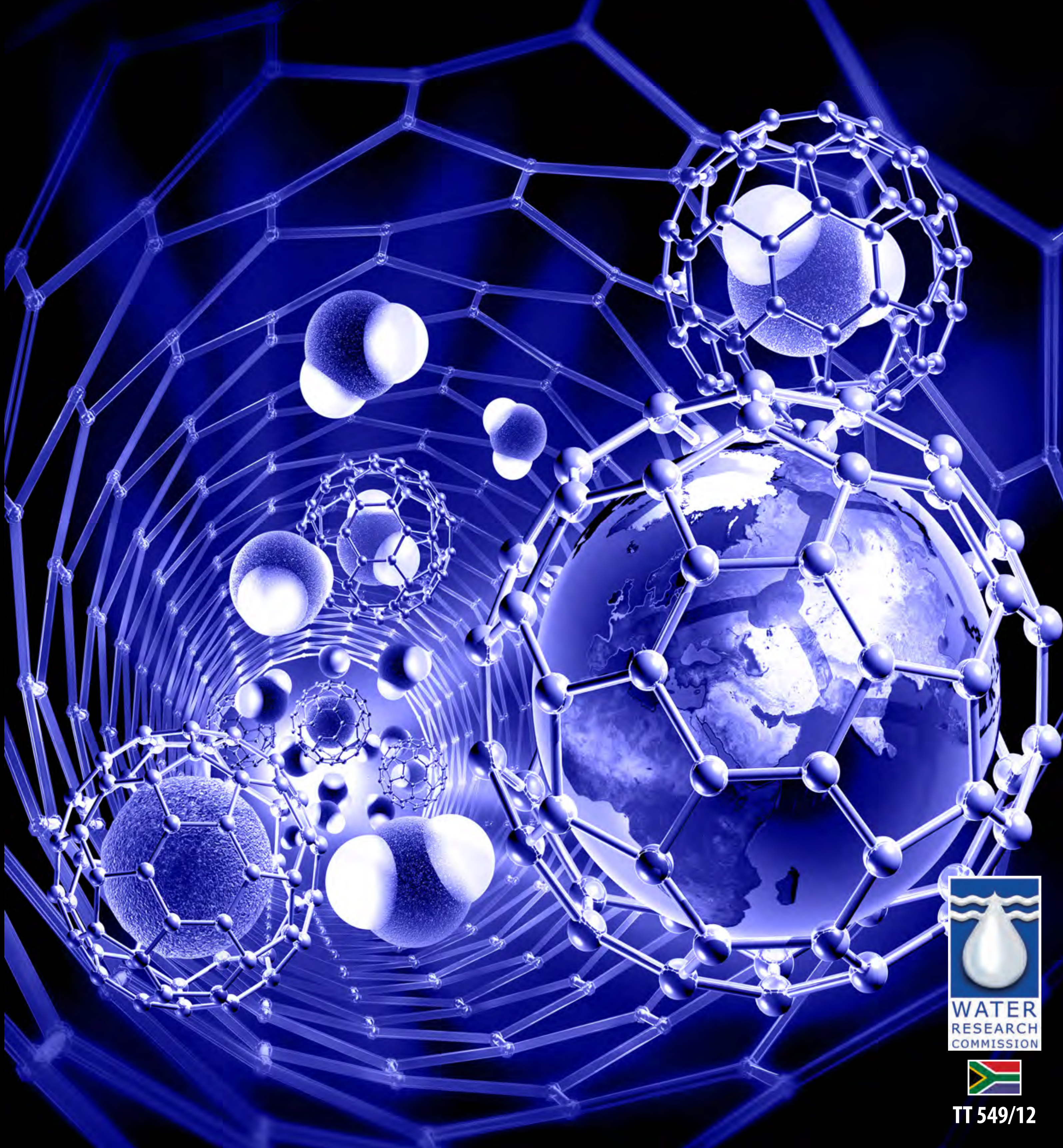
Framework Document for a WRC Research Programme on

ENGINEERED NANOMATERIALS

Victor Wepener, Bhekie Mamba & Ndeke Musee



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Framework Document for a WRC Research Programme on Engineered Nanomaterials

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EXECUTIVE SUMMARY

Nanotechnology has taken the world of science by storm since it allows for the development of new materials with extraordinary properties. Nanomaterials are defined as objects with one, two, or three external dimensions in the size range of 1-100 nanometres (nm). Examples of novel nanotechnology applications include the development of highly accurate and sensitive medical diagnostic devices, new ways of disease therapy, and the monitoring and remediation of basic water supplies. South Africa, through the National Nanotechnology Strategy (NSS), has initiated a national coordinated effort to guide the country's nanoscience and nanotechnology to ensure that we remain competitive within the international research community in this fast-developing field. The NNS broadly groups the benefits of nanotechnology of national importance in South Africa in six focus areas, namely: water, energy, health, chemical and bio-processing, mining and minerals, and advanced materials and manufacturing to ensure the country derives social- and industrial-related benefits.

With the rapid progression of nanotechnology from laboratory to industrial applications and commercialisation of products, it is imperative that risk that may be associated with engineered nanomaterials (ENMs) requires attention at its infancy phase to ensure safe and responsible long-term development of this novel technology. With the widening gap on the understanding and knowledge on the risks of ENMs to the environment and the increasing application of nanotechnology it is not surprising that there is a growing volume of international scientific literature expressing concern towards environmental distribution and effects of these materials. The reason being, the unique inherent physical and chemical properties of nanomaterials that make them suitable for successful application. e.g. in water treatment, medicine, etc. also provides them with potential for biological uptake and effects in non-target organisms.

Risk is a function of both the hazard and the exposure potency. To fully elucidate the potential impacts of ENMs, risk profiling these materials requires scrutiny at different life cycle phases. By using the toxicological data from the studies of nanomaterials in aquatic systems and the exposure potency, risk profiles can be determined. Such profiles will indicate which materials needs further attention, and also provide basis for deriving suggestions on how such ENMs can be re-engineered to function as intended but with minimized potential to cause adverse effects in ecological organisms. It is generally accepted that the existing methods and framework for aquatic toxicity hazard assessment, i.e. use of standard test organisms with mortality, growth, and reproduction as endpoints are generally adequate to identify hazard associated with ENMs exposure. However there is also consensus that within each group of tests, modifications relevant to ENMs would be required.

Therefore, in this document, an outline on the development of a research framework and a motivation as to why the different components were selected to address the research needs into risk assessment of ENMs in waters of South Africa are presented. To stay in line with current national initiatives, a research programme is required to increase our collective understanding on the potential risks and mechanisms of addressing such risks adequately. Ecological risk assessment (ERA) is a structured approach that describes, explains and organizes scientific facts, laws and relationships to provide a sound basis to develop protection measures for the environment. It is the key concepts of the ERA process, i.e. identification of the hazard, exposure assessment, assessment of the dose-response relationship and risk characterisation that provides guidance into identification of the research priorities for understanding environmental exposures, environmental dose and bioavailability and effects following internal exposure to ENMs in South African waters. These five research priorities are to 1) Identify principle sources of exposure and exposure routes; 2) Determine the dominant physico-chemical properties that affect environmental transportation of ENMs; 3) Understand transformation under different environments; 4) Determine the applicability of effects tests on individual species; and 5) Determine ecosystem effects. The key to the successful understanding and development of nanotechnology is in interdisciplinary and international collaboration. This will require the development of a new generation of both analytical infrastructure and adequately trained human resources.

