Impact and effectiveness of risk mitigation strategies on the insurability of nanomaterial production: evidences from industrial case studies

Focus Article

Impact and effectiveness of risk mitigation strategies on the insurability of nanomaterial production: evidences from industrial case studies

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Workers involved in producing nanomaterials or using nanomaterials in manufacturing plants are likely to have earlier and higher exposure to manufactured/engineered nanomaterials (ENM) than the general population. This is because both the volume handled and the probability of the effluence of 'free' nanoparticles from the handled volume are much higher during a production process than at any other stage in the lifecycle of nanomaterials and nanotechnology-enabled products. Risk assessment (RA) techniques using control banding (CB) as a framework for risk transfer represents a robust theory but further progress on implementing the model is required so that risk can be transferred to insurance companies. Following a review of RA in general and hazard measurement in particular, we subject a Structural Alert Scheme methodology to three industrial case studies using ZrO_2, TiO_2, and multi-walled carbon nanotubes (MWCNT). The materials are tested in a pristine state and in a remediated (coated) state, and the respective emission and hazard rates are tested alongside the material performance as originally designed. To our knowledge, this is the first such implementation of a CB RA in conjunction with an ENM performance test and offers both manufacturers and underwriters an insight into future applications.

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INTRODUCTION

Ensuring safe exposure scenarios should be considered as an ethical obligation toward all workers involved in the production of nanomaterials. There is also, in many jurisdictions, a legal obligation [e.g., Control of substances hazardous to health (COSHH) in the UK] for employers to protect these workers from any work-related harm. An insurance provider is an important stakeholder and, to a great extent, functions as a proxy regulator by influencing work practices and standard operating protocols (SOP). If

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insufficient efforts are made by the employer to protect its workforce, it may be difficult or prohibitively expensive to obtain insurance. Given the multiplicity of stakeholders and the associated cost/benefits, a strong case can be made that the development of effective risk mitigation strategies offer an economic opportunity as well as assisting in the responsible development of nanotechnology.[1] The International Risk Governance Council recommends a corrective and adaptive approach that takes into account the level and extent of available knowledge of nanomaterials so that a societal balance of the predicted risks and benefits can be achieved.[2] More recently, ISO standards provide a pragmatic approach for the control of occupational exposures to Engineered NanoMaterials (ENMs).[3]

Risk Assessment (RA) is a process that integrates the identification and collection of data related to hazard and exposure to enable the characterization of risk in a population (e.g., workers) in order to provide information needed for effective Risk Management (RM). RA is increasingly applied not just within the occupational setting but across the life cycle of a product or technology and in relation to ENMs, there remains substantial uncertainties which makes risk prediction difficult.[4] For example, conventional RA frameworks currently fail to estimate the risks from the exposure to ENMs due to the methodological limitations and epistemological uncertainties. The lack of quantitative health data can potentially lead to ambiguous, qualitative risk estimations, and this could result in a failure to adequately support timely regulatory decisions—all of which are critical for effective RM.[4]

Studies that reduce the uncertainty in these key factors would influence the estimates of the risks present, improve RA, and lead to more effective risk mitigation strategies in the industrial manufacturing of ENM.[5] To comply with this objective, there are two main types of recommendations addressing firstly, hazards and secondly exposures as the critical components of RA. In terms of hazards, there is a need to:

1 implement testing strategies for assessing toxicity and ecotoxicity;
2 find the best metrics for assessing particle toxicity and ecotoxicity;
3 use nomenclature which includes novel relevant attributes driving adverse effects, such as surface area, surface reactivity and morphology; and
4 identify handling and dispersion protocols'methods for ENMs.

In terms of exposure, there is a need to:

1 develop exposure monitoring methodologies including use of most relevant metrics (e.g., particle number, surface area, etc.);
2 employ methods for reducing exposure such as engineering containment and protection;
3 implement closed-loop production with recycling of effluent nanomaterials; and
4 implement personal protection equipment.

RM, on the other hand, comprises the selection of a strategy or strategies that are designed to avoid, prevent, reduce, transfer, or self-contain risks. This may include the development of 'safety by molecular or process design' approaches, applied to single nano-phases or to more critical process steps such as waste treatment and disposal. Such an approach to safety by design has the chance to form a preventive and robust RM measure to prevent risks rather than address them when they occur. Such an approach becomes an effective and sustainable RM tool only if supported by a sound and practical RA in conjunction with performance evaluation of the nanomaterials and process. Finally, improved communication and dialogue on RA and RM actions are absolutely required.

While workers involved in the manufacture of nanomaterials or nano-enabled products are at the frontline in terms of exposure potential, there is a paucity of research on the assessment of the risks of exposure to ENMs within the nano-manufacturing setting. RA techniques such as the use of control banding (CB) have been suggested by many[6] as a potential framework that can be used for this purpose as a proxy for specific testing such as on-site measurements of emission/exposure; however, further progress on developing and implementing such models is required to improve their predictive power and reliability so that risk associated with nanomaterial production can be effectively quantified and transferred to insurance companies. This is important as it provides a pragmatic and cost-effective approach RA and RM to ensure long-term sustainability of nanomaterials production.[6] While CB tools are not being primarily designed for underwriters, they can, in many instances, help to categorize both exposure and hazard. The resultant risk location can then be used to rank the acceptability of certain risks or can be associated with certain production processes in the nanotechnology sector so that an insurance premium can be applied to specific scenarios. The economic burden of insurance could encourage companies to adopt a proactive behavior toward RM procedures,

demonstrating that safety practices have been put in place and proven to be effective to reduce emission and exposure and/or to reduce the toxicity (if any) of a given ENM. However, the success of CB critically depends on identifying hazards, and here, despite the effort of the scientific community[7,8] there remains a high degree of ambiguity.

In this article, we increase the general applicability and relevance of hazard information data related to a specific nanoparticle by developing a Structural Alert Scheme (SAS) which may also be used by non-specialists. The scheme has been developed within the framework of a European Commission-funded research Project 'Sanowork' on the basis of a collation of known, biologically effective doses apparent in particle toxicology.[9] The SAS is based on recognized commonalities in the mechanisms of toxicity, which has been identified over decades of research by the toxicology community and linked with particle physicochemical properties. Such physicochemical properties, known as 'structural indicators' of particles are known to infer a hazard. Therefore, the hazard identification scheme uses 'structural alerts' such as classification of the bulk material as a carcinogenic, mutagenic, or reproductive toxin, or on the basis of its size, surface area, chemical reactivity, surface charge, solubility and morphology—to evaluate the toxicological profile of a given material. Such key properties enable a rapid classification of particles based upon common physicochemical characterization data.

Herein, the SAS scheme to real case studies that involves the manufacture of three ENMs that are currently produced on an industrial scale; namely zirconia (ZrO_2), titania (TiO_2), and multi-wall carbon nanotubes (MWCNT). These materials have been following a safety-by-design strategy and tested both in a pristine state and in a remediated state, and the respective emission and hazard rates have been tested alongside the testing of the desired material performance. We then test the effectiveness and impact of the adopted risk mitigation strategies in terms of the change in risk relocation and finally the impact that risk remediation strategies have on material performances. To our knowledge, this is the first such implementation of a CB RA in conjunction with an ENM performance test and offers both manufacturers and underwriters an insight into future applications.

