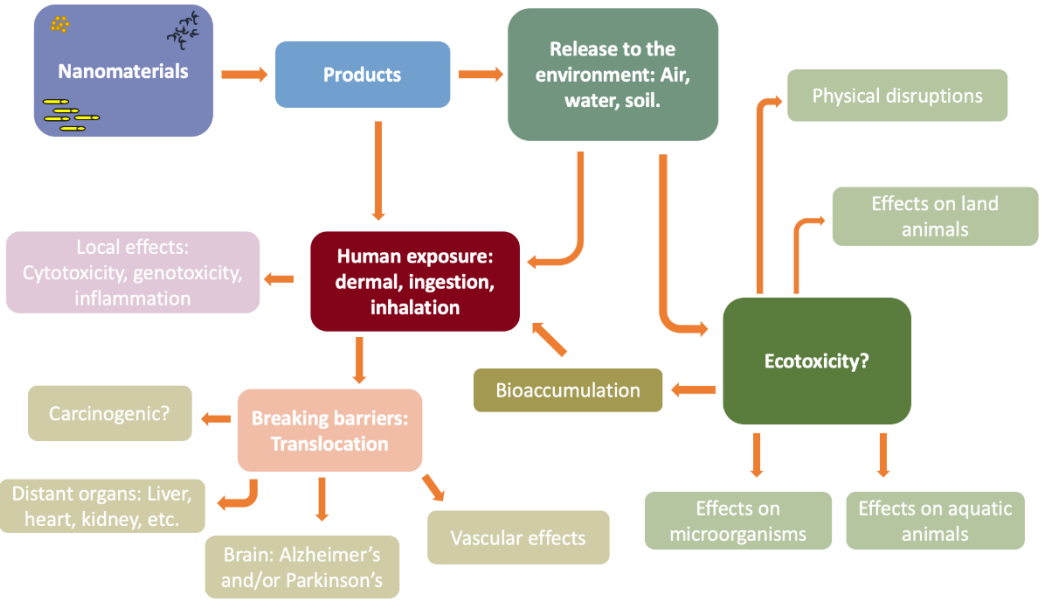

Figure 1. General view of possible interactions, routes of exposure and adverse outcomes that can be triggered by nanomaterials exposure on humans and the environment.

The impact of nanotechnology and nanomaterials is transforming the world, and the following numbers can give us an overview of how these are becoming central players in our economies. In recent years, the development of nanotechnology and the use of nanomaterials have experienced exponential growth, and it is estimated that in 2022 the market of these nanomaterials may reach revenue of 9.1 billion USD in Europe only [20], and other estimations calculate that by 2024 the worldwide market of nanomaterials may reach 125 billion USD [21]. In 2018 there were more than 11,000 new applications for patents related to nanotechnology in the United States and more than 1,700 in the European Patent Office [22].

Since ancient times it has been known that inhalation, ingestion, and skin exposure to certain substances can be deleterious for human health and probably the miners are the best example in history of occupational exposure to materials leading to health problems. Reports of this exist since the time of the Roman Empire [23]. It is told that *those who cannot remember the past are condemned to repeat it* and, in this regard, there has been a growing concern of how nanomaterials could be deleterious for workers that get exposed to these materials [24], hence the need to address their safety. There is also a growing concern

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related to how the final users of these products may be exposed and potentially experience adverse outcomes. In order to tackle these issues, different strategies have been taken to evaluate the toxicity of nanomaterials, which is the first step of assessing the potential risk that workers, final users, and the environment, may encounter. Environmental [25], animal [26], *in vitro* [27] and *in silico* [28] models are used to assess the potential effects of nanomaterials, and many of these models are adaptations of previous strategies used to evaluate chemicals or larger particles. This type of adaptations has created problems of accuracy and confidence on the obtained results, considering that nanomaterials may interfere with the traditional methods.

In this review, our aim is to give an overview of the state of the art of the main approaches related to the evaluation of nanomaterials in relation to toxicity and safety, and what are the emerging strategies related to the field. For this, we present the advances in nanotoxicology, immunotoxicology, epigenetics and genotoxicity, advanced models including organ-on-chip, cheminformatics/*in silico* tools and LCA.

2. Nanotoxicology

The assessment of the detrimental effects of nanomaterials to living organisms, known as nanotoxicology, is still mostly an extension of the conventional methods used to assess the toxicity of other chemicals (including drugs) *in vivo* and *in vitro*. An accurate assessment of the toxicological effects of nanomaterials depends on having good knowledge of their characteristics [29], namely composition, size, shape, dispersity, surface charge, surface functionality, protein corona formation, etc. These are well-known determinants of a materials' ability to cross biological barriers, as well as of their agglomeration/ aggregation status [30], which, in turn, bring additional challenges. These manifest, for example, as interference with the optical readouts or direct chemical reaction with assay components of conventional *in vitro* techniques, among others [31]. Table 1 summarizes reported interferences of nanomaterials with conventional *in vitro* assays and suggests possible solutions for the identified interference.

Table 1. Main interferences from nanomaterials on some of the most widely used conventional toxicological assays identified so far.

Conventional methodology	Observed interference		Proposed solution
	Cause	Result/interpretation	
MTT reduction	NMs optical density; NMs aggregation in cell medium	Falsely increased viability	Sample centrifugation after cell lysis
LDH leakage	NMs redox activity	Falsely decreased viability	None
WST reduction	Protein adsorption to NMs	Falsely decreased cytokine production	Add serum proteins to NMs suspension
ELISA (cytokine release)	Interference enzyme activity	Falsely decreased genotoxicity	None
Comet assay	NMs redox activity	Falsely increased ROS levels	None
ROS quantification (H_2DCF-DA)	NMs quench fluorescence; NMs scatter emitted fluorescence	Falsely decreased ROS levels	Sample centrifugation after cell lysis

NMs: nanomaterials; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; LDH: lactate dehydrogenase; WST: water-soluble tetrazolium salts; ELISA: enzyme-linked immunosorbent assay; ROS: reactive oxygen species; H_2DCF-DA: 2',7'-dichlorodihydrofluorescein diacetate

Besides the methodological issues identified above, the reported exposure/dose metrics is another major concern which has been subject of debate [32]. This is because a mass-based dose metric (e.g., $\mu g.cm^{-3}$), a concept introduced in the context of *in vivo* exposure to airborne particulate matter, is still conventionally used in nanotoxicology. However, this metric experiences substantial impact from small fluctuations dispersity values of the

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nanomaterials, confirming its inappropriateness for the purpose. Reporting the number of nanoparticles or the particles´ surface area, is seemingly more representative in some cases [29,33–36]. In fact, these metrics were found to correlate well with increased mortality [34] and with the occurrence of adverse outcomes such as inflammation, both *in vivo* [36] and *in vitro* [33,35]. More recently, a combination of two criteria, namely particle size together with mass or surface area, was identified as more predictive of the occurrence of lung toxicity derived from nanoparticles, as it allows to overcome the limitations of individual dose metrics [37].

Still, differences in nanomaterials size, density, and surface reactivity entail that they dissolve, settle, diffuse or agglomerate differently as well [38]. Therefore, while the metrics referred above describe well the exposure conditions, they do not adequately reflect the dose of nanomaterials that effectively interacts with tissues or cells. For that, the contact surface area (of the cells in culture or of the exposed tissue *in vivo*) needs to be accounted for, to reflect the delivered dose per surface area or mass [39,40]. Ultimately, this will allow the estimation of the cellular dose with the help of highly sensitive analytical methods and computational tools [38,41,42]. Noteworthy, all the above cited works relate to pulmonary toxicity either *in vivo* or *in vitro*, as the lung is the subject of most of the studies on both particulate matter and nanomaterials (Figure 2). Notwithstanding inhalation being considered the main route of exposure to nanomaterials, these can be translocated to the systemic circulation [43], reaching other organs where they end up accumulating [44–46]. Therefore, it would be important to verify whether similar correlations between the surface area metric and the toxicological outcomes are also observed for other important target organs, such as the liver and the brain, and other exposure settings, types of nanomaterials, and outcomes of interest. The conflicting, sometimes even contradictory, results obtained from different laboratories additionally highlight an urgent need for the development of standard protocols for handling nanomaterials and testing them in biological systems detailed to the level of how to apply nanoparticles on the cell cultures [47]. This would be a crucial step to improve the current status of nanotoxicological assessments.

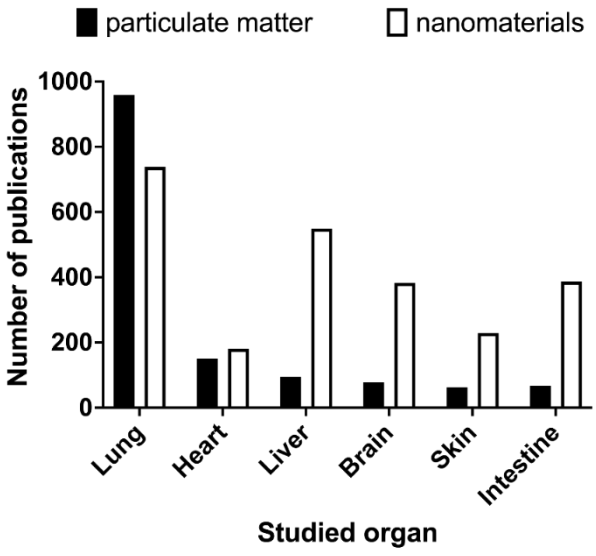

Figure 2. The main target organs studied in the context of (nano)toxicology. A PubMed search for "particulate matter toxicology" or "nanomaterial toxicology" followed by the organ identified in the X axis was performed in April 2022. No other filters were applied. Of note, using the words "pulmonary", "cardiovascular" and "gastrointestinal", instead of the specific organ yields even higher numbers for the 3 categories, but lung-related toxicology still prevails by far.

The levels of exposure to nanomaterials in occupational, accidental, or other scenarios, is yet another fundamental aspect to adequately identify proper dose-ranges to be

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tested for nanotoxicology purposes. For that, a multidisciplinary approach is required in order to develop tools that allow for such characterization. In line with this, the Horizon Europe program has already granted funding to develop low-cost sensors to monitor the levels of ultrafine particles in closed environments [48]. Despite the great step forward, this will still be a conservative approach and an estimated internal exposure-dose would be ultimately desired [49], as not the whole exposure dose will effectively interact with the target tissues. For now, most published studies mainly address acute exposures to unrealistically high doses of nanomaterials. While that data is extremely important to understand the hazard and the mechanisms by which nanomaterials may exert toxicity, the lack of translatability to real-life exposure scenarios fails to convey the real risk posed by nanomaterials. Important first steps are being already taken as attempts to overcome this issue, by undertaking longer-term, repeated exposures to lower-than-usually-tested concentrations of nanomaterials both *in vivo* [50,51] and *in vitro* [52–54]. Moreover, the current investment in the development of new, human-based, more advanced *in vitro* models with prolonged cultivation times (see section 5) will allow for repeated exposures to low concentrations, which would more closely resemble the most common exposure scenarios. Still, we are far from testing realistic conditions, where we are continuously and simultaneously exposed not only to nanomaterials but to many different chemicals, that may synergize, potentiate, or inhibit each other [55]. Thinking forward, predicting internal exposure doses to multi-chemicals mixtures and their effects would be a tremendous achievement for (nano)toxicology. Because it is realistically impossible to experimentally test every nanomaterial type, size, and shape, it becomes fundamental that experimentalists and computational scientists closely collaborate in the search for common terminology, paving the way towards a more predictive nanotoxicology.

A major ambition of nanotoxicology is the understanding of the mechanistic basis behind the adverse effects induced by nanomaterials. From the knowledge collected so far, some mechanisms stand out as the most frequently uncovered for different types of nanomaterials, namely oxidative stress, inflammation, and deoxyribonucleic acid (DNA) damage leading to cell death [45]. Even though the sequence of events is not yet clear or fully understood, it is likely that the overproduction of reactive oxygen species (ROS) is, in fact, the trigger leading to a sequence of events that culminates with cell death (Figure 3).

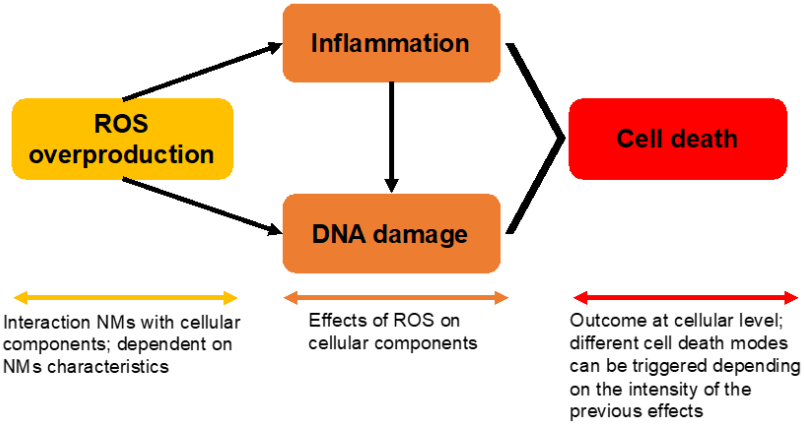

Figure 3. Predominant mechanisms of nanomaterials-induced toxicity identified so far and their presumed interaction.

The described mechanisms have been associated with nanomaterials-induced adverse effects in the liver [56] and the brain [57], among other organs, with important repercussions observed in the offspring as well [58]. Comparatively to the bigger sized homologous materials, the enlarged surface area of nanomaterials comes with increased reactivity, which seems to be the main driver for the observed overproduction of ROS. The

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impact of nanomaterials characteristics on their cytotoxicity is subject of extensive *in silico* studies and is further addressed in section 6.

Together with a robust characterization of the nanomaterials [59], other recognized primary needs for the advancement of nanotoxicology are higher throughput assays and the development of standards and test guidelines to fulfil regulatory demands [60]. Certainly, it is also fundamental to properly communicate the findings to the non-scientific community to avoid a general perception of risk that is yet to be fully substantiated, preventing bias towards fear of and a still unwarranted fight against the use of nanomaterials.

3. Immunotoxicity

As mentioned earlier, the inflammatory response, driven by the innate immune system, plays a major role in nanomaterials-induced toxicity. During the last couple of decades, a wealth of compelling evidence has been gathered to support the idea that immune responses to biomaterials has a great impact on their biological activity and toxicological effects [61–64]. Industries are designing more advanced materials, which means that more workers and consumers will be exposed to nanoscale particles, posing a risk of detrimental effects in natural systems and ultimately to humans. For instance, it is well documented that exposure to fine inhalable pollution particles is directly linked to millions of deaths [65,66] and Shears et al. showed that exposure to diesel exhaust particles increases susceptibility to invasive pneumococcal disease [67]. Yet there are large knowledge gaps regarding the potential long-term health effects associated with these particles. It is imperative to reassess the impact of nanoparticulate material on short- and longstanding detrimental health effects, thus requiring a more in-depth knowledge of nanoparticle interaction with the immune system (Figure 4). Immunotoxicology is a recent subfield of toxicology that studies the deleterious immunomodulatory effects (e.g., immunosuppression, immunostimulation, hyperinflammation) of newly developed and existent (nano)materials. The growing relevance of this field is patent on the recently published technical report ISO/TR 10993-22:2017 that has a subsection devoted to immunotoxicity, on the toxicological evaluation of medical devices that are composed of or contain nanomaterials [68].

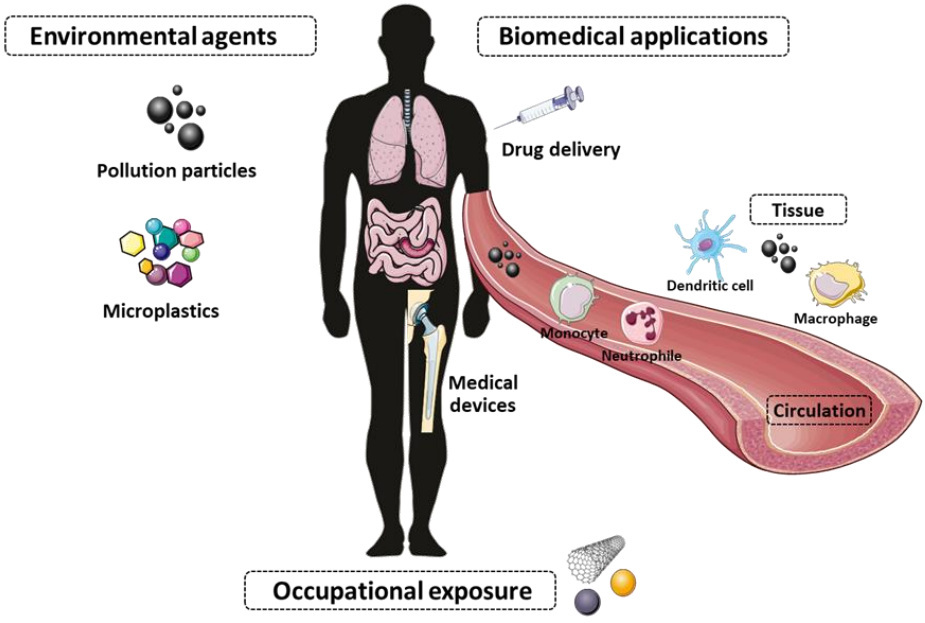

Figure 4. Exposure to nanomaterials activates the immune surveillance system. Nanomaterials used for industrial and biomedical applications or present in the environment, can have a major impact on human, animal, and plant health. If nanoparticles penetrate anatomical barriers, cells of the innate immune system (e.g., macrophages, monocytes), found in circulation or locally in different tissues, recognize them. This may lead to nanoparticle degradation/elimination or modulate the body towards beneficial or detrimental responses. (Servier Medical Art, smart.servier.com)

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While studying the immunomodulatory effect of a nanomaterial is of the uttermost importance to be aware of a possible endotoxin contamination, a fundamental barrier that can cause serious toxic effects in humans [69,70], and may give rise to confounding or even opposite results in the literature. To give an example, one group showed that chitosan had the ability to inhibit lipopolysaccharides (LPS)-induced secretion of proinflammatory cytokines [71], while a separate group observed the reversed effect [72]. However, when using endotoxin-free chitosan, the polymer on its own was incapable of generating proinflammatory cytokine secretion [73]. The release of LPS, present on the outer membrane of Gram-negative bacteria [74], takes place after death and lysis of the cell; since microorganisms are ubiquitous present during nanomaterial preparation, unless precautionary measures are taken into consideration, the risk of inadvertent contamination is high. At the present, the limulus amebocyte lysate (LAL) assay is considered the golden standard to assess and quantify endotoxin content in pharmaceutical products and medical devices, nevertheless there are a few limitations for its application [75]. For that reason, it is important to use a second method to validate our results, such as the monocyte activation test [76], especially in the case of preparations containing nanomaterials, considering that interferences with toxicological assays are a concern [77], as discussed in the previous section.

It is imperative to design more sophisticated *in vitro* models that can better recapitulate complex aspects of the human physiology. This is of particular importance in the cancer field, where it was shown that a staggering 97% of drugs that are on clinical trials fail, as they may not work on the targets researchers intended [78]. On reason that helps to explain these results, and that will be addressed in more detail on section 5, is the use of simplified static two-dimensional (2D), monoculture models (e.g., NCI-60 cell line screening panel [79]), which fail to recapitulate physiologically relevant *in vivo* mechanism, thus worsening *in vitro* translation to clinical data. In this context, inclusion of cells of the immune system (e.g., macrophages, monocytes, lymphoid cells) during preclinical studies, has the huge potential to facilitate the identification of clinical candidates with cytotoxic capacities, minimizing iterative steps or large and prolonged trials, increasing the probability of regulatory success, since many immune cells are involved in events that support tumorigenesis [80]. This is highly appealing for pharmaceutical industry, since low successful translation of a drug candidates in research into an approved product, has a major economic impact [81,82]. Thus, inclusion of immune cells in more preclinical models, should become a routine methodology. Of note, the selection of cells lines as models of cells of the immune system is still challenging. For instance, macrophages have a pivot role in the host response to foreign materials; the 2 most used cell lines for macrophage studies are THP-1 and U937 cells. While they are generally good models to study gene expression and cytokine secretion upon exposure to a myriad of materials, they fail to recapitulate important aspects present in blood-derived macrophages [83,84].

Recently, a connection between nanomaterials and immune training has emerged. Vertebrate immunity is classically divided into innate and adaptive immune responses. The innate system is typically characterized as a non-specific and rapid first line of defense, yet recent findings have challenged this classical view, supporting the idea that the innate immune system is able to be programmed to generate an enhanced non-specific response upon a later challenge, in a process termed “trained immunity”. It has been demonstrated that this protection is achieved through epigenetic and metabolic reprograming which allows for stable, long-term chemical alterations in the DNA that modify the transcriptional potential of a cell by regulating gene expression [85,86]. This idea that innate cells can retain some memory of past immunological insults, allowing for enhanced cellular responses to secondary infections is well documented in the case of microbial stimuli [87,88] and endogenous molecules [89,90], but only recently demonstrated for exogenous molecules [91,92]. Little information is available about the capacity of nanomaterials to promote this phenomenon, making it an exciting field, with immense possibilities. It is fair to hypothesize that more nanomaterials are able to induce innate immune

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cells reprogramming. While trained immunity can prime the body to generate more effective immune responses, it can also have deleterious consequences contributing to hyper-inflammatory states or inadequate responses to future challenges, thus modifying the capacity of an organism to adapt to the environment. Nanomaterials innate training capacity has been overly neglected. Systematically assessing the immune profile generated by distinct nanomaterials attributes and correlate those to the overall impact on immune system, might endow us with the capacity to predict the immunosafety of a novel and existing materials more accurately and lay the basis to generate three-dimensional (3D) models incorporating multiple cell types, including immune cells, that better recapitulate *in vivo* complexity.

Addressing the immunomodulatory abilities of nanomaterials adds scientific and economical value, shedding a light on potential harmful consequences of existing substances, guiding the design of novel and safer materials, providing fertile ground for the development of new therapeutic agents.

4. Genotoxicity and epigenetics

4.1.Nanogenotoxicity

Genotoxicity, the damage in genetic materials, may lead to carcinogenesis and other chronic diseases. If germ cell DNA is compromised, it will affect the individual health and could also have an impact on the next generations [93–95]. Therefore, genotoxicity assessment is considered a crucial aspect of nanomaterial hazard identification. The mode of nanomaterial induced genotoxicity can be classified as direct primary (interaction between nanomaterial and genetic materials directly), indirect primary (nanomaterial induced reactive nitrogen species (RNS)/ROS species affects the genetic materials) and secondary (damage of genetic materials due to nanomaterial-induced inflammation) mechanism [93,96–98]. Oxidative stress has been widely considered as an underlying mechanism of nanomaterial induced genotoxicity [93,95,99,100].