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1. INTRODUCTION

Nanotechnology is defined as the design, characterisation, production and application of structures, devices and systems by controlling shape and size at the nanometre scale. This emerging technology is set to revolutionize some of the fundamental features of everyday life in the 21st Century. Nanotechnology finds wide applications in the fields of medicine, manufacturing, energy production, water purification, and remediation of contaminated environments. For instance, the fabrication of nanoscale materials with distinct properties (e.g. optical, electrical, or magnetic) are presently being exploited in consumer nanoproducts and industrial applications such as; sunscreens and cosmetics, coatings and surfaces, remediation, and water purification, in fuel cells, batteries and as fuel additives, catalysis, lubrication, or even in medical implants, diagnostics and drug delivery.

Owing to the high potential societal and economic benefits of nanotechnologies has attracted governments and major industrial companies worldwide resulting to commitment of significant resources towards supporting research and development (Musee *et al*., 2010a). This has resulted in the expansion of nanoscale-based processes, materials, and products. For example, there are large scale national- and continental-wide research and development programmes on nanotechnology in Europe, the United States, and Asia (e.g. Japan, China, and South Korea) – with financial support in order of billions of dollars (EU 2006; NNI 2008; Roco, 2004; 2005; Meridian Institute, 2005; Holman *et al*., 2006) as well as in developing countries such as South Africa (DST, 2005; DST, 2010; Musee *et al*., 2010a).

In the USA, for example, the funding request for nanotechnology research and development in 13 Federal departments and agencies for 2010/11 financial year was $1.64 billion (Roco, 2010) –and has grown to $1.76 billion in 2011/12 FY (Roco, 2011). This means that the funding for nanotechnology R&D in USA has grown by 255% since the National Nanotechnology Initiative (NNI) started in 2001. Guzmán *et al*. (2006) analyzed data and information on the funding levels by the USA federal government and industry and showed that only a small portion of the funds were invested in fundamental research in understanding the human health- and the environmental-related risks of nanotechnology. It was also estimated that the funding in all environmental nanotechnology studies – $88.2 million – constituted merely 2.7% of the $3.26 billion of the federal grant funds coordinated by the NNI from 2001 to 2005. Moreover, the study noted that most of the funds (approximately 1.24%) were allocated for investigating the novel environmental applications of the nanotechnologies.

In addition, the environmental risks of nanotechnology only received about 0.5% of the accumulated NNI funding from 2000-2004. This funding model in the USA was not much different from those of Japan and the European Union, though in the former, the funding priorities after 2004 changed considerably in favour of understanding the implications of NMs in biological and environmental systems (Guzmán *et al*., 2006). Notably, none of these studies directly focused on fate and impacts of nanowaste streams, stability, fate, and behaviour of ENMs in different ecological systems as a consequence of industrial production processes as well as post-customers' use of nanoproducts.

However, in the recent years, many countries have recognized that the applications of ENMs may potentially pose risks in causing safety, health, and environmental effects that may comprise the welfare of humans and the environment. Consequently, these countries are supporting nanotechnology risk assessment programmes though the funding provided is significantly below 1% with respect to the entire R&D funding in the field of nanotechnology. Similar activities on nanotechnology are presently under implementation phase in South Africa where government investment in nanotechnology foci entails the support of; developing research platforms, collaborative national and international networks, and human capital development (Musee, 2009; Musee *et al*., 2010a) – and support on risk assessment of ENMs concerning their potential health, safety and environmental research currently at the infancy phase.

According to capital venture predictions for the year 2014, manufactured goods will account for 15% of the global manufacturing output that are based on the incorporation of nanotechnologies with an estimated economic value of US$2.6 trillion dollars (Lux Research, 2004; Roco, 2005). To contextualize the potential impact of nanotechnology, this economic volume represents both the information technology and telecom industries, which combined, are approximately 10 times larger than the revenues from biotechnology. In spite of the large expenditures on R&D for synthesis of ENMs and the commercialization of nanoproducts – the funding for other crucial research such as the risk assessment of ENMs and the evaluation of suitable waste management approaches, are comparatively small (Meridian Institute, 2005; Maynard 2006).

On the other hand, there has been a steady growth in venture capital and patenting of intellectual property (IP) in the nanotechnology field (Paull *et al*., 2003; Wolfe *et al*., 2003; Huang *et al*., 2004; Li *et al*., 2007). Nonetheless, due to fierce domestic and global market competition – firms are under enormous pressure to rapidly develop and introduce new nanotechnologically-enabled materials and products into the marketplace, or face huge losses in form of: market share, revenue, customers, and even strategic position. In this context, the business climate in which companies are operating provides limited opportunities to screen the potential toxicity of ENMs – and therefore, other funding mechanisms to support risk assessment research through funding organizations such as WRC will positively contribute in supporting responsible, safe and sustainable exploitation of nanotechnology capabilities without compromising biological life forms in different ecological systems.

1.1 Definition of terminologies

Aggregates of nanoparticles are groups of heterogeneous particles in which the various components are held together by relatively strong forces and thus not easily broken apart where the resulting external surface area may be significantly smaller than the sum of calculated surface areas of the individual components.

Agglomerates of nanoparticles, on the other hand, are group of particles held together by relatively weak forces, including van der Waals forces, electrostatic forces and surface tension where the resulting surface area is similar to the sum of the surface areas of the individual components.

Nanoparticles are particles that have structural features with at least one dimension of 100 nm or less, and exhibit novel characteristics in comparison to their counterpart bulk materials. Nanoparticles may also differ from their larger counterparts by their propensity to aggregate or agglomerate (Ju-Nam and Lead, 2008).

Nanomaterials (NMs) are generally classified in terms of the dimensions of their constituent nanostructures involved (Klaine *et al*., 2008). Primarily there are three classes of NMs. Nanomaterials with 1-D nanometric dimensions examples include surface coatings used in lithographic depositing of nano-scale layers of materials on silicon wafers in the development of computer chips or the thin films such as surface treatments for glass used in filling microscopic depressions and production of surfaces that prevents dirt from attaching. Nanomaterials with 2-D nanometric dimensions include nanotubes, nanowires, fibres and fibrils; and finally, NMs with 3-D nanometric dimensions comprise of engineered quantum dots, nanocrystals, fullerenes, and particles of metals such as gold and silver or of metallic oxides such as titanium and zinc oxides.

1.2 Types of engineered nanomaterials

Nanoparticles naturally widely exist in the environment from sources such as photochemical and volcanic activities, or are created by plants and algae. In addition, they are generated from anthropogenic processes as non-intentional by-products from processes like combustion, welding fumes, and vaporization and from diesel and petrol- fuelled vehicles (Shi *et al*., 2007). Other terms frequently used in the literature to describe these type of incidental nanoparticles are -ultrafine‖ in the air (NIOSH, 2009) and -colloid‖ for particles with slightly different size range particularly in the soil and water environment (Lead and Wilkinson 2006).

Engineered nanomaterials (ENMs) are broadly classified as carbon-based materials (e.g. fullerenes, carbon nanotubes – singe walled carbon nanotubes [SWNCT] or multi-walled carbon nanotubes [MWCNT]) and inorganic engineered nanoparticles (ENPs) fabricated from metal oxides (e.g. zinc oxide, yttrium iron oxide, nickel zinc iron oxide, titaniumoxide, indiumtin oxide, samarium (III) oxide, erbium (III) oxide, aluminium oxide, etc.) and metals (gold, silver, iron, copper, palladium, etc.). Other forms of NMs include semiconductor nanocrystals known as quantum dots (QDs; e.g. cadmium selenide [CdSe], indium phosphide [InP] cadmium telluride [CdTe], zinc selenide [ZnSe], etc.). In addition, mixtures of different phases of NMs are also fabricated at laboratory and industrial scales. It is the high diversity and rapid development of ENMs that has raised concerns regarding their potential impact in the environment including the wastewater treatment systems (Musee *et al*., 2011).