METHODOLOGY

A case study method has been used to test the effectiveness and impact of the risk mitigation strategies on the insurability of three selected ENMs: ZrO_2, TiO_2, and MWCNTs. These were chosen because of their industrial and societal relevance due to the fact that they are currently being mass-produced by a number of manufacturers around the world. The case studies were built up through an epistemological discourse of risk characteristics and associated terminologies so that a common theoretical framework is set up for insurance risk analysis. The SAS was then developed and implemented for each of the three ENMs. Our central hypothesis is that by tuning both the emission potential/exposure and the hazardous properties identified on the basis of the SAS and assessed by using toxicologically relevant tests, different situations can be related to the cases studied. This would, in turn, support the validity of the proposed remediation strategies for risk mitigation, which could then be implemented for RM. Below we describe •these two steps, epistemological discourse and SAS implementation, in detail.

Epistemological Discourse of Risks Associated with ENM Production

CB and RA

For RA, CB is used to categorize risk levels as a surrogate marker of exposure and hazard. The main components and information required for a CB approach are:

1. the severity score, which is estimated on the basis of known or suspected hazardous properties. In the case of ENMs, these hazardous properties can include surface chemistry, surface area, particle shape, size and morphology, solubility, carcinogenicity/reproductive toxicity, mutagenicity, dermal toxicity and if it acts as a sensitizer (respiratory or dermal); and
2. the probability score (i.e., the exposure probability), which includes the estimated amount of material used, dustiness/mistiness, number of employees with similar exposure, frequency of operation, and duration of operation.

In the absence of quantitative data, representing an effective strategy of risk communication to insurers by using a generally accepted risk ranking, the CB approach can be adapted to become a tool to suggest behavior-based RM practices. In particular, the bands related to the exposure can surrogate for the lack of field measurements.

Control Banding and Exposure and Hazard

In the CB approach, both exposure and toxicity potential can be categorized and appropriate risk weightings

are applied to these categories. The exposure assessment can be performed by scoring different factors such as the physical state of the materials/products containing ENM (e.g., as an aerosol, dry powder, liquid form, and so on), quantity (experimental or mass production), emission potential (cleanroom environment or open space and the emissivity of the ENM), frequency, and duration of use. The overall score can then be used to determine the level of exposure potential. Similarly, toxicity levels can be assigned with the help of a scientific literature review and lab analysis (i.e., *in vivo* and *in vitro* tests) as well as applying scores to parameters such as chemical composition, particle shape, size, surface charge, solubility, aggregation, and agglomeration of nanomaterials. Typically, nanotechnology manufacturers• familiar with data reporting on quantitative exposure levels can check whether such levels are within the Occupational Exposure Limits (OELs). This has implications in terms of the implementation of the CB approach by insurers for it is along the toxicity axis of a two-dimensional toxicity-exposure CB, that any movement is liable to have consequence in health and safety concerns.

Emission and Exposure

In every step of ENM production and handling, there is the need to identify critical stages of emission potential, perhaps restricted to some selected operations. The human exposure potential of a nanoparticle depends on the probability of becoming airborne and entering the body by inhalation, ingestion, or dermal pathways. From this exposure potential, possible harmful effects are determined by its intrinsic toxicity, its bioavailability, and the ability to accumulate, persist, and translocate within the environment and the human body. Products that contain hazardous nanoparticles may create potential health and safety risks throughout the product life cycle, including material processing, transportation, manufacture, use, and disposal/ recycling of products containing ENMs. While in theory it is possible to obtain some form of quantitative information related to ENMs, current analytical instruments are generally inadequate in establishing an appropriate quantitative workplace personal exposure measurements, especially using unconventional metrics such as surface area.[10] Thus, exposure represents a weak link in terms of the estimation of possible health outcomes. A minimum set of data that should be reported for all ENM exposure studies has been proposed by a group of scientists concerned with exposure assessment and characterization.[11] This set of data includes, but may not be limited to, both nano-specific (e.g., physicochemical characteristics of airborne released and measured particles, emissivity, and flow dynamics) and non-nano-specific information (e.g., on processes, description of sites, and presence of RM tools put in place).

Significantly more research is needed to obtain the comprehensive exposure scenarios and associated exposure estimates for ENMs. In occupational/environmental settings, harmful effects from particles are consistently associated with changes that occur at their surfaces from the early stages of their life, i.e., from the point of creation or emission. Mechanical, thermal, and chemical processes lead to different types and extent of emissions. Furthermore, agglomerations or aggregations of nanoparticles, including nanoparticles of heterogeneous compositions, lead to exposures that were otherwise unlikely to occur with single nanoparticles.[12,13] Such changes in chemical identity can originate from, but not limited to, contaminants arising from the synthesis process. Notwithstanding, nanomaterials' surfaces can adsorb airborne and ground molecules, e.g., bacterial endotoxins,[14] which are able to induce inflammatory responses, e.g., via NLRP3 inflammosome.[15] These contaminants of biological origin are among the most powerful inflammatory stimuli and are able to modulate biological responses by immune-competent cells, such as airways macrophages. Recent research has demonstrated that ENM, such as TiO2, can absorb LPS•, a component of gram-negative bacterial wall, thus enhancing the inflammatory response *in vitro* of macrophages.[16] Such changes in both chemical and biological identities, and the consequences therefrom in terms of safety of ENM, have been recently emphasized[17–19] and represent a challenge in predicting health hazards resulting from exposure to ENM in the real exposure scenarios.

Another important consideration is that current nanoparticle hazard is typically assessed in experimental conditions that are far from mimicking real exposure scenarios. In spite of the intrinsic characteristics of particles, the environment nanoparticles inhabit can modulate biological responses well above what is expected during the standardized approach used for *in vitro* studies utilizing 'clean' particles. In real life, such as in manufacturing and handling conditions, the surface of nano-objects can collect airborne and ground molecules, such as bacterial endotoxins, which are able to induce inflammatory responses.

Emission versus Exposure

Nevertheless, the reliability of information contained in a CB approach and the subsequent usability of such information by underwriters largely depends on the expertise of industrial hygienists who are

usually in charge of application of such a scheme. Such professionals are typically more familiar with exposure parameters than the hazardous properties of newly synthesized chemicals that usually lack safety information. Indeed, emission potential parameters alone do not predict the worker exposure potential. That said, they are a good starting point in disclosing the likelihood that ENMs become airborne during production. Currently, the toxicity potential of many ENMs is substantially unknown. Such a lack of information has been overcome by the application of the precautionary principle that does not represent a proactive strategy, instead is quite a conservative approach marred by uncertainties.

