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Current situation and future prognosis of health, safety and environment risk assessment of nanomaterials in South Africa

The commercialisation and everyday use of nanomaterials and nanomaterial-enabled products (NEPs) is rising year-on-year. Responsible development of nanotechnology includes understanding their potential implications on health, safety, and the environment (HSE). The health risk assessment of nanomaterials has therefore become one of the major activities of international agencies including the Organisation for Economic Co-operation and Development and the Environmental Protection Agency for protection of human health and the environment. Nationally, with the foresight and the leadership of the Department of Science and Innovation, a HSE programme was initiated to establish the necessary infrastructure to conduct the tests in the hazard identification and exposure assessment that are needed in the risk assessment of nanomaterials synthesised as well as NEPs available in South Africa. Here we present the advances that have been made in elucidating the different facets that are required when undertaking risk assessments of nanomaterials, i.e. physicochemical characterisation, hazard identification, exposure assessment and effects assessment. These facets are increasingly being considered throughout the nanomaterials present in the life cycles of NEPs. South Africa's research contribution to an international understanding of HSE risks of nanomaterials is highlighted and the future direction to generate the necessary information for effective risk communication and management is provided. This will assist in ensuring safer innovation of nanotechnology in South Africa and support the export of locally manufactured nanomaterials as per international requirements.

Significance:
- Significant contributions of South Africa to the nanomaterial HSE knowledge base are highlighted.
- Development of standardised testing methodologies in nanomaterial HSE and protection of human and ecological health through risk assessment of nanomaterials are discussed.
- This paper contributes to ensuring safer innovation of nanotechnology in South Africa.

Background

Nanomaterials are defined as "material with any external dimension in the nanoscale or having an internal structure or surface structure in the nanoscale"[1]. For new commercial nanomaterials (and respective applications) and nanomaterial-enabled products (NEPs), risk assessments are required to provide science-based information to predict or estimate risk associated with exposure. We anticipate that the health risk assessment of nanomaterials and NEPs will follow the traditional risk assessment paradigm for chemicals involving hazard identification, dose-response assessment, exposure assessment and risk characterisation.[2] A similar approach was proposed for the health risk assessment of nanomaterials to include the identification of their physicochemical properties, the assessment of their hazard and dose-response relationship, and the determination of exposure (occupational, consumer, environment), to facilitate robust and efficient evaluation of their associated risks during their entire life cycle. The health risk assessment of nanomaterials has therefore become one of the major activities globally to develop standardised testing procedures led by the Organisation for Economic Cooperation and Development (OECD).[3] Moreover, international initiatives such as the US National Nanotechnology Initiative Research Strategy[4] and the European Commission[5] were established to ensure nanosafety in the United States of America and Europe.

The need for the development of a focused research strategy for health, safety and environment (HSE) aspects in support of the South African National Nanotechnology Strategy was realised and initial research areas were proposed.[6] Gulumian and others[7] pointed out that these research activities should not be undertaken in isolation and that internationally derived best practice guidelines should be adopted so that research could be focused specific to South Africa's requirements. To this end, the South African Department of Science and Innovation (DSI) established the *Nanotechnology HSE Research Platform* in 2015. It is within this platform that the bulk of the scientific information pertaining to Nano HSE, nationally, has been produced. This platform has enabled South Africa to establish and grow the necessary infrastructure required for the hazard identification and exposure assessment necessary in the risk assessment of nanomaterials or NEPs. The aim of this paper is therefore to describe the major contributions thus far by South Africa in the field of nanomaterial HSE, within the context of current international developments. We further evaluate the research needs in relation to national and international development needs in the field. It is anticipated that the achievements reached thus far and new directions identified will aid in the risk assessment, communication and management of nanomaterials and NEPs in South Africa.