In addition, ENMs exhibit diverse differences due to their shape, size, surface charge, and chemical composition mostly due to the mode of their production (Maynard and Aitken, 2007). In this section, we summarize the most dominant types of ENPs currently being fabricated or researched in the South African context.

1.3 Nanotechnology research in South Africa

South Africa is listed among the middle-ground countries in terms of nanotechnological advancement (Schutte and Focke, 2007). The activity timeline of nanotechnology and nano-related activities in South Africa to support research and development in the nanoscience and nanotechnology fields are presented below:

i. The South African Nanotechnology Initiative (SANi) formed in 2002 between twelve universities, four industrial councils and ten industrial companies.
ii. South Africa's Advanced Manufacturing Technology Strategy (AMTS) was launched in 2003.
iii. The Department of Science and Technology (DST) published the National Strategy on Nanotechnology in 2005.
iv. A Nanotechnology Innovation Centre is in the process of being established on initiative of DST/Mintek, the water Research Commission and the Medical Research Council (2007).
v. The High-Performance Computing Facility was officially launched by the Department of Science and Technology at the North West University's Potchefstroom Campus in October 2009 (Eish!, 2009). The facility was established in response to the ongoing demand for computing power, particularly in the field of natural-sciences research. It will enhance the university's capacity to deliver research excellence, ensuring that it remains one of the country's top research institutions.
vi. The National Nanotechnology Strategy prescribes that instruments be put in place to ensure that Nanotechnology is applied according to international best practice. To this end, a Nano Heath Safety and Environment Committee was constituted by DST in 2010/2011. Its main responsibilities will be to:
    - Investigate global approaches to risk and health issues in the research and application of Nanoscience and Nanotechnology,
    - prescribe frameworks for handling of such issues locally and
    - develop policy framework governing research, manufacture and application of nanomaterials and monitoring implementation.

The DST published the National Nanotechnology Strategy (DST, 2005) preceded by the South African Nanotechnology Initiative (SANi) – which was formed in 2002 (SANi, 2002) – with membership drawn from universities, industrial companies and science councils (DST 2005). These include National Centre for Nanostructured Materials, CSIR (Nanocomposite R&D, Silicon nanoparticle synthesis, Quantum dot synthesis, International collaboration, Nano-Biotech, Nano Drug Encapsulation), Mintek Nanotechnology Innovation Centre (Project AuTEK creating gold-based chemo-therapeutics), University of Cape Town (Silicon Nanoparticle Inks and Printing of devices), Tshwane University of Technology (Carbon Nanotubes), University of Johannesburg (Carbon Nanotubes and various other materials), University of the Witwatersrand (Various nanomaterials), University of Limpopo (Nano modelling) and University of Zululand (Quantum Dots). Industrial companies include the three major South African gold mining houses – AngloGold Ashanti, Gold Fields and Harmony Gold. In addition, to the authors' knowledge, only a single company is presently producing nanomaterials at commercial scale in South Africa – and due to commercial concerns the company could not even divulge the types of nanomaterials and the intended applications.

The implementation of the National Nanotechnology Strategy invigorated R & D development activities towards synthesis and characterization of nanomaterials in among different research centres and universities in South Africa. The research foci is to find the potential application areas from the nanosciences and nanotechnologies activities supported by the DST particularly to address the country's socially-oriented problems such as health, water and energy security, and enhancing economic value of industrially-oriented aspects such as; chemical and bio-processing, minerals and mining, and advanced materials and manufacturing. And secondly, to justify the need for a well-directed and targeted research programme towards understanding potential safety, health, and environmental impacts of nanomaterials to humans and other ecological organisms.

Focus areas of support

i. The establishment of characterization centres which are geographically distributed and contain multi user facilities to provide researchers with: advanced instruments for the creation of Research and Innovation Networks that will serve to enhance collaboration among traditional disciplines, research teams and institutions;
ii. Capacity building initiatives that are aimed at developing human capital resources; and
iii. A number of Flagship Projects that are aimed at demonstrating the benefits of nanotechnology towards an enhanced quality of life and increased economic growth. These will initially focus on: – water, energy, health and chemical and bioprocessing, mining, minerals and advanced manufacturing.

To contextualize the potential complex challenges nanotechnologies are likely to pose to both occupational and environmental health – different nanomaterials synthesized from research or anticipated for potential applications in South Africa are summarized below.

The pressing issues of water resources and water supply systems of South Africa with respect to water quantity as well as water quality has made researchers to investigate nanotechnology to provide viable alternatives to current purification methods that may require improvement (Molapisi, 2007). Demands on water sources are increasing at an accelerated rate due to population growth as well as increasing industrial activities. The rapid rate of urbanisation since independence in 1994 causes increasing demands for safe drinking water in urban areas, thus resulting in the need to upgrade and expand water supply systems on a continuous basis in order to meet these demands.

Human health and living conditions are other aspects which the South African government is making a determined effort to improve across the nation, with great emphasis on the most impoverished and marginalized citizens and is pursuing these goals on several fronts. It is hoped that investments in nanoscience and nanotechnology will bring forth the desired overall results. Nanotechnology, for example, could spur the production of cheaper, more effective water filtration systems that would increase access to safe drinking water, and the development of more targeted, slow-release nano-encapsulated pills that could prove beneficial in the treatment of HIV/AIDS, tuberculosis and other infectious diseases (Molapisi, 2007; Rosi and Mirkin, 2005).

Examples of active institutions in nanotechnology research, their respective fields of expertise and/or envisaged applications are presented in Table 1 (these are not comprehensive but are for illustrative purposes only). Generically, R&D appear to be concentrated on electronic materials, energy management, catalysis, electrolytic processes, membranes, nanotubes and fibres, strong materials, drug delivery and modelling. The applications employ diverse nanomaterials such as carbon nanotubes, quantum dots, organic and inorganic nanoparticles like dendrimers and titanium dioxide, aluminium oxides, nanocomposites among other.

Table 1. List of institutions nanotechnology associated activities and materials.

Category	Institution	Research/activity focus
Universities	University of the Witwatersrand	Metals nanoparticles (gold, silver, copper, palladium), polymers, CNTs.
	University of Cape Town	Dendrimers, polymers, silicon nanoparticles, printing of devices
	Tshwane University of Technology	CNTs (e.g. SWCNTs), nanocomposites such as nanoporous activated carbons (NPACs)
	University of Johannesburg	CNTs (e.g. MWCNTs,), polymers(e.g. β- cyclodextrin), bimetallic nanoparticles (e.g. silicon carbide (SiC) nanorods)
	University of Stellenbosch	Nanofibres of different polymers, e.g. cellulose acetate, nylon, polyacrylonitrile, polyvinyl alcohol, etc. and nanoparticles of various forms, CNTs (SWCNTs)
	University of Zululand	Quantum dots, (e.g. CdSe and CdS), nanoparticles, composites, etc.
	University of Limpopo	Nano modelling. Various forms of potential applications and novel nanomaterials are modelled.
	University of KwaZulu-Natal	Polyelectrolyte carboxymethyl konjac glucomannan- chitosan, CNTs (e.g. MWCNTs), numerical modelling
	Rhodes University	Gold nanofibres, CNTs, nanostructured metallophthalocyanines. Sensor detectors development.
	University of the Western Cape	Gold nanoparticles, carbon nanopipes, carbon nanotubes (e.g. MWCNTs, etc.).
Science Councils	MINTEK	Nanoscale materials produced include: Nano Au, Ag, Pt, Pd, TiO_2, Fe_2O_3, Fe_2O_3, Au, etc. Particularly, Gold nanoparticles are anticipated for chemo-therapeutics applications
	CSIR	Nanocomposites, metal nanoparticles (e.g. silicon, TiO_2, ZnO, SnO_2, etc.), quantum dots, nano drug encapsulation, nano-biotech, CNTs, polymers.