The current state of genotoxicity assessments still depends on those genotoxicity tests designed to screen general chemical agents and are being adapted for nanomaterial (Table 2) and no nanoparticle-specific positive controls have been established [101] in this regard. The genotoxicity assessment mainly includes DNA damage (strand break, adduct formation), gene mutation and chromosomal damage (clastogenicity and aneugenicity) as endpoints [93–95,99]. *In vitro* (human and murine cultured cell lines) system, are the most widely used model, followed by *in vivo* (mainly rats and mice) ones, for nanogenotoxicity assessment [94,99]. *In vitro* models are appropriate for primary genotoxicity assessment, while *in vivo* systems are more suitable for secondary genotoxicity. The ongoing efforts on advanced *in vitro* system (e.g., 3D co-culture of different cell types) development have the potential to simulate the *in vivo* microenvironment and successfully assess secondary genotoxicity [95,96]. In addition, ecotoxicological models, namely, fish [102,103], drosophila [104], nematode [105], yeast, bacteria [106] etc. are also in use for nanogenotoxicity assessment (Figure 5). The largely applied assays for nanogenotoxicity assessment are comet assay followed by micronucleus (MN) assays. The confirmation and standardization of nanomaterials specific genotoxicity assessments are still ongoing process, however, in the interim *in vitro* based comet assays, MN assay, hypoxan-thine-guanine phosphoribosyl transferase (HPRT) assays are recommended to be useful as standard battery test method of nano-genotoxicity assessment with the confirmation of uptake of nanomaterials [94,99,100]. Furthermore, a modified comet with DNA repair enzymes (8-oxoguanine DNA glycosylase (OGG1) and formamidopyrimidine-DNA glycosylase (FPG)) has been used to address oxidative stress-mediated DNA damage [99]. The nanogenotoxicity filed and related test systems continuously evolve, improve [94], and adapt from basic biology. For instance, i) detection of alterations of sensors (genes/proteins) expressions for DNA damage response and check-point pathways (namely phosphorylation of the Ser-139 residue of the histone variant (γH2AX), protein p53 binding protein 1 (53BP1), ataxia-telangiectasia mutated serine/threonine kinase (ATM), ataxia telangiectasia and Rad3 related serine/threonine kinase (ATR), tumor protein p53 (p53), cyclin dependent kinase inhibitor

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1A (p21), checkpoint kinase 1 (CHK1), CHK2; ii) establishment and application of reporter cell lines related to DNA damage response and DNA repair pathways, such as, Tox-Tracker reporter assay [107,108]. The one obvious future potentiality is not only applying new genotoxicity assays but also, up-gradation of existing assays to high-throughput (HT) and high-content (HC) platform, such as, HT comet assay, HT *in vitro* MN assay, and HC γH2AX or 53BP1 assays [107,109]. In addition, the multiplex detection (for e.g., Luminex based platform) of biomarkers from DNA damage response and repair pathways could potentially improve the future nanogenotoxicity assessment. A great diversity in testing strategies, model systems and results were evident in nanotoxicity assessment. Some studies also highlighted that the selection of cell lines could affect the results of the same nanomaterials [99].

Table 2. Current testing strategies for nanomaterial induced genotoxicity assessment

Genotoxicity Marker	Assays	References
Gene mutation	Bacterial reverse mutation (Ames test)	OECD TG 471
	In vitro mammalian mutagenicity assay: Mouse lymphoma (L5178Y) TK+/-assay	OECD TG 490
	In vitro mammalian mutagenicity assay: HPRT assay	OECD TG 476
	In vivo gene mutation assay (transgenic rodent somatic and germ cell gene mutation	OECD TG 488
Chromosomal damage assays	*In vitro* chromosomal aberration assay	OECD TG 473
	In vitro MN assay	OECD TG 487
	In vivo (mammalian bone marrow) chromosomal aberration test	OECD TG 475
	In vivo MN assay (mammalian erythrocyte MN)	OECD TG 474
DNA damage (strand-break and DNA-adduct)	*In vitro* comet assay	JaCVAM EURL-ECVAM/ICCVAM [95,100]
	Modified *in vitro* comet assay with DNA repair enzymes (e.g., OGG1, FPG)	
	In vivo (mammalian alkaline) Comet Assay	OECD TG 489
DNA damage (DNA-adduct)	HPLC/MS; ELISA	[105,110,111]
DNA damage response and repair	The γH2AX and 53BP1 foci count assay	[109,112]
	Multiplex array for DNA repair activity	[110,111]
	FM-HCR assay	[113]

OECD TG: organization for economic cooperation and development test guidelines; HPRT: hypoxanthine-guanine phosphoribosyl transferase; MN: micronucleus; JaCVAM: Japanese center for the validation of alternative methods; EURL-ECVAM: European Union reference laboratory for alternatives to animal testing; ICCVAM; interagency coordinating committee on the validation of alternative methods; HPLC/MS: high performance liquid chromatography mass spectrometer; ELISA: enzyme-linked immunosorbent assay; FM-HCR: fluorescence multiplex–host-cell reactivation.

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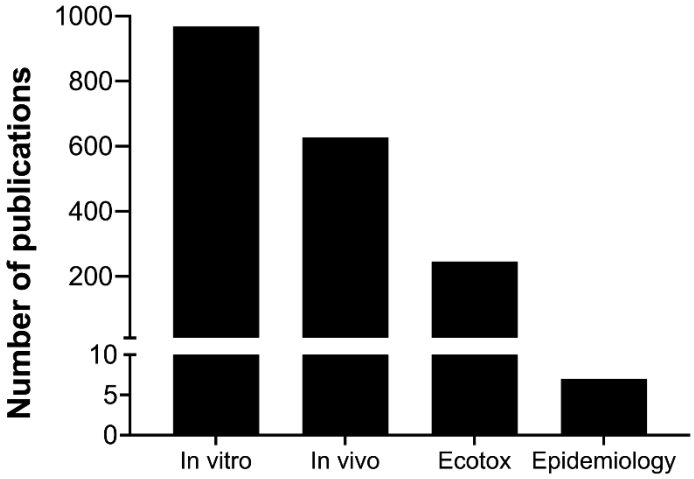

Figure 5: Nanomaterial induced genotoxicity on various model systems (the figure is generated with the numbers of published papers appear in PubMed data base with specific key word search; epidemiology mainly representing 'occupational exposure' related studies; ecotoxicology model species include mainly fish species, drosophila, bivalve molluscs, *C.elegans*, white worms, yeast etc.)

It is noteworthy that most focus has been given to DNA damage (strand break analyzed by comet assay) or oxidative stress mode action (8-hydroxydeoxyguanosine (8-OHdG) detection or modified comet assay with OGG1 and FPG enzymes). The other mode of DNA damage mechanisms, including perturbation of DNA repair or synthesis processes, has been widely neglected in the nanogenotoxicity field [99,113,114]. Altered DNA repair (enhanced or reduced) has been linked to various diseases, including cancer [113]. Among the few studies focused on the adverse effects of engineered nanomaterials in DNA repair process are silver nanoparticles (AgNPs) induced nuclear factor erythroid-related factor 2 (Nrf-2) mediated down-regulation of OGG1 gene expression [115] and affects non-homologous end joining (NHEJ) repair pathway through targeting DNA-dependent protein kinase, catalytic subunit (DNA-PKcs) [116] in human cell lines. In addition, perturbed base excision repair (BER) and nucleotide excision repair (NER) abilities in TiO_2 and AgNP exposed human cell lines were also reported recently [110,111]. In same line of evidence, AgNP exposure affects BER repair pathway (OGG1, nudix hydrolase 1 (MTH1)) in human cell lines and Bis(5'-nucleosyl)-tetraphosphatase (NDX-4) in *Caenorhabditis elegans*, *C.elegans*) as a function of p38 mitogen-activated protein kinases (p38MAPK) [105]. Recently, fluorescence multiplex–host-cell reactivation (FM-HCR) assay system has been applied to evaluate the nanomaterial induced altered DNA repair capacity which can assess all the six major DNA repair pathways in single assay platform [113,117]. Assay development and refinement is vital to understand the underlying mechanism of genotoxicity. The nanogenotoxicity assessment can be improved by integrating the methodological approaches based on the evaluation DNA repair capacity and DNA strand break as well as upgradation to the HT platform.

Understanding the test nanomaterial, their physicochemical properties and interactive behavior in exposed system can be critical to the genotoxic potentiality [97,100]. Hence, the selection of the appropriate assay can be based on the nanomaterial intrinsic materials' characteristics and exposure system-dependent properties [99].

4.2.Nanoepigenetics

Epigenetics is the field of study which investigates the modification of gene expression without changes in DNA sequences. The term 'epigenetics' has been widened by National Institutes of Health (NIH) as "both heritable changes in gene activity and expression but also stable, long-term alterations in the transcriptional potential of a cell that are not

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necessarily heritable" [118]. As the changes can be stable, reversible, or heritable, the exposure/effects of early life can influence later life disease susceptibility or even pass through the successive unexposed generations. The primary epigenetic marks which involve in gene regulations are DNA methylation, histone modifications, and noncoding ribonucleic acid (ncRNAs) (e.g., long non-coding RNA (lncRNA), microRNA (miRNA), small interfering RNA (siRNA), piwi-interacting RNA (piRNA), circular RNA (circRNA), etc.) and chromatin remodeling. Recent studies indicate that the epigenome and epigenetic regulatory mechanisms play a pivotal role in gene-environment interaction to shape the phenotype, including adaptive response and adverse outcomes, including various diseases [119–121]. The alterations of epigenetic biomarkers are highly dynamic and depends on various factors such as, species, exposure, time and even cell/tissue type. Epigenetic biomarkers' dynamic nature and plasticity makes the testing strategies highly challenging. At present, there is no single platform available to screen all the known epigenetic modification to identify the most important or altered epigenetic marker linked to particular chemical exposure. Alterations in targeted epigenetic markers can be measured with existing standard molecular biology techniques and whole genome sequencing (Table 3).

Table 3: Common methodologies for epigenetic endpoints applied for nanomaterial studies

Epigenetic endpoints	Specific epigenetic markers	Analytic methods	References
DNA methylation	Global DNA methylation screening (5mc, 5hmC, 6mA etc.)	HPLC/MS; ELISA; Methylation sensitive comet assay; Pyrosequencing (repetitive sequences LINE-1 or Alu)	[122–125]
	Gene specific promoter methylation	Methylation specific PCR	[122,126]
	Differentially methylated regions (whole genome sequencing)	MPS; DNA methylation specific microarrays; MeDIP followed by sequencing	[122,127]
Histone modification	Whole genome (specific histone marker)	ChIP with DNA microarray, ChIP-Seq, ChIP-Chip	[128,129]
	Gene-specific histone (specific) modification	ChIP-qPCR	[129,130]
	Global histone modification markers	HPLC/MS; ELISA; Immunostaining; Immunoblotting	[131,132]
Non-coding RNAs	Whole genome	RNA-seq, Microarray	[133,134]
	Gene specific	qPCR	[135]

DNA: deoxyribonucleic acid; HPLC/MS: high performance liquid chromatography mass spectrometer; ELISA: enzyme-linked immunosorbent assay; 5mC: 5-methylcytocine; 5hmC: 5-hydroxymethyl cytosine; 6mA: 6-adenine methylation; PCR: polymerase chain reaction; MPS: massively parallel DNA sequencing; MeDIP: Methylated DNA immunoprecipitation; ChIP: Chromatin immunoprecipitation (ChIP); qPCR: quantitative PCR; RNA: ribonucleic acid.

Accumulating evidence supports incorporating epigenetic biomarkers as toxicity endpoints, including nanomaterial risk assessment. Nonetheless, the current knowledge is insufficient in respect of the role of each epigenetic machinery in gene expressions in normal and complex diseased conditions. In other words, there is still lack of clarity on the role of epigenetic biomarkers with the causality of the adverse health outcome [136]. Therefore, better understanding of the nanomaterials adverse effects on epigenome are necessary. Currently, most nanomaterial induced epigenetic toxicity studies focus on DNA methylation changes and its related enzymes machineries. The DNA methylation mainly include 5-methylcytocine (5mC), 5-hydroxymethyl cytosine (5hmC) and 6-adenine methylation (6mA) markers (Figure 6). All the nanomaterials induced DNA methylation alterations were mainly reported about 5mC and 5hmC markers, specifically, two studies

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demonstrated altered 6mA level in carbon black nanoparticles exposed rats [137,138]. The other most studied epigenetic biomarkers are miRNA profiling changes due to nanomaterial exposure (Figure 6). In particular, some studies reported integrative analysis of ncRNA with transcriptome (mRNA) [133,139] or proteomes [140].

Conversely, only a handful of studies focused on histone modification or lncRNA expressions profiling in nanomaterial exposed conditions. The circRNA, another type of ncRNA, is just getting momentum in toxicity field, so as in nanomaterial hazard identification assessment [134,141]. The increasing number of studies demonstrating the epigenetic alterations resulting from nanomaterial exposure still lacks the connection with potential health risks. This is due to the unavailability of epigenetic modifications in epidemiological studies, except only a few related to workplace nanomaterial exposure [124,133,142–144]. Likewise, nanogenotoxicity, epigenetic toxicity of nanomaterials is mostly based on *in vitro* followed by *in vivo* (mice or rats) models (Figure 6). Besides those, DNA methylation studies were also reported in ecotoxicology models species (such as white worms [145] and zebrafish [146,147]), while histone modification [148] and ncRNA (lncRNA and miRNA) were mainly reported in nanomaterial exposed roundworm (*C. elegans*) (Figure 6). In the epigenetic study design, one must consider the development of long-term impact with multigenerational or transgenerational effects, preferably with alternative *in vivo* models (e.g., zebrafish, drosophila, and *C. elegans* etc.) [148] to reduce animal use. Furthermore, epigenetic alterations in adverse effects vs adaptive response in nanomaterial treated conditions also need to be considered; however, it can only possibly be achieved with future technological advancement and improved testing strategies.

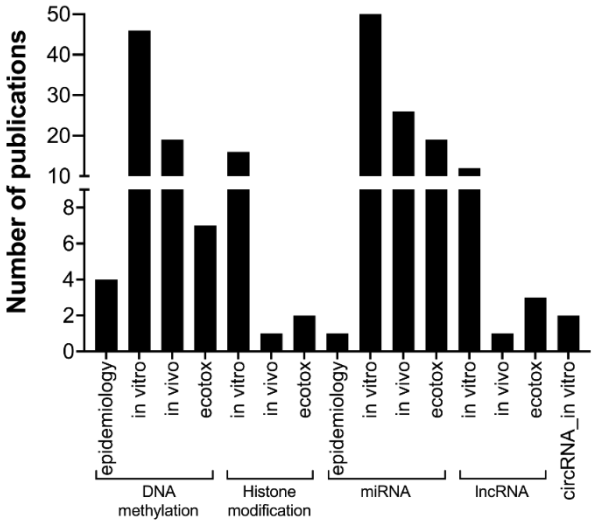

Figure 6: The nanomaterial induced alterations in different epigenetic biomarkers based on various model systems (the figure is generated with the numbers of published papers appear in the PubMed database with specific keyword search; epidemiology mainly representing 'occupational exposure' related studies; in ecotoxicology model species include mainly zebrafish, yeast and *C.elegans*)

In summary, integrating epigenetic endpoints in nanomaterial risk and safety assessment, with all its current limitations and existing challenges, needs considerable attention. However, a sufficient amount of reproducible data is required on the causal relationship between the nanomaterial exposure, adverse phenotype, and the specific epigenetic marker [95,149]. Therefore, we have to travel a long way to select the most representative epigenetic biomarkers to be evaluated with the most reliable testing strategies in the best model system for nanomaterial safety assessment.

 doi:10.20944/preprints202205.0229.v1

5. Advanced models for *in vitro* testing

5.1. 3D cultures

Although animal studies are considered physiologically relevant, their limited predictability, longer experimentation times, high costs, lack of high-throughput screening associated with the implementation of the 3Rs (reduction, replacement, and refinement) principle and novel regulations that ban animal experimentation (e.g., in the cosmetics field) led to the need of developing advanced *in vitro* models [150–153]. So, worldwide research groups are dedicating efforts to develop a new generation of advanced *in vitro* models capable of recapitulating organ functions becoming effective tools for toxicology, pharmacology (investigate drug metabolism, pharmacokinetics, and toxicity) as well as for the mechanistic understanding of organ physiology and pathophysiology [151,154–156]. Several promising advanced models have been already established addressing superior physiological relevance, correlation, and validation against *in vivo* models [150–152,154,155,157–159]. However, it is important to stress that the priority in models' development was not for nanosafety purposes.

Nanomaterials safety assessment starts traditionally with *in vitro* cultures using 2D systems that cannot mimic events observed in 3D structures as native tissues [153]. Actually, the significant biological barriers and organs for nanotoxicological studies are the skin for dermal exposure, the gastrointestinal tract for oral uptake, lungs (bronchial and alveolar epithelium) for inhalation, and endothelium for intravenous exposure, while liver, lung, kidney, bone marrow and spleen are important organs to study nanomaterials accumulation [150,151,153]. It is widely reported that 3D models provide a closer and more realistic *in vivo*-like approximation being more sensitive to identify cellular responses to nanomaterials, wherein information on barrier penetration and translocation capabilities can be accomplished [151]. In general, 2D cell monolayers usually overestimate the extent of nanotoxicity whereas the 3D models provide a closer and more realistic *in vivo*-like approximation. An interesting example is skin organotypic 3D models that are already commercially available (e.g., EpiDerm™, epiCS®, EpiSkin™ and SkinEthic™) in which several Organization for Economic Cooperation and Development (OECD) guidelines are employed to evaluate chemicals safety. These seems to be suited to the assessment of skin-related nanomaterials risk [160]. Three-dimensional skin models exposed to nanomaterials provided more realistic analyses with lesser nanomaterials penetration due to an enhanced barrier function [159,160]. Oral epithelium and urogenital tract tissues are already commercially available; however, few companies provide relevant essential organs for nanomaterials safety such as the liver, kidney, respiratory epithelium, and intestinal epithelium [150–154,161]. An amazing effort was done in a European Union (EU) project that was fully dedicated to the development of reliable and robust physiologically anchored tools for nanomaterial hazard assessment (PATROLS). The developed advanced models included the lung (co-culture constituted of immune cells, interstitial cells, or barrier cell types using permeable transwell membrane inserts), intestine (cell triple co-culture differentiated epithelial and immuno-competent macrophage-like cell line), and liver (hepatocyte-based microtissue and a HepG2 human hepatocyte spheroid) that were specially fabricated to allow cytotoxicity, genotoxicity, and inflammatory responses to nanomaterials in more realistic exposure scenarios [162].

Spheroid and organoid technology have already started to contribute to nanotoxicity assessment, seemingly filling the gap between 2D and *in vivo* models demonstrating a good predictive value and significant data correlation with clinical trials (in the case of nanomaterials for therapy) [151]. Liver, kidney, brain, skin, lung, and intestinal organoids among others were already established and they are also considered promising tools to predict nanomaterials toxicity to organs [151]. They provide organotypic cytoarchitectures with a spatially organized structure, *in vivo* phenotypic and extracellular matrix (ECM) expression mimicking important cellular functions such as cell migration, differentiation, and apoptosis among other advantages [159]. In nanotoxicological studies organoids seem to offer a barrier to nanomaterial distribution and cytotoxicity [153,163].

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Lately, attention is being paid to the development of patient-specific and disease realistic models that illustrate vulnerable individuals within the populations. Excitingly it was observed that the diseased hepatic spheroid model is more sensitive to nanomaterials toxicological stress than the equivalent healthy models [164]. Alternatively, 3D biomaterial-based models also present multiple benefits, since they are constituted by synthetic or naturally derived biomaterials, mimicking cell-ECM interactions [157,165,166]. With the latest improvements in materials science, microfabrication techniques and bioreactor-based spheroid and organoid technology [154], new efforts are being made to combine self-organizing 3D biological structures into organ-on-chips (OoCs) to emulate both the structural and dynamic complexity of tissues and organs [154,159,167–169].

5.2. Organ-on-chip

OoCs are one of the top ten emerging technologies considered a revolutionary tool that may substitute animal experimentation in the future [154,155,169]. In recent years, microfluidic devices have demonstrated tremendous potential for developing *in vivo*-like cellular or tissue structures on a chip that may be leveraged for examining the safety assessment of nanomaterials in highly dynamic conditions. OoCs are normally designed using a reductionist approach (focus on key cellular constituents, structural organization, and biochemical and/or mechanical cues of the basic anatomical element responsible for organ/tissue function) with the versatility to increase the complexity of the system [168]. They employ bioengineering technologies to organize cells into "tissues" and facilitate fluid flow with the goal of constructing miniaturized tissue/organ testing devices that try to mimic the human body in all aspects (e.g., function, metabolism, architecture) [155]. They provide a 3D microenvironment that can be constructed with the aid of biocompatible materials (support cell growth) that together with biomechanical and biochemical cues and occasionally electrical signals are synchronized to model *in vivo*-like responses [156]. OoCs began to emerge at the turn of the century when Ingber et al., demonstrated the essential elements for lung organotypic function, showing that nanomaterials uptake from the air interface only occurred with the application of cyclic stretch [170]. A decade later, great advancements were seen in OoCs models as a result of the major developments in microfabrication technologies, sensors, imaging, and biology. Currently available OoCs examples are the brain, heart, lungs, liver, gut, pancreas, kidneys, skeletal muscle, adipose tissue, skin, cornea, cervix, amnion, placenta, blood vessels and bone [156]. Numerous of these models comprise two overlapping perusable channels separated by a polymeric permeable membrane, which permits the culture of two or more cell types in fluidically independent chambers [168].

Although significant advances were already observed in the past decades, there is still a significant lack in using OoCs to understand key biological mechanisms related to nanomaterials exposure and uptake, with most studies remaining in the proof-of-principle stage (Table 4). OoC examples of skin, lung, and gut are being used to test nanomaterials entrance, liver and kidney for metabolism and clearance as well as bone- marrow, blood-vessel, and spleen as toxicity-susceptible organs [154]. Microfluidic-based systems seem to be a versatile tool allowing the recreation of physiologically relevant measurement conditions, and more relevant the nonstop monitoring of nanomaterials adverse effects, maintaining stable suspensions during cell exposure [152]. Scientific evidence proposes that biochemical and biophysical (e.g., stretch/strain forces for actuated tissues, or hemodynamic shear forces for vascular tissues) [167] cues formed in the complex biological microenvironment can have a profound impact on nanomaterials comportment, which are not considered in most of the conventional 2D *in vitro* models [154].

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Table 4. Single and multiple-organ-on-chip models employed in nanomaterials' safety assessment.