Assessment of physicochemical properties

The physicochemical properties of nanomaterials determine their environmental fate and interaction with biological systems.[7,8] Their significance became apparent with the recognition that small changes in these properties may influence environmental behaviour and subsequent biological uptake of nanomaterials. Nationally, the

infrastructure to determine dissolution properties has been established[9], and, internationally, contributions have been made to determine the biodurability and dissolution of nanomaterials[10,11]. For example, the dissolution of gold nanoparticles (AuNPs) has been determined in different biological and environmental media.[12]

Hazard identification

For hazard identification, it became crucial for international agencies to develop *in vitro* and *in vivo* assays that characterise acute and chronic toxicity.[13] The OECD Working Party on Manufactured Nanomaterials therefore launched the Sponsorship Programme in November 2007 to standardise testing, with South Africa being the Lead Sponsor for the safety testing of AuNPs.[13]

For *in vitro* tests, South African research has demonstrated the interference of nanomaterials in optical read-out tests[14] and has contributed to international development of an interference-free *in vitro* colony-forming ability test[15]. Moreover, researchers have recommended the use of label-free techniques to assess toxicity of nanomaterials[16] and investigated their interference in genotoxicity and mutagenicity assays[17] and with the RNA analyses[18]. More recently, research demonstrated the interference of AuNPs with *in vitro* endotoxin detection assays[19] and provided guidance in the sterilisation of nanomaterials[20] and proposed alternative testing strategies[21]. South Africa also contributed to elucidating the mechanism involved in the cellular uptake of AuNPs[22,23] and in the mechanisms involved in the possible use of nanomaterials in nanomedicine[24,25].

As for *in vivo* tests, the derivation of no observed adverse effect levels (NOAELs) requires sub-chronic (90 d) or long-term chronic (>2 years) studies. Due to ethical concerns, sub-acute (28 d)[26] studies were suggested as an alternative to ensure sufficient recovery time following exposure. This revised 90-day OECD Test Guideline 413[27] further requires that retained lung burdens should be determined.

South Africa, in collaboration with leading international research groups, has conducted *in vivo* inhalation studies to assess the lung burden of high dissolution rate silver nanoparticles (AgNPs) and relatively lower dissolution rate AuNPs.[28-31] Such collaborations also illustrated that the even lobar deposition of poorly soluble AuNPs and soluble AgNPs are similar[28-30] and thus could propose the reduction of experimental animals to be used in the said 28-day inhalation toxicity and 90-day inhalation toxicity OECD guidelines. These inhalation studies also showed the size-dependent clearance from lungs after short-term inhalation exposure.[32] South Africa further contributed to inhalation studies related to nano aerosol generation as part of the development of an international standard (ISO TR19601). Collaborative work was also conducted to investigate the tissue distribution of AuNPs and AgNPs after sub-acute intravenous co-administration of similarly sized counterparts[33] as well as their effect on the blood biochemical and haematological parameters[34].

With regard to ecological hazard assessment of nanomaterials, standardised toxicity testing methodologies and test organisms were initially utilised to understand the effects of exposure. Tests were carried out using traditional standardised aquatic test species (i.e. algae, macro-invertebrates and fish) to determine the hazards of, for example, double-walled carbon nanotubes[35], induction of oxidative stress in the floating macrophyte *Spirodela punctuta* following exposure to AgNPs and zinc oxide (ZnO) nanoparticles[36], and the mortality and behaviour effects of aluminium oxide and titanium dioxide (TiO_2) to the early life stages of a freshwater snail (*Physa acuta*)[37].

Subsequently, South African and other international researchers have been evaluating the applicability of standardised toxicity tests for *inter alia* nematodes[38], enchytraeid potworms[39], aquatic invertebrates and fish[40,41]. Using these standardised OECD protocols, local studies conducted as part of the safety testing of AuNPs revealed that nanomaterials had lower toxicity than their chemical equivalents.[39-41] These and other South African studies on the three most commonly used toxicity bioassays – i.e. the 72-h algal growth inhibition test, 48-h *Daphnia* immobilisation test, and 96-h fish mortality test – contributed towards adaptations needed for nanomaterial toxicity testing. For example, CytoViva Dark field imaging was used[41] to demonstrate that the disposal of surface-bound AuNPs

by *Daphnia* occurs through increased moulting. Moreover, South Africa developed a standardised screening procedure to assess the hazard of nanomaterials in saline environments using brine shrimp (*Artemia* sp.)[42] and proposed a new method to assess cell toxicity in real time using the xCELLigence real-time cell analyser to evaluate the effects of AuNPs and AgNPs to three different mammalian cells lines[43].

As part of the call for further development of sub-lethal endpoints of chronic (long-term) exposure, Botha et al.[44] used an integrated physiological response (i.e. swimming behaviour) in zebra fish (*Danio rerio*) that showed sub-lethal dose-response effects of AuNPs where gene expression and oxidative stress enzymes did not reveal any effects. The sensitivity of this endpoint was further demonstrated following exposure of *D. rerio* to sub-lethal concentrations of CdTe quantum dots and nanodiamonds[45].