Standardization

There are control-banding tools available for RM that can be adopted by the insurer but with some modifications.[20–25] The current lack of standardization in the safety issues posed by the nanotechnology development is a significant obstacle to the effectiveness of CB as an underwriting tool. Recently, the International Standards Organization (ISO) has made efforts to define both nanomaterial characteristics and provide nanomaterial characterization methodologies in this regard.[3] ISO/TS 12901–2:2014 'Occupational risk management applied to engineered nanomaterials – Part 2: Use of the control banding approach' is intended for use by competent personnel, such as health and safety managers, production managers, environmental managers, industrial/occupational hygienists, and others with a responsibility for the safe operation of facilities engaged in production, handling, processing, and disposal of ENMs. We also see the development of ISO/TS 12901–2:2014 is applicable to engineered materials that consist of nano-objects such as nanoparticles, nanofibres, nanotubes, and nanowires, as well as their aggregates and agglomerates (NOAA).

Hazard Axis

The development of a severity score based on hazardous properties or even hazard data for a given ENM is certainly the most involved part of any effort aimed at assessing the reliability and validity of a CB approach as an underwriting tool and, in general, for health and safety managers. Understanding the potential hazards of these novel materials before the occurrence of any widespread exposure should be seen as mandatory. However, obtaining such an understanding is not an easy undertaking as it requires in-depth toxicological investigations to ascertain a solid, generally accepted basis of any hazard analysis related to ENM. Conducting primary toxicological analysis on all new materials or products, in particular those subject to surface modification (e.g., by coating), imposes a substantial and often unaffordable burden on industry, in particular, on small and medium enterprises, which either have limited toxicological testing facility and/or the financial resources to outsource. This limitation stifles innovation and the potential benefits of nanotechnology. Therefore, a more cost-effective approach, especially during the research and development phase of new nanoparticles or nano-containing products, could be the use of secondary sources of information, such as the toxicological literature to inform us of potential hazards. However, this often requires careful interpretation and extrapolation of data by experts as the available literature data may not necessarily be tailored to extract hazard data for the particle in question. Additionally, there can be variability within one single kind of material. This variability may originate from many transformations during their production, handling, and analysis. This signifies the huge scale of effort that is required to create and share protocols of analysis, but with specific information on nano-manufacturing steps and main transformations occurred at different levels. This makes it very difficult to make a comparison between the same material from different sources. Quantitative hazard assessment of the type suitable for extrapolation to humans typically requires *in vivo* testing to internationally accepted standards (such as OECD Test guidelines) using relevant routes of exposure (e.g., inhalation testing). However, such testing can be prohibitively expensive and also comes with an ethical burden over the use of animal testing. Ideally, hazard data would be taken from the well-designed *in vitro* tests of nanoparticles with the appropriate biological systems (cell lines, medium, etc.) mimicking the *in vivo* situations as much as possible with a validated route of *in vitro* to *in vivo* extrapolation. Ultimately, a number of industrial applications may actually need *in vivo* data primarily from appropriate animal models to establish safety, efficacy, bioavailability, and fate of nanoparticles. Needless to say, the associated cost, complexity, and ethical burden increase accordingly.

Hazard Banding

Another approach for determining hazard potential of ENMs is through the identification of the key physicochemical characteristics that represent the 'structural determinants' of toxicity. For example, a fibrous shape of sufficient length is known to increase the pathogenicity of a material. A key example of this is a study whereby titanium dioxide (TiO_2) nanoparticles were shown to be of low toxicity to the lungs, yet their toxicity increased markedly when formed into

long fibers (nanobelts).[26] In the above study, the toxicity and pathogenic potential changed dramatically as the shape of the material is altered into one that a phagocytic cell has difficulty processing. Therefore, we can see the fibrous shape as a 'structural indicator' of potential toxicity. This approach thus relies on establishing a correlation between the hazard potential and its structural determinants and, if properly developed, can indeed support the development of 'safer' ENMs, allowing the appropriate modification of their aspect ratio and synthesis so as to lower their toxic potential while preserving their useful and innovative features.

As elimination, i.e., avoiding the hazardous substance or the process which causes exposure, is unlikely to be an option if an ENM has been selected for its specific properties, it might be possible to reduce the likelihood of exposure by, for example, binding powder ENMs in liquid or solid media or increasing the agglomeration state. Dispersions, pastes, or pelletized forms should be used instead of dry and dusty powder substances wherever this is technically feasible. Moreover, surface modification addressed to decrease hazard specific properties by preserving nanoscale reactivity should be investigated as an 'elimination/substitution' primary prevention strategy.[27]

However, more effective control measures can rely on the 'Prevention through Design' (PtD) approach, a proactive tool to prevent possible hazard and exposure potential to mitigate risks rather than address them when they occur. PtD is an approach (and in the United States, a national initiative) to design out hazards rather than address them as an exposure. This approach can be applied both at the molecular and at process scale levels. On a molecular scale, it is indeed possible to modify the nanomaterial to suppress toxicity while preserving its innovative properties to commercial purposes. It is foreseen that such an approach toward the management of safety issues should influence the hazard management, as the *a priori* mitigation of potential toxicity can avoid the need to manage possible health consequences coming from exposure, especially if prolonged. The above strategy represents a proactive approach to the RM (in the framework of a global scenario still adopting a precautionary principle).[27]

Hazard Identification using Materials Safety Data Sheets

The first step in the identification of a hazard, i.e., of intrinsic properties making a conventional chemical harmful, relies on information which is usually listed in material safety data sheets (MSDS). MSDS are critical sources of information for a diverse range of professionals including workers, employers, product manufacturers, vendors, importers as well as regulating authorities, and insurance companies. The quality and accuracy of information provided by these documents are important in order to fulfill their main purpose, i.e., communicating risk. However, several pitfalls have been highlighted within the MSDS in relation to nanomaterials. For example, current MSDS do not allow for the description of nanomaterials in comparison to their bulk forms. As the hazard potential of nanomaterials is related to its physicochemical properties, information on important physicochemical parameters such as size, size distribution, surface area, zeta potential are important from the point of a proper consideration of potential hazards. While such physicochemical measurements are neither necessarily difficult to obtain nor excessive in terms of investment, they are usually not included in current MSDS.

Another deficiency of MSDS is that the recommendations for OELs are relevant for the bulk material and have not been validated for the 'nano' forms of the material. Extrapolating OELs for bulk to that for 'nano' scale is potentially misleading especially without considering detailed physicochemical information of the 'nano' form as exemplified by the recommended exposure limits (REL) proposed by the National Institute for Occupational Safety and Health (NIOSH) for TiO_2. Here based on *in vivo* studies of both micron (fine) and nano-sized (ultra-fine) TiO_2 particles, NIOSH proposed a REL of 2.4 mg/m^3 for fine TiO_2 and a much lower level of 0.3 mg/m^3 for ultrafine including engineered nanoscale form as time-weighted average (TWA).[28] It is also worth noting that due to the observed relationship between TiO_2 particle surface area dose and toxicity, NIOSH proposed that the measurement of aerosol surface area would be the most appropriate metric (and allow a single exposure limit) for evaluating workplace exposures to TiO_2.[28] However, as mentioned earlier, personal sampling devices suitable for routine workplace for measuring particle surface area are not currently available leading NIOSH to propose a dual REL reflecting the differential toxicity of fine versus ultrafine TiO_2. While the lack of information in a MSDS is nothing new and certainly not restricted to nanomaterials only, a general recommendation for appropriate risk communication could be that, in the absence of sufficient data, a precautionary principle should be applied and always be emphasized within MSDS.