1.4 Institutions involved in the above activities

Most local universities have some ongoing research initiatives in the nanotechnology field. Mintek has been steadily developing the critical mass in nano-science and nanotechnology, specifically in the field of biomedical diagnostics. The resources that have been created are unique in South Africa and include a team of interdisciplinary researchers, ranging from drug researchers, chemists and chemical engineers, materials scientists to physicists.

a. University of Johannesburg

The focus is to develop novel solutions of treating water cheaply and efficiently that meets drinking, industrial, and environmental water quality standards. The nanomaterials used to achieve this objective by removing inorganic and organic pollutants include; carbon nanotubes polymerised with Cyclodextrin polyurethanes for removing organic pollutants from water. Collaborative work with Mintek is currently ongoing where electrochemical cells which will be used for early detection and monitoring water-borne pollutants (Mintek Report, 2010).

b. University of Western Cape

Research is conducted into gold nanoparticles in biosensors (Owino *et al*., 2008).

c. Tshwane University of Technology

Silver nanoparticles mounted on different substrates such as zeolites, carbon and polymers have been developed for water disinfection applications.

d. University of the Witwatersrand

This institution is involved in the synthesis of nanoparticles of gold, silver, copper, and palladium as well as the synthesis of single wall and MWCNTs, nanospheres (fullerenes), nanowires and nanobamboos. The core group research objective is to develop high performance catalysts in addition to the use of nanoparticles for the removal of hexavalent chromium from industrial wastes. The synthesis and characterization of a carbon nanotube (CNT) catalyst support system enhanced with docking stations along the exterior which limit the surface mobility of ultra-small iron catalyst particles on CNT surfaces during Fisher-Tropsch synthesis and therefore prevent decrease in effectiveness of the catalytic behaviour of the metal NPs over time. The technology has also been extended into neuro-pharmaceutics.

e. University of Stellenbosch

Preparation of magnetite nanoparticle is the core research activity of this university with anticipated applications for effluent processing, therapeutic and diagnostic testing and densimetric separation. The group also investigates the polymer-clay nanocomposites. In 2010 the ―nano-teabag‖ was developed, which is anticipated for use in water purification and is already being piloted in the Limpopo province.

f. University of Cape Town

The university has formed collaborations with Mintek in nanoprecious metal (nanogold particle) catalysis and health research. Most of the research activities under the malaria programme take place at the University of Cape Town (UCT) with Mintek acting as the link to development of promising results.

g. Rhodes University

At Rhodes University, the nanotechnology research is carried out at the DST/Mintek Nanotechnology Innovation Centre housed at the Department of Chemistry. Their research involves the synthesis of nanomaterials such as carbon nanotubes, quantum dots and magnetic fluids for use in medical applications (in combination with dyes such as metallophthalocyanines) and for the development of sensors.

h. University of Zululand

The research group has mainly focused on quantum dots and other forms of nanoparticles. In South Africa the University of Zululand can be regarded as the leader in the research and fabrication of quantum dots as these materials finds applications in diagnostics, security systems, biological probes, and optics.

i. University of KwaZulu-Natal

The syntheses of novel polyelectrolyte carboxymethyl konjac glucomannan-chitosan nanoparticles for drug delivery were synthesised. Other materials synthesised include carbon nanomaterials such as nanotubes and nanofibres.

j. University of Limpopo

Nanotechnology research at the University of Limpopo focuses on applying modelling tools to investigate the properties of nanomaterials as material strength, electrical conductivity, crystal structure, etc. as well as theoretically simulating nanoparticles.

k. Research organizations

The Council for Scientific and Industrial research (CSIR), Council for Mineral Technology (Mintek) and iThemba Laboratories are the research organizations involved in nano- research.

i. The CSIR hosts one of the multi-user facilities for synthesis and characterization of nanomaterials in South Africa. Recent acquisition of modern facilities has enhanced the centre's capability for fabricating a wide range of nanomaterials for both research and industrial applications. These comprise of carbon nanotubes, nanocomposites, polymers, quantum dots and metal-based nanoparticles, e.g. titanium dioxide.

ii. Mintek established AuTEK (gold TEK), a joint venture between Mintek and South Africa's three major gold mining houses: AngloGold Ashanti, Gold Fields and Harmony Gold. The programme on nanotechnology has concentrated on gold catalysis for the oxidation of carbon monoxide to carbon dioxide to be used in air purification.

iii. Materials Research Group iThemba LABS National Research Foundation laboratories' research focus is on the mechanism of formation of nano-clusters.

l. Private sector

There is an increasing interest by different industrial sectors towards harnessing the commercial benefits of novel nanomaterials through fabrication of consumer products and industrial applications has also contributed in enhancing nanotechnology research activity in South Africa. The involvement of Anglo gold, SASOL, ESKOM, DENEL, De Beers, Gold mines, Harmony Gold, Goldfields, Prime Products Manufacturing, Plascon, Dulux SAPI has been witnessed (Eish!, 2009).

1.5 A yard-stick for South Africa's performance in nanotechnology

The state of nanotechnology in South Africa was investigated through a cientometric analysis of nano-scale research in South Africa during the period 2000-2005 (Pouris and Anastassios, 2007, Schutte and Focke, 2007). Schutte and Focke (2007) documents the trends in South African nano-research over time, major institutional contributors and journals in which South African authors publish their research, international collaborators and performance in comparison to four countries (Brazil, India, South Korea and Australia).

The major findings of the investigation were:

i. Nano-scale research in South Africa is driven by individual researcher's interests and it is in its early stages of development;

ii. The country's nano-scale research is below what one would expect in light of its overall publication output;

iii. The country's nano-research is distributed at a number of universities with a sub critical concentration of researchers.

2. NANOMATERIALS AND THE ENVIRONMENT

According to ENNSATOX (2011) the current worldwide sales of products incorporating nanomaterials are €1.1 trillion and are expected to rise to €4.1 trillion by 2015. A major portion of this growth is represented by ENPs. This exponential growth in research into the synthesis, characterization and application of ENPs has not had a concomitant understanding in the environmental and toxicological properties of the particles. Due to the lack of basic toxicological information on ENPs it is difficult to set environmental quality standards or perform risk assessments (Lubick, 2008). This is clearly demonstrated in the available scientific literature (as of mid-2009 when the study was conducted) on nanomaterials, with close to 100,000 papers published on nanomaterials in general of which less than 40 was related to ecotoxicological issues (Kahru and Dubourguier, 2010). In their review paper Peralta-Videa *et al*. (2011) they provide a useful synthesis of the reviews that have been carried out on different components related to nanomaterials in the environment, e.g. composition, characterization and stability of ENPs; toxicity of ENPs, including aquatic ecotoxicity; and environmental fate and transport of ENPs. Most of the topics of the reviews are bound together by a framework for risk assessment, which is needed to inform policy and provide guidance in managing ENPs (Klaine *et al*., 2008; Lubic, 2008; Wiesner *et al*., 2009; Kahru and Dubourguier, 2010; Savolainen *et al*., 2010). It is therefore not surprising that the risk assessment paradigm forms the backbone of nanomaterial research strategies in a number of countries, e.g. the United States Nanomaterial Research Strategy (USEPA, 2009), European Union (SCCP, 2007), individual member countries such as Germany (BAUA, 2007) and now South Africa (Gulumian, 2011).