These different *in vitro* cell models and *in vivo* animal models described above, contribute to the techniques that are used in hazard assessment and regulation of nanoparticles before they are released into the market. This means that, for the safe development and commercialisation of nanotechnologies in South Africa, there are existing test systems that have successfully been validated and established to achieve the objectives for hazard assessment.

Assessment of exposure

Occupational exposure

Exposure to nanomaterials may occur directly through occupational and consumer exposure or indirectly through environmental exposure (Figure 1). There is thus the need for lifecycle risk assessment. The exposure assessment under the different scenarios critically requires that the nature and extent of contact with nanomaterials under different conditions and identified activities is determined. The identification of the routes of exposure such as inhalation, digestion, dermal or intravenous injection with dose and duration is also of great importance. The fact that nanomaterials come in various sizes, shapes, functionalities, concentrations, and chemical compositions must be borne in mind when undertaking exposure assessments.

Assessment of occupational exposure

Studies by the OECD and US National Institute for Occupational Safety and Health (NIOSH) provide guidance on strategies, techniques, and sampling protocols for determining nanomaterial concentrations in air. The three-tiered approach recommended by the OECD for occupational exposure assessment is as follows[46]:

- Tier 1: On-site inspection and questionnaire to determine if the nanomaterials can be released from the processes/tasks.

- Tier 2: Determine potential exposure in the workplace through screening and/or task specific measurements using the correct metrics (mass, number, surface area) with specialised online instrumentation. The establishment of background concentrations and levels in the personal breathing zone of the worker needs to be determined.

- Tier 3: Tier 2 with concurrent particle sampling for offline analysis of particle morphology, mass or fibre concentration and chemical composition. These are related to particle control values in order to ascertain whether controls are sufficient or need to be improved.

Recommended exposure limits, another term for occupational exposure levels (OELs), for carbon nanotubes and nanofibres were determined to be 1 $\mu g/m^3$ elemental carbon as a respirable mass 8-h time-weighted average concentration[47] and for nano-TiO_2 to be 0.3 mg/m^3 as time-weighted average concentrations for up to 10 h per day during a 40-h work week[48]. These recommended exposure limits have already been accepted by the US Occupational Safety and Health Administration (OSHA).[49] The aforementioned examples demonstrate that there is advancement in deriving OELs for nanomaterials. However, with the rapid expansion of nanotechnology and development of new types of nanomaterials, the development of OELs in the workplace is lagging. Subsequently, for
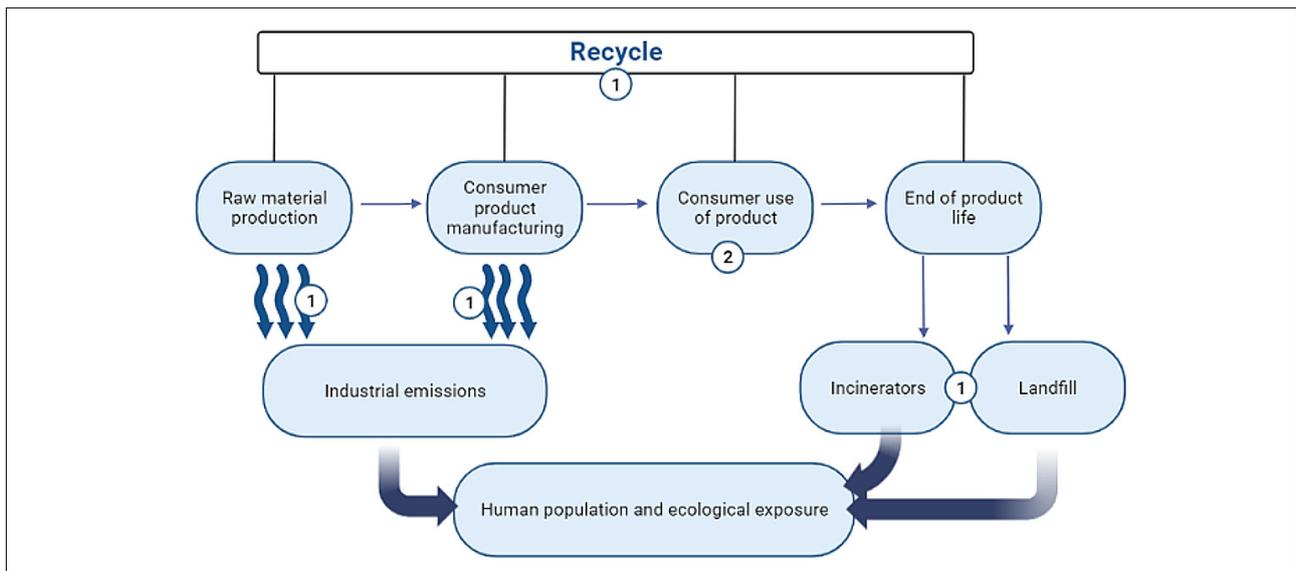
Figure 1: Diagram depicting the human and ecological exposure to nanomaterials during different product lifecycle stages of the materials in environmental, health and safety evaluations (1 = worker exposure, 2 = consumer exposure).