Advanced cell models	Cell types	Nanomaterials exposure conditions	Sensorization	Toxicological assays	Key biological outcomes
Heart microphysiological system	NRVMs	TiO_2 NPs at 10 and 100 $\mu g.mL^{-1}$ and Ag NPs at 50 $\mu g.mL^{-1}$	Electrical sensors	LDH assay, MTT assay	The high-dose exposure of TiO_2 NPs (100 $\mu g.mL^{-1}$) demonstrated impaired contractile function and damaged tissue structure after 48 h of exposure. Ag NPs exposure caused cytotoxicity [171]
Blood-brain-barrier-on-a-chip	HAs and HUVECs	INPM exposure at 0, 5, 10, 20, and 40 $\mu g.mL^{-1}$	---	ROS detection assay, CCK8 assay	The INPM could potentially activate several inflammatory pathways that directly damage brain structures which further lead to neurological diseases [172]
Liver-on-a-chip	PRH	10 nm Fe_3O_4 NPs	---	---	Perfusion of Fe_3O_4 NPs results in the reduction of albumin and urea production, indicating potential liver injury [173]
Lung-on-a-chip	HPAEpiC, HUVECs, and THP-1	PM2.5 exposure at 200 and 400 $\mu g.mL^{-1}$	---	Immunofluorescence staining assay, FITC-dextran permeability assay, ELISA	A low concentration of PM2.5 causes limited cytotoxicity, but a higher concentration of PM2.5 (>200 $\mu g.mL^{-1}$) could significantly increase the ROS generation, apoptosis, and inflammation responses of epithelial cells and endothelial cells on the barrier and attachments of monocytes to the vessels [174]
	BEAS-2B and HUVECs	CSE at 10, 20, and 50 $\mu g.mL^{-1}$	---	RT-PCR, ELISA, western blotting	Lung-on-a-chip enables the study of nanoparticle adsorption during various breathing frequencies, puff profiles of smoking, breath-holding patterns during inhalation and exhalations and the particle deposition in the lungs and the respiratory tracts [175]
Placenta barrier-on-a-chip	BeWo	20 nm SiO_2 and TiO_2 NPs, and 80 nm ZnO NPs for 24 h	Membrane-bound impedance sensor array	ROS detection assay	SiO_2 and TiO_2 NPs induced no loss in barrier integrity. In contrast, ZnO NPs displayed severe acute cytotoxicity already after 4 h [176]
	BeWo and HUVECs	TiO_2 NPs exposure at 50 and 200 $\mu g.mL^{-1}$	---	Immunofluorescence staining assay, ROS detection assay	Gradually increased cell death with increasing concentrations of NPs, thereby potentially leading to placental membrane rupture [177]
Gut/Liver-on-a-chip	Caco-2, HT29-MTX + HepG2, C3A	50 nm carboxylated PS NPs	---	AST assay	Gut/liver chip model demonstrates compounding effects of inter-organ crosstalk between gut and the liver in facilitating NP toxicity [173]
Lung/Liver/Kidney-on-a-chip	A549 + HepG2 + TH-1	Ag, Au-PEG, TiO_2, and SiO_2-FITC NPs	TEER measurements	Live/dead assay	The interconnection of the different modules aims at the simulation of whole-body exposure and response. SiO_2-FITC NPs showed a cytotoxic effect on TH-1 after 12 h, which could be due to the interaction of NPs with cancerous cells releasing a substance that may have induced a cytotoxic effect [178,179]

NRVMs: neonatal rat ventricular myocytes cells; TiO_2: titanium dioxide; NPs: nanoparticles; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; LDH: lactate dehydrogenase; HAs: human astrocytes cells; HUVECs: human umbilical vein endothelial cells; INPM: indoor nanoscale

 doi:10.20944/preprints202205.0229.v1

particulate matter; ROS: reactive oxygen species; PRH: primary rat hepatocytes cells; CCK8: cell counting kit 8; Fe3O4: iron oxide; HPAEpiC: human alveolar epithelial cells; THP-1: human acute leukemia monocytic cells; PM2.5: fine inhalable particles, with diameters less than 2.5 μm; FITC: fluorescein-5-isothiocyanate; ELISA: enzyme-linked immunosorbent assay;BEAS-2B: human bronchial epithelial cells; CSE: cigarette smoke extract; RT-PCR: reverse transcription polymerase chain reaction; BeWo: human choriocarcinoma cells; SiO_2: silicon dioxide; ZnO: zinc oxide; Caco-2: human colorectal adenocarcinoma cells; HT29-MTX: human colorectal adenocarcinoma cells with epithelial morphology; HepG2: human liver hepatoma cells; C3A: clonal derivative of HepG2; PS: polystyrene; AST: aspartate aminotransferase activity; PEG: polyethylene glycol; A549: adenocarcinoma human alveolar basal epithelial cells; TH-1: Type 1 T helper cells; TEER: transepithelial electrical resistance

Modulating the fluid flow in the model by a microfluidic device can assist to enhance the bioavailability of the nanomaterials to the cells leaving little opportunity for off-target nanomaterials deposition since localized nanomaterials deposition induces ROS production and inflammatory response leading to toxicity [168]. Although the presence of flow plays a pivotal role in nanomaterials toxicity in the human body, the interaction of nanomaterials with cells and their uptake and translocation under continuous perfusion is not clear and even controversial [169]. The high variety of nanomaterials, the lack of standardization in nanomaterials preparation and dosage control together with alteration in the volume of the cell culture media, and cell number cause the controversial and significant discrepancies in nanomaterials safety assessment in microphysiological models [152]. Efforts should be done to improve *in vitro* oriented simulation and optimize fluidic design considering the physicochemical properties of nanomaterials [152,154,169]. Another challenge is the small sizes of the chip associated with nanomaterials′ surface reactivity which can lead to nanomaterials surface adsorption on the small size of the channels. Nanomaterials absorption into the device can result in a misrepresentation of nanomaterials toxicity due to the reduction of nanomaterials concentration and consequent concentration-response interpretation. Alternative polymeric materials or chemical modifications of the chip surface are being proposed to minimize nanomaterials adsorption [169].

5.3. Multiple-organ-on-chip

Nowadays technological innovations are conducting the scientific community in developing multiple-organ-on-chip (MOoCs) devices. These are constituted by multi-tissue fabricated using two or more tissue chips or by incorporating multiple interconnected chambers representative of tissue/organ on one chip to recapitulate communications among different tissues [154]. Integrated multi-organoid models have been proposed trying to mimic complex procedures of metabolism and responses at the multi-organoid level, where flow rates resemble blood circulation in the human body [180]. Therefore, MOoCs can be exploited to evaluate nanomaterials systemic toxicity from the dynamic process of distribution, absorption, metabolism, and excretion features, nonetheless, systemic predictions are still challenging [158,159]. Future studies should take advantage of the development of engineered perfusable vasculature into microphysiological platforms making it possible to mimic the delivery of nanomaterials in a more physiologically relevant way, granting for accurate prediction of their performance *in vivo* [154,168]. We believe that in the near future these platforms will provide new insights not only into systemic nanomaterials effects on different organs but also their metabolites and subsequent secondary toxicity.

Besides many other advantages, MOoCs models might allow nanotoxicity signatures at metabolite, protein, or gene levels that can be explored by combining them with diverse cutting-edge analytical approaches (e.g., fluorescence methods, microfluidics, artificial intelligence, multi-omics, and single-cell analyses) [159]. The capability to integrate parallel streams on the same chip will allow high-throughput screening, as well as will decreases analytes and the overall testing time. Efforts should also be made to explore how single-

 doi:10.20944/preprints202205.0229.v1

cell analysis and bioinformatics tools can advantage the mechanistic knowledge of adverse biological responses to nanomaterials in the physiological context of OoC and MOoCs models.

5.4. Sensors integration with microphysiological models

The advancement of the *in vitro* models used for nanotoxicology calls for the monitorization of important parameters related to the organ that is being emulated. Most approaches explored so far rely on off-chip and endpoint measurements, and imaging, therefore, not taking full advantage of all the possibilities offered by OoC platforms [181]. The integration of sensing strategies to enable real-time and *in situ* analysis of biological molecules, and continuous detection of cellular functional changes, boosts the utility of OoC systems. Additionally, integrated sensing technologies offer label-free, non-invasive approaches, with multiplexing, customization, and automation potential.

While most of the integrated sensing OcC platforms have been developed for the purpose of physiology or disease modelling (including cancer) and drug testing, their application to (nano)toxicology is possible and foreseen. So far, the focus has been mostly placed on the integrated monitoring of the microenvironment conditions of the OoC, such as temperature, pH, and oxygen levels, as well as of the establishment of a cohesive barrier function (transepithelial electric resistance (TEER)) (Table 4) [171,176,178,182–185]. Interestingly, HT measurements of some of these parameters have recently been achieved [186]. The analytical techniques mostly employed for these purposes are electrical (e.g., for TEER), electrochemical (e.g., for oxygen and pH), and optical (e.g., also for oxygen and pH), even though the latter ones rely on the use of indicator dyes [187]. The study of the metabolic function and the real-time release of tissue biomarkers in OoC provides evidence of the tissue dynamics, and their functional competence and maturity. The monitorization of some parameters, such as glucose levels or lactate production, supplies useful information regardless of the organ under analysis [188–190]. On the other hand, for certain tissues, the secretion of specific biomarkers benchmarks their utility for specific ends. For example, the online monitoring of transforming growth factor beta (TGF-β) production has shed light on the role of the communication between hepatocytes and stellate cells on the onset of liver injury [191]. Likewise, the continuous tracking of albumin, glutathione-S-transferase, as well as creatine kinase-myocardial band production from a human heart-liver-on-chip platform shows great potential to understand the crosstalk between both organs [192]. More recently, measuring insulin secretion in a pancreatic islet-on-a-chip *in situ* supports the application of the device for diabetes-related research [193]. These are representative examples of the infinite possibilities offered by OoC with integrated sensing abilities, which are expected to cover any secreted biomarkers of relevance in the near future. Within the (nano)toxicology field, in particular, extracellular biomarkers of adversity can be targeted and tracked in real-time, preferably in medium- to HT formats. Their continuous footmark can help unravel the mechanisms by which nanotoxicity is triggered and propagated. Thus far, most of these advanced human-based sensing devices have been largely put to the test with drugs, so it seems a good time to start challenging them with nanomaterials.

6. *In silico* tools in nanotoxicology

As has been previously mentioned, nanotoxicology and engineered nanomaterials production have seen rapid advancements and innovation over the last two decades. Despite the continuous effort from the scientific community, understanding the potential environmental and human health hazards of engineered nanomaterials still is a crucial challenge. In this regard, one of the current research frontiers in nanosafety re-lates to the use of *in silico* tools, such as quantitative structure-activity relationships (QSAR) and the closely related quantitative property-activity relationships (QSPR) models. Such research interest is explained by the ability of QSAR/QSPR models to determine the relationship between an endpoint/target variable, i.e., biological activity or property, and some relevant structural characteristics (designated as descriptors) of the system.

 doi:10.20944/preprints202205.0229.v1

Overall, the classical QSAR/QSPR workflow concerns (i) compilation of data from public databases or experimental procedures, (ii) data curation, (iii) descriptor calculation, (iv) model construction, and (v) statistical measures [194]. As in other fields related to nanosafety, the development of QSAR/QSPR models has been guided by a trade-off between predictive performance and interpretability. On one side, the model can comprise trivial molecular descriptors, such as physicochemical properties, and present a simple learning method such as linear regression [195]. On the other side, the model can contain continuous and data-driven molecular descriptors, which offer high predictive power, and present complex black-box approaches such as artificial neural networks [196]. Currently, it is argued that a simple and informative model is more valuable to experimentalists [194].

Given that the developed models have been mainly applied to small molecules and traditional materials, the application of this process to nanomaterials requires adapting the methodologies to a higher level of complexity. One of the possible avenues to fulfill this requirement is applying new computational representations that include the necessary information to describe the structure and composition of such complex systems. Besides, as it is realistically impossible to test every nanomaterial experimentally, it is paramount to pave the way to a more predictive nanotoxicology. Therefore, Table 5 summarizes the most recent nanotoxicology predictive models focusing on the impact of nanomaterials' properties, structure, and composition on their cytotoxicity and inflammatory potential. As a complement, a dynamic visual representation (Figure 7) was obtained by GEPHI (software version 0.9.2). The detailed pipeline to design the network is described elsewhere [197]. Overall, the network focuses on (i) the type of nanomaterial, (ii) descriptors, and (iii) the used QSAR model to predict a defined endpoint.

Table 5. Overview of the most recent nanotoxicology predictive models. A PubMed search for "QSAR and nanoparticles" from 2020 to 2022 was performed in April 2022. No other filters were applied.

Nanomaterials	Descriptors	Models[1]	Main Goal
FD	204 molecular descriptors generated from the QSAR analyzing tools of BIOVIA Discovery Studio	LinReg	Predict the physicochemical properties of FD that promote their cytotoxic effects/anticancer activity [198].
Metal NPs	24 physicochemical descriptors and toxicity data	MLR	Predict the toxicity and design the structures of metal NPs with low toxicity [199].
Metal oxide NPs	61 periodic table descriptors	MLR	Predict and investigate the essential descriptors responsible for the cytotoxicity of metal oxide NPs on *E. coli* cells under different conditions [200].
Gold NPs	Structural information (i.e., Dragon descriptors) of the surface ligands	MLR	Predict possible relationships between the oxidative reactivity of gold NPs and their cytotoxicity [201].
Carbon NPs	Physicochemical descriptors (molecular weight, overall surface area, volume, specific surface area, and sum of degrees)	Orthogonal PLS regression	Predict the interaction between carbon NPs and SARS-CoV-2 RNA fragment [202].
Amine-containing heterolipids NPs	116 physicochemical descriptors	PLS regression coupled with stepwise forward algorithm	Predict the pKa of the amine-containing heterolipids NPs [203].

[1] All models included follow the standardization and validation principles established by the Organization for Economic Cooperation and Development (OECD) [194].

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Metal oxide NPs	Quantum-mechanical computations (such as molecular geometries), physicochemical descriptors (such as zeta-potential in water), and periodic table descriptors (such as electronegativity of each atom).	PLS regression, decTrees, SVM, and logReg	Predict the inflammatory potential of metal oxide NPs [204].
Functionalized magneto-fluorescent NPs	Norm index descriptors (describe the structure characteristics of the involved NPs)	RF	Predict the cellular uptake of functionalized magneto-fluorescent NPs to Pa-Ca2 cells. Provide guidance for the design and manufacture of safer nano-materials [205].
Metal and metal oxide NPs	Structural information (such as core structure and material type), supported by physicochemical descriptors (such as zeta potential, average agglomerate size in media, among others)	decTrees, GBR, KNN, linReg, RF, SVM, and XGBoost	Predict the cytotoxicity of metal and metal oxide NPs in Zebrafish embryos [206].
Virtual carbon NPs library	126 nanodescriptors (such as electronegativity of each atom)	KNN and RF	Predict cytotoxicity and inflammatory responses induced by PM2.5 [207].
Functionalized magneto-fluorescent NPs	Improved optimal quasi-smiles-based descriptors	MC	Predict the cellular uptake of functionalized magneto-fluorescent NPs to Pa-Ca2 and HUVEC cell lines [208].
Gold NPs	Optimal quasi-smiles-based descriptors	MC	Predict the cellular uptake of gold NPs to A549 cells [209].
Functionalized magneto-fluorescent NPs	Optimal quasi-smiles-based descriptors	MC	Develop self-consistent predictive models for the cellular uptake of functionalized magneto-fluorescent NPs to PaCa2 cells [210].
Metal oxide NPs	Optimal quasi-smiles-based descriptors	MC	Predict the cell viability of different cells lines when exposed to metal oxide NPs [211].
ZnO NPs	Optimal quasi-smiles-based descriptors	MC	Predict the toxicity of ZnO NPs in rats via intraperitoneal injections [212].
Metal oxide NPs	Optimal quasi-smiles-based descriptors	MC	Predict the cell viability (expressed in %) and cytotoxicity (categorized as true or false) of different cell lines when exposed to 7 types of metal oxide NPs [213].
Cadmium QD	Optimal quasi-smiles-based descriptors	MC	Predict hepatic cells viability when exposed to cadmium QD [214].
FD	Structural information (such as polarizability), optimal quasi-smiles-based descriptors, and physicochemical properties (obtained from data warrior)	MC and CPANN	Predict the binding scores activity for 169 FD related to 5 proteins classified as anti-diabetes targets [215].
Metal-based nanomaterials	Optimal quasi-smiles-based descriptors	MC	Predict the response of *Daphnia magna* when exposed to metal-based nanomaterials [216].

FD: fullerene derivatives; QSAR: quantitative structure-activity relationships; NPs: nanoparticles; MLR: Multiple linear regression; GNP: gold nanoparticle; CNP: carbon nanoparticles; PLS: partial least squares; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; SW-PLSR: PLS regression coupled with stepwise; SVM: Support vector machine; RF: random forest; PaCa2: pancreatic cancer cells; KNN: k-nearest neighbors; MC: Monte Carlo; HUVEC: human umbilical vein endothelial cells; MO: Metal oxide; ZnO: Zinc Oxide; CPANN: Counter Propagation Artificial Neural Network

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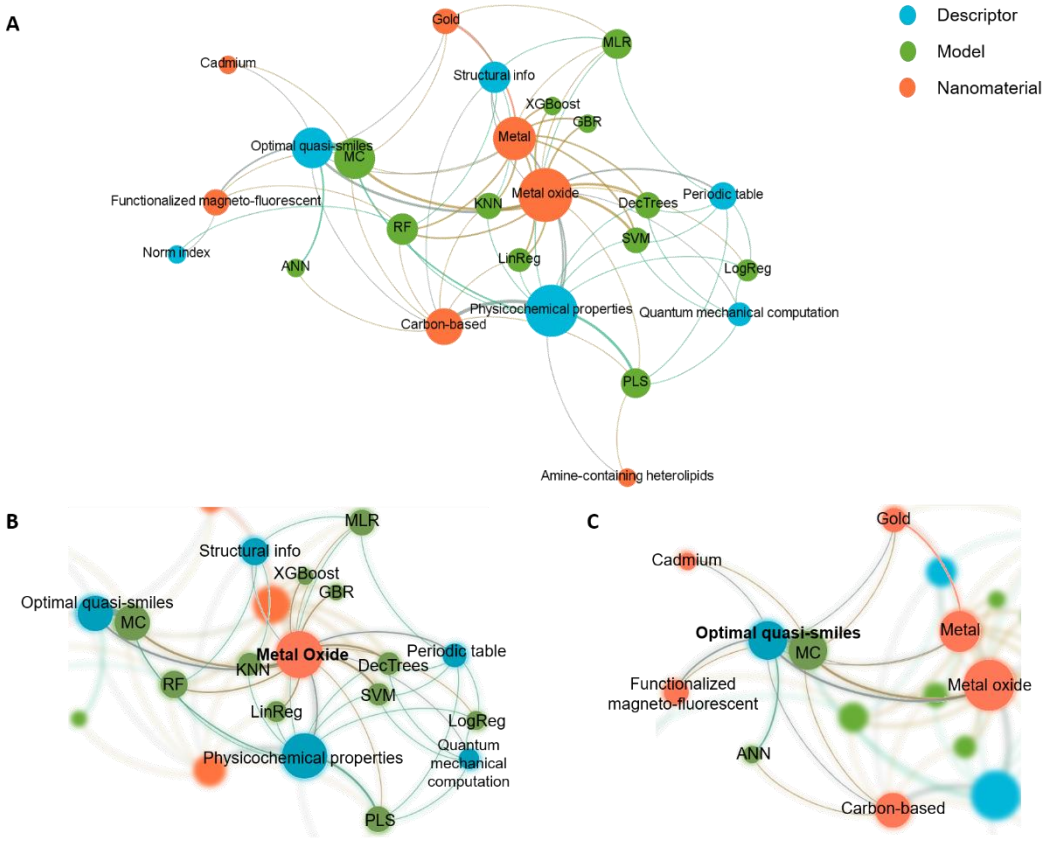

Figure 7. Graphical network of the 19 articles that meet the criteria to be included in Table 1. (A) Network with all the connections between the type of nanomaterial, their relevant structural characteristics (descriptors), and the QSAR model used to predict a defined endpoint. (B) Most relevant connections of metal oxide class. (C) Most relevant connections of optimal quasi-smiles-based descriptors and the closely related Monte Carlo (MC) algorithm. ANN: artificial neural networks; DecTrees: decision trees; GBR: gradient boosting regressor; KNN: k-nearest neighbors; LinReg: linear regression; LogReg: logistic re-gression; MC: Monte Carlo; MLR: multiple linear regression; PLS: partial least squares; RF: Random Forest; SVM: support vector machine; XGBoost: extreme gradient boosting.

As a first interpretation of the network (Figure 7A), it is possible to identify that metal oxide, metal, and carbon-based nanomaterials are currently the most studied. Regarding the descriptors, the most appealing are the physicochemical properties (such as molecular weight and octanol-water partition coefficient), the optimal quasi-smiles (i.e., a sequence of symbols that represent the nanomaterials by their properties and the experimental conditions involved in their experiments), and the structural information (namely the electronegativity of each atom). Concerning the models, the complexity varies from linear regression to an artificial neural network. Nonetheless, the most used models are Monte Carlo (MC), multiple linear regression (MLR), and partial least squares (PLS) regression.

By taking advantage of the interactivity of the network, it is possible to highlight a specific class and understand the existing connections between the nanomaterial type, the descriptors, and the model used to predict a defined endpoint. Hence, Figure 7B highlights the metal oxide class and shows that several descriptors successfully represented such nanomaterials. Moreover, it identifies that this information set allowed to train and implement several QSAR predictive models, going from linear regression to tree-based ensemble algorithms such as decision trees and random forest.

As a representative example, Roy, J. et al. [200] used the second-generation periodic table-based descriptors to represent metal oxide NPs, such as TiO_2 and ZnO. Ultimately, an MLR model was trained and used to predict the cytotoxicity of the nanoparticles on

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Escherichia coli under different conditions, achieving an R^2 of $\approx$ 0.77. Additionally, Figure S1A and Figure S1B highlight the metal and carbon-based nanomaterials, respectively.

As mentioned above, one of the exciting approaches to represent nanomaterials is to use quasi-smiles. Such research interest is explained by the ability of quasi-smiles to encode the structure, composition, and physicochemical properties of a specific nanomaterial. Moreover, if reasonably, quasi-smiles can also encode the experimental conditions involved in the nanomaterials experiment. Thus, quasi-smiles have become a simple but powerful tool to represent nanomaterials to QSAR approaches. In this regard, Figure 8C highlights the optimal quasi-smiles-based descriptors and the closely related MC algorithm, showing that quasi-smile has been used to represent metal oxide, metal, and carbon-based nanomaterials. As a representative example, Toropova et al. [209] developed an MC model to predict the cellular uptake of gold NPs by A549 lung carcinoma cells. To do so, gold NPs were represented by quasi-smiles that considered the size of the NPs, the first and second ligand interacting with the NPs, and the cellular uptake in A549 cells. Then, these optimal descriptors were collected and used to train an MC model, achieving an R^2 of 0.7-0.9.

In summary, the most recent works covering predictive nanotoxicology and different representations of relevant structural characteristics of nanomaterials have been briefly presented and discussed in this section. Interestingly, it is noticeable that most of the works focus their attention on nanoparticles. Despite being a recent frontier in nanosafety, the *in silico* tools have been increasing their predictive efficiency to specific problems and consequently the popularity among the scientific community. Nonetheless, achieving a broader predictive nanotoxicology, where an *in silico* tool can be applied to different problems with similar efficiency, is still a crucial challenge. To overcome such a challenge, the scientific community needs to reinforce the relationship between experimentalists and computational scientists and to start or keep implementing the FAIR - Findability, Accessibility, Interoperability, and Reusability - data principles [217]. Currently, there are interfaces, such as eNanoMapper [218], that provide and collect FAIR data to support nanosafety assays. Following these principles and using the data collection templates makes it possible to collect experimental data standardly. Consequently, the amount of data would exist in greater quantity and diversity, which would allow the development of *in silico* tools with a broader domain of applicability.