The focus of the risk assessment strategies is predominantly human risk based and Stone *et al*. (2010) caution that there is still a considerable lack of knowledge on uptake, biological fate, effects and modes of action of nanomaterials in species other than rodent and mammalian models. This is particularly the case for exposure and uptake routes other than the air (e.g. waterborne exposure, sediment exposures). Behra and Krug (2008) identified three main research priorities that need to be addressed: (i) the choice of nanoparticles to use in biological experiments, and the tests (analysis of physico- chemical properties, aggregation, sedimentation, etc.) needed to characterize them before, during and after these experiments, need to be determined; (ii) the need to examine the route of uptake of synthetic NPs by organisms in different environments (important for the behaviour of synthetic NPs in the food-chain); (iii) the choice of organisms and endpoints measured.

These are not components that can be dealt with in isolation and therefore current research projects addressing ecological risk assessment of nanomaterials are multidisciplinary research programmes. For example the NanoImpactNet programme is funded under European Commission FP7 (CSA-CA218539) and brings together a multidisciplinary network of 24 different European research groups that are actively involved in studies of the potential health and environmental impacts of nanomaterials. One of the objectives of NanoImpactNet is to devise strategies for the investigation of nanomaterial exposure, hazard and hence risk in the environment. This will be achieved by addressing the following three key questions (Stone *et al*. 2010):

- What properties should be characterized for nanomaterials used in environmental and ecotoxicological studies?
- What reference materials should be used for environmental and ecotoxicology studies?
- Is it possible to group different nanomaterials into categories/ groups for consideration in environmental studies?

The ENNSATOX Programme also funded under European Commission FP7 (NMP4-SL-2009-229244) aims to study and relate the structure and functionality of well characterised engineered nanoparticles (e.g. zinc oxide, titanium dioxide and silicon dioxide) to their biological activity in the aquatic environment. This will be done by taking into account the impact of the nanoparticles on environmental systems from their initial release to uptake by organism using a series of biological models of increasing complexity from single cells to fish (ENNSATOX, 2011). Thus to provide scientifically relevant research results it is essential that nanomaterial risk assessment research programmes are designed to address the topic in an integrated manner, i.e. from environmental fate, biological uptake to effect at different levels of biological organization.

2.1 Risk assessment of ENPs in South Africa: ecotoxicology studies

Recently the need for a parallel R&D into risk assessment of ENMs with current developments in nanosciences and nanotechnology was recognized by the DST (Gulumian *et al*., 2012). This was mainly related to the potential safety and health issues of ENMs to humans at various phases of these materials lifecycle. However, thus far limited scientific studies have been published and those that have been deal with isolated components (e.g. single species and limited effects endpoints). This makes it difficult to extrapolate to provide adequate knowledge on the fate and behaviour of ENMs in different environmental compartments such as water, soil and sediments. In this section, the current studies on the ecotoxicology of ENMs in South Africa are summarized. It should be noted that to the knowledge of the authors the only few organizations in SA are actively involved in this field – e.g. the CSIR and the University of Johannesburg.

a. The CSIR risk assessment research initiatives of ENMs

The CSIR research team has investigated potential toxicological effects of ENMs in the aquatic and sediment-water environments using invertebrates (Musee *et al*., 2010b; Musee Oberholster *et al*., 2011) as well as marine organisms to elucidate the potential effects of ENMs to biological systems in different ecological systems. The findings suggest that exposure to ENMs present in the sediment may cause profound impacts in the environment. However, there are several scientific aspects that need to be considered such as the impact of the physicochemical properties and abiotic factors – and what factors offers synergistic or antagonistic mechanisms with respect to the observed toxicological effects.

On the other hand, the aquatic plants such as algae are an important component of aquatic ecosystems, and the impact of ENMs on these species are currently being investigated in collaboration with the University of Johannesburg. In these studies, the thrust of the study is to elucidate the effects of ENMs on several end-points – such as growth rates, survival rates, degree of DNA damage, and enzymatic activities – and the mechanisms in which the effects of the end- points are influenced by physicochemical properties and abiotic factors. Generation of data and knowledge is envisaged in this field is envisaged to enhance our ability of undertaking effective and realistic assessment of the risks posed by ENMs to different biological life forms in the environment. Other answered questions is whether plants can uptake ENMs – how this will critically affect transportation and exposure pathways of the materials in the environment – and if plant accumulated ENMs may be transferred through food chains to higher trophic levels (e.g. humans).

Fate and behaviour of ENMs in aquatic environments

In pursuit of understanding the potential adverse effects of ENMs to different biological life forms, it is increasingly becoming clear that one of the significant influencing aspects could be due to their fate, behaviour, and interactions once they reach the environment. In this context, there is increasing scientific quest to address several key questions. For example, what is the fate and behaviour of ENMs in freshwater and wastewater environments? What are the dominant factors that control the fate, behaviour and transportation of ENMs once they have been released into an aquatic environment?

To address these questions among others, it is important to understand the mechanisms that determine the stability of a given ENM when it is present in either freshwater or wastewater systems. It is also important to understand if ENMs can be bioaccumulated, biomagnified or persist when they are present in organisms. Similarly, it is important to understand what might be the effects of different treatment techniques in removing ENMs from freshwater or wastewater. This is one of the areas where there is a serious lack of risk data that are needed to substantiate the effect of

ENMs in the environment. A laboratory-scale, simulated wastewater system will be developed to study the behaviour of ENMs in a treatment system and will also use samples from an actual wastewater treatment plant to study how various forms of ENMs will behave under such conditions. The studies are currently in the planning phase and commenced in 2011/12 financial year.

Moreover, the disposal of ENMs and the risks of leakage and spillages during their manufacture, packaging and transport are also not known understood and remain causes of great concern. Therefore, an improved understanding of the fate and behaviour of ENMs will allow the development of practical mechanisms to deal with nanoscale materials in case of spillages, or treat them effectively after their release into wastewater treatment plants.

Development of risk assessment models

In the scientific literature, there is dramatic increase in the volume of data and information concerning risk assessment of ENMs in the peer-reviewed articles. However, a key question remains: to what extent are these data valuable or directly applicable in informing decision-making to manage adequately the diverse array of potential risks of ENMs in South Africa and elsewhere in the world? One way of exploring and exploiting the value of these data is by developing decision models using modelling tools. Notably, the most challenging aspect of this research is lack of methods that can properly and reliably translate raw scientific data into usable predictions that can support justifiable and transparent decision-making processes to allow orderly governance of the nanotechnology industry. In addressing part of this challenge, In the CSIR modelling approaches have been applied to elucidate the potential risk of ENPs (Musee, 2010c; 2010d).

b. University of Johannesburg risk assessment research initiatives of ENMs

Three large scale research projects are currently underway on the aquatic ecotoxicology of selected metal nanoparticles, i.e. CNTs, cyclodextrins and nanogold. The characterization of the studied ENMs under different physicochemical conditions (i.e. pH, conductivities and organic matter) are carried out by the Department of Chemical Technology. The biological responses using the standard accepted suite of test organisms in South Africa (Ansara-Ross *et al*., 2009) are undertaken in the environmental room so of the Centre for Aquatic Research in the Department of Biology. Studies are currently underway on the assessment of the toxicity of phosphorylated multi-walled carbon nanotubes (MWCNTs) and cyclodextrin derived polymers on bacteria, algae, invertebrates and fish. The effects of ENM preparation methods, functionalisation, surface chemistry and interaction with other ionic species at various environmental parameters are related to changes in toxicity endpoints of the selected species.