nanomaterials where no limit values are available, nano-reference values have been developed as provisional substitutes for health-based OELs or NOAELs. These nano-reference values are based on a precautionary approach and have been developed for four classes of nanomaterials: Class 1 – rigid, biopersistent nanofibre (e.g. carbon nanotubes, metal oxide fibres), Class 2 – biopersistent granular nanomaterials (e.g. Au, Fe, CoO), Class 3 – biopersistent granular and fibre nanomaterials (e.g. TiO_2, ZnO, C60), Class 4 – non-persistent granular nanomaterials (e.g. fats, NaCl.[50]

Using the nano-reference values approach, South African researchers assessed exposure to AuNPs in a pilot scale facility where the measured nanoparticle emission was below the recommended nano-reference values.[51] Using the tiered approach, exposure assessment was conducted in various research laboratories and in different industrial settings in South Africa to assess exposure to several types of nanomaterials utilising the established infrastructure. The values calculated from the measurements are used to calculate the 8-h time-weighted average exposure concentration to compare it to proposed OELs. Thereafter, proposed actions need to be taken to ensure the protection of workers, including engineering controls and personal protective equipment, to further minimise the risk of exposure.

Together with the identification of suitable biomarkers of internal exposure and indicators of toxicological responses[52], it is also important to develop surveillance programmes to protect the workers dealing with nanomaterials[53]. To this end, South Africa contributed towards the development of World Health Organization guidelines to protect workers from potential risks of nanomaterials.[54]

Assessment of environmental exposure

Most of the information related to the fate and transport of nanomaterials in the environment has been obtained from modelling studies. These approaches were applied to quantify the levels of AgNPs and TiO_2NPs in terrestrial and aquatic ecosystems from the cosmetics industry passing through wastewater treatment plants.[55] Further studies were conducted on simulated wastewater treatment plants to determine the fate and effect of AgNPs and ZnONPs[56,57] and concluded that these materials predominantly remain in the sludge. Other studies found that aggregation and dissolution kinetics of aluminium oxide (Al_2O_3) and CuO nanoparticles were strongly influenced by source-specific physicochemical characteristics such as pH, natural organic matter and solutes.[58] The latter physicochemical characteristics also influenced the toxicity of ZnO and iron oxide (FeO_x) nanoparticles to the bacterium *Bacillus subtilis*.[59]

South African researchers conducted a comprehensive review of the existing approaches used to predict the bioaccumulation of nanomaterials. They concluded that the octanol-water partition coefficient (log K_{ow}) may not be applicable but that kinetic models such as the physiologically based pharmacokinetic model showed the greatest promise in predicting bioaccumulation and biological exposure.[60] South African researchers were further involved in a meta-analysis of existing nanomaterial bioaccumulation studies in fish to assess the bioaccumulation potential of nanomaterials.[61] The authors found that a tiered approach that makes use of *in vitro*, *in silica*, *ex vivo* and, at the final tier, *in vivo* data shows promise as a new standardised protocol for nanomaterial bioaccumulation testing. It is currently being applied to assess CuO and quantum dots bioaccumulation in both terrestrial (i.e. earthworms) and aquatic (i.e. invertebrates and fish) organisms.