7. Life Cycle Assessment and nanosafety

The current paradigm of the nanotoxicology, which relies mainly on *in vitro* and *in silico* models, is aiming to develop safe nanomaterials at an early stage of the innovation process in order to minimize potential human and environmental hazards. Therefore, as previously stated, it is imperative to ensure that the generated data is FAIR and accessible, so it can be used in an efficient away, especially bearing in mind the current expectations for safer and sustainable emerging technologies. This transition is crucial, and it has been rising in the last years, as a result of the ambitious roadmap of the recent EU policy initiatives and the sustainable development goals of the United Nations. The plan involves a recognized demand for a competitive system on the efficient use of resources and energy, while minimizing emissions and waste production, simultaneously promoting a circular economy [219–221].

Nanotechnology is an emerging field promising innovative solutions in a wide range of applications that are expected to greatly contribute to a sustainable development [222]. Nevertheless, the fast growth of nanotechnology research associated with the increased interest on the novelty systems and nanomaterials has not been completely followed by sufficient knowledge about safety and sustainability issues [223–227]. Hence, over the last years, the scientific community from the diverse fields of the nanosafety framework, did an effort to focus the research into identifying and understanding the potential risks and impacts that this emerging field may pose to humans and to the environment [226], as it has been pointed out throughout this review. For instance, and as outcome example, a series of tools have been developed under the scope of different European projects (e.g.,

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NanoFase, NanoReg2, SUNSHINE, SbD4Nano, ASINA and SAbyNA), some of which are still ongoing. Most of these tools are based on a life cycle concept, in which the idea is to establish an optimized safety strategy, preferentially starting at an early stage of the nanotechnology innovation process development [228–230]. Additionally, this new framework intends not only to guarantee the safety but also to ensure sustainability concerns [231]. The plan is, therefore, to maximize or at least maintain the functionality, as well as the lifetime of nanomaterials and nano-enabled products in a circularity system, together with the efficient design of the production process that ensures a minimization of the environmental impacts while guaranteeing the safety across their life cycle [222,232]. In this sense, the LCA is a powerful tool that can be applied in a wide range of areas as a cross-cutting approach, providing a comprehensive understanding of the potential environmental impacts while identifying the main hotspots for a potential improvement and ensuring the environmental sustainability of such products and processes. The LCA is an international standardized methodology comprising four main phases (Figure 8), as defined in the ISO 14040 series: (i) goal and scope definition, (ii) life cycle inventory (LCI), (iii) life cycle impact assessment (LCIA) and (iv) interpretation.

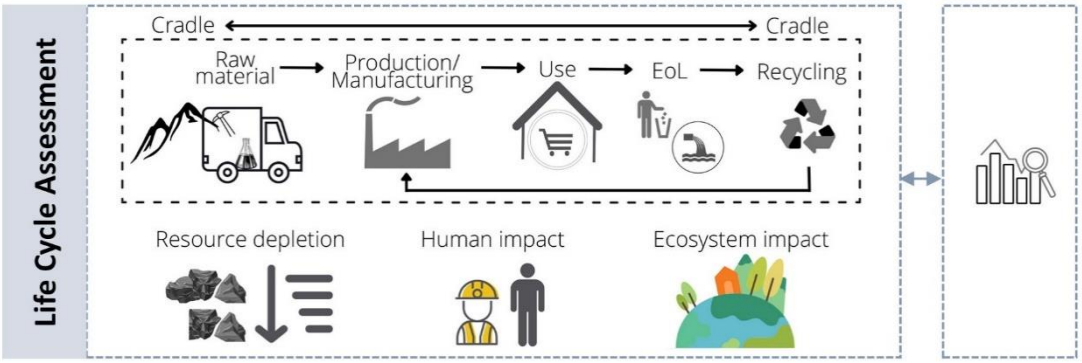

Figure 8. General conceptual framework of LCA. EoL: end-of-life,

The idea is to use the LCA along with the risk assessment perspectives, to integrate both safety and environmental issues, with functionality and socio-economic indicators (e.g., Social LCA (S-LCA) and Life Cycle Cost (LCC)) into a decision support system, as so-called Safe-and-Sustainable-by-Design (SSbD) [222]. The general concept is basically a multi-objective optimization process, and a novel integration approach for decision-making was recently developed under the scope of the ASINA project through mathematical algorithms solving a Multi Criteria Decision Analysis (MCDA) model [233]. Nevertheless, even with the advances on the SSbD approach, there are still challenges on how to properly provide strategic guidelines regarding safety and sustainability issues among all the stakeholders, from the nanotechnology innovators to the regulatory policy-makers [230,234,235].

A crucial factor is essentially related to the complexity and dynamic behavior of the nanomaterials which then leads, as it was previously stated, to a lack of tools and proficient measurements of quantitative and qualitative data. The ongoing development of analytical techniques to detect and characterize nanomaterials is even more critical in the case of complex natural systems [236], especially due to the problems in the differentiation between natural and engineered nanomaterials [237,238].

The releases of nanomaterials can occur during any stage of the life cycle of a nano-enabled product, and, for instance, humans can be exposed through different exposure routes (e.g., inhalation, dermal and ingestion) linked to a wide range of situations (e.g., occupational workplaces, medical treatments, use or consumption) [239]. Therefore, it is essential to understand, not only those releases of the nanomaterials connected to the pristine form, but also the ones from the further transformations that may occur due to the ageing and/or altered processes [238,240,241], as well as the probable interactions with the surrounding setting, which could affect the potential fate and toxicity effect [242]. Thus, a

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holistic analysis should be considered, comprising aspects from the extraction to the end-of-life management scenarios and, therefore, the LCA has been considered as an appropriate tool to use [243,244].

In the last two decades, a number of studies related to the LCA application in a wide range of nanotechnology fields, by using different types of nanomaterials, have been conducted in a total of 128 works (Figure 9). The reference studies are from 2001 to 2022 and were mostly recompiled from a few review studies [245–247] and from the following publications [227,248–260]. From this analysis it is possible to observe a wide variety of nanomaterials and application fields. The main studied nanomaterials were TiO_2 and CNT, including single-wall CNT and multi-wall CNT, while the synthesis and production were the major requested fields to be assessed through the LCA.

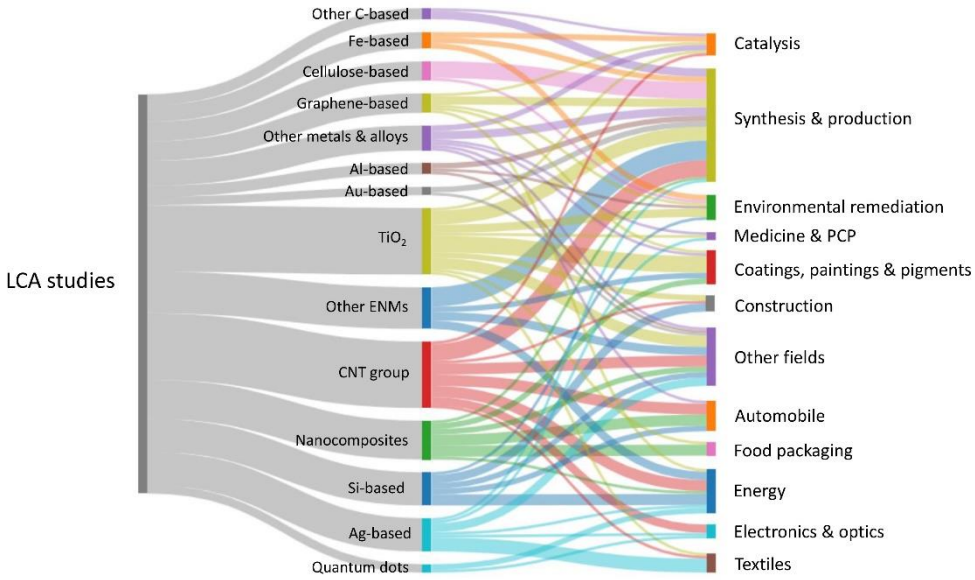

Figure 9. LCA application in different nanotechnology fields, including the nanomaterials studied. ENMs: engineered nanomaterials; PCP: personal care products; other metals and alloys: some of the most studied were Zn-based and Cu-based; other ENMs: some of the most studied were tungsten-based and zirconia-based.

Although many studies have been published in the last years, there are still many limitations as in the beginnings of LCA application [243]. This has been stated in recent reviews [245,247] and it was also debated in a LCA Discussion Forum a few years ago [261]. Some of those limitations are related to the system boundary considerations. The most comprehensive ones are the cradle-to-grave and cradle-to-cradle analysis, which comprise all the life cycle stages of a system – the raw material extraction, production, transport, use and the end-of-life management, including, recycling/upcycling. However, a critical problem is that most of the existing LCA studies do not consider the end-of-life management scenarios (e.g., wastewater treatment plants (WWTPs)), incineration and landfill) that could act as sink compartments where the nanomaterials potentially accumulate [19]. Actually, to the best of our knowledge, there is no international regulation on the disposal management of nanomaterials [247]. This could be a concern accounting for the increased availability of nano-enabled products which will eventually enter into various waste treatment processes with no specific treatment being considered [262]. Taking into account the wide array of product categories in which nanomaterials could be present, like plastics, for instance, inevitable exposure pathways to nanomaterials through the waste management scenarios, are foreseeable. For instance, through the incineration of non-recyclable fractions and, consequently, the landfilling of the produced ashes [263]. In the case of those recyclable fractions the persistence of nanomaterials could represent a restriction in terms of safety reuse, which could be a problem considering the need to maintain a circular economy. Furthermore, other important exposure pathways are linked

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to the increased use of the personal care products - containing nanomaterials - that could enter directly into the water compartment (freshwater and marine water) and, indirectly, through the WWTPs and, from there, probably reaching the soil compartment through the sludge valorization [263,264]. Previous studies stated that the waste treatment of those products containing large amounts of carbon-based nanomaterials could be eliminated during incineration and in the WWTPs, whereas for those containing metals and metal oxides a persistent potential was observed [265]. In addition, some efforts focusing on the detection and characterization of nanomaterials on the waste streams have been made [266,267] but more studies are needed to elucidate the potential risk of the waste management treatments.

Another important constraint is the lack of LCI datasets even those not related to the nanomaterials. Most of the available data could be obtained from the literature, databases, lab and pilot scale studies but, most of the times, the information is still limited [268]. The accuracy of the data collected has an important effect on the reliability of the results and the use of primary data is also recommended. Besides, for those emerging technologies that are not yet established at an industrial scale, the potential impacts - mainly in terms of energy and materials supplies - could be overestimated, considering that the optimization is usually attained with the upscaling of such processes [268]. Therefore, it is fundamental the involvement of the manufacturing industries for those well-established processes. Nevertheless, the scarcity of exchange of information from the industry sector has been identified as a general problem due to confidentiality issues and also associated with the difficulty of tracking the final commercial application of the nanomaterials. Hence, the need for a transparent exchange of information among all the stakeholders is urgently needed.

An additional limitation relates to the assessment of the potential impacts, especially those on human health and the environment, triggered from the released nanomaterials. This is because there is no consensus on how to approach an appropriate modelling in the LCA framework and there is a challenge on how to account for those releases on the LCI modelling. For instance, the Material Flow Analysis (MFA) model has been used for tracking the flows of nanomaterials across their entire possible life cycle, from the technosphere to their relocation into several compartments of the environment [269,270]. Some recent improvements have been attained, especially those related to the inclusion of different sizes and forms of the nanomaterials on the dynamic modelling [271,272]. This could be an important advance for the incorporation of the nanomaterial's releases in the LCI modelling, considering the recommendation on having specific features of the nanomaterials (e.g., shape, size, crystalline structure, surface charge and surface area), in addition to mass and chemical composition, that are the ones considered for conventional chemicals [273]. This approach will be an essential step forward for tracking the releases of nanomaterials across the life cycle of nano-enabled products considering that, for instance, the loss or gain of surface functional groups will determine their environmental fate, exposure, and toxicity potential [274].

Another critical challenge, also related to the possible impacts on human health and the environment - linked to the releases of the nanomaterials - is the lack of characterization factors (CF). In the LCIA, different models are used to translate the quantity of each emission in order to evaluate the environmental impacts through multiple impact categories (e.g., human toxicity, global warming potential and resource depletion). Therefore, the emissions are converted into environmental damages through the three different models (fate, exposure, and effect) to obtain a specific CF. This requires both a qualitative and quantitative assessment along with a mechanistic understanding for each of these components. From the analysis of the previous LCA studies in the field of nanotechnology, it is evident the lack of information related to the inclusion of those impacts caused from the releases of nanomaterials, due to the absence of specific CF (Figure 10).

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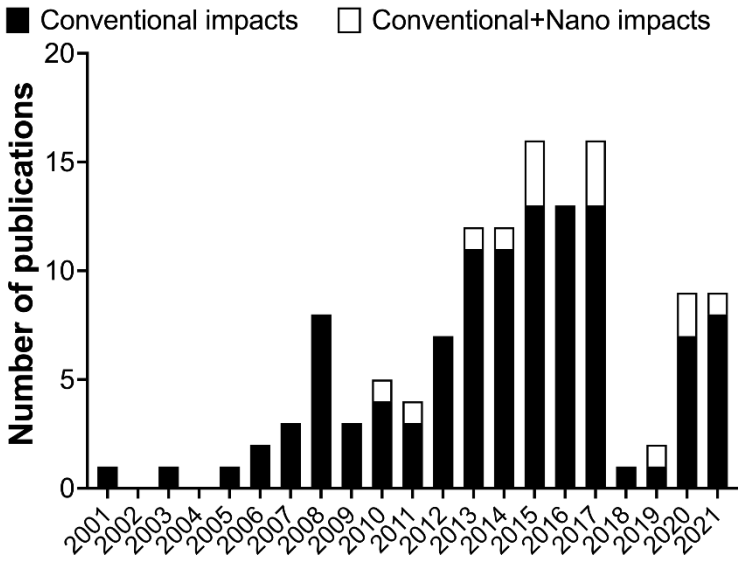

Figure 10. Number of publications related to the LCA in nanotechnology accounting for conventional impacts and also for those impacts linked to the releases of nanomaterials.

To characterize the toxicity impacts that are required in the LCA calculations, the OECD guidelines recommend the use of the USEtox model once it is appropriate to apply it to nanomaterials [275]. In fact, and although the USEtox model was firstly oriented to chemicals, some efforts have been done in order to attempt the establishment of CF for specific nanomaterials, including the most suitable one - so far - for the specific case of TiO_2 [276]. Within the USEtox framework, apart from the fate and exposure factors, the effect factors to account for the particular case of freshwater ecotoxicity impact are calculated through the fraction of species (representative of the three trophic levels – algae, crustacean, and fish) that are potentially affected by the exposure to a specific substance. The recommendation for nanomaterials is to apply an approach based on the geometric mean at the trophic level, rather than the species level, in order to provide a proper distribution of the toxicity data [245]. On the other hand, for the human toxicity assessment, the effect factor reflects the potential to increase human disease due to the change in lifetime intake of a particular substance, extrapolated from animal *in vivo* models [277]. This could also be another critical point - in a good way - considering the new paradigm for toxicology, wherein there is a need to replace animal testing by alternative *in vitro* models, as previously stated in this review. This transition could solve some problematics with the use of animal data during the LCIA [275]. In fact, a recent method to determine human toxicological effect factors for some nanomaterials, by using *in vitro* data, was proposed [278]. Nevertheless, more strategies are needed for comparison purposes.

Despite all the progress, there is still no agreement on which specific nano fate descriptors (e.g., agglomeration, aggregation, and sedimentation), physicochemical properties, particle characteristics, among other features, need to be integrated into specific mechanistic models [279]. This is challenging, especially considering the amount and the wide variety of nanomaterials that should be studied and, therefore, the use of *in silico* analysis will provide a crucial information of those relevant characteristics of the exposure related to a particular or to a group of effects, as discussed in the previous section. Therefore, further improvements in this topic are needed, considered that probably some nanomaterials, including their forms, cannot be clustered and read-across due to their specificity in terms of both environmental release profile (i.e., amounts, form, and compartment) and adverse effects [280].

Noteworthy, and considering that it is even more imperative to determine the environmental impacts of the emerging technologies at an initial stage of development, some shortcomings are expectable during the assessment [268,281]. Nevertheless, even with all the challenges and uncertainties, a prospective LCA should be performed in a useful way

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that could lead into a decisive strategy to orient the investments and to predict the upcoming environmental impacts [282] as, for instance, those linked to the critical raw materials, in order to ensure the sustainability of such technologies.

8. Conclusions

Nanosafety is a very broad concept related to many different approaches to evaluate the potential deleterious effects of nanomaterials to reduce the potential impact on environment and health. For this, traditional methods of toxicology (i.e., cytotoxicity, immunotoxicity, genotoxicity) have been adapted to evaluate nanomaterials. New approaches using high throughput methods allowing to evaluate multiple outcomes in a single sample, are also part of the new trends, aiming to tackle the rapid growth of the nanomaterials field. Also, new technologies, such as OoCs and MOoCs have emerged as new platforms to predict drug efficacy but the scientific development for nanosafety assessment is still delayed. We consider that the expansion of OoC with novel development in fields such as bioimaging will improve label-free analysis on chips bringing information to tissue as well as at the cellular level. Innovative detection methods and analysis possibilities will help to accurately study cellular responses and communication among tissues or organs, prolonging culture timeframes, to better detect the long-term performance and predictability of nanomaterials toxicity. All these adaptations of traditional and new methods are critical to feed the *in silico* models aiming to predict the effects of nano-materials, becoming critical for the Safe by Design concept. Finally, going beyond the effects of nanomaterials in living organisms, the LCA assessment must be performed to have a clear image of how the production, use and disposal of nanomaterials may have an impact on the sustainable use of nanomaterials by industry.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Figure S1: Graphical network of the 19 articles that meet the criteria to be included in Table 5.

Author Contributions: Conceptualization, E.A.M..; writing—original draft preparation, F.L., N.C., S.C., E.F.G., C.L., J.M., L.O., A.R., V.V.B., and E.A.M.; writing—review and editing, F.L. N.C., S.C., C.L., J.M., L.O., A.R., V.V.B., and E.A.M.; visualization, F.L. N.C., S.C., C.L., J.M., L.O., A.R., V.V.B., and E.A.M.; funding acquisition, E.A.M.. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the European Union's H2020 project Sinfonia (N.857253). SbDToolBox, with reference NORTE-01-0145-FEDER-000047, supported by Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund.

Data Availability Statement: Data is contained within the article or supplementary material

Acknowledgments: Luís Ortiz acknowledge the Erasmus Mundus Joint Master Degree in Environmental Contamination and Toxicology (ECT+), with an Erasmus Mundus scholarship from the ECT+ program (ref: 2019-1485)

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Tripathi, G.K. Engineered Nanomaterials And Their Properties : A Review. *Bioscience Biotechnology Research Communications* **2019**, *12*, 764–771, doi:10.21786/bbrc/12.3/32.
2. Wu, Q.; Miao, W.; Zhang, Y.; Gao, H.; Hui, D. Mechanical Properties of Nanomaterials: A Review. *Nanotechnology Reviews* **2020**, *9*, 259–273, doi:10.1515/ntrev-2020-0021.
3. Schaming, D.; Remita, H. Nanotechnology: From the Ancient Time to Nowadays. *Foundations of Chemistry* **2015**, *17*, 187–205, doi:10.1007/s10698-015-9235-y.
4. Feynman, R.P. Plenty of Room at the Bottom Available online: https://web.archive.org/web/20170105015142/http:/www.its.caltech.edu/~feynman/plenty.html (accessed on 15 April 2022).
5. Taniguchi, N. N the Basic Concept of "Nano-Technology." *Proceedings of the International Conference on Production Engineering, Tokyo, Part II, Japan Society of Precision Engineering* **1974**.
6. Roduner, E. Size Matters: Why Nanomaterials Are Different. *Chemical Society Reviews* **2006**, *35*, 583–592, doi:10.1039/b502142c.
7. Subhan, M.; Choudhury, K.; Neogi, N. Advances with Molecular Nanomaterials in Industrial Manufacturing Applications. *Nanomanufacturing* **2021**, *1*, 75–97, doi:10.3390/nanomanufacturing1020008.
8. National Institute for Occupational Safety and Health *Approaches to Safe Nanotechnology Managing the Health and Safety Concerns Associated with Engineered Nanomaterials*; 2009;
9. Zielińska, A.; Costa, B.; Ferreira, M. v.; Miguéis, D.; Louros, J.M.S.; Durazzo, A.; Lucarini, M.; Eder, P.; v. Chaud, M.; Morsink, M.; et al. Nanotoxicology and Nanosafety: Safety-by-Design and Testing at a Glance. *International Journal of Environmental Research and Public Health* **2020**, *17*, 4657, doi:10.3390/ijerph17134657.
10. Leslie, H.A.; van Velzen, M.J.M.; Brandsma, S.H.; Vethaak, A.D.; Garcia-Vallejo, J.J.; Lamoree, M.H. Discovery and Quantification of Plastic Particle Pollution in Human Blood. *Environment International* **2022**, *163*, 107199, doi:10.1016/J.ENVINT.2022.107199.
11. Nemmar, A.; Hoet, P.H.M.; Vanquickenborne, B.; Dinsdale, D.; Thomeer, M.; Hoylaerts, M.F.; Vanbilloen, H.; Mortelmans, L.; Nemery, B. Passage of Inhaled Particles Into the Blood Circulation in Humans. *Circulation* **2002**, *105*, 411–414, doi:10.1161/hc0402.104118.
12. Raftis, J.B.; Miller, M.R. Nanoparticle Translocation and Multi-Organ Toxicity: A Particularly Small Problem. *Nano Today* **2019**, *26*, 8–12, doi:10.1016/j.nantod.2019.03.010.
13. Oberdörster, G.; Elder, A.; Rinderknecht, A. Nanoparticles and the Brain: Cause for Concern? *Journal of Nanoscience and Nanotechnology* **2009**, *9*, 4996–5007, doi:10.1166/jnn.2009.GR02.
14. Calderón-Garcidueñas, L.; Pérez-Calatayud, Á.A.; González-Maciel, A.; Reynoso-Robles, R.; Silva-Pereyra, H.G.; Ramos-Morales, A.; Torres-Jardón, R.; Soberanes-Cerino, C. de J.; Carrillo-Esper, R.; Briones-Garduño, J.C.; et al. Environmental Nanoparticles Reach Human Fetal Brains. *Biomedicines* **2022**, *10*, 410, doi:10.3390/biomedicines10020410.
15. Piccardo, M.; Renzi, M.; Terlizzi, A. Nanoplastics in the Oceans: Theory, Experimental Evidence and Real World. *Marine Pollution Bulletin* **2020**, *157*, 111317, doi:10.1016/J.MARPOLBUL.2020.111317.
16. Kugiya, H. Hoovering the Ocean Available online: https://www.washingtonpost.com/climate-solutions/2020/05/13/climate-solutions-plastics/ (accessed on 15 April 2022).
17. Carrington, D. Microplastics Found in Human Blood for First Time Available online: https://www.theguardian.com/environment/2022/mar/24/microplastics-found-in-human-blood-for-first-time (accessed on 10 April 2022).