Implementation of the Structural Alert System

Hazard identification of selected ENMs is articulated around three key points:

1 Critical analysis of the SDS of the nanomaterials, as provided by the company supplying the ENM. The appraisal is based upon the literature resources and guidance documents describing best practices such as that from ISO as well as the Swiss State Secretariat for Economic Affairs[29].
2 Screening of the literature for the development of a practical hazard identification scheme (i.e., the SAS tool).
3 Integration of the above scheme with an efficient (toxicological) testing strategy.

Using physicochemical data provided either by the suppliers of nanomaterials or gathered from reference laboratories, each material and its remediated form were analyzed using the hazard identification scheme. This scheme aims at providing a practical and accessible approach for hazard identification and evaluation of remediation from a toxicological point of view so that it can be readily understood and used by a diversity of users such as insurance companies, risk assessors, and toxicologists. Table 1 summarizes the nanomaterial's physicochemical characteristics, which are appraised from the literature and known to strongly influence the particle's toxicological profile. Scientific arguments/evidences for toxicological consequences for each of these particles can be found in the literature and are summarized in Table 1. The specific question underlying the use of a SAS is to identify the hazard accurately without carrying out any toxicological testing. The physicochemical characteristics influencing toxicity, or 'structural alerts', were drawn together into a simple, intuitive flow decision tree that raise specific questions on materials' intrinsic properties to identify and inform on their potential hazards. The aim and the vision of such scheme is to identify key 'structural indicators' of nanoparticle toxicity, as identified and supported by the scientific literature, and form these into a single scheme or 'tool'. The 'structural indicators' are simply physicochemical properties of particles that are known to infer a hazard. For example, a fibrous shape of sufficient length is known to increase the pathogenicity of a material. Therefore, we can see fibrous shape as a 'structural indicator' of potential toxicity, and this can be achieved without toxicological testing.

For each ENM produced and its remediated form, the following straightforward questions, relevant for identifying structural determinants of hazard, were answered:

1 Is the median particle size in the upper or lower portion of the 100 nm size range?
2 Is the bulk material classified as carcinogenic, mutagenic, or toxic for reproduction (CMR) or sensitizer?
3 Is the nanomaterial reactive? (because: (a) contaminated with reactive contaminants, or (b) intrinsically reactive for the presence of chemical groups or photo-reactive)
4 Is the nanomaterial highly acidic/basic?
5 Does the nanomaterial have a charged surface?
6 Is the nanomaterial soluble? If so, does dissolution lead to the release of toxic or reactive components such as ionic species?
7 Is the nanomaterial a High Aspect Respirable Particle (HARP)?

Median values are common determinants in defining the statistical size of a particle which has a heterogeneous size distribution. A typical example for measuring a particle size distribution is to use the so-called D50 value, which is the point where half of the size distribution resides above this value and the remaining half below. The use of the median value is thus compliant with the EU recommendation 2011/696/EU. This is particularly appropriate for a distribution which deviates significantly from a Gaussian distribution and possesses long outlier tails.

Once the basic premises of the tool were defined, the next step was to implement the tool to identify potential hazards associated with the intrinsic properties of a selection of targeted nanomaterials used within a manufacturing environment. If a given material being evaluated triggers a 'Yes' response, then this material may present a hazard and the nature of this hazard and potential effect (subject to dose) it may cause are reported in the scheme. A negative response still requires all other questions to be answered. A 'Yes' answer does not necessarily mean that the nanoparticle will pose a significant hazard. Indeed, the structural indicators are not given in terms of quantitative ranges, i.e., detailing the level of hazard associated with a measurable quantity of a certain property (e.g., iron contamination), instead it should be considered that within each 'Yes' response, there may be a spectrum of toxicity from low/none to high and testing would be required to quantify this. In the absence of testing, a precautionary approach should be adopted. The reason why there is not a quantitative range and instead, only qualitative properties are that sufficient detailed information is not available to define such parameters. However, if such an approach is to be considered by the industrial, regulatory, and research communities to be of value in screening nanomaterials

TABLE 1 | The Relationship Between Structural Alerts and Physicochemical Features Relevant in terms of Hazard and Biological Effects

Structural Alert	Physicochemical characteristics	Hazard/Effects Consequences
Size distribution: nanometric (1–100 nm range)	• Large surface area • High surface-to-volume ratio • Large amount of less coordinated and more reactive atoms/ions exposed at the particle surface	• Potential for translocation • Increased deposition along the respiratory tract, in particular gas-exchange region • Different cell penetration routes and retention in many cells and organs to a larger extent than larger particles • Enhanced surface reactivity • Foster dissolution of the materials thus lead to the release of potentially toxic ions
Bulk material classified as a carcinogenic, mutagenic or toxic for reproduction (CMR) or sensitizer	• It cannot be excluded that the NM is a CMR or skin/respiratory sensitizer until tested	• Potential for repeated dose toxicity, carcinogenesis, mutagenesis, sensitization and/or reproductive toxicity
Purity/Contaminations	• Presence of reactive Transition metals used as catalysts • Amorphous carbon • PAHs etc. • Biological contaminants (e.g., endotoxins)	• Potential for ion driven cytotoxicity/inflammation/ Oxidative stress, leading to acute toxicity, repeated dose toxicity (e.g. fibrosis), sensitization and/or carcinogenicity • Enhanced inflammatory potential (*in vivo*)
Intrinsic reactivity of the material	• Photo-reactivity • Chemical reactivity • Presence of surface defects • Importance of surface reactivity relative to surface area	• Potential phototoxicity (*infrequent*) • Potential for inflammogenic effects and/ or genotoxicity leading to acute toxicity, repeated dose toxicity, and/or carcinogenesis • Modulated by the interactions with biomolecules
Intrinsic acidity/basicity	pH alterations away from the normal range (for tissues/biological systems)	• A substantial pH deviation away from the normal range of the biological environment at the site of deposition could cause local effects such as skin irritation/corrosion, or cell death within the lungs leading to inflammation/oedema/fibrosis.
Surface charge	Propensity to agglomerate or aggregate in various fluids Zeta-potential as proxy for particle charge giving an idea of the level of agglomeration/aggregation of the material	• Potential for translocation • Reduced reactivity (i.e., agglomeration into large particles, will decrease the biologically accessible surface area) • Biological membrane and protein interactions (*charged biomolecules*) • Uptake by cells • Potential for cytotoxicity/inflammation leading to acute toxicity, repeated dose toxicity
Solubility	• Release of ions in different matrices • Bio-durability • (*Note: the bio-durability of carbon nanotubes has been shown to depend on many parameters such as their structure mono or multi-wall, the presence of surface defects, their functionalization etc.*)	• Cell uptake and release of toxic ions inside cells • Potential for ion driven cytotoxicity/ inflammation/Oxidative stress/leading to acute toxicity (*Note: soluble particle that does not release toxic ions or other components could result in the overall progressive reduction/removal of dose as the particle dissolves ultimately removing any toxic stimulus (if caused) or be intrinsically non-toxic. However, a particle that is soluble but releases toxic/reactive ions or other components may generate localized or even systemic toxicant accumulation and, hence, toxicity*). • Bio-persistence of the dose