Assessment of exposure from consumer products

In terms of turnover, the pharmaceutical sector is currently the most important of the six considered nanotechnology markets, but all of them are expected to grow significantly in the future.[62] The potential therefore exists for consumer and environmental exposure to nanomaterials present in NEPs at different product lifecycle phases, i.e. production, use, and end-of-life. Assessment of consumer exposure is therefore complex.[63] An approach was developed to obtain sufficient quantities of materials (e.g. released from products, weathered fragmented products and sieved fragmented products) in order to study these nanomaterials during different lifecycle stages.[64] Environmental exposure assessment due to release of nanomaterials has largely been dominated by pristine nanomaterial type, compared to those incorporated in NEPs.[63] Because the functionalisation of nanomaterials into products alters their pristine state[65], there are limitations in applying data obtained from pristine nanomaterials to elucidate exposure arising from the various lifecycle phases.

Prior to assessment of nanomaterials exposure in NEPs, it is important to establish the type of NEPs in the market. The global and local NEP markets are dominated by health and fitness products (e.g. sporting goods, active wear, personal care, and sunscreen products), being 42–81% of the identified or examined NEPs.[66] Hence the potential for environmental exposure to occur consistently and likely to increase with future demand for more superior products preferred by consumers. In South Africa, it has been illustrated that NEPs extend beyond the products that are declared by manufacturers.[67] There is increased need for regional and ultimately global databases to enhance value to industry, consumers, researchers, and government authorities, and at a lower cost than the current country-specific registries.[68]

It is impractical, and in principle unnecessary, to analyse nanomaterial emissions from all NEPs; numerous studies have adopted a model that, at a lower tier, guides to priority emission-potential NEPs based on nanomaterials loci or fixation in the product.[66] In brief, NEPs with nanomaterials suspended in liquid (e.g. shower gels, body creams), surface bound (e.g. toothbrush, fabrics), airborne (e.g. air conditioner) and suspended in solid gel (e.g. eye shadow, make-up sticks), exhibit elevated nanomaterials environmental exposure potential relative to counterparts where the nanomaterials are fixed in a solid matrix or nanostructured surface.

Overall, information pertaining to nanomaterials environmental exposure has greatly improved compared to a decade ago. Locally, studies have proposed priority groups of NEPs exhibiting considerable pollution potential[55,66,69] as well as steps that enrich the information gap raised by authorities concerning emerging environmental pollutants[70]. Additionally, through platforms such as the Nanotechnology Industries Association, prioritisation of NEPs that raise HSE concerns have been highlighted.[71] In the USA, the Food and Drug Administration has also set regulations pertaining to NEPs falling within food and drug classes.[72] Whilst such examples highlight efforts to identify and minimise NEP cases of nanomaterial concern, many exposure dynamics remain poorly understood or complex, hence considerable challenges remain in the regulation of commercial items. Closer cooperation between authorities, industry, research, and public communities on nanomaterial HSE matters can enrich and advance the debate in this matter; South Africa still needs to enhance such a robust approach.

Application of models and/or *in silico* approaches

The aim of computational *in silico* approaches is to develop predictive models that can replace *in vitro* and *in vivo* testing for the purposes of human and ecological risk assessment of nanomaterials.

Computational approaches and the prediction of toxicity

This involves the development of computational models of nanomaterial structure property/activity relationships (QSAR) to predict toxicity of nanomaterials and then to assist in safety by design considerations. These studies done in conjunction with EU partners are aimed at identifying relevant response descriptors in relation to toxicological, transcriptomic, and toxicogenomic endpoints that will assist in developing QSARs for predicting the toxicity of nanomaterials.[73-77]

Computational approaches and the prediction of dose

Dosimetry refers to estimating or measuring the amount (in terms of mass, number, surface area, volume, etc.) of a nanomaterial at a specific biological target site at a particular point in time.[78] The assessment of the dose delivered to the cells and the internalised dose (i.e. the dosimetry) is essential for interpretation of both *in vitro* and *in vivo* toxicity data. South Africa therefore uses the sedimentation, diffusion and dosimetry (ISDD) and volumetric centrifugation method (VCM) modelling platforms to calculate cellular delivered dose[79] for the hazard identification of nanoparticles.