18. Salem, S.S.; Hammad, E.N.; Mohamed, A.A.; El-Dougdoug, W. A Comprehensive Review of Nanomaterials: Types, Synthesis, Characterization, and Applications. *Biointerface Research in Applied Chemistry* **2022**, *13*, 41, doi:10.33263/BRIAC131.041.
19. Part, F.; Berge, N.; Baran, P.; Stringfellow, A.; Sun, W.; Bartelt-Hunt, S.; Mitrano, D.; Li, L.; Hennebert, P.; Quicker, P.; et al. A Review of the Fate of Engineered Nanomaterials in Municipal Solid Waste Streams. *Waste Management* 2018, *75*, 427–449.
20. Inshakova, E.; Inshakov, O. World Market for Nanomaterials: Structure and Trends. *MATEC Web of Conferences* **2017**, *129*, 02013, doi:10.1051/matecconf/201712902013.
21. Research and Markets Global Nanotechnology Market 2018-2024: Market Is Expected to Exceed US$ 125 Billion Available online: https://www.prnewswire.com/news-releases/global-nanotechnology-market-2018-2024-market-is-expected-to-exceed-us-125-billion-300641054.html (accessed on 15 April 2022).
22. StatNano Nanotechnology Patents of 2018 at the USPTO and EPO through the Lens of Statistics Available online: https://statnano.com/news/65159 (accessed on 14 April 2022).
23. Gochfeld, M. Chronologic History of Occupational Medicine. *Journal of Occupational and Environmental Medicine* **2005**, *47*, 96–114, doi:10.1097/01.jom.0000152917.03649.0e.
24. Sousa, M.; Arezes, P.; Silva, F. Occupational Exposure to Incidental Nanoparticles: A Review on Control Banding. *Journal of Physics: Conference Series* **2021**, *1953*, 012008, doi:10.1088/1742-6596/1953/1/012008.
25. Suhendra, E.; Chang, C.-H.; Hou, W.-C.; Hsieh, Y.-C. A Review on the Environmental Fate Models for Predicting the Distribution of Engineered Nanomaterials in Surface Waters. *International Journal of Molecular Sciences* **2020**, *21*, 4554, doi:10.3390/ijms21124554.
26. Valic, M.S.; Halim, M.; Schimmer, P.; Zheng, G. Guidelines for the Experimental Design of Pharmacokinetic Studies with Nanomaterials in Preclinical Animal Models. *Journal of Controlled Release* **2020**, *323*, 83–101, doi:10.1016/j.jconrel.2020.04.002.
27. Kathawala, M.H.; Xiong, S.; Richards, M.; Ng, K.W.; George, S.; Loo, S.C.J. Emerging In Vitro Models for Safety Screening of High-Volume Production Nanomaterials under Environmentally Relevant Exposure Conditions. *Small* **2013**, *9*, 1504–1520, doi:10.1002/smll.201201452.
28. Oberlintner, A.; Likozar, B.; Novak, U. Hydrophobic Functionalization Reactions of Structured Cellulose Nanomaterials: Mechanisms, Kinetics and in Silico Multi-Scale Models. *Carbohydrate Polymers* **2021**, *259*, 117742, doi:10.1016/J.CARBPOL.2021.117742.
29. Oberdörster, G. Safety Assessment for Nanotechnology and Nanomedicine: Concepts of Nanotoxicology. In Proceedings of the Journal of Internal Medicine; January 2010; Vol. 267, pp. 89–105.
30. Gatoo, M.A.; Naseem, S.; Arfat, M.Y.; Mahmood Dar, A.; Qasim, K.; Zubair, S. Physicochemical Properties of Nanomaterials: Implication in Associated Toxic Manifestations. *BioMed Research International* 2014, *2014*.
31. Kumar, A.; Singh, S.; Shanker, R.; Dhawan, A. Chapter 1: Nanotoxicology: Challenges for Biologists. In *Issues in Toxicology*; Royal Society of Chemistry, 2018; Vol. 2018-January, pp. 1–16.
32. Hull, M.; Kennedy, A.J.; Detzel, C.; Vikesland, P.; Chappell, M.A. Moving beyond Mass: The Unmet Need to Consider Dose Metrics in Environmental Nanotoxicology Studies. *Environmental Science and Technology* 2012, *46*, 10881–10882.
33. Duffin, R.; Tran, L.; Brown, D.; Stone, V.; Donaldson, K. Proinflammogenic Effects of Low-Toxicity and Metal Nanoparticles in Vivo and in Vitro: Highlighting the Role of Particle Surface Area and Surface Reactivity. *Inhalation Toxicology* **2007**, *19*, 849–856, doi:10.1080/08958370701479323.
34. Hennig, F.; Quass, U.; Hellack, B.; Küpper, M.; Kuhlbusch, T.A.J.; Stafoggia, M.; Hoffmann, B. Ultrafine and Fine Particle Number and Surface Area Concentrations and Daily Cause-Specific Mortality in the Ruhr Area, Germany, 2009–2014. *Environmental Health Perspectives* **2018**, *126*, doi:10.1289/EHP2054.

35. Monteiller, C.; Tran, L.; MacNee, W.; Faux, S.; Jones, A.; Miller, B.; Donaldson, K. The Pro-Inflammatory Effects of Low-Toxicity Low-Solubility Particles, Nanoparticles and Fine Particles, on Epithelial Cells in Vitro: The Role of Surface Area. *Occupational and Environmental Medicine* **2007**, *64*, 609–615, doi:10.1136/oem.2005.024802.
36. Sager, T.M.; Castranova, V. Surface Area of Particle Administered versus Mass in Determining the Pulmonary Toxicity of Ultrafine and Fine Carbon Black: Comparison to Ultrafine Titanium Dioxide. *Particle and Fibre Toxicology* **2009**, *6*, doi:10.1186/1743-8977-6-15.
37. Noël, A.; Truchon, G.; Cloutier, Y.; Charbonneau, M.; Maghni, K.; Tardif, R. Mass or Total Surface Area with Aerosol Size Distribution as Exposure Metrics for Inflammatory, Cytotoxic and Oxidative Lung Responses in Rats Exposed to Titanium Dioxide Nanoparticles. *Toxicology and Industrial Health* **2017**, *33*, 351–364, doi:10.1177/0748233716651560.
38. Teeguarden, J.G.; Hinderliter, P.M.; Orr, G.; Thrall, B.D.; Pounds, J.G. Particokinetics in Vitro: Dosimetry Considerations for in Vitro Nanoparticle Toxicity Assessments. *Toxicological Sciences* 2007, *95*, 300–312.
39. Braakhuis, H.M.; Cassee, F.R.; Fokkens, P.H.B.; de La Fonteyne, L.J.J.; Oomen, A.G.; Krystek, P.; de Jong, W.H.; van Loveren, H.; Park, M.V.D.Z. Identification of the Appropriate Dose Metric for Pulmonary Inflammation of Silver Nanoparticles in an Inhalation Toxicity Study. *Nanotoxicology* **2016**, *10*, 63–73, doi:10.3109/17435390.2015.1012184.
40. Jeong, J.; Han, Y.; Poland, C.A.; Cho, W.S. Response-Metrics for Acute Lung Inflammation Pattern by Cobalt-Based Nanoparticles. *Particle and Fibre Toxicology* **2015**, *12*, doi:10.1186/s12989-015-0089-1.
41. Cohen, J.M.; Teeguarden, J.G.; Demokritou, P. An Integrated Approach for the in Vitro Dosimetry of Engineered Nanomaterials. *Particle and Fibre Toxicology* **2014**, *11*, 20, doi:10.1186/1743-8977-11-20.
42. Rischitor, G.; Parracino, M.; la Spina, R.; Urbán, P.; Ojea-Jiménez, I.; Bellido, E.; Valsesia, A.; Gioria, S.; Capomaccio, R.; Kinsner-Ovaskainen, A.; et al. Quantification of the Cellular Dose and Characterization of Nanoparticle Transport during in Vitro Testing. *Particle and Fibre Toxicology* **2016**, *13*, doi:10.1186/s12989-016-0157-1.
43. Nemmar, A.; Hoet, P.H.M.; Vanquickenborne, B.; Dinsdale, D.; Thomeer, M.; Hoylaerts, M.F.; Vanbilloen, H.; Mortelmans, L.; Nemery, B. Passage of Inhaled Particles Into the Blood Circulation in Humans. *Circulation* **2002**, *105*, 411–414, doi:10.1161/hc0402.104118.
44. Shabbir, S.; Fakhar-E-, M.; Kulyar, A.; Bhutta, Z.A.; Boruah, P.; Asif, M. Toxicological Consequences of Titanium Dioxide Nanoparticles (TiO 2 NPs) and Their Jeopardy to Human Population., doi:10.1007/s12668-021-00836-3/Published.
45. Vlastou, E.; Gazouli, M.; Ploussi, A.; Platoni, K.; Efstathopoulos, E.P. Nanoparticles: Nanotoxicity Aspects. In Proceedings of the Journal of Physics: Conference Series; Institute of Physics Publishing, December 1 2017; Vol. 931.
46. Xiong, P.; Huang, X.; Ye, N.; Lu, Q.; Zhang, G.; Peng, S.; Wang, H.; Liu, Y. Cytotoxicity of Metal-Based Nanoparticles: From Mechanisms and Methods of Evaluation to Pathological Manifestations. *Advanced Science* **2022**, 2106049, doi:10.1002/advs.202106049.
47. Moore, T.L.; Urban, D.A.; Rodriguez-Lorenzo, L.; Milosevic, A.; Crippa, F.; Spuch-Calvar, M.; Balog, S.; Rothen-Rutishauser, B.; Lattuada, M.; Petri-Fink, A. Nanoparticle Administration Method in Cell Culture Alters Particle-Cell Interaction. *Scientific Reports* **2019**, *9*, doi:10.1038/s41598-018-36954-4.
48. European Commission Environment and Health (2021) (HORIZON-HLTH-2021-ENVHLTH-02) Available online: https://ec.europa.eu/info/funding-tenders/opportunities/portal/screen/opportunities/topic-details/horizon-hlth-2021-envhlth-02-02;callCode=HORIZON-HLTH-2021-ENVHLTH-02;freeTextSearchKeyword=;matchWholeText=true;typeCodes=1;statusCodes=31094501,31094502,31094503;programmePeriod=null;programCcm2Id=null;programDivisionCode=null;focusAreaCode=null;destination=null;

mission=null;geographicalZonesCode=null;programmeDivisionProspect=null;startDateLte=null;startDateGte=null;crossCuttingPriori-ty-Code=null;cpvCode=null;performanceOfDelivery=null;sortQuery=sortStatus;orderBy=asc;onlyTenders=false;topicListKey=callTopicSearchTableState (accessed on 17 April 2022).

49. Cohen Hubal, E.A.; Wetmore, B.A.; Wambaugh, J.F.; El-Masri, H.; Sobus, J.R.; Bahadori, T. Advancing Internal Exposure and Physiologically-Based Toxicokinetic Modeling for 21st-Century Risk Assessments. *Journal of Exposure Science and Environmental Epidemiology* **2019**, *29*, 11–20, doi:10.1038/s41370-018-0046-9.
50. Skalska, J.; Dąbrowska-Bouta, B.; Strużyńska, L. Oxidative Stress in Rat Brain but Not in Liver Following Oral Administration of a Low Dose of Nanoparticulate Silver. *Food and Chemical Toxicology* **2016**, *97*, 307–315, doi:10.1016/j.fct.2016.09.026.
51. Tentschert, J.; Laux, P.; Jungnickel, H.; Brunner, J.; Estrela-Lopis, I.; Merker, C.; Meijer, J.; Ernst, H.; Ma-Hock, L.; Keller, J.; et al. Organ Burden of Inhaled Nanoceria in a 2-Year Low-Dose Exposure Study: Dump or Depot? *Nanotoxicology* **2020**, *14*, 554–576, doi:10.1080/17435390.2020.1736355.
52. Comfort, K.K.; Braydich-Stolle, L.K.; Maurer, E.I.; Hussain, S.M. Less Is More: Long-Term in Vitro Exposure to Low Levels of Silver Nanoparticles Provides New Insights for Nanomaterial Evaluation. *ACS Nano* **2014**, *8*, 3260–3271, doi:10.1021/nn5009116.
53. Llewellyn, S. v.; Conway, G.E.; Zanoni, I.; Jørgensen, A.K.; Shah, U.K.; Seleci, D.A.; Keller, J.G.; Kim, J.W.; Wohlleben, W.; Jensen, K.A.; et al. Understanding the Impact of More Realistic Low-Dose, Prolonged Engineered Nanomaterial Exposure on Genotoxicity Using 3D Models of the Human Liver. *Journal of Nanobiotechnology* **2021**, *19*, doi:10.1186/s12951-021-00938-w.
54. Torres, A.; Dalzon, B.; Collin-faure, V.; Rabilloud, T. Repeated vs. Acute Exposure of RAW264.7 Mouse Macrophages to Silica Nanoparticles: A Bioaccumulation and Functional Change Study. *Nanomaterials* **2020**, *10*, doi:10.3390/nano10020215.
55. Tsatsakis, A.M.; Docea, A.O.; Tsitsimpikou, C. New Challenges in Risk Assessment of Chemicals When Simulating Real Exposure Scenarios; Simultaneous Multi-Chemicals' Low Dose Exposure. *Food and Chemical Toxicology* **2016**, *96*, 174–176, doi:10.1016/j.fct.2016.08.011.
56. Vilas-Boas, V.; Vinken, M. Hepatotoxicity Induced by Nanomaterials: Mechanisms and in Vitro Models. *Archives of Toxicology* **2021**, *95*, 27–52, doi:10.1007/s00204-020-02940-x.
57. Hussain, Z.; Thu, H.E.; Elsayed, I.; Abourehab, M.A.S.; Khan, S.; Sohail, M.; Sarfraz, R.M.; Farooq, M.A. Nano-Scaled Materials May Induce Severe Neurotoxicity upon Chronic Exposure to Brain Tissues: A Critical Appraisal and Recent Updates on Predisposing Factors, Underlying Mechanism, and Future Prospects. *Journal of Controlled Release* 2020, *328*, 873–894.
58. Teng, C.; Jiang, C.; Gao, S.; Liu, X.; Zhai, S. Fetotoxicity of Nanoparticles: Causes and Mechanisms. *Nanomaterials* 2021, *11*, 1–25.
59. Ribeiro, A.R.; Leite, P.E.; Falagan-Lotsch, P.; Benetti, F.; Micheletti, C.; Budtz, H.C.; Jacobsen, N.R.; Lisboa-Filho, P.N.; Rocha, L.A.; Kühnel, D.; et al. Challenges on the Toxicological Predictions of Engineered Nanoparticles. *NanoImpact* **2017**, *8*, 59–72, doi:10.1016/j.impact.2017.07.006.
60. Shatkin, J.A. The Future in Nanosafety. *Nano Letters* **2020**, *20*, 1479–1480, doi:10.1021/acs.nanolett.0c00432.
61. Irvine, D.J.; Swartz, M.A.; Szeto, G.L. Engineering Synthetic Vaccines Using Cues from Natural Immunity. *Nature Materials* **2013**, *12*, 978–990, doi:10.1038/nmat3775.
62. Kyriakides, T.R.; Raj, A.; Tseng, T.H.; Xiao, H.; Nguyen, R.; Mohammed, F.S.; Halder, S.; Xu, M.; Wu, M.J.; Bao, S.; et al. Biocompatibility of Nanomaterials and Their Immunological Properties. *Biomedical Materials* **2021**, *16*, 042005, doi:10.1088/1748-605X/abe5fa.

63. Lebre, F.; Hearnden, C.H.; Lavelle, E.C. Modulation of Immune Responses by Particulate Materials. *Advanced Materials* **2016**, *28*, 5525–5541, doi:10.1002/adma.201505395.
64. Muñoz-Wolf, N.; Lavelle, E.C. Promotion of Trained Innate Immunity by Nanoparticles. *Seminars in Immunology* **2021**, *56*, 101542, doi:10.1016/j.smim.2021.101542.
65. Burnett, R.; Chen, H.; Szyszkowicz, M.; Fann, N.; Hubbell, B.; Pope, C.A.; Apte, J.S.; Brauer, M.; Cohen, A.; Weichenthal, S.; et al. Global Estimates of Mortality Associated with Long-Term Exposure to Outdoor Fine Particulate Matter. *Proceedings of the National Academy of Sciences* **2018**, *115*, 9592–9597, doi:10.1073/pnas.1803222115.
66. Vohra, K.; Vodonos, A.; Schwartz, J.; Marais, E.A.; Sulprizio, M.P.; Mickley, L.J. Global Mortality from Outdoor Fine Particle Pollution Generated by Fossil Fuel Combustion: Results from GEOS-Chem. *Environmental Research* **2021**, *195*, 110754, doi:10.1016/j.envres.2021.110754.
67. Shears, R.K.; Jacques, L.C.; Naylor, G.; Miyashita, L.; Khandaker, S.; Lebre, F.; Lavelle, E.C.; Grigg, J.; French, N.; Neill, D.R.; et al. Exposure to Diesel Exhaust Particles Increases Susceptibility to Invasive Pneumococcal Disease. *Journal of Allergy and Clinical Immunology* **2020**, *145*, 1272-1284.e6, doi:10.1016/j.jaci.2019.11.039.
68. International Organization for Standardization Biological Evaluation of Medical Devices — Part 22: Guidance on Nanomaterials (ISO/TR 10993-22:2017) 2017.
69. Burrell, R. Human Responses to Bacterial Endotoxin. *Circ Shock* **1994**, *43*, 137–153.
70. Copeland, S.; Warren, H.S.; Lowry, S.F.; Calvano, S.E.; Remick, D. Acute Inflammatory Response to Endotoxin in Mice and Humans. *Clinical and Vaccine Immunology* **2005**, *12*, 60–67, doi:10.1128/CDLI.12.1.60-67.2005.
71. Ma, P.; Liu, H.-T.; Wei, P.; Xu, Q.-S.; Bai, X.-F.; Du, Y.-G.; Yu, C. Chitosan Oligosaccharides Inhibit LPS-Induced over-Expression of IL-6 and TNF-α in RAW264.7 Macrophage Cells through Blockade of Mitogen-Activated Protein Kinase (MAPK) and PI3K/Akt Signaling Pathways. *Carbohydrate Polymers* **2011**, *84*, 1391–1398, doi:10.1016/j.carbpol.2011.01.045.
72. Park, J.; Babensee, J.E. Differential Functional Effects of Biomaterials on Dendritic Cell Maturation. *Acta Biomaterialia* **2012**, *8*, 3606–3617, doi:10.1016/j.actbio.2012.06.006.
73. Bueter, C.L.; Lee, C.K.; Wang, J.P.; Ostroff, G.R.; Specht, C.A.; Levitz, S.M. Spectrum and Mechanisms of Inflammasome Activation by Chitosan. *The Journal of Immunology* **2014**, *192*, 5943–5951, doi:10.4049/jimmunol.1301695.
74. Gorbet, M.B.; Sefton, M. v. Endotoxin: The Uninvited Guest. *Biomaterials* **2005**, *26*, 6811–6817, doi:10.1016/j.biomaterials.2005.04.063.
75. Fujita, Y.; Nabetani, T. Iron Sulfate Inhibits *Limulus* Activity by Induction of Structural and Qualitative Changes in Lipid A. *Journal of Applied Microbiology* **2014**, *116*, 89–99, doi:10.1111/jam.12349.
76. Brown, J. Using the Monocyte Activation Test as a Stand-Alone Release Test for Medical Devices. *ALTEX* **2021**, 151–156, doi:10.14573/altex.2012021.
77. Mangini, M.; Verde, A.; Boraschi, D.; Puntes, V.F.; Italiani, P.; de Luca, A.C. Interaction of Nanoparticles with Endotoxin *Importance in Nanosafety Testing and Exploitation for Endotoxin Binding*. *Nanotoxicology* **2021**, 1–19, doi:10.1080/17435390.2021.1898690.
78. Lin, A.; Giuliano, C.J.; Palladino, A.; John, K.M.; Abramowicz, C.; Yuan, M. lou; Sausville, E.L.; Lukow, D.A.; Liu, L.; Chait, A.R.; et al. Off-Target Toxicity Is a Common Mechanism of Action of Cancer Drugs Undergoing Clinical Trials. *Science Translational Medicine* **2019**, *11*, doi:10.1126/scitranslmed.aaw8412.
79. Chabner, B.A. NCI-60 Cell Line Screening: A Radical Departure in Its Time. *J Natl Cancer Inst* **2016**, *108*, djv388, doi:10.1093/jnci/djv388.
80. Roma-Rodrigues, C.; Mendes, R.; Baptista, P.; Fernandes, A. Targeting Tumor Microenvironment for Cancer Therapy. *International Journal of Molecular Sciences* **2019**, *20*, 840, doi:10.3390/ijms20040840.