TABLE 1 | Continued

Structural Alert	Physicochemical characteristics	Hazard/Effects Consequences
Morphology and size/ classification as a High Aspect Ratio Particle (HARP)	• Aerodynamic diameter • Aspect ratio • Fibrous aspect/bundle-like spherical morphology	• Potential for impaired clearance, lung, and pleural retention • Potential for cytotoxicity/inflammation/oxidative stress leading to acute toxicity, repeated dose • toxicity (e.g., Fibrosis) and/or carcinogenicity

the deriving of such quantitative parameters could be achieved by targeted research.

Finally, the scheme does not consider what is the most crucial component of toxicology, which is dose, which can only be derived from considering exposure. Testing of the particles to identify a threshold dose resulting in a significant toxicological effect (i.e., via a dose response) must be compared to relevant exposure levels to ascertain whether the quantified toxicological results are of relevance (i.e., a positive result at a very high dose, far above what can reasonably expected during human exposure could be considered irrelevant).

Further refinements of the tool were thus conducted through an intelligent testing strategy which addressed—by a panel of *in vitro* models that relied on relevant biological effects such as the production of Reactive Oxygen Species (ROS), assessment of cell viability, genotoxicity, cell transformation, functional endpoints (e.g., cytokine secretion or Nitric Oxide production), and alteration of biological barriers. The test methods used correlate to a specific structural alert/hazard/effect.

RESULTS AND DISCUSSIONS

Case 1 – ZrO_2 nanopowders

ZrO_2 is one of the most important materials used in the industry because of its high melting point, mechanical properties, low thermal conductivity, and high ionic conductivity as well as antibacterial activity.[30,31] ZrO_2 nano-powders can become airborne during maintenance operations, such as reactor washing, disposal or during recovery, and processing of the nano-powders. To reduce the likelihood of exposure to the pristine nano-powders in waste water environment, forced aggregation of ZrO_2 has been triggered using surface charge reduction to induce powder gelification. Emission potential was assessed before and after the gelification process and the success of forced aggregation strategy evaluated. The performance of the remediated ZrO_2 in reducing the emission potential was estimated in terms of an increase in the sedimentation rate and waste reduction (by 99%) and the quality or technological properties of recycled uncoated ZrO_2, which was considered as comparable to the pristine form of ZrO_2. On the hazard control side, nano-powders have been coated with inert materials such as SiO_2 (inorganic coating) or citrate (organic coating) by heterocoagulation or chemical synthesis and health hazard of pristine and remediated form assessed.

Health hazards from ZrO_2 nano-powders are relatively less known. The MSDS sheet supplied by the manufacturer (Company 1) only included data for the identification of the substance/mixture, firefighting and first aid measures, but did not include toxicological information on ZrO_2 nano-powders useful to characterize the hazard. From the literature analysis, ZrO_2 is known as a photoreactive material[32] and this property is likely to be enhanced at the nano size. ZrO_2 is not classified as a carcinogen but the study by Mohr et al.[33] did find an increased tumor incidence upon exposure to the bulk material. No evidence of ion release upon particle dispersion in aqueous environment (i.e., cell culture medium) is available.

The application of the SAS on the basis of available information (Table 1) leads to the following conclusions: while is not listed as a carcinogen by ACGIH, IARC, or NTP, limited evidence in animals does suggest that at high doses (overload conditions) of ZrO_2 is potentially carcinogenic. ZrO_2 is in the lower portion of the 100 nm size range (20 nm), not a sensitizer, potentially photo-reactive, water insoluble, has a high positive surface charge (+50 mV) yet low intrinsic reactivity based on ROS production measured in dark condition by Electron paramagnetic resonance (EPR) with the spin trap, Tempone-H, the dispersion has a low acid pH of 1.62 which may pose a hazard, and cannot be classified as a HARP. The citrate-coated ZrO_2 nanoparticles (3% wt ZrO_2 and 3% wt trisodium citrate dihydrate) showed a pH = 6.5 which does not pose a hazard, but the other characteristics were substantially unchanged with the exception of surface charge which reduced from a positive charge of 50 mV to a negative charge of −32.8 mV.

To fill in the gaps in hazard information, the interaction of ZrO_2 with biological systems was assessed along an established range of doses *in vitro*. The toxicity of pristine ZrO_2 NP was tested and compared with the cytotoxicity of Aeroxide P25 TiO_2 NP used as a benchmark control. ZrO_2 NPs appear endowed with slight cytotoxicity (10% loss viability) in two cell lines (human alveolar cells A549 and murine macrophages RAW 264.7) and scarce capability to activate macrophages (Nos2 gene induction), cell-specific ability to elicit oxidative stress, reduced proliferation (RI), enhanced apoptosis (CBMN cyt) and necrosis (MTT). Using the DCFH assay, ZrO_2 nano-powders were found to significantly increase oxidative stress in A549 cells treated at a dose of 40 μg/cm^2 culture area. Taken together, these data suggest a very low bioreactivity of ZrO_2 nano-powder.

Citrate-coated ZrO_2 showed a slight reduction in cell viability and in the ability to elicit oxidative stress, but was indifferent for other endpoints. The most interesting finding was that ZrO_2 nanopowders caused substantial red blood cell lysis (39.9%) indicating an adverse effect on the cell membrane, whereas the citrate-coated ZrO_2 did not (12.4%). The hemolytic potential has been suggested to correlate with the lung inflammogenicity of metal oxide nanoparticles[34] and merits further study.

These results suggest that this remediation of ZrO_2 nano-powders using citrate coating is effective mainly in reducing oxidative stress generated by the particle's surface reactivity and reducing hemolytic potential via a reduction in surface charge. As a result, in combination with the significant reduction in emission potential deriving from surface coating, the remediation strategy also seems to affect the hazardous properties allowing a shift along the 'severity axis' from high to low toxicity even in possible accidental exposure scenarios. Other biological endpoints relevant to long-term effects (e. g. cell transformation) were collected. However, no difference was seen between the pristine and the modified ENMs.

Case 2. TiO_2 Nanosols

TiO_2 nanosols are increasingly used as photocatalytic additives for ceramic industries. One of the most cost-effective technologies for impregnation and surface coating is represented by spray coating. However, spray coating results in an appreciable emission potential of airborne particles[35] with the potential for exposure to workers. In many manufacturing plants enclosure and general ventilation are not feasible as engineering control tools. For these reasons the control of hazard potential can be a useful strategy to mitigate the risk in the presence of unavoidable exposure. The same strategy used for ZrO_2 has been used for TiO_2 and the effect of SiO_2 (inorganic coating) or citrate (organic coating) evaluated.