Preliminary results on the toxicity of polymerised cyclodextrin (*pol*CD), pristine multi- walled carbon nanotubes (pristine MWCNT) and oxidised multi-walled carbon nanotubes (*ox*MWCNTs) to the water flea *Daphnia pulex*, using mortality as a toxicological endpoint has been determined (Nyembe *et al*., 2010). The results show that the toxicities of the tested nanomaterials are dose dependent. The most toxic material was *ox*MWCNTs. The acid oxidation introduced functional groups that contributed to its toxicity. Also its particle size distribution (14.5-93.7 nm) and zeta potential (-3.4 mV) worked synergistically towards the material's toxicity. The presence of residual Fe catalyst, agglomeration, particle size (14.5-93.7 nm) contributed to the toxicity of pristine MWCNTs. *pol*CD were toxic whereas the pure CDs were not toxic at all to the *Daphnia* even though their particle sizes lower than those of the MWCNTs. The order of toxicity was *ox*MWCNT > *pol*CD> pristine MWCNT. Studies have also been completed on the influence of CNTs on growth of the algal test species (Schwab *et al*., 2011). The information generated through the ENM characterisation and toxicity tests will be incorporated into the risk models that are being developed by the CSIR.

3. NANOMATERIALS IN SOUTH AFRICAN WATER SYSTEMS

Engineered nanomaterials may be released into water systems via diverse point and diffuse sources. These releases may occur at any stage in the ENMs product lifecycle. It is therefore not surprising that the most recent international initiatives such as the National Nanotechnology Initiative's Environmental Health and Safety Research Strategy in the USA (NNI, 2011) assessed environmental releases across the nanomaterial product life sources. According to Nowack *et al*. (2012) the largest published body of ecological risk assessment literature is on pristine ENMs (P-ENM), and that greater classification of ENMs merit to be considered. This is to reflect the diversity of ENM as a function of surface properties during different life cycle stages. The authors indicated that the P-ENMs are embedded into products to form product-modified ENM (PM-ENM). The PM-ENM are in turn weathered through environmental processes while still embedded within a product (product-weathered ENM; PW-ENM) or are removed from the product and acted on by environmental processes (transformed ENM; ET-ENM). The relationship between the ENMs life cycle, ENM category, and sources is presented in Figure 1.

Generally, most laboratory-based stability and toxicity tests have been conducted using P-ENMs with highly varied surface coatings; however, these products will only reach the environment through unintentional or diffuse sources. The industrially modified PM-ENMs undergo changes, e.g. hydrophilic-hydrophobic properties in organic solvents, changes in surface charge and reactivity, which will change the physical, chemical, and biological properties when compared to the parent P-ENM (Nowack *et al*., 2012). In most instances the manufacturing process does not result to intentional release of PM-ENMs into the aquatic environment. However, in the case of the nanotechnology application in wastewater treatment, PM-ENMs are released into the treatment process intentionally (Savage and Diallo, 2005).

Based on modelling predictions by Gottschalk *et al*. (2009) most surface waters could be considered to be driven by wastewater discharges. Consequently the ENMs that form part of water purification, waste water treatment or move through the treatment plants pose a risk to the receiving water environment. Given most ENMs are expected to be as a result of breakdown and weathering of products containing ENMs, Nowack *et al*. (2012) indicated that a major challenge is to determine whether the environmentally relevant PW-ENMs and subsequent ET-ENMs will be more or less reactive in the environment. The interactions of ENMs with the receiving aquatic environment have not been well studied and large portion of published literature makes use of hypothetical examples, which are based on extrapolations from laboratory-based bioassays (Musee *et al*., 2011; Nowack *et al*., 2012).

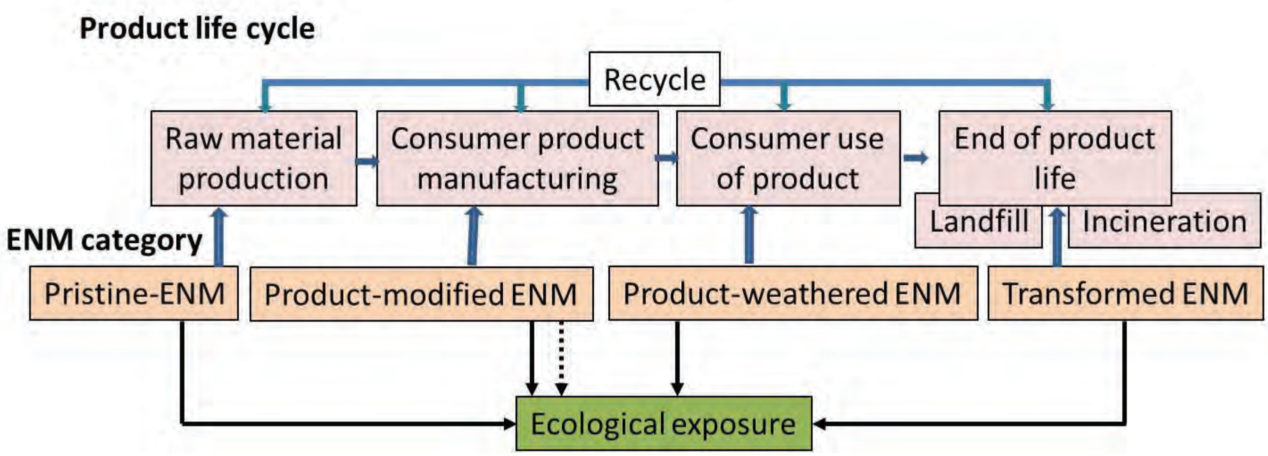

Figure 1. Diffuse (solid line) and point (dashed line) releases of different category ENMs during the different life stages of the ENM into the environment (adapted from NNI, 2011; Nowack *et al*., 2012).

Two distinctive groups of ENMs could enter the aquatic environment. The first and by far the largest group are ENMs that may enter water systems through diffuse sources during production, use and disposal stages (Musee *et al*., 2011). This group comprises of all P-ENMs, PM-ENMs, PW-ENMs, and

ET-ENMs. The second group of ENMs is those that are applied specifically within water treatment processes such as nanosorbants (e.g. carbon nanotubes, zeolites, etc.), nanocatalysts and redox active nanoparticles (e.g. nanoscale metals – TiO_2, etc.), nanostructured and reactive membranes (e.g. carbon nanotubes), bioactive nanoparticles (e.g. AgO, MgO, etc.) and dendrimer enhanced ultrafiltration (e.g. dendritic nanopolymers). Comprehensive reviews on the application of ENMs in water purification have been summarised elsewhere (Savage and Diallo, 2005; Schutte and Focke, 2007), and will not receive attention in this report. For the purposes of this report, we will make use of the generic term ENMs but keeping in mind that the category of ENM may change depending on the underpinning factors that causes their fate, transport, and persistence in different environmental compartments.

3.1 Current state of knowledge on the methods to assess risk posed by ENMs to the aquatic environment

The risk assessment process is guided by four key concepts (Figure 2), namely; identification of the hazard, exposure assessment, assessment of the dose-response relationship, and risk characterization (NRC, 1983; Suter *et al*., 2003). Therefore, to assess risk requires the linking of physical and chemical behaviour (exposure) to effects on organisms in the aquatic environment (effects). Most reported literature concerned with assessing exposure and effects assessment of ENMs suggests the unacceptably high degree of uncertainty in risk assessment is largely due to the paucity on usable environmental hazard data and detailed knowledge on both their fate and the behaviour that are critical in enhancing our collective understanding of the environmental effects (Klaine *et al*., 2012).