81. Paul, S.M.; Mytelka, D.S.; Dunwiddie, C.T.; Persinger, C.C.; Munos, B.H.; Lindborg, S.R.; Schacht, A.L. How to Improve R&D Productivity: The Pharmaceutical Industry's Grand Challenge. *Nature Reviews Drug Discovery* **2010**, *9*, 203–214, doi:10.1038/nrd3078.
82. Shih, H.-P.; Zhang, X.; Aronov, A.M. Drug Discovery Effectiveness from the Standpoint of Therapeutic Mechanisms and Indications. *Nature Reviews Drug Discovery* **2018**, *17*, 19–33, doi:10.1038/nrd.2017.194.
83. Chanput, W.; Peters, V.; Wichers, H. THP-1 and U937 Cells. In *The Impact of Food Bioactives on Health*; Springer International Publishing: Cham, 2015; pp. 147–159.
84. Tedesco, S.; de Majo, F.; Kim, J.; Trenti, A.; Trevisi, L.; Fadini, G.P.; Bolego, C.; Zandstra, P.W.; Cignarella, A.; Vitiello, L. Convenience versus Biological Significance: Are PMA-Differentiated THP-1 Cells a Reliable Substitute for Blood-Derived Macrophages When Studying in Vitro Polarization? *Frontiers in Pharmacology* **2018**, *9*, doi:10.3389/fphar.2018.00071.
85. Netea, M.G.; Domínguez-Andrés, J.; Barreiro, L.B.; Chavakis, T.; Divangahi, M.; Fuchs, E.; Joosten, L.A.B.; van der Meer, J.W.M.; Mhlanga, M.M.; Mulder, W.J.M.; et al. Defining Trained Immunity and Its Role in Health and Disease. *Nature Reviews Immunology* **2020**, *20*, 375–388, doi:10.1038/s41577-020-0285-6.
86. Riksen, N.P.; Netea, M.G. Immunometabolic Control of Trained Immunity. *Molecular Aspects of Medicine* **2021**, *77*, 100897, doi:10.1016/j.mam.2020.100897.
87. Kleinnijenhuis, J.; Quintin, J.; Preijers, F.; Joosten, L.A.B.; Ifrim, D.C.; Saeed, S.; Jacobs, C.; van Loenhout, J.; de Jong, D.; Stunnenberg, H.G.; et al. Bacille Calmette-Guérin Induces NOD2-Dependent Nonspecific Protection from Reinfection via Epigenetic Reprogramming of Monocytes. *Proceedings of the National Academy of Sciences* **2012**, *109*, 17537–17542, doi:10.1073/pnas.1202870109.
88. Schrum, J.E.; Crabtree, J.N.; Dobbs, K.R.; Kiritsy, M.C.; Reed, G.W.; Gazzinelli, R.T.; Netea, M.G.; Kazura, J.W.; Dent, A.E.; Fitzgerald, K.A.; et al. Cutting Edge: *Plasmodium Falciparum* Induces Trained Innate Immunity. *The Journal of Immunology* **2018**, *200*, 1243–1248, doi:10.4049/jimmunol.1701010.
89. Bekkering, S.; Quintin, J.; Joosten, L.A.B.; van der Meer, J.W.M.; Netea, M.G.; Riksen, N.P. Oxidized Low-Density Lipoprotein Induces Long-Term Proinflammatory Cytokine Production and Foam Cell Formation via Epigenetic Reprogramming of Monocytes. *Arteriosclerosis, Thrombosis, and Vascular Biology* **2014**, *34*, 1731–1738, doi:10.1161/ATVBAHA.114.303887.
90. Christ, A.; Günther, P.; Lauterbach, M.A.R.; Duewell, P.; Biswas, D.; Pelka, K.; Scholz, C.J.; Oosting, M.; Haendler, K.; Baßler, K.; et al. Western Diet Triggers NLRP3-Dependent Innate Immune Reprogramming. *Cell* **2018**, *172*, 162-175.e14, doi:10.1016/j.cell.2017.12.013.
91. Lebre, F.; Boland, J.B.; Gouveia, P.; Gorman, A.L.; Lundahl, M.L.E.; I Lynch, R.; O'Brien, F.J.; Coleman, J.; Lavelle, E.C. Pristine Graphene Induces Innate Immune Training. *Nanoscale* **2020**, doi:10.1039/C9NR09661B.
92. Swartzwelter, B.J.; Barbero, F.; Verde, A.; Mangini, M.; Pirozzi, M.; de Luca, A.C.; Puntes, V.F.; Leite, L.C.C.; Italiani, P.; Boraschi, D. Gold Nanoparticles Modulate BCG-Induced Innate Immune Memory in Human Monocytes by Shifting the Memory Response towards Tolerance. *Cells* **2020**, *9*, 284, doi:10.3390/cells9020284.
93. Shukla, R.K.; Badiye, A.; Vajpayee, K.; Kapoor, N. Genotoxic Potential of Nanoparticles: Structural and Functional Modifications in DNA. *Frontiers in Genetics* **2021**, *12*, doi:10.3389/fgene.2021.728250.
94. Doak, S.H.; Manshian, B.; Jenkins, G.J.S.; Singh, N. In Vitro Genotoxicity Testing Strategy for Nanomaterials and the Adaptation of Current OECD Guidelines. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* **2012**, *745*, 104–111, doi:10.1016/j.mrgentox.2011.09.013.
95. Dusinska, M.; Tulinska, J.; el Yamani, N.; Kuricova, M.; Liskova, A.; Rollerova, E.; Rundén-Pran, E.; Smolkova, B. Immunotoxicity, Genotoxicity and Epigenetic Toxicity of Nanomaterials: New Strategies for Toxicity Testing? *Food and Chemical Toxicology* **2017**, *109*, 797–811, doi:10.1016/j.fct.2017.08.030.

96. Evans, S.J.; Clift, M.J.D.; Singh, N.; Wills, J.W.; Hondow, N.; Wilkinson, T.S.; Burgum, M.J.; Brown, A.P.; Jenkins, G.J.; Doak, S.H. In Vitro Detection of in Vitro Secondary Mechanisms of Genotoxicity Induced by Engineered Nanomaterials. *Particle and Fibre Toxicology* **2019**, *16*, 8, doi:10.1186/s12989-019-0291-7.
97. Samadian, H.; Salami, M.S.; Jaymand, M.; Azarnezhad, A.; Najafi, M.; Barabadi, H.; Ahmadi, A. Genotoxicity Assessment of Carbon-Based Nanomaterials; Have Their Unique Physicochemical Properties Made Them Double-Edged Swords? *Mutation Research/Reviews in Mutation Research* **2020**, *783*, 108296, doi:10.1016/j.mrrev.2020.108296.
98. Donaldson, K.; Poland, C.A.; Schins, R.P.F. Possible Genotoxic Mechanisms of Nanoparticles: Criteria for Improved Test Strategies. *Nanotoxicology* **2010**, *4*, 414–420, doi:10.3109/17435390.2010.482751.
99. Elespuru, R.; Pfuhler, S.; Aardema, M.J.; Chen, T.; Doak, S.H.; Doherty, A.; Farabaugh, C.S.; Kenny, J.; Manjanatha, M.; Mahadevan, B.; et al. Genotoxicity Assessment of Nanomaterials: Recommendations on Best Practices, Assays, and Methods. *Toxicological Sciences* **2018**, *164*, 391–416, doi:10.1093/toxsci/kfy100.
100. Karlsson, H.L.; di Bucchianico, S.; Collins, A.R.; Dusinska, M. Can the Comet Assay Be Used Reliably to Detect Nanoparticle-Induced Genotoxicity? *Environmental and Molecular Mutagenesis* **2015**, *56*, 82–96, doi:10.1002/em.21933.
101. Warheit, D.B.; Donner, E.M. Rationale of Genotoxicity Testing of Nanomaterials: Regulatory Requirements and Appropriateness of Available OECD Test Guidelines. *Nanotoxicology* **2010**, *4*, 409–413, doi:10.3109/17435390.2010.485704.
102. Ali, D.; Falodah, F.A.; Almutairi, B.; Alkahtani, S.; Alarifi, S. Assessment of DNA Damage and Oxidative Stress in Juvenile *Channa Punctatus* (Bloch) after Exposure to Multi-walled Carbon Nanotubes. *Environmental Toxicology* **2020**, *35*, 359–367, doi:10.1002/tox.22871.
103. Boran, H.; Şaffak, S. Comparison of Dissolved Nickel and Nickel Nanoparticles Toxicity in Larval Zebrafish in Terms of Gene Expression and DNA Damage. *Archives of Environmental Contamination and Toxicology* **2018**, *74*, 193–202, doi:10.1007/s00244-017-0468-8.
104. Carmona, E.R.; Escobar, B.; Vales, G.; Marcos, R. Genotoxic Testing of Titanium Dioxide Anatase Nanoparticles Using the Wing-Spot Test and the Comet Assay in Drosophila. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* **2015**, *778*, 12–21, doi:10.1016/j.mrgentox.2014.12.004.
105. Chatterjee, N.; Eom, H.J.; Choi, J. Effects of Silver Nanoparticles on Oxidative DNA Damage-Repair as a Function of P38 MAPK Status: A Comparative Approach Using Human Jurkat T Cells and the Nematode *Caenorhabditis Elegans*. *Environmental and Molecular Mutagenesis* **2014**, *55*, 122–133, doi:10.1002/em.21844.
106. Butler, K.S.; Peeler, D.J.; Casey, B.J.; Dair, B.J.; Elespuru, R.K. Silver Nanoparticles: Correlating Nanoparticle Size and Cellular Uptake with Genotoxicity. *Mutagenesis* **2015**, *30*, 577–591, doi:10.1093/mutage/gev020.
107. Nelson, B.C.; Wright, C.W.; Ibuki, Y.; Moreno-Villanueva, M.; Karlsson, H.L.; Hendriks, G.; Sims, C.M.; Singh, N.; Doak, S.H. Emerging Metrology for High-Throughput Nanomaterial Genotoxicology. *Mutagenesis* **2017**, *32*, 215–232, doi:10.1093/mutage/gew037.
108. Karlsson, H.L.; Gliga, A.R.; Calléja, F.M.; Gonçalves, C.S.; Wallinder, I.O.; Vrieling, H.; Fadeel, B.; Hendriks, G. Mechanism-Based Genotoxicity Screening of Metal Oxide Nanoparticles Using the ToxTracker Panel of Reporter Cell Lines. *Particle and Fibre Toxicology* **2014**, *11*, 41, doi:10.1186/s12989-014-0041-9.
109. Collins, A.R.; Annangi, B.; Rubio, L.; Marcos, R.; Dorn, M.; Merker, C.; Estrela-Lopis, I.; Cimpan, M.R.; Ibrahim, M.; Cimpan, E.; et al. High Throughput Toxicity Screening and Intracellular Detection of Nanomaterials. *WIREs Nanomedicine and Nanobiotechnology* **2017**, *9*, doi:10.1002/wnan.1413.
110. Bobyk, L.; Tarantini, A.; Beal, D.; Veronesi, G.; Kieffer, I.; Motellier, S.; Valsami-Jones, E.; Lynch, I.; Jouneau, P.-H.; Pernet-Gallay, K.; et al. Toxicity and Chemical Transformation of Silver Nanoparticles in A549 Lung Cells: Dose-Rate-Dependent Genotoxic Impact. *Environmental Science: Nano* **2021**, *8*, 806–821, doi:10.1039/D0EN00533A.

111. Biola-Clier, M.; Beal, D.; Caillat, S.; Libert, S.; Armand, L.; Herlin-Boime, N.; Sauvaigo, S.; Douki, T.; Carriere, M. Comparison of the DNA Damage Response in BEAS-2B and A549 Cells Exposed to Titanium Dioxide Nanoparticles. *Mutagenesis* **2017**, *32*, 161–172, doi:10.1093/mutage/gew055.
112. Armand, L.; Tarantini, A.; Beal, D.; Biola-Clier, M.; Bobyk, L.; Sorieul, S.; Pernet-Gallay, K.; Marie-Desvergne, C.; Lynch, I.; Herlin-Boime, N.; et al. Long-Term Exposure of A549 Cells to Titanium Dioxide Nanoparticles Induces DNA Damage and Sensitizes Cells towards Genotoxic Agents. *Nanotoxicology* **2016**, *10*, 913–923, doi:10.3109/17435390.2016.1141338.
113. Toprani, S.M.; Bitounis, D.; Huang, Q.; Oliveira, N.; Ng, K.W.; Tay, C.Y.; Nagel, Z.D.; Demokritou, P. High-Throughput Screening Platform for Nanoparticle-Mediated Alterations of DNA Repair Capacity. *ACS Nano* **2021**, *15*, 4728–4746, doi:10.1021/acsnano.0c09254.
114. Singh, N.; Nelson, B.; Scanlan, L.; Coskun, E.; Jaruga, P.; Doak, S. Exposure to Engineered Nanomaterials: Impact on DNA Repair Pathways. *International Journal of Molecular Sciences* **2017**, *18*, 1515, doi:10.3390/ijms18071515.
115. Piao, M.J.; Kim, K.C.; Choi, J.-Y.; Choi, J.; Hyun, J.W. Silver Nanoparticles Down-Regulate Nrf2-Mediated 8-Oxoguanine DNA Glycosylase 1 through Inactivation of Extracellular Regulated Kinase and Protein Kinase B in Human Chang Liver Cells. *Toxicology Letters* **2011**, *207*, 143–148, doi:10.1016/j.toxlet.2011.09.002.
116. Lim, H.K.; Asharani, P. v.; Hande, M.P. Enhanced Genotoxicity of Silver Nanoparticles in DNA Repair Deficient Mammalian Cells. *Frontiers in Genetics* **2012**, *3*, doi:10.3389/fgene.2012.00104.
117. Bitounis, D.; Huang, Q.; Toprani, S.M.; Setyawati, M.I.; Oliveira, N.; Wu, Z.; Tay, C.Y.; Ng, K.W.; Nagel, Z.D.; Demokritou, P. Printer Center Nanoparticles Alter the DNA Repair Capacity of Human Bronchial Airway Epithelial Cells. *NanoImpact* **2022**, *25*, 100379, doi:10.1016/j.impact.2022.100379.
118. Casati, L.; Sendra, R.; Sibilia, V.; Celotti, F. Endocrine Disrupters: The New Players Able to Affect the Epigenome. *Frontiers in Cell and Developmental Biology* **2015**, *3*, doi:10.3389/fcell.2015.00037.
119. Norouzitallab, P.; Baruah, K.; Vanrompay, D.; Bossier, P. Can Epigenetics Translate Environmental Cues into Phenotypes? *Science of The Total Environment* **2019**, *647*, 1281–1293, doi:10.1016/j.scitotenv.2018.08.063.
120. Bowers, E.C.; McCullough, S.D. Linking the Epigenome with Exposure Effects and Susceptibility: The Epigenetic Seed and Soil Model. *Toxicological Sciences* **2017**, *155*, 302–314, doi:10.1093/toxsci/kfw215.
121. Thomson, J.P.; Moggs, J.G.; Wolf, C.R.; Meehan, R.R. Epigenetic Profiles as Defined Signatures of Xenobiotic Exposure. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* **2014**, *764–765*, 3–9, doi:10.1016/j.mrgentox.2013.08.007.
122. Ren, N.; Atyah, M.; Chen, W.-Y.; Zhou, C.-H. The Various Aspects of Genetic and Epigenetic Toxicology: Testing Methods and Clinical Applications. *Journal of Translational Medicine* **2017**, *15*, 110, doi:10.1186/s12967-017-1218-4.
123. Chatterjee, N.; Yang, J.; Choi, J. Differential Genotoxic and Epigenotoxic Effects of Graphene Family Nanomaterials (GFNs) in Human Bronchial Epithelial Cells. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* **2016**, *798–799*, 1–10, doi:10.1016/j.mrgentox.2016.01.006.
124. Ghosh, M.; Öner, D.; Poels, K.; Tabish, A.M.; Vlaanderen, J.; Pronk, A.; Kuijpers, E.; Lan, Q.; Vermeulen, R.; Bekaert, B.; et al. Changes in DNA Methylation Induced by Multi-Walled Carbon Nanotube Exposure in the Workplace. *Nanotoxicology* **2017**, *11*, 1195–1210, doi:10.1080/17435390.2017.1406169.
125. Ghosh, M.; Öner, D.; Duca, R.C.; Bekaert, B.; Vanoirbeek, J.A.J.; Godderis, L.; Hoet, P.H.M. Single-Walled and Multi-Walled Carbon Nanotubes Induce Sequence-Specific Epigenetic Alterations in 16 HBE Cells. *Oncotarget* **2018**, *9*, 20351–20365, doi:10.18632/oncotarget.24866.
126. Gong, C.; Tao, G.; Yang, L.; Liu, J.; Liu, Q.; Li, W.; Zhuang, Z. Methylation of PARP-1 Promoter Involved in the Regulation of Nano-SiO2-Induced Decrease of PARP-1 MRNA Expression. *Toxicology Letters* **2012**, *209*, 264–269, doi:10.1016/j.toxlet.2012.01.007.

127. Chen, Y.; Xu, M.; Zhang, J.; Ma, J.; Gao, M.; Zhang, Z.; Xu, Y.; Liu, S. Genome-Wide DNA Methylation Variations upon Exposure to Engineered Nanomaterials and Their Implications in Nanosafety Assessment. *Advanced Materials* **2017**, *29*, 1604580, doi:10.1002/adma.201604580.
128. Stapleton, P.A.; Hathaway, Q.A.; Nichols, C.E.; Abukabda, A.B.; Pinti, M. v.; Shepherd, D.L.; McBride, C.R.; Yi, J.; Castranova, V.C.; Hollander, J.M.; et al. Maternal Engineered Nanomaterial Inhalation during Gestation Alters the Fetal Transcriptome. *Particle and Fibre Toxicology* **2018**, *15*, 3, doi:10.1186/s12989-017-0239-8.
129. Cuvier, O.; Fierz, B. Dynamic Chromatin Technologies: From Individual Molecules to Epigenomic Regulation in Cells. *Nature Reviews Genetics* **2017**, *18*, 457–472, doi:10.1038/nrg.2017.28.
130. Choi, J.H.; Lee, H.; Lee, H.; Lee, H. Dopant-Dependent Toxicity of CeO2 Nanoparticles Is Associated with Dynamic Changes in H3K4me3 and H3K27me3 and Transcriptional Activation of NRF2 Gene in HaCaT Human Keratinocytes. *International Journal of Molecular Sciences* **2021**, *22*, 3087, doi:10.3390/ijms22063087.
131. Jadhav, V.; Ray, P.; Sachdeva, G.; Bhatt, P. Biocompatible Arsenic Trioxide Nanoparticles Induce Cell Cycle Arrest by P21WAF1/CIP1 Expression via Epigenetic Remodeling in LNCaP and PC3 Cell Lines. *Life Sciences* **2016**, *148*, 41–52, doi:10.1016/j.lfs.2016.02.042.
132. Li, L.; Ma, N.; Zhou, H.; Wang, Q.; Zhang, H.; Wang, P.; Hou, H.; Wen, H.; Gao, F. Zinc Oxide Nanoparticles-Induced Epigenetic Change and G2/M Arrest Are Associated with Apoptosis in Human Epidermal Keratinocytes. *International Journal of Nanomedicine* **2016**, *Volume 11*, 3859–3874, doi:10.2147/IJN.S107021.
133. Shvedova, A.A.; Yanamala, N.; Kisin, E.R.; Khailullin, T.O.; Birch, M.E.; Fatkhutdinova, L.M. Integrated Analysis of Dysregulated NcRNA and MRNA Expression Profiles in Humans Exposed to Carbon Nanotubes. *PLOS ONE* **2016**, *11*, e0150628, doi:10.1371/journal.pone.0150628.
134. Liu, L.; Jia, Y.; Zhang, X.; Chen, S.; Wang, S.; Zhu, J.; Zheng, L.; Chen, Z.; Huang, L. Identification of the Function and Regulatory Network of Circ_009773 in DNA Damage Induced by Nanoparticles of Neodymium Oxide. *Toxicology in Vitro* **2022**, *78*, 105271, doi:10.1016/j.tiv.2021.105271.
135. Eom, H.-J.; Chatterjee, N.; Lee, J.; Choi, J. Integrated MRNA and Micro RNA Profiling Reveals Epigenetic Mechanism of Differential Sensitivity of Jurkat T Cells to AgNPs and Ag Ions. *Toxicology Letters* **2014**, *229*, 311–318, doi:10.1016/j.toxlet.2014.05.019.
136. Chappell, G.; Pogribny, I.P.; Guyton, K.Z.; Rusyn, I. Epigenetic Alterations Induced by Genotoxic Occupational and Environmental Human Chemical Carcinogens: A Systematic Literature Review. *Mutation Research/Reviews in Mutation Research* **2016**, *768*, 27–45, doi:10.1016/j.mrrev.2016.03.004.
137. Zhang, S.; Meng, P.; Cheng, S.; Jiang, X.; Zhang, J.; Qin, X.; Tang, Q.; Bai, L.; Zou, Z.; Chen, C. Pregnancy Exposure to Carbon Black Nanoparticles Induced Neurobehavioral Deficits That Are Associated with Altered M6A Modification in Offspring. *NeuroToxicology* **2020**, *81*, 40–50, doi:10.1016/j.neuro.2020.07.004.
138. Han, B.; Chu, C.; Su, X.; Zhang, N.; Zhou, L.; Zhang, M.; Yang, S.; Shi, L.; Zhao, B.; Niu, Y.; et al. N ^{6} -Methyladenosine-Dependent Primary MicroRNA-126 Processing Activated PI3K-AKT-MTOR Pathway Drove the Development of Pulmonary Fibrosis Induced by Nanoscale Carbon Black Particles in Rats. *Nanotoxicology* **2020**, *14*, 1–20, doi:10.1080/17435390.2019.1661041.
139. Oh, J.-H.; Son, M.-Y.; Choi, M.-S.; Kim, S.; Choi, A.; Lee, H.-A.; Kim, K.-S.; Kim, J.; Song, C.W.; Yoon, S. Integrative Analysis of Genes and MiRNA Alterations in Human Embryonic Stem Cells-Derived Neural Cells after Exposure to Silver Nanoparticles. *Toxicology and Applied Pharmacology* **2016**, *299*, 8–23, doi:10.1016/j.taap.2015.11.004.
140. Srivastava, A.K.; Yadav, S.S.; Mishra, S.; Yadav, S.K.; Parmar, D.; Yadav, S. A Combined MicroRNA and Proteome Profiling to Investigate the Effect of ZnO Nanoparticles on Neuronal Cells. *Nanotoxicology* **2020**, *14*, 757–773, doi:10.1080/17435390.2020.1759726.