TiO_2 is classified by the International Agency for Research on Cancer[36] as possibly carcinogenic to humans (Group 2B) based on sufficient evidence in experimental animals and inadequate evidence from epidemiological studies and it is also a well-known photocatalyst triggering the formation of radicals in biological systems.[37,38] There is a body of literature reporting that ultraviolet (UV)-light irradiation promotes the release of reactive oxidative species (ROS) from TiO_2 nanoparticles in aqueous or humid conditions. ROS have been known to induce cell damage and cell death both *in vitro* and *in vivo*. In addition, published reports point to genotoxicity and ROS production in connection to the exposure to some forms of TiO_2 nanoparticles.[38,39]

The MSDS sheet supplied by the manufacturer (Company 2) included data for the identification of the substance/mixture, but the crystal phase information (anatase) was originally missing and furthermore, the product was not labeled as a nano product. Useful information for identifying associated hazards was, however, included as was information on ingredients in the product. Neither the physical and chemical properties nor the information on size distribution were available [e.g., those that can be obtained by Dynamic Light Scattering (DLS) tests]. The OELs were given for bulk materials while the material was in a nano-form and there was no indication of whether these bulk values were appropriate or not; although based on the recommendations of NIOSH[28] described above, the use of the bulk OEL would not be appropriate. In terms of the toxicological information, one of the major omissions from the MSDS is that TiO_2 has been classified as a Group 2B carcinogen by the International Agency for Research on Cancer.[40]

It is well known that any change on the surface acidity influences the zeta potential, colloidal stability, and surface hydrophylicity of metal oxides dispersed in water and, in turn, can be related to particle toxicity, independently from pH medium. We have found that while pristine TiO_2 colloidal suspension in water is highly acidic pH (1.58), citrate-coated TiO_2 becomes slightly basic with improved hydrophilicity[41] although there is little modification in hydrodynamic diameter (45 and 58 nm, respectively). In addition pristine TiO_2 colloid suspension possesses a very high positive charge of +41.2 mV which is abrogated by citrate coating (−37.4 mV) but not by SiO_2 coating (+32.2 mV). It is well known that TiO_2 is insoluble in water and ions are not released when particles are

dispersed in an aqueous environment (e.g., cell culture medium). Therefore, based on the physicochemical properties reported in the literature, it appears that the main point of alteration between the pristine and remediated TiO_2, i.e., the citrate coating, poses the lowest hazard in terms of the deviation away from an acid/base neutral solution and a reduction in surface charge.

The toxicity of TiO_2 nanosols (both pristine and coated) has been tested and compared with the cytotoxicity of Aeroxide P25 TiO_2 NP used as a benchmark control. Pristine TiO_2 nanosols did not induce overt cytotoxicity in A549 cells and in RAW264.7 cell lines and did not affect proliferative activity (e.g., the inhibiting concentration of 20% of cell population—IC_{20}—was over 80 $\mu g/cm^2$). They showed a slight capability to induce oxidative stress and macrophage activation (Nos2 gene) and induction of TNF-alpha (a marker of inflammogenicity) owing to the low cytotoxicity. Other biological endpoints were investigated, e.g., whether the ENMs are able to affect the epithelial permeability (by the assessment of transepithelial electric resistance – TEER) in monolayers of human airway epithelial cells (Calu-3) undergoing a sub-acute exposure to the materials (up to 12 days). The results indicate the absence of any significant effects. In relation to the hemolytic potential of the pristine and remediated forms, pristine TiO_2 caused high levels of red blood cell lysis (68.4%), yet this was significantly reduced by surface treatment with either citrate (14.1%; $P < 0.01$) or SiO_2 (3.7%; $P < 0.001$). In the case of citrate coating, this led to a substantial reduction in surface charge which could account for the reduction in hemolysis yet this reduction in surface was not seen with SiO_2 coating suggesting an alternative mechanism of mitigation. As a result, it is evident that evaluation of the SiO_2 particles using SAS scheme would result in a 'Yes' answer to the question 'Does the nanomaterial have a highly charged surface?' indicating potential toxicity through membrane interactions but testing using the hemolysis assay suggest this may not be the case resulting in a potential false positive.

TiO_2 is photo-active and as such the intrinsic reactivity of the TiO_2 nanoparticle preparations was investigated in the presence of UV-light to assess the effect of remediation strategies on ROS generation. The results indicate that, on equal mass basis, coating pristine TiO_2 nanoparticles with SiO_2 or citrate does not affect the ability of TiO_2 nanoparticles to generate the ROS. However, if the dilutive effect of the addition of SiO_2 (or citrate) is accounted for and the results normalized to the TiO_2 content, the outcome can be different. For example, coating with SiO_2 leads to an increase in the surface photo-reactivity and increasing levels of SiO_2 lead to the generation of higher ROS. Otherwise the citrate coating caused a decrease of photo-reactivity when assessed both in a TiO_2 floating and immobilized system.[42]

SiO_2-coated TiO_2 NPs did not exert significant cytotoxicity in the cell models tested. On the contrary, citrate-coated TiO_2 in RAW 264.7 and A549 cells showed a slight albeit significant decrease (up to 15% at 80 $\mu g/cm^2$; $P < 0.001$) in cell viability thus indicating cytotoxicity. There was also a significant (fivefold) enhancement in micro-nucleus frequency, a genotoxicity parameter, compared to pristine TiO_2 NP.

To sum up, although pristine TiO_2 NP seems endowed with low cytotoxicity, showing no effect on epithelial permeability and a mild capability to induce oxidative stress and macrophage activation, silica or citrate coatings remediation seems to be ineffective. Materials undergoing surface modifications through heterocoagulation with silica NP apparently exhibited greater effects than the pristine form, both in terms of nitric oxide (NO) production and cytokine induction. The solvent control generally gives negative results while silica NP, used for remediation, also exhibit a mild pro-inflammogenic activity. Citrate coating, however, increased the toxicity potential. Silica coatings provides a reduction in surface toxicity, only if coupled with granulation step (spray-drying) that improve the interaction between heterocoagulated TiO_2 and SiO_2. In this case the remediation of TiO_2 nanosols seems effective mainly in reducing oxidative stress generated by nanoparticles' surface reactivity. The data suggest that a combination of approaches (e.g., silica coating and spray drying) could improve the biocompatibility of TiO_2 nanoforms, thus reducing the hazard.