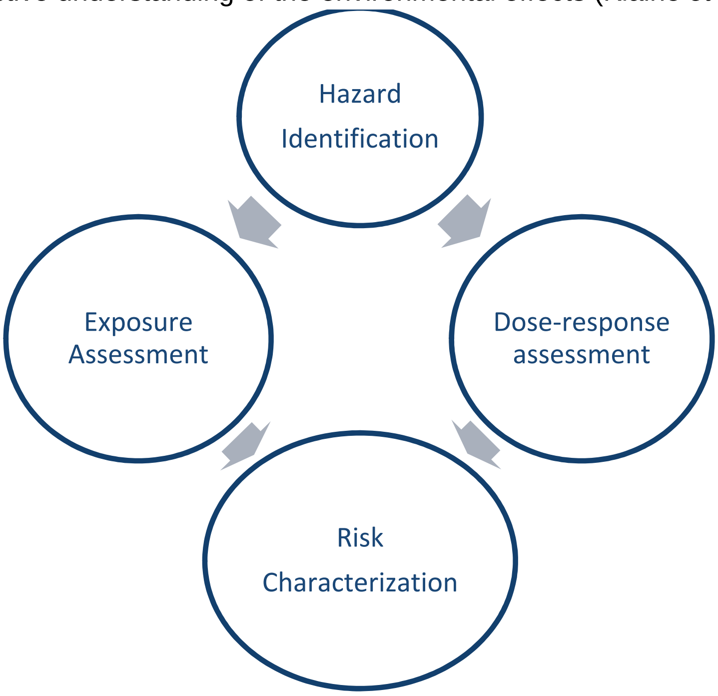

Figure 2. Diagrammatic representation of the risk assessment process (adapted from NNI, 2011).

According to Klaine *et al*. (2012), ENMs ecological hazard assessment has not kept pace with the advances in nanotechnology. To address this shortfall in information, aquatic ecotoxicologists have resorted to using standardized testing methodologies and test organisms in an attempt to understand the effects of ENM exposure. This implies that currently ENM hazard assessment is conducted using similar approaches applied for traditional chemicals. Crane and co-workers (Crane *et al*., 2008) were of the opinion that the existing methods and framework for hazard assessment, i.e. use of standard test organisms with mortality, growth, and reproduction as endpoints are generally adequate to identify hazard associated with ENMs exposure. However there is consensus that within each group of tests, modifications relevant to ENMs would be required. The European standards organization, the OECD, has initiated a process whereby the standard regulatory tests for new ENMs are being validated (OECD, 2010).

3.2 Factors to consider when using standardized toxicity tests in ENM hazard assessment

Handy *et al*. (2012) synthesized the generic methodological issues that are common to different types of ecotoxicity tests with specific reference to ENMs based on the collective experience of a group of leading scientists with extensive hands on experience in ENMs toxicity testing. For the purposes of this report, only the most salient issues addressed in their report are highlighted in addition to aspects currently being considered by aquatic ecotoxicologists in South Africa.

Setting up the toxicity bioassay

The current methods of preparing glassware and setting up the bioassays are acceptable but in selecting the type of test (e.g. static, static-renewal, flow-through, etc.) and controls; there are a number of positive and negative factors associated with each of the tests that merit careful consideration. For example, pitfalls associated with measuring the general water quality conditions during the exposures are highlighted, e.g. the influence of carbon nanotubes on oxygen and pH readings due to their absorbance to the electrode glass probes, and the interferences of ENMs with colorimetric assays for ammonia determination, etc.

Characterization of exposure

There are a number of factors that need to be considered when confirming and maintaining concentrations during the exposure period, e.g. when to replace exposure medium, the physico-chemical conditions during the exposures, the influence of these conditions of the ENMs characteristics, etc. Characterization of the test media during experiments is an essential requirement for exposure characterisation within the risk assessment process. General consensus has been reached on the measurement requirements for ENMs in the stock and exposure solutions (Table 2). While there is still debate on which dose metric should be used routinely in ENMs ecotoxicity testing (e.g. particle size, particle number, or mass concentration) it is generally accepted that for ecotoxicity tests, minimally mass concentration (mg/l) and measurement of particle size distribution in the exposure medium should be taken. In addition, the dissolved and particulate metal concentrations should be measured for metal-based ENMs. The authors also provided an in depth assessment of positive and negative factors associated with different dispersion methods that are available for exposing the ENMs. It was stressed that care should be used with terminology, e.g. in ENMs ecotoxicology we do not deal with aqueous solutions but rather a colloidal dispersions.

Characterisation of effect

The current endpoints applied in standard toxicity testing (mortality, growth inhibition, reproduction, etc.) are deemed sufficient for the purposes of assessing the effects of ENMs. The authors also indicated that at present there is no evidence of nano-specific sub-lethal endpoints. Since ENMs elicit many fundamental toxic responses, endpoints such as enzymatic biomarkers, histopathology, etc. (Wepener *et al*., 2011) could be used in the interim to provide information on biological hazard. Klaine *et al*. (2012) indicated that rapid development of effects assessment endpoints such as the “omics” approach that relies on computational ecotoxicology may evolve hypotheses of toxicological mechanisms useful in providing insights of the ENMs effects. Handy *et al*. (2012) pointed out that it should be endeavoured to identify a nano-specific biomarker response similar to the development of the vitellogenin assay, which is now routinely used to identify exposure to endocrine disrupting chemicals.

Furthermore, the report of Handy *et al*. (2012) also provided detailed assessment of the three most commonly used toxicity bioassays, i.e. the 72 hour algal growth inhibition test, the 48 hour *Daphnia* immobilization test, and the 96 hour fish mortality test. The authors pointed out some of the variations that need to be applied to these standard protocols to take into account the toxic responses related to the differences between the physical characteristics of ENMs and traditional dissolved chemicals. For instance the shading effect of carbon nanotubes due to aggregation can inhibit photosynthetic activity in the algal test without necessarily exerting a toxic effect (Schwab *et al*., 2011). Similarly, Handy *et al*. (2012) refer to a number of studies where the precipitation of high concentrations of ENMs result in e.g.

particle induced inhibition of respiration in *Daphnia magna*, whilst the clouding of water by the ENMs resulted in increased aggressive behaviour in fish bioassays.

Table 2. Properties to characterize ENMs in stock solution[a] and in environmental media[b] proposed by a range of authors.

Property	Oberdorster *et al*. (2005)[a]	Powers *et al*. (2006, 2007)[a]	Thomas *et al*. (2006)[a]	Warheit (2008)[a]	Klaine *et al*. (2008)[a]	Stone *et al*. (2010)[b]	Von der Kramer *et al*. (2012)[b]
Mass concentration							**
Size distribution	*	**	**	**	**	**	**
Agglomeration state/dispersion	*	**	*	**	*	**	
Crystal structure	*	*	*	**			
Chemical Composition	*	*	*		**	**	
Surface area and Porosity	*	**	*	**	**		
Surface chemistry		**	*	**	*	**	
Surface charge		*	*	**	*	**	
Shape and Morphology		**	**		*		
Dissolution/ Solubility		*	*		**	**	
Physical/chemical properties (purity)		**		**			
Methods of synthesis				**			

*Of importance; **Priority.