141. Wu, T.; Li, Y.; Liang, X.; Liu, X.; Tang, M. Identification of Potential CircRNA-MiRNA-MRNA Regulatory Networks in Response to Graphene Quantum Dots in Microglia by Microarray Analysis. *Ecotoxicology and Environmental Safety* **2021**, *208*, 111672, doi:10.1016/j.ecoenv.2020.111672.
142. Rossnerova, A.; Honkova, K.; Chvojkova, I.; Pelclova, D.; Zdimal, V.; Hubacek, J.A.; Lischkova, L.; Vlckova, S.; Ondracek, J.; Dvorackova, S.; et al. Individual DNA Methylation Pattern Shifts in Nanoparticles-Exposed Workers Analyzed in Four Consecutive Years. *International Journal of Molecular Sciences* **2021**, *22*, 7834, doi:10.3390/ijms22157834.
143. Liou, S.-H.; Wu, W.-T.; Liao, H.-Y.; Chen, C.-Y.; Tsai, C.-Y.; Jung, W.-T.; Lee, H.-L. Global DNA Methylation and Oxidative Stress Biomarkers in Workers Exposed to Metal Oxide Nanoparticles. *Journal of Hazardous Materials* **2017**, *331*, 329–335, doi:10.1016/j.jhazmat.2017.02.042.
144. Rossnerova, A.; Honkova, K.; Pelclova, D.; Zdimal, V.; Hubacek, J.A.; Chvojkova, I.; Vrbova, K.; Rossner, P.; Topinka, J.; Vlckova, S.; et al. DNA Methylation Profiles in a Group of Workers Occupationally Exposed to Nanoparticles. *International Journal of Molecular Sciences* **2020**, *21*, 2420, doi:10.3390/ijms21072420.
145. Bicho, R.C.; Scott-Fordsmand, J.J.; Amorim, M.J.B. Multigenerational Exposure to WCCo Nanomaterials—Epigenetics in the Soil Invertebrate Enchytraeus Crypticus. *Nanomaterials* **2020**, *10*, 836, doi:10.3390/nano10050836.
146. Zhou, W.; Tian, D.; He, J.; Yan, X.; Zhao, J.; Yuan, X.; Peng, S. Prolonged Exposure to Carbon Nanoparticles Induced Methylome Remodeling and Gene Expression in Zebrafish Heart. *Journal of Applied Toxicology* **2019**, *39*, 322–332, doi:10.1002/jat.3721.
147. Hu, J.; Lin, W.; Lin, B.; Wu, K.; Fan, H.; Yu, Y. Persistent DNA Methylation Changes in Zebrafish Following Graphene Quantum Dots Exposure in Surface Chemistry-Dependent Manner. *Ecotoxicology and Environmental Safety* **2019**, *169*, 370–375, doi:10.1016/j.ecoenv.2018.11.053.
148. Wamucho, A.; Heffley, A.; Tsyusko, O. v. Epigenetic Effects Induced by Silver Nanoparticles in Caenorhabditis Elegans after Multigenerational Exposure. *Science of The Total Environment* **2020**, *725*, 138523, doi:10.1016/j.scitotenv.2020.138523.
149. Kohl, Y.; Rundén-Pran, E.; Mariussen, E.; Hesler, M.; el Yamani, N.; Longhin, E.M.; Dusinska, M. Genotoxicity of Nanomaterials: Advanced In Vitro Models and High Throughput Methods for Human Hazard Assessment—A Review. *Nanomaterials* **2020**, *10*, 1911, doi:10.3390/nano10101911.
150. Cao, Y.; Li, S.; Chen, J. Modeling Better in Vitro Models for the Prediction of Nanoparticle Toxicity: A Review. *Toxicology Mechanisms and Methods* 2021, *31*, 1–17.
151. Tirumala, M.G.; Anchi, P.; Raja, S.; Rachamalla, M.; Godugu, C. Novel Methods and Approaches for Safety Evaluation of Nanoparticle Formulations: A Focus Towards In Vitro Models and Adverse Outcome Pathways. *Frontiers in Pharmacology* 2021, *12*.
152. Mahto, S.K.; Charwat, V.; Ertl, P.; Rothen-Rutishauser, B.; Rhee, S.W.; Sznitman, J. Microfluidic Platforms for Advanced Risk Assessments of Nanomaterials. *Nanotoxicology* 2015, *9*, 381–395.
153. Wick, P.; Chortarea, S.; Guenat, O.T.; Roesslein, M.; Stucki, J.D.; Hirn, S.; Petri-Fink, A.; Rothen-Rutishauser, B. In Vitro-Ex Vivo Model Systems for Nanosafety Assessment. *European Journal of Nanomedicine* **2015**, *7*, 169–179, doi:10.1515/ejnm-2014-0049.
154. Ashammakhi, N.; Darabi, M.A.; Çelebi-Saltik, B.; Tutar, R.; Hartel, M.C.; Lee, J.; Hussein, S.M.; Goudie, M.J.; Cornelius, M.B.; Dokmeci, M.R.; et al. Microphysiological Systems: Next Generation Systems for Assessing Toxicity and Therapeutic Effects of Nanomaterials. *Small Methods* 2020, *4*.
155. Joseph, X.; Akhil, V.; Arathi, A.; Mohanan, P. v. Comprehensive Development in Organ-On-A-Chip Technology. *Journal of Pharmaceutical Sciences* 2022, *111*, 18–31.

156. Donoghue, L.; Nguyen, K.T.; Graham, C.; Sethu, P. Tissue Chips and Microphysiological Systems for Disease Modeling and Drug Testing. *Micromachines (Basel)* **2021**, *12*, 1–35, doi:10.3390/mi12020139.
157. Fröhlich, E. Comparison of Conventional and Advanced in Vitro Models in the Toxicity Testing of Nanoparticles. *Artificial Cells, Nanomedicine and Biotechnology* 2018, *46*, 1091–1107.
158. Watson, D.E.; Hunziker, R.; Wikswo, J.P. Fitting Tissue Chips and Microphysiological Systems into the Grand Scheme of Medicine, Biology, Pharmacology, and Toxicology. *Experimental Biology and Medicine* 2017, *242*, 1559–1572.
159. Prasad, M.; Kumar, R.; Buragohain, L.; Kumari, A.; Ghosh, M. Organoid Technology: A Reliable Developmental Biology Tool for Organ-Specific Nanotoxicity Evaluation. *Frontiers in Cell and Developmental Biology* 2021, *9*.
160. Sanches, P.L.; Geaquinto, L.R.D.O.; Cruz, R.; Schuck, D.C.; Lorencini, M.; Granjeiro, J.M.; Ribeiro, A.R.L. Toxicity Evaluation of Tio2 Nanoparticles on the 3d Skin Model: A Systematic Review. *Frontiers in Bioengineering and Biotechnology* **2020**, *8*, doi:10.3389/fbioe.2020.00575.
161. Lee, E.M.; Singh, D.; Singh, D.; Choi, S.M.; Zo, S.M.; Park, S.J.; Han, S.S. Novel Alginate-Gelatin Hybrid Nanoparticle for Drug Delivery and Tissue Engineering Applications. *Journal of Nanomaterials* **2014**, *2014*, 1–7, doi:10.1155/2014/124236.
162. Doak, S.H.; Clift, M.J.D.; Costa, A.; Delmaar, C.; Gosens, I.; Halappanavar, S.; Kelly, S.; Pejinenburg, W.J.G.M.; Rothen-Rutishauser, B.; Schins, R.P.F.; et al. The Road to Achieving the European Commission's Chemicals Strategy for Nanomaterial Sustainability—A PATROLS Perspective on New Approach Methodologies. *Small* **2022**, 2200231, doi:10.1002/smll.202200231.
163. Zielińska, A.; Costa, B.; Ferreira, M. v.; Miguéis, D.; Louros, J.M.S.; Durazzo, A.; Lucarini, M.; Eder, P.; Chaud, M. v.; Morsink, M.; et al. Nanotoxicology and Nanosafety: Safety-by-Design and Testing at a Glance. *International Journal of Environmental Research and Public Health* 2020, *17*, 1–22.
164. Kermanizadeh, A.; Valli, J.; Sanchez, K.; Hutter, S.; Pawlowska, A.; Whyte, G.; Moritz, W.; Stone, V. Particulate and Drug-Induced Toxicity Assessed in Novel Quadruple Cell Human Primary Hepatic Disease Models of Steatosis and Pre-Fibrotic NASH. *Archives of Toxicology* **2022**, *96*, 287–303, doi:10.1007/s00204-021-03181-2.
165. Vanderburgh, J.; Sterling, J.A.; Guelcher, S.A. 3D Printing of Tissue Engineered Constructs for In Vitro Modeling of Disease Progression and Drug Screening. *Annals of Biomedical Engineering* **2017**, *45*, 164–179, doi:10.1007/s10439-016-1640-4.
166. Vijayavenkataraman, S.; Yan, W.-C.; Lu, W.F.; Wang, C.-H.; Fuh, J.Y.H. 3D Bioprinting of Tissues and Organs for Regenerative Medicine. *Advanced Drug Delivery Reviews* **2018**, *132*, 296–332, doi:10.1016/j.addr.2018.07.004.
167. Ahmed, H.M.M.A.M.; Moreira Teixeira, L.S. New Endeavors of (Micro)Tissue Engineering: Cells Tissues Organs on-Chip and Communication Thereof. *Cells Tissues Organs* 2021.
168. Kang, S.; Park, S.E.; Huh, D.D. Organ-on-a-Chip Technology for Nanoparticle Research. *Nano Convergence* 2021, *8*.
169. Lu, R.X.Z.; Radisic, M. Organ-on-a-Chip Platforms for Evaluation of Environmental Nanoparticle Toxicity. *Bioactive Materials* 2021, *6*, 2801–2819.
170. Huh, D.; Matthews, B.D.; Mammoto, A.; Montoya-Zavala, M.; Yuan Hsin, H.; Ingber, D.E. Reconstituting Organ-Level Lung Functions on a Chip. *Science (1979)* **2010**, *328*, 1662–1668, doi:10.1126/science.1188302.
171. Ahn, S.; Ardoña, H.A.M.; Lind, J.U.; Eweje, F.; Kim, S.L.; Gonzalez, G.M.; Liu, Q.; Zimmerman, J.F.; Pyrgiotakis, G.; Zhang, Z.; et al. Mussel-Inspired 3D Fiber Scaffolds for Heart-on-a-Chip Toxicity Studies of Engineered Nanomaterials. *Analytical and Bioanalytical Chemistry* **2018**, *410*, 6141–6154, doi:10.1007/s00216-018-1106-7.
172. Li, Y.; Liu, Y.; Hu, C.; Chang, Q.; Deng, Q.; Yang, X.; Wu, Y. Study of the Neurotoxicity of Indoor Airborne Nanoparticles Based on a 3D Human Blood-Brain Barrier Chip. *Environment International* **2020**, *143*, 105598, doi:10.1016/j.envint.2020.105598.

173. Lu, R.X.Z.; Radisic, M. Organ-on-a-Chip Platforms for Evaluation of Environmental Nanoparticle Toxicity. *Bioactive Materials* **2021**, *6*, 2801–2819, doi:10.1016/j.bioactmat.2021.01.021.
174. Xu, C.; Zhang, M.; Chen, W.; Jiang, L.; Chen, C.; Qin, J. Assessment of Air Pollutant PM2.5 Pulmonary Exposure Using a 3D Lung-on-Chip Model. *ACS Biomaterials Science & Engineering* **2020**, *6*, 3081–3090, doi:10.1021/acsbiomaterials.0c00221.
175. Joseph, X.; Akhil, V.; Arathi, A.; Mohanan, PV. Comprehensive Development in Organ-On-A-Chip Technology. *Journal of Pharmaceutical Sciences* **2022**, *111*, 18–31, doi:10.1016/j.xphs.2021.07.014.
176. Schuller, P.; Rothbauer, M.; Kratz, S.R.A.; Höll, G.; Taus, P.; Schinnerl, M.; Genser, J.; Bastus, N.; Moriones, O.H.; Puntes, V.; et al. A Lab-on-a-Chip System with an Embedded Porous Membrane-Based Impedance Biosensor Array for Nanoparticle Risk Assessment on Placental Bewo Trophoblast Cells. *Sensors and Actuators B: Chemical* **2020**, *312*, 127946, doi:10.1016/j.snb.2020.127946.
177. Yin, F.; Zhu, Y.; Zhang, M.; Yu, H.; Chen, W.; Qin, J. A 3D Human Placenta-on-a-Chip Model to Probe Nanoparticle Exposure at the Placental Barrier. *Toxicology in Vitro* **2019**, *54*, 105–113, doi:10.1016/j.tiv.2018.08.014.
178. Kohl, Y.; Biehl, M.; Spring, S.; Hesler, M.; Ogourtsov, V.; Todorovic, M.; Owen, J.; Elje, E.; Kopecka, K.; Moriones, O.H.; et al. Microfluidic In Vitro Platform for (Nano)Safety and (Nano)Drug Efficiency Screening. *Small* **2021**, *17*, 2006012, doi:10.1002/smll.202006012.
179. Bendre, A.; Bhat, M.P.; Lee, K.-H.; Altalhi, T.; Alruqi, M.A.; Kurkuri, M. Recent Developments in Microfluidic Technology for Synthesis and Toxicity-Efficiency Studies of Biomedical Nanomaterials. *Materials Today Advances* **2022**, *13*, 100205, doi:10.1016/j.mtadv.2022.100205.
180. Li, M.; Gong, J.; Gao, L.; Zou, T.; Kang, J.; Xu, H. *Advanced Human Developmental Toxicity and Teratogenicity Assessment Using Human Organoid Models*;
181. Clarke, G.A.; Hartse, B.X.; Asli, A.E.N.; Taghavimehr, M.; Hashemi, N.; Shirsavar, M.A.; Montazami, R.; Alimoradi, N.; Nasirian, V.; Ouedraogo, L.J.; et al. Advancement of Sensor Integrated Organ-on-Chip Devices. *Sensors (Switzerland)* 2021, *21*, 1–44.
182. Zoio, P.; Lopes-Ventura, S.; Oliva, A. Barrier-on-a-Chip with a Modular Architecture and Integrated Sensors for Real-Time Measurement of Biological Barrier Function. *Micromachines (Basel)* **2021**, *12*, doi:10.3390/mi12070816.
183. Alexander, F.; Eggert, S.; Wiest, J. A Novel Lab-on-a-Chip Platform for Spheroid Metabolism Monitoring. *Cytotechnology* **2018**, *70*, 375–386, doi:10.1007/s10616-017-0152-x.
184. Shaegh, S.A.M.; de Ferrari, F.; Zhang, Y.S.; Nabavinia, M.; Mohammad, N.B.; Ryan, J.; Pourmand, A.; Laukaitis, E.; Sadeghian, R.B.; Nadhman, A.; et al. A Microfluidic Optical Platform for Real-Time Monitoring of PH and Oxygen in Microfluidic Bioreactors and Organ-on-Chip Devices. *Biomicrofluidics* **2016**, *10*, doi:10.1063/1.4955155.
185. Bendre, A.; Bhat, M.P.; Lee, K.H.; Altalhi, T.; Alruqi, M.A.; Kurkuri, M. Recent Developments in Microfluidic Technology for Synthesis and Toxicity-Efficiency Studies of Biomedical Nanomaterials. *Materials Today Advances* **2022**, *13*, doi:10.1016/j.mtadv.2022.100205.
186. Azizgolshani, H.; Coppeta, J.R.; Vedula, E.M.; Marr, E.E.; Cain, B.P.; Luu, R.J.; Lech, M.P.; Kann, S.H.; Mulhern, T.J.; Tandon, V.; et al. High-Throughput Organ-on-Chip Platform with Integrated Programmable Fluid Flow and Real-Time Sensing for Complex Tissue Models in Drug Development Workflows. *Lab on a Chip* **2021**, *21*, 1454–1474, doi:10.1039/d1lc00067e.
187. Fuchs, S.; Johansson, S.; Tjell, A.; Werr, G.; Mayr, T.; Tenje, M. In-Line Analysis of Organ-on-Chip Systems with Sensors: Integration, Fabrication, Challenges, and Potential. *ACS Biomaterials Science and Engineering* 2021, *7*, 2926–2948.
188. Ehrlich, A.; Tsytkin-Kirschenzweig, S.; Ioannidis, K.; Ayyash, M.; Riu, A.; Note, R.; Ouedraogo, G.; Vanfleteren, J.; Cohen, M.; Nahmias, Y. Microphysiological Flux Balance Platform Unravels the Dynamics of Drug Induced Steatosis. *Lab on a Chip* **2018**, *18*, 2510–2522, doi:10.1039/c8lc00357b.

189. Weltin, A.; Hammer, S.; Noor, F.; Kaminski, Y.; Kieninger, J.; Urban, G.A. Accessing 3D Microtissue Metabolism: Lactate and Oxygen Monitoring in Hepatocyte Spheroids. *Biosensors and Bioelectronics* **2017**, *87*, 941–948, doi:10.1016/j.bios.2016.07.094.
190. Dornhof, J.; Kieninger, J.; Muralidharan, H.; Maurer, J.; Urban, G.A.; Weltin, A. Microfluidic Organ-on-Chip System for Multi-Analyte Monitoring of Metabolites in 3D Cell Cultures. *Lab on a Chip* **2022**, *22*, 225–239, doi:10.1039/d1lc00689d.
191. Zhou, Q.; Patel, D.; Kwa, T.; Haque, A.; Matharu, Z.; Stybayeva, G.; Gao, Y.; Diehl, A.M.; Revzin, A. Liver Injury-on-a-Chip: Microfluidic Co-Cultures with Integrated Biosensors for Monitoring Liver Cell Signaling during Injury. *Lab on a Chip* **2015**, *15*, 4467–4478, doi:10.1039/c5lc00874c.
192. Zhang, Y.S.; Aleman, J.; Shin, S.R.; Kilic, T.; Kim, D.; Shaegh, S.A.M.; Massa, S.; Riahi, R.; Chae, S.; Hu, N.; et al. Multisensor-Integrated Organs-on-Chips Platform for Automated and Continual in Situ Monitoring of Organoid Behaviors. *Proc Natl Acad Sci U S A* **2017**, *114*, E2293–E2302, doi:10.1073/pnas.1612906114.
193. Glieberman, A.L.; Pope, B.D.; Zimmerman, J.F.; Liu, Q.; Ferrier, J.P.; Kenty, J.H.R.; Schrell, A.M.; Mukhitov, N.; Shores, K.L.; Tepole, A.B.; et al. Synchronized Stimulation and Continuous Insulin Sensing in a Microfluidic Human Islet on a Chip Designed for Scalable Manufacturing. *Lab on a Chip* **2019**, *19*, 2993–3010, doi:10.1039/c9lc00253g.
194. Roy, K. Advances in QSAR Modeling. *Applications in Pharmaceutical, Chemical, Food, Agricultural and Environmental Sciences; Springer: Cham, Switzerland* **2017**, *555*, 39.
195. Hitaoka, S.; Chuman, H. Revisiting the Hansch–Fujita Approach and Development of a Fundamental QSAR. *Journal of Pesticide Science* **2013**, *38*, 60–67, doi:10.1584/jpestics.D12-082.
196. Winter, R.; Montanari, F.; Noé, F.; Clevert, D.A. Learning Continuous and Data-Driven Molecular Descriptors by Translating Equivalent Chemical Representations. *Chemical Science* **2019**, *10*, 1692–1701, doi:10.1039/C8SC04175J.
197. Lima, T.S.M.; Souza, W.; Geaquinto, L.R.O.; Sanches, P.L.; Stepień, Ewa.L.; Meneses, J.; Gortari, E.F.; Meisner-Kober, N.; Himly, M.; Granjeiro, J.M.; et al. Nanomaterial Exposure, Extracellular Vesicle Biogenesis and Adverse Cellular Outcomes: A Scoping Review. *Nanomaterials 2022, Vol. 12, Page 1231* **2022**, *12*, 1231, doi:10.3390/NANO12071231.
198. Huang, H.J.; Kraevaya, O.A.; Voronov, I.I.; Troshin, P.A.; Hsu, S.H. Fullerene Derivatives as Lung Cancer Cell Inhibitors: Investigation of Potential Descriptors Using QSAR Approaches. *Int J Nanomedicine* **2020**, *15*, 2485–2499, doi:10.2147/IJN.S243463.
199. Gao, Y.; Zhai, H.; She, X.; Si, H. Quantitative Structure-Activity Relationships; Studying the Toxicity of Metal Nanoparticles. *Current Topics in Medicinal Chemistry* **2020**, *20*, 2506–2517, doi:10.2174/1568026620666200722112113.
200. Roy, J.; Roy, K. Modeling and Mechanistic Understanding of Cytotoxicity of Metal Oxide Nanoparticles (MeOxNPs) to Escherichia Coli: Categorization and Data Gap Filling for Untested Metal Oxides. *Nanotoxicology* **2022**, doi:10.1080/17435390.2022.2038299.
201. Wang, S.; Yan, X.; Su, G.; Yan, B. Cytotoxicity Induction by the Oxidative Reactivity of Nanoparticles Revealed by a Combinatorial GNP Library with Diverse Redox Properties. *Molecules* **2021**, *26*, doi:10.3390/MOLECULES26123630.
202. Zhang, F.; Wang, Z.; Vijver, M.G.; Peijnenburg, W.J.G.M. Probing Nano-QSAR to Assess the Interactions between Carbon Nanoparticles and a SARS-CoV-2 RNA Fragment. *Ecotoxicology and Environmental Safety* **2021**, *219*, 112357, doi:10.1016/J.ECOENV.2021.112357.

203. Dhumal, D.M.; Patil, P.D.; Kulkarni, R. v.; Akamanchi, K.G. Experimentally Validated QSAR Model for Surface PKaPrediction of Heterolipids Having Potential as Delivery Materials for Nucleic Acid Therapeutics. *ACS Omega* **2020**, *5*, 32023–32031, doi:10.1021/ACSOMEGA.0C04931/ASSET/IMAGES/LARGE/AO0C04931_0007.JPEG.
204. Huang, Y.; Li, X.; Xu, S.; Zheng, H.; Zhang, L.; Chen, J.; Hong, H.; Kusko, R.; Li, R. Quantitative Structure-Activity Relationship Models for Predicting Inflammatory Potential of Metal Oxide Nanoparticles. *Environ Health Perspect* **2020**, *128*, 1–13, doi:10.1289/EHP6508.
205. Shi, H.; Pan, Y.; Yang, F.; Cao, J.; Tan, X.; Yuan, B.; Jiang, J. Molecules Nano-SAR Modeling for Predicting the Cytotoxicity of Metal Oxide Nanoparticles to PaCa2. **2021**, doi:10.3390/molecules26082188.
206. Gousiadou, C.; Marchese Robinson, R.L.; Kotzabasaki, M.; Doganis, P.; Wilkins, T.A.; Jia, X.; Sarimveis, H.; Harper, S.L. Machine Learning Predictions of Concentration-Specific Aggregate Hazard Scores of Inorganic Nanomaterials in Embryonic Zebrafish. *Nanotoxicology* **2021**, *15*, 446–476, doi:10.1080/17435390.2021.1872113.
207. Liu, G.; Yan, X.; Sedykh, A.; Pan, X.; Zhao, X.; Yan, B.; Zhu, H. Analysis of Model PM 2.5-Induced Inflammation and Cytotoxicity by the Combination of a Virtual Carbon Nanoparticle Library and Computational Modeling. *Ecotoxicol Environ Saf* **2020**, *191*, doi:10.1016/J.ECOENV.2020.110216.
208. Qi, R.; Pan, Y.; Cao, J.; Jia, Z.; Jiang, J. The Cytotoxicity of Nanomaterials: Modeling Multiple Human Cells Uptake of Functionalized Magneto-Fluorescent Nanoparticles via Nano-QSAR. *Chemosphere* **2020**, *249*, doi:10.1016/J.CHEMOSPHERE.2020.126175.
209. Toropova, A.P.; Toropov, A.A.; Leszczynska, D.; Leszczynski, J. Application of Quasi-SMILES to the Model of Gold-Nanoparticles Uptake in A549 Cells. *Computers in Biology and Medicine* **2021**, *136*, 104720, doi:10.1016/J.COMPBIOMED.2021.104720.
210. Toropov, A.A.; Toropova, A.P. The System of Self-Consistent Models for the Uptake of Nanoparticles in PaCa2 Cancer Cells. *Nanotoxicology* **2021**, *15*, 995–1004, doi:10.1080/17435390.2021.1951387.
211. Ahmadi, S.; Toropova, A.P.; Toropov, A.A. Correlation Intensity Index: Mathematical Modeling of Cytotoxicity of Metal Oxide Nanoparticles. *Nanotoxicology* **2020**, *14*, 1118–1126, doi:10.1080/17435390.2020.1808252.
212. Toropov, A.A.; Toropova, A.P. Quasi-SMILES as a Basis for the Development of Models for the Toxicity of ZnO Nanoparticles. *Science of The Total Environment* **2021**, *772*, 145532, doi:10.1016/J.SCITOTENV.2021.145532.
213. Toropova, A.P.; Toropov, A.A.; Leszczynski, J.; Sizochenko, N. Using Quasi-SMILES for the Predictive Modeling of the Safety of 574 Metal Oxide Nanoparticles Measured in Different Experimental Conditions. *Environmental Toxicology and Pharmacology* **2021**, *86*, 103665, doi:10.1016/J.ETAP.2021.103665.
214. Kumar, P.; Kumar, A. Unswerving Modeling of Hepatotoxicity of Cadmium Containing Quantum Dots Using Amalgamation of QuasiSMILES, Index of Ideality of Correlation, and Consensus Modeling. *Nanotoxicology* **2021**, *15*, 1199–1214, doi:10.1080/17435390.2021.2008039.
215. Fjodorova, N.; Novič, M.; Venko, K.; Drgan, V.; Rasulev, B.; Türker Saçan, M.; Sağ Erdem, S.; Tugcu, G.; Toropova, A.P.; Toropov, A.A. How Fullerene Derivatives (FDs) Act on Therapeutically Important Targets Associated with Diabetic Diseases. *Comput Struct Biotechnol J* **2022**, *20*, 913–924, doi:10.1016/J.CSBJ.2022.02.006.
216. Bunmahotama, W.; Vijver, M.G.; Peijnenburg, W. Development of a Quasi–Quantitative Structure–Activity Relationship Model for Prediction of the Immobilization Response of Daphnia Magna Exposed to Metal-Based Nanomaterials. *Environmental Toxicology and Chemistry* **2022**, *00*, 0–1, doi:10.1002/ETC.5322.
217. Wilkinson, M.D.; Dumontier, M.; Aalbersberg, Ij.J.; Appleton, G.; Axton, M.; Baak, A.; Blomberg, N.; Boiten, J.-W.; da Silva Santos, L.B.; Bourne, P.E.; et al. The FAIR Guiding Principles for Scientific Data Management and Stewardship. *Scientific Data* **2016**, *3*, 160018, doi:10.1038/sdata.2016.18.
218. Jeliazkova, N.; Chomenidis, C.; Doganis, P.; Fadeel, B.; Grafström, R.; Hardy, B.; Hastings, J.; Hegi, M.; Jeliazkov, V.; Kochev, N.; et al. The ENanoMapper Database for Nanomaterial Safety Information. *Beilstein Journal of Nanotechnology* **2015**, *6*, 1609, doi:10.3762/BJNANO.6.165.