Case 3. MWCNT

MWCNT have generated a great deal of interest due to their peculiar mechanical and electrical properties as reinforcing agents in novel hybrid or polymeric composites combining the beneficial properties of multiple materials. Recent studies have investigated in depth the consequences of the interaction of MWCNT with biological systems, highlighting severe toxic effects induced by these materials both *in vivo* and *in vitro*.[43–47] Due to structural similarities in terms of the 'needle-like' shape, in combination with their high aspect ratio and low dispersion of some forms of carbon nanotubes, it has been hypothesized, that where MWCNT exhibit fiber-type morphology their respiratory toxic properties may be similar to those of other fibrous materials (e.g., asbestos and nickel nanowires), the toxicity mechanisms of which

are related to the fiber pathogenicity and the frustrated phagocytosis paradigms.[48–50] Indeed, numerous *in vivo* studies have already demonstrated that MWCNT, when inhaled or instilled into the lungs of rodents, have the potential to cause transient inflammatory changes, granuloma formation, and fibrosis in the lung tissue[48,51,44] as well as translocate from the lung to the pleural cavity.[52] Long (>20 μm), straight MWCNT have also been shown to have the potential to cause inflammation and granuloma formation in the mesothelial lining of the pleura, consistent with the pathogenic behavior of asbestos.[53,54]

Exposure to MWCNT can occur during the synthesis and recovery phase of the carbon nanotube production process, mainly during the feed preparation, degassing molted polymers, and cleaning processes. Thus, the assessment of the main parameters affecting the ability of MWCNT to become airborne, such as the dustiness and the shape, along with the aerodynamic diameter (respirability), are mandatory.

MWCNT produced to be incorporated in plastic composites were assessed before and after the application of the proposed remediation strategy. The main physicochemical characteristics of pristine MWCNT, as declared in the SDS provided by the manufacturer (Company 3), are: (1) nominal composition >98% carbon (Real, amorphous carbon, <0.2%); (2) Purity (Redox Active Metals <1.8%); (3) size: 20–40 nm (no method of measurement specified); (4) surface area by N_2 adsorption/desorption, 40 m^2/g.

Applying the SAS showed that although MWCNT is a form of carbon nanotube and the SDS indicate a diameter of 20–40 nm and a fiber length of more than 10 μm, when analyzed using electron microscopy (SEM and TEM) the majority of particles did not have a fibrous aspect instead had a bundle-like spherical morphology. Wherever the free fibers• were observed, they were typically far shorter than the claimed 10 μm length. When dispersed in cell culture medium, these MWCNT display a spherical aspect, with only 50% of the particles shorter than 2 μm and an aspect ratio smaller than 2. The World Health Organization (WHO) defines a respirable fiber as having a length greater than 5 μm, a diameter less than 3 μm and a length to width ('aspect') ratio of greater than 3:1.[55] Therefore based on this criterion, the MWCNT preparation is not classified as HARP.

Pristine MWCNT were modified by dispersing them with a nonionic surfactant, (poloxamer Pluronic® F-127)• followed by ball milling for 24 h and then (1) freeze granulation for cold solvent removal or (2) spray drying for hot solvent removal. The main difference between the two forms of modification lies in the solvent removal method, specifically hot or cold.

No specific information relative to the effect of spray drying versus freeze granulation on CNT physicochemical properties could be found from the literature although physiochemical characterization showed that neither freeze granulation nor spray drying dramatically altered the mean agglomerate size or the MWCNT tube diameter. The main alterations observed was a reduction in specific surface area from 40 m^2/g in the case of the pristine to 13.7 m^2/g after freeze granulation and 1.8 m^2/g after spray drying. This corresponded to a change in agglomeration state as freeze granulation and spray drying are two methods of granulation which causes an intentional agglomeration of fine particles into larger particles and it was noted that the spray-dried preparation possessed a tightly packed rather than weakly packed structure compared to pristine control. It is generally admitted that freeze granulation leads to improved granule homogeneity with less cavities in the granules and also it reduces oxidation of the material and potential contaminants.

Analysis of the intrinsic reactivity of the MWCNT preparations by EPR showed a highly significant generation of free radicals by all of the materials ($P < 0.001$ vs. vehicle control) which was significantly reduced by freeze granulation although still remained very high.

The cytotoxicity of pristine MWCNT was tested and compared with the cytotoxicity of crocidolite asbestos, used as benchmark fibrous particle. Pristine MWCNT did not induce any significant cytotoxicity in treated cells compared to untreated cells when assessed using the LDH assay; however, with the alternative methods (resazurin assay) differential cytotoxic effects on the two cell lines were tested. In RAW 264.7 cells pristine MWCNT did not affect cell viability at all the experimental times even at the highest doses tested (no significant difference in viability between control and treated at the maximal dose; IC_{20} >80 μg/cm^2 at 24, 48, and 72 h). In A549 cells a significant decrease in cell viability was observed at 48 h (at 80 μg/cm^2 41• $P < 0.001$; $IC_{20} = 6.0$ μg/cm^2) remaining stable at 72 h (32% $P < 0.001$; $IC_{20} = 5.9$ μg/cm^2). Moreover, these MWCNT caused low levels of hemolysis (5.3%). Despite the high levels of free radicals detected by EPR using the spin trap Tempone-H, incubation of the MWCNT samples before and after remediation did not lead to appreciable levels of oxidative stress within the cells tested. Therefore, measurement of particle reactivity in acellular conditions by EPR, in the case of MWCNT, could lead to a false positive concern of hazardous properties

although this would be inherently conservative. The inflammation-related endpoint (NO production at 48 and 72 h) in macrophages showed a threefold induction at the highest dose (80 μg/cm^2) at 72 h, with a No Observed Effect Level (NOEL) = 40 μg/cm^2.

With the both remediated MWCNT forms, no significant induction in NO production has been observed indicating a significant mitigation of the toxicity in comparison with the pristine form. Overall, in alveolar epithelial cells and macrophages these MWCNT showed no or very low cytotoxicity as assessed by the LDH assay. In contrast, viability data, obtained with a different method (the resazurin assay), indicated that pristine MWCNT caused a significant loss of viability, which was more evident in airway epithelial cells than in macrophages. The RRS partially mitigate these effects of the materials.

EPR analysis showed that all three MWCNT were able to generate substantial amount of ROS in this acellular assay, although modified forms produce slightly less ROS. Similarly, increased production of ROS could be observed in the acellular DCFH assay compared to control medium. In terms of oxidative stress, all the MWCNT preparations induced oxidative stress as assessed by the DCFH-DA assay and alterations in cellular glutathione levels. Similar effects were observed in alveolar epithelial cells but to a slightly lower extent than in macrophages.

Overall the three MWCNT tested displayed very similar toxicological profiles and no difference in terms of response could be observed among the materials tested. However, toward macrophages the modified materials exhibit a smaller activating effect and, therefore, are expected to exert a smaller pro-inflammatory activity *in vivo*.

It is known that aspect ratio nanomaterials can pose a risk for health, and size modifications or size control have been suggested to have a beneficial impact on hazard. These MWCNT are tangled CNT and as such and due to the ball milling process, it is more likely to behave like a particle rather than a HARP.