Aspects related to the detection of ENMs in biological and environmental matrices, predicting the environmental fate of ENMs and hazard assessment of ENMs (Table 3) are some of the challenges identified by Klaine *et al*. (2012) associated with developing quantitative risk assessment methodologies for ENMs. These challenges together with the research needs identified by the National Nanotechnology Initiative's Environmental Health and Safety Research Strategy (NNI, 2011) form the basis of the research priorities that should be addressed when applying an ecological risk assessment framework to assess risks posed by ENMs in South African waters. This framework is discussed in Section 4.2 of this report.

a. ENMs effects characterization facilities

South Africa has a well-developed aquatic toxicity testing framework based on a whole effluent toxicity (WET) testing approach (Wepener, 2009). It was initially implemented to evaluate the acceptability of potentially hazardous effluents for discharge into receiving waters (Slabbert *et al*., 1998). The methodologies applied in WET focus on acute and chronic toxicity testing using standardized laboratory-based bioassays involving laboratory-reared organisms. These WET methodologies have been incorporated into the Direct Estimation of Ecological Effects Potential (DEEEP) toxicity tests (Slabbert, 2004), the current choice for application within the National Toxicity Monitoring Programme. Slabbert and Murray (2011) further identified all the components of the National Water Act that would require the application of standardised toxicity tests.

There is a well-established and formalised network of aquatic toxicity practitioners in South Africa (Aquatox Forum) consisting of groups, laboratories, and individuals from academia, industry and government. Members of the Aquatox Forum were actively involved in the development of guideline documents for accreditation of a range of different standardized toxicity tests within the South African framework (Chapman *et al*., 2011a). Chapman *et al*. (2011a) report synthesized extensively the standard and other fundamental research-based toxicity tests and institutions that conduct aquatic toxicology tests in South Africa. At present there are 28 such institutions or individuals with the majority (18) located in Gauteng Province. The laboratories involved in aquatic toxicity testing are situated in a number of academic institutions, private companies, water utilities and government. Table 3 presents a summary of the main aquatic toxicology laboratories with the toxicity testing capabilities at these institutions. These standardized toxicity tests are potential candidates of forming the mainstay of ENMs hazard assessment. In addition, through the centralized involvement of the Aquatox Forum and its toxicity practicing membership offers a suitable platform of modifying the tests to suite the certain range of unique needs for ENMs toxicity assessment.

Table 3. Considerations on moving ENMs nanoparticle environmental research forward: A framework to assess the environmental impact of ENMs (reprinted from Klaine *et al*. 2012 with permission from John Wiley and Sons).

	Current state of science	**Gaps**	**Framework**
Detecting ENMs	• In pristine conditions	• In complex media • At realistic concentrations • For aged or weathered materials • Relative to background materials	• Develop colloid science techniques for environmental matrices • Gather input from toxicologists on appropriate metrics
Predicting fate	• Behaviour of pristine, unaltered nanomaterials under laboratory conditions	• The nature of released particles • Information on nanoparticle being altered and aging in the environment • Product-specific particle processes and time scales	• Assess exposure for product and/or altered nanoparticle-specific categories of nanomaterials
Assessing hazard	• Use of traditional aquatic toxicology endpoints and relevant species	• Sufficiently fast and targeted analytical methodology to meet data needs during testing • Appropriate controls • Addressing time-dependent exposure • Dispersion methods • Scale (volume) problems	• Apply newly developed technology for exposure monitoring and control • Account for time-varying exposure • Prioritize toxicology tests most likely to identify risks • Develop minimum toxicology recommendations
Developing risk assessment methodologies	• Existing framework available and applicable • Limited scientific information • First global approaches for screening assessment	• Exposure uncertainty because of uncertain fate processes • Uncertain effects thresholds • Uncertainty of risk characterization metrics • Tools for location-specific assessment	• Examine product *vs.* nanoparticle *vs.* aged nanoparticle • Address physical form and spatial variability • Investigate interactions with toxic chemicals • Consider nanoparticle-type specific metrics

4. NEED FOR AN ECOLOGICAL RISK ASSESSMENT FRAMEWORK TO STRUCTURE RESEARCH PRIORITIES OF ENMS IN SOUTH AFRICAN WATERS

Until very recently there has been a lack of support in South Africa in establishing the potential risks of ENMs and products to both humans and the environment (Musee *et al*., 2010; OECD, 2012). This phenomenon is however similar to what has been observed in other countries during their initial phases of implementing nanotechnology strategies and programmes. In 2010, the DST launched an initiative towards establishing a research platform on aspects related to health, safety and environmental of nanotechnology. A national steering committee consisting of representatives from the National Institute for Occupational Health (NIOH), Council for Scientific and Industrial research (CSIR), University of Johannesburg (UJ), and North West University (NWU) were tasked with examining the ethical and risks associated with nanotechnology. Rather than come up with an independent framework, a decision was made (Musee *et al*., 2010; Gulumian, 2011) to develop a framework that will be aligned with the current initiatives in the United States, European Union and member countries, Japan (NNI, 2011; SCCP, 2007; Thomas *et al*., 2006).

Similar to the situation experienced internationally, nanomaterials synthesized in South Africa encompass a multitude of classes (see section 1), which contain different subclasses in countless modified versions. According to Gulumian *et al*. (2012) it is impractical and virtually impossible to conduct risk assessment of each individual type of ENMs, and therefore, the committee recommended that risk assessment strategies should be based on different categories of ENMs. This would require prioritization strategies for toxicity testing and risk assessment and also take into account the commercial production and exposure potential of the ENMs (OECD, 2009). Once consensus is reached on those ENMs that would qualify for comprehensive risk assessment, detailed data should be generated to support robust and effective risk assessment (Gulumian *et al*., 2012).

4.1 The ecological risk assessment concept

Risk assessment can be defined as the process of assigning magnitudes and probabilities to the adverse effects of anthropogenic activities or natural catastrophes (Suter, 1993). The effects are termed as hazards, and the existence of a hazard and the related uncertainty of the hazards' effects, result in the formulation of risk. Therefore, risk is the probability or likelihood of a prescribed undesired effect occurring and impacting on an environment (Suter, 1993; USEPA, 1998).

Ecological risk assessment is a structured approach that describes, explains and organizes scientific facts, laws and relationships, thereby providing a sound basis to develop sufficient protection measures for the environment, which facilitates the development of utilisation strategies for the environment (Claassen *et al*., 2001). It is therefore the process that evaluates the likelihood that adverse effects may occur or are occurring as a result of exposure to one or more stressors (Suter, 2001). As a result, it is concerned with the causal relationship between stressors and receptors with the consequent effects (Figure 3). Ecological risk assessment is the dominant framework for technical support to environmental regulation endeavours in industrialized democracies (Suter, 2001). Moreover, it is generally considered among the most preferred and commonly used decision-making tools in modern times (Landis, 2003).

As a result, it is concerned with the causal relationship between stressors and effects, and also deals with the consequences of alternative decisions. The risk estimates for such an assessment are normally calculated by measuring the exposure and the effects in relation to defined assessment endpoints (Landis and Wiegers, 1997).

Table 4. Selected institutions currently involved in aquatic toxicity testing and the types of tests that can be carried out.

Institution	Standardised tests	Other fundamental research endpoints
Centre for Aquatic Research (University of Johannesburg)	Bacteria, Algal, *Daphnia* (acute and chronic), Fish (acute and growth) using standard test organisms and indigenous species.	Enzymatic and non-enzymatic biomarkers, DNA damage (Comet assay), Omics approach (genomics, proteonomics, toxicogenomics), histopathology.
Institute for Water Research (Rhodes University)	*Daphnia* (acute and chronic) and other indigenous macro-invertebrate species.	Enzymatic and non-enzymatic biomarkers in fish.
North-West University	Acute and chronic soil toxicity tests using standard earthworm bioindicators.	
University of Stellenbosch	Acute and chronic soil toxicity tests using standard earthworm bioindicators.	Enzymatic and non-enzymatic biomarkers, DNA damage (Comet assay), toxicogenomics.
University of Zululand	Acute and chronic estuarine sediment toxicity test using indigenous amphipods.	
University of the Orange Free State	Bacteria, algal, *Daphnia* (acute and chronic), fish (acute and growth) using standard test organisms	