219. European Commission *Communication from the Commission to the European Parliament, the European Council, the Council, the European Economic and Social Committee and the Committee of the Regions the European Green Deal, COM(2019) 640 Final*; Brussels, 2019;
220. European Commission *Communication from the Commission to the European Parliament, The Council, The European Economic and Social Committee and The Committee of The Regions A New Circular Economy Action Plan For a Cleaner and More Competitive Europe. COM(2020) 98 Final.*; Brussels, 2020;
221. United Nations United Nations Transforming Our World: The 2030 Agenda for Sustainable Development Available online: https://sdgs.un.org/2030agenda (accessed on 26 April 2022).
222. Gottardo, S.; Mech, A.; Drbohlavová, J.; Małyska, A.; Bøwadt, S.; Riego Sintes, J.; Rauscher, H. Towards Safe and Sustainable Innovation in Nanotechnology: State-of-Play for Smart Nanomaterials. *NanoImpact* 2021, *21*.
223. Friedersdorf, L.E.; Bjorkland, R.; Klaper, R.D.; Sayes, C.M.; Wiesner, M.R. Fifteen Years of NanoEHS Research Advances Science and Fosters a Vibrant Community. *Nature Nanotechnology* 2019, *14*, 996–998.
224. Dekkers, S.; Wijnhoven, S.W.P.; Braakhuis, H.M.; Soeteman-Hernandez, L.G.; Sips, A.J.A.M.; Tavernaro, I.; Kraegeloh, A.; Noorlander, C.W. Safe-by-Design Part I: Proposal for Nanospecific Human Health Safety Aspects Needed along the Innovation Process. *NanoImpact* **2020**, *18*, doi:10.1016/j.impact.2020.100227.
225. Shandilya, N.; Marcoulaki, E.; Barruetabeña, L.; Llopis, I.R.; Noorlander, C.; Jiménez, A.S.; Oudart, Y.; Puelles, R.C.; Pérez-Fernández, M.; Falk, A.; et al. Perspective on a Risk-Based Roadmap towards the Implementation of the Safe Innovation Approach for Industry. *NanoImpact* **2020**, *20*, doi:10.1016/j.impact.2020.100258.
226. Pomar-Portillo, V.; Park, B.; Crossley, A.; Vázquez-Campos, S. Nanosafety Research in Europe – Towards a Focus on Nano-Enabled Products. *NanoImpact* **2021**, *22*, doi:10.1016/j.impact.2021.100323.
227. Tsalidis, G.A.; Soeteman-Hernández, L.G.; Noorlander, C.W.; Saedy, S.; van Ommen, J.R.; Vijver, M.G.; Korevaar, G. Safe-and-Sustainable-by-Design Framework Based on a Prospective Life Cycle Assessment: Lessons Learned from a Nano-Titanium Dioxide Case Study. *International Journal of Environmental Research and Public Health* **2022**, *19*, 4241, doi:10.3390/ijerph19074241.
228. Jantunen, A.P.K.; Gottardo, S.; Rasmussen, K.; Crutzen, H.P. An Inventory of Ready-to-Use and Publicly Available Tools for the Safety Assessment of Nanomaterials. *NanoImpact* **2018**, *12*, 18–28, doi:10.1016/j.impact.2018.08.007.
229. Soeteman-Hernandez, L.G.; Apostolova, M.D.; Bekker, C.; Dekkers, S.; Grafström, R.C.; Groenewold, M.; Handzhiyski, Y.; Herbeck-Engel, P.; Hoehener, K.; Karagkiozaki, V.; et al. Safe Innovation Approach: Towards an Agile System for Dealing with Innovations. *Materials Today Communications* **2019**, *20*, doi:10.1016/j.mtcomm.2019.100548.
230. Sánchez Jiménez, A.; Puelles, R.; Perez-Fernandez, M.; Barruetabeña, L.; Jacobsen, N.R.; Suarez-Merino, B.; Micheletti, C.; Manier, N.; Salieri, B.; Hischier, R.; et al. Safe(r) by Design Guidelines for the Nanotechnology Industry. *NanoImpact* **2022**, *25*, doi:10.1016/j.impact.2022.100385.
231. Organisation for Economic Co-operation and Development (OECD) *Moving Towards a Safe(r) Innovation Approach (SIA) for More Sustainable Nanomaterials and Nano-Enabled Products. Series on the Safety of Manufactured Nanomaterials No. 96. ENV/JM/MONO(2020)36/REV1*; 2020;
232. Keijer, T.; Bakker, V.; Slootweg, J.C. Circular Chemistry to Enable a Circular Economy. *Nature Chemistry* 2019, *11*, 190–195.
233. Furxhi, I.; Perucca, M.; Blosi, M.; Lopez de Ipiña, J.; Oliveira, J.; Murphy, F.; Costa, A.L. ASINA Project: Towards a Methodological Data-Driven Sustainable and Safe-by-Design Approach for the Development of Nanomaterials. *Frontiers in Bioengineering and Biotechnology* **2022**, *9*, doi:10.3389/fbioe.2021.805096.
234. Salieri, B.; Barruetabeña, L.; Rodríguez-Llopis, I.; Jacobsen, R.; Manier, N.; Trouiller, B.; Chapon, V.; Hadrup, N.; Sánchez Jiménez, A.; Micheletti, C.; et al. Integrative Approach in a Safe by Design Context Combining Risk, Life

Cycle and Socio-Economic Assessment for Safer and Sustainable Nanomaterials. *NanoImpact* **2021**, *23*, 100335, doi:10.1016/j.impact.2021.100335ï.

235. Sánchez Jiménez, A.; Puelles, R.; Pérez-Fernández, M.; Gómez-Fernández, P.; Barruetabeña, L.; Jacobsen, N.R.; Suarez-Merino, B.; Micheletti, C.; Manier, N.; Trouiller, B.; et al. Safe(r) by Design Implementation in the Nanotechnology Industry. *NanoImpact* **2020**, *20*, doi:10.1016/j.impact.2020.100267.
236. Schaumann, G.E.; Philippe, A.; Bundschuh, M.; Metreveli, G.; Klitzke, S.; Rakcheev, D.; Grün, A.; Kumahor, S.K.; Kühn, M.; Baumann, T.; et al. Understanding the Fate and Biological Effects of Ag- and TiO2-Nanoparticles in the Environment: The Quest for Advanced Analytics and Interdisciplinary Concepts. *Science of the Total Environment* 2015, *535*, 3–19.
237. Nowack, B.; Baalousha, M.; Bornhöft, N.; Chaudhry, Q.; Cornelis, G.; Cotterill, J.; Gondikas, A.; Hassellöv, M.; Lead, J.; Mitrano, D.M.; et al. Progress towards the Validation of Modeled Environmental Concentrations of Engineered Nanomaterials by Analytical Measurements. *Environmental Science: Nano* 2015, *2*, 421–428.
238. Mitrano, D.M.; Nowack, B. The Need for a Life-Cycle Based Aging Paradigm for Nanomaterials: Importance of Real-World Test Systems to Identify Realistic Particle Transformations. *Nanotechnology* 2017, *28*.
239. Cai, X.; Liu, X.; Jiang, J.; Gao, M.; Wang, W.; Zheng, H.; Xu, S.; Li, R. Molecular Mechanisms, Characterization Methods, and Utilities of Nanoparticle Biotransformation in Nanosafety Assessments. *Small* 2020, *16*.
240. Nowack, B.; Mitrano, D.M. Procedures for the Production and Use of Synthetically Aged and Product Released Nanomaterials for Further Environmental and Ecotoxicity Testing. *NanoImpact* 2018, *10*, 70–80.
241. Mitrano, D.M.; Motellier, S.; Clavaguera, S.; Nowack, B. Review of Nanomaterial Aging and Transformations through the Life Cycle of Nano-Enhanced Products. *Environment International* 2015, *77*, 132–147.
242. Klaine, S.J.; Koelmans, A.A.; Horne, N.; Carley, S.; Handy, R.D.; Kapustka, L.; Nowack, B.; von der Kammer, F. Paradigms to Assess the Environmental Impact of Manufactured Nanomaterials. *Environmental Toxicology and Chemistry* **2012**, *31*, 3–14, doi:10.1002/etc.733.
243. Hischier, R.; Walser, T. Life Cycle Assessment of Engineered Nanomaterials: State of the Art and Strategies to Overcome Existing Gaps. *Science of the Total Environment* **2012**, *425*, 271–282, doi:10.1016/j.scitotenv.2012.03.001.
244. Hischier, R.; Nowack, B.; Gottschalk, F.; Hincapie, I.; Steinfeldt, M.; Som, C. Life Cycle Assessment of Façade Coating Systems Containing Manufactured Nanomaterials. *Journal of Nanoparticle Research* **2015**, *17*, doi:10.1007/s11051-015-2881-0.
245. Salieri, B.; Turner, D.A.; Nowack, B.; Hischier, R. Life Cycle Assessment of Manufactured Nanomaterials: Where Are We? *NanoImpact* 2018, *10*, 108–120.
246. Cucurachi, S.; Rocha, C.F.B. Life-Cycle Assessment of Engineered Nanomaterials. In *Nanotechnology in Eco-efficient Construction: Materials, Processes and Applications*; Elsevier, 2018; pp. 815–846 ISBN 9780081026410.
247. Nizam, N.U.M.; Hanafiah, M.M.; Woon, K.S. A Content Review of Life Cycle Assessment of Nanomaterials: Current Practices, Challenges, and Future Prospects. *Nanomaterials* 2021, *11*.
248. Chong, W.C.; Chung, Y.T.; Teow, Y.H.; Zain, M.M.; Mahmoudi, E.; Mohammad, A.W. Environmental Impact of Nanomaterials in Composite Membranes: Life Cycle Assessment of Algal Membrane Photoreactor Using Polyvinylidene Fluoride – Composite Membrane. *Journal of Cleaner Production* **2018**, *202*, 591–600, doi:10.1016/j.jclepro.2018.08.121.
249. Fernandes, S.; da Silva, J.C.G.E.; da Silva, L.P. Life Cycle Assessment of the Sustainability of Enhancing the Photodegradation Activity of TiO2 with Metal-Doping. *Materials* **2020**, *13*, doi:10.3390/ma13071487.
250. Fernandes, S.; Esteves da Silva, J.C.G.; Pinto da Silva, L. Comparative Life Cycle Assessment of High-Yield Synthesis Routes for Carbon Dots. *NanoImpact* **2021**, *23*, doi:10.1016/j.impact.2021.100332.

251. Pallas, G.; Vijver, M.G.; Peijnenburg, W.J.G.M.; Guinée, J. Life Cycle Assessment of Emerging Technologies at the Lab Scale: The Case of Nanowire-Based Solar Cells. *Journal of Industrial Ecology* **2020**, *24*, 193–204, doi:10.1111/jiec.12855.
252. Pallas, G.; Vijver, M.G.; Peijnenburg, W.J.G.M.; Guinée, J. Ex Ante Life Cycle Assessment of GaAs/Si Nanowire–Based Tandem Solar Cells: A Benchmark for Industrialization. *International Journal of Life Cycle Assessment* **2020**, *25*, 1767–1782, doi:10.1007/s11367-020-01791-z.
253. Beloin-Saint-Pierre, D.; Hischier, R. Towards a More Environmentally Sustainable Production of Graphene-Based Materials: Building on Current Knowledge to Offer Recommendations. *International Journal of Life Cycle Assessment* **2021**, *26*, 327–343, doi:10.1007/s11367-020-01864-z.
254. Garas, G.; Sayed, A.M.; Bakhoum, E.S.H. Application of Nano Waste Particles in Concrete for Sustainable Construction: A Comparative Study. *International Journal of Sustainable Engineering* **2021**, *14*, 2041–2047, doi:10.1080/19397038.2021.1963004.
255. Lin, S.; Ng, S.F.; Ong, W.J. Life Cycle Assessment of Environmental Impacts Associated with Oxidative Desulfurization of Diesel Fuels Catalyzed by Metal-Free Reduced Graphene Oxide. *Environmental Pollution* **2021**, *288*, doi:10.1016/j.envpol.2021.117677.
256. Mio, A.; Bertagna, S.; Cozzarini, L.; Laurini, E.; Bucci, V.; Marinò, A.; Fermeglia, M. Multiscale Modelling Techniques in Life Cycle Assessment: Application to Nanostructured Polymer Systems in the Maritime Industry. *Sustainable Materials and Technologies* **2021**, *29*, doi:10.1016/j.susmat.2021.e00327.
257. Musino, D.; Rosset, A.; Lelong, C.; Luche, S.; Bergé, V.; Brochard, G.; Plumail, M.; Trouiller, B.; Auger, A.; Guiot, A.; et al. CNC/AgNP Hybrids as Safer-by-Design Biocides in Paints. *Environmental Science: Nano* **2021**, *8*, 3673–3684, doi:10.1039/d1en00407g.
258. Rezvani Ghomi, E.; Khosravi, F.; Saedi Ardahaei, A.; Dai, Y.; Neisiany, R.E.; Foroughi, F.; Wu, M.; Das, O.; Ramakrishna, S. The Life Cycle Assessment for Polylactic Acid (PLA) to Make It a Low-Carbon Material. *Polymers (Basel)* 2021, *13*.
259. Ponnusamy, P.G.; Mani, S. Life Cycle Assessment of Manufacturing Cellulose Nanofibril-Reinforced Chitosan Composite Films for Packaging Applications. *International Journal of Life Cycle Assessment* **2022**, doi:10.1007/s11367-022-02035-y.
260. Garcia Gonzalez, M.N.; Quiroga-Flores, R.; Börjesson, P. Life Cycle Assessment of a Nanomaterial-Based Adsorbent Developed on Lab Scale for Cadmium Removal: Comparison of the Impacts of Production, Use and Recycling. *Cleaner Environmental Systems* **2022**, *4*, 100071, doi:10.1016/j.cesys.2022.100071.
261. Beloin-Saint-Pierre, D.; Turner, D.A.; Salieri, B.; Haarman, A.; Hischier, R. How Suitable Is LCA for Nanotechnology Assessment? Overview of Current Methodological Pitfalls and Potential Solutions: 65th LCA Discussion Forum, Swiss Federal Institute of Technology, Zürich, May 24, 2017. In Proceedings of the International Journal of Life Cycle Assessment; Springer Verlag, January 1 2018; Vol. 23, pp. 191–196.
262. Organisation for Economic Co-operation and Development (OECD) *Nanomaterials in Waste Streams*; OECD, 2016; ISBN 9789264240612.
263. Zahra, Z.; Habib, Z.; Hyun, S.; Sajid, M. Nanowaste: Another Future Waste, Its Sources, Release Mechanism, and Removal Strategies in the Environment. *Sustainability (Switzerland)* 2022, *14*.
264. Brar, S.K.; Verma, M.; Tyagi, R.D.; Surampalli, R.Y. Engineered Nanoparticles in Wastewater and Wastewater Sludge - Evidence and Impacts. *Waste Management* 2010, *30*, 504–520.
265. Gottschalk, F.; Nowack, B. The Release of Engineered Nanomaterials to the Environment. *Journal of Environmental Monitoring* 2011, *13*, 1145–1155.

266. Part, F.; Zecha, G.; Causon, T.; Sinner, E.K.; Huber-Humer, M. Current Limitations and Challenges in Nanowaste Detection, Characterisation and Monitoring. *Waste Management* **2015**, *43*, 407–420, doi:10.1016/j.wasman.2015.05.035.
267. Huang, Y.; Keller, A.A.; Cervantes-Avilés, P.; Nelson, J. Fast Multielement Quantification of Nanoparticles in Wastewater and Sludge Using Single-Particle ICP-MS. *ACS ES&T Water* **2021**, *1*, 205–213, doi:10.1021/acsestwater.0c00083.
268. Thonemann, N.; Schulte, A.; Maga, D. How to Conduct Prospective Life Cycle Assessment for Emerging Technologies? A Systematic Review and Methodological Guidance. *Sustainability (Switzerland)* 2020, *12*.
269. Gottschalk, F.; Scholz, R.W.; Nowack, B. Probabilistic Material Flow Modeling for Assessing the Environmental Exposure to Compounds: Methodology and an Application to Engineered Nano-TiO2 Particles. *Environmental Modelling and Software* **2010**, *25*, 320–332, doi:10.1016/j.envsoft.2009.08.011.
270. Adam, V.; Caballero-Guzman, A.; Nowack, B. Considering the Forms of Released Engineered Nanomaterials in Probabilistic Material Flow Analysis. *Environmental Pollution* **2018**, *243*, 17–27, doi:10.1016/j.envpol.2018.07.108.
271. Zheng, Y.; Nowack, B. Size-Specific, Dynamic, Probabilistic Material Flow Analysis of Titanium Dioxide Releases into the Environment. *Environmental Science and Technology* **2021**, *55*, 2392–2402, doi:10.1021/acs.est.0c07446.
272. Adam, V.; Wu, Q.; Nowack, B. Integrated Dynamic Probabilistic Material Flow Analysis of Engineered Materials in All European Countries. *NanoImpact* **2021**, *22*, doi:10.1016/j.impact.2021.100312.
273. Hischier, R. Framework for LCI Modelling of Releases of Manufactured Nanomaterials along Their Life Cycle. *International Journal of Life Cycle Assessment* **2014**, *19*, 838–849, doi:10.1007/s11367-013-0688-8.
274. Gilbertson, L.M.; Wender, B.A.; Zimmerman, J.B.; Eckelman, M.J. Coordinating Modeling and Experimental Research of Engineered Nanomaterials to Improve Life Cycle Assessment Studies. *Environmental Science: Nano* **2015**, *2*, 669–682, doi:10.1039/c5en00097a.
275. Organisation for Economic Co-operation and Development (OECD) *Guidance Manual Towards The Integration of Risk Assessment into Life Cycle Assessment of Nano-Enabled Applications. Series on the Safety of Manufactured Nanomaterials No. 57 ENV/JM/MONO(2015)30.*; 2015;
276. Salieri, B.; Hischier, R.; Quik, J.T.K.; Jolliet, O. Fate Modelling of Nanoparticle Releases in LCA: An Integrative Approach towards "USEtox4Nano." *Journal of Cleaner Production* **2019**, *206*, 701–712, doi:10.1016/j.jclepro.2018.09.187.
277. Fantke, P.; Aylward, L.; Bare, J.; Chiu, W.A.; Dodson, R.; Dwyer, R.; Ernstoff, A.; Howard, B.; Jantunen, M.; Jolliet, O.; et al. Advancements in Life Cycle Human Exposure and Toxicity Characterization. *Environmental Health Perspectives* **2018**, *126*, doi:10.1289/EHP3871.
278. Salieri, B.; Kaiser, J.P.; Rösslein, M.; Nowack, B.; Hischier, R.; Wick, P. Relative Potency Factor Approach Enables the Use of in Vitro Information for Estimation of Human Effect Factors for Nanoparticle Toxicity in Life-Cycle Impact Assessment. *Nanotoxicology* **2020**, *14*, 275–286, doi:10.1080/17435390.2019.1710872.
279. Meesters, J.A.J.; Peijnenburg, W.J.G.M.; Hendriks, A.J.; van de Meent, D.; Quik, J.T.K. A Model Sensitivity Analysis to Determine the Most Important Physicochemical Properties Driving Environmental Fate and Exposure of Engineered Nanoparticles. *Environmental Science: Nano* **2019**, *6*, 2049–2060, doi:10.1039/c9en00117d.
280. Wigger, H.; Nowack, B. Material-Specific Properties Applied to an Environmental Risk Assessment of Engineered Nanomaterials–Implications on Grouping and Read-across Concepts. *Nanotoxicology* **2019**, *13*, 623–643, doi:10.1080/17435390.2019.1568604.
281. Hetherington, A.C.; Borrion, A.L.; Griffiths, O.G.; McManus, M.C. Use of LCA as a Development Tool within Early Research: Challenges and Issues across Different Sectors. *International Journal of Life Cycle Assessment* **2014**, *19*, 130–143, doi:10.1007/s11367-013-0627-8.

 doi:10.20944/preprints202205.0229.v1

282. Adrianto, L.R.; van der Hulst, M.K.; Tokaya, J.P.; Arvidsson, R.; Blanco, C.F.; Caldeira, C.; Guillén-Gonsálbez, G.; Sala, S.; Steubing, B.; Buyle, M.; et al. How Can LCA Include Prospective Elements to Assess Emerging Technologies and System Transitions? The 76th LCA Discussion Forum on Life Cycle Assessment, 19 November 2020. In Proceedings of the International Journal of Life Cycle Assessment; Springer Science and Business Media Deutschland GmbH, August 1 2021; Vol. 26, pp. 1541–1544.