In this case study, the remediation strategy was mainly based on size modification to alter the exposure potential. Dustiness testing showed that granulation indeed changed the risk of exposure by forming less emissive powders when agitated and therefore less dusty yet with minimal effect on surface reactivity. While remediation in this case did not address the overall reactivity of the material or drastically alter the toxicological profile, the strategy was effective at reducing emissivity and thereby the exposure potential. As risk is a function of hazard and exposure, a reduction in either of these components can have a beneficial impact on risks faced.

Summary of Case Studies and Limitations of the Study

This approach has a great relevance and applicability to hazard identification in relation to nanoparticles and thus provides a tool for nonspecialists for rapid identification of the potential basis of toxicity for nanoparticles based on physicochemical characterization data with zero to minimum toxicological data. This is important because if CB can be linked to the SAS scheme, it can work as a robust qualitative and also rapid instrument for the underwriting community to ascertain risks without getting into the detailed scientific knowledge of nanoparticle toxicity. A safe occupational exposure scenario can then be identified through the exposure assessments conducted under real conditions and at all stages of nanomaterial production, use, and disposal. By basing the hazard identification on the presence or absence of key physicochemical characteristics influencing toxicity, rather than considering the nanoparticle as a whole single entity, this study provides a flexible approach in identifying hazards in a multitude of samples with numerous physicochemical modifications.

Such an approach provides a useful support to the development and evaluation of PtD risk remediation strategies. It is indeed prudent for NM manufacturers to try to mitigate the potential risks of nanoparticles *a priori* during the design stage in preference to downstream measures put in place during manufacturing or customer use. By applying the 'design approach' for nanomaterials and products that incorporate nanoparticles, the health risk of the nanoparticle may be mitigated by potentially lowering the hazard and/or the exposure potential. Nevertheless, in spite of the increasing interest in such a strategy, the proposed design principles cannot be independent from the evaluation of the performance of 'remediated' ENMs, making the approach attractive for manufacturers.[56]

As shown by the MWCNT case study, the intrinsic harmful potential of some nanomaterials can be only in part modulated by material manipulation and therefore to reduce risk, the exposure to such materials must be controlled and reduced.

A key challenge to SAS approach is that while toxicological data are not required to screen a particle against the structural alerts, data from physicochemical characterization are required. Without data on the physicochemical properties of a test material, the SAS is inoperable, however, much of the requested data

TABLE 2 | Table Summarizing the Ideal Physicochemical Data Critical for Hazard Assessment of NM Through the Structural Alert Scheme.

Structural Alert	Ideal Physicochemical Data/Parameters and Relevant Analytical Techniques
Size distribution: nanometric (1–100 nm)	• Size distribution represented by the median value (e.g., D_{50})
Bulk material classified as a CMR	Information on the bulk in terms of carcinogenicity, mutagenicity, and repro-toxicity
Purity/Contaminations:	Multiple analytical techniques
• transition metals • amorphous carbon • PAHs etc. • biological contaminants	• Absorption spectrometry (atomic, ultraviolet–visible, infrared)
Intrinsic reactivity of the material	
• photo-reactivity, • chemical reactivity • presence of surface defects	• Importance of surface reactivity (>surface area) • Multiple analytical techniques such as liquid chromatography, gas chromatography, mass spectrometry • For example : EPR + light to assess the photo-reactivity
Intrinsic acidity/basicity	pH measurements of dispersions in biologically relevant fluids
Surface charge	Zeta-potential measurements of dispersions in biologically relevant fluids (e.g., pH 7 and at pH 4)
Solubility	Analysis in biologically relevant media : • Release of toxic components • Bio-durability
Morphology and size (ideally D_{ae}): classification as a HARP	• SEM • TEM • Aerosol studies (D_{ae})

would be gathered as part of routine quality control by manufacturers (e.g., size distribution, purity, etc.) or is relatively inexpensive to obtain.

Table 2 summarizes the physicochemical properties that should ideally be reported for nanoparticles to allow a full comparison to the SAS and can be presented on MSDS as an initial aide for an effective hazard/RA. A thorough discussion of techniques is avoided here as there are so many complementary techniques. Techniques listed in Table 2 are rather indicative and simplistic than being comprehensive. In practice, actual assessment of chemical purity and intrinsic reactivity would largely depend on the material to be tested and the preparation procedures taken to produce the materials. In any preparation technique there can be products of synthesis, additives and stabilizers, coatings, carbonaceous substances (e.g., amorphous carbon for carbon nanotubes), reaction by-products. The purity could be tested in terms of the amount of metallic elements, amorphous carbon (e.g., for CNTs), polycyclic aromatic hydrocarbons and so on. Intrinsic reactivity can be assessed, e.g., by EPR under UV light to assess the photo-reactivity. It has to be noted that not all of the physicochemical methods are underpinned by standardizations, e.g., through methods such as those published by ISO or OECD. Physicochemical properties listed in Table 2 are initial guidelines and their standardization, when endorsed by end-users as of relevance, use and importance, should be considered as an achievable research priority.

The ambiguity raised by a lack of information could be seen as a loose loose situation whereby if key information is not available, the precautionary principle should be enacted. The result would be the need for more stringent control measures which may have cost and productivity implications for industry until physicochemical and/or toxicological analysis

provides more definitive information. Only then can control measures be judged as being sufficient or excessive. If the precautionary principle is not enacted and the 'not expected to present a hazard' approach taken in the absence of corroborative evidence, then it is possible that workers (and potentially consumers) could be put at risk if such an assumption proves incorrect.

CONCLUSION

Using physicochemical data provided either by the suppliers (e.g., in the form of safety data sheets) or gathered from EU FP7 Project Sanowork (grant agreement no. 280716), each material and its remediated forms were screened using a structural alerts scheme. In doing so, the results of the hazard identification were compiled into a hazard matrix table to more clearly see the role of remediation strategies on the intrinsic hazard potential of the different nanomaterials. Following a safety-by-design approach, the surface or structure of the nanoparticle was changed and the effect on hazard and/or exposure potential was evaluated, together with the expected functional properties. However, while in part successful, this approach highlighted the knowledge gaps in terms of physicochemical characterization which hampered making strong conclusions in terms of efficiency of some remediations. This lack of information is challenging but if deemed sufficiently useful, could form a base set of recommended physicochemical characterization required from the point of hazard identification in the absence of toxicological testing as shown in Table 2.

By basing the hazard identification on the presence or absence of key physicochemical characteristics influencing toxicity, rather than considering the nanoparticle as a whole single entity, this provided a flexible approach in identifying hazards in a multitude of samples with numerous physicochemical modifications. For instance, it became evident that coating of particles such as TiO_2 and ZrO_2 with citrate resulted in a shift from a highly acidic suspension (representing a hazard) to a more neutral suspension. In addition, the action of granulation systems on MWCNTs provided the expected more handling and less emitting nano-powder with a significant induction in NO production.

The demonstrated possibility to tune hazardous properties and exposure determinant allows ENM production risk to reside within acceptability, a shift toward lower exposure and hazard bands categories should be implemented and pursued as a best practice and proactive behavior against the simple precautionary approach.

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