Dosimetry is also important for *in vivo* studies where the delivered dose to internal organs needs to be determined. The physiologically based pharmacokinetic model is standard procedure that is applied to simulate the absorption, distribution, metabolism, and elimination of chemical substances in organisms. In collaboration with international organisations, South African partners recently outlined future directions in the physiologically based pharmacokinetic modelling of nanomaterials.[80] A recent sub-acute inhalation study demonstrated how this approach could be applied to assess the lung retention and particokinetics of AgNPs and AuNPs co-exposure in rats.[81]

Chemoinformatics and chemical structures

Chemoinformatics has solved the issue of representing chemical structures for small molecules as simple 1D codes, such as SMILES and InChI, which are machine-readable chemical identifiers. South Africa has contributed to a recent collaborative work, which considered the issues involved in developing an InChI for nanomaterials (NInChI).[82]

Risk assessment and risk management methods

To understand the risk of nanomaterials, it is essential to obtain basic information on the following aspects of nanomaterials: physicochemical properties, *in vitro* and *in vivo* toxicity, dose-effect relationships and exposure scenarios for workers, consumers and the general environment (i.e. determining levels, frequency and duration of exposure). Therefore, risk assessment and risk management considerations have formed the core research areas for the DSI Nanotechnology HSE Risk Assessment programme. The aim of the programme is to integrate the quantitative exposure and hazard data obtained from all the HSE programme projects into risk assessment and other *in silico* models to predict nanomaterial behaviour and risks across the different life cycles of NEPs. Through data generated in the HSE programme, South Africa has been able to contribute to the integration of safety testing measures across the innovation chain of nanomaterials using new approach methodologies.[77]

Future prognosis

Nanomaterials and NEPs are increasingly being synthesised and commercialised in South Africa. In the past 5 years, there have been significant advances in research related to the components of the risk assessment process. By and large, these research activities were not undertaken in isolation but formed part of international nanomaterial HSE research programmes.

The achievements of the HSE programme could therefore be summarised as:

1. Support of regulation and decision-making through evidence-based data derived from a broad-base nanotechnology HSE research platform.

2. Establishment of the required tests and the necessary infrastructure to assess the hazardous nature of and determine exposure to nanomaterials that are being synthesised and soon to be commercialised in South Africa.

3. Establishment of the necessary human capital development to conduct such tests.

4. Continued collaborative research efforts in international research initiatives that are aimed at developing nanotechnology HSE testing methodologies and regulatory approaches, e.g. the OECD Working Party on Manufactured Nanomaterials programme and EU Horizon 2020 supported research projects.

5. Continued support of the development of international standards through ISO 229 Nanotechnologies where South Africa is represented by the South African Bureau of Standards (SABS) and the appointed experts contribute to the development of such nano-safety guidelines and standards for nanomaterials and nanotechnologies.

Through the participation and contributions of South African scientists in large-scale EU FP7 and Horizon 2020 funded nano-research programmes (e.g. Nanosolutions, Nanoharmony, caLIBRAte, NanoSolveIT), significant amounts of data have been generated. The challenge that now faces international and South African researchers is how to validate these predictions from cell lines to whole organisms and indeed other species (i.e. read-across extrapolation) and determine how these *in vitro* mode of action predictions influence higher level biological responses such as growth, reproduction, etc. Therefore, both local and international focus is on the use of additional knowledge-based tools such as the development of adverse outcome pathways that can be implemented in the risk assessment of nanomaterials. The necessity of implementing tools such as adverse outcome pathways arises from the fact that it may not be possible to conduct separate risk assessments for every nanomaterial and NEP.

Furthermore, a glaring void that needs urgent attention in South Africa is nanomaterial HSE discussions between industry and authorities, as these have not yet been consistent. This partnership will facilitate in the risk management of NEPs produced in the country. Important work that still needs to be completed in this regard is:

1. Facilitate partnerships with industry to provide guidance on process-related exposures and worker protection.

2. Develop guidelines for the development of safe handling and use (industry).

3. Develop guidelines and standards to train researchers and workers for activities involving nanomaterials in the research and workplace environments in South Africa.

4. Identify, characterise, and communicate risks to all stakeholders through appropriate risk communication and risk management strategies. This will require research into risk communication strategies and integration into risk management frameworks. Thus, in line with international initiatives, risk communication needs to form an integral component of all nanotechnology research programmes.

5. Facilitate communication between stakeholders by providing support for industry partnerships and informed regulatory decision-making.

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Competing interests

We have no competing interests to declare.

Authors' contributions

M.G.: Conceptualisation; writing – initial draft; writing – revisions; project leadership. M.T.: Writing – initial draft; writing – revisions. X.M.: Project management; funding acquisition; writing – revisions. V.W.: Writing – initial draft; writing – revisions; corresponding author.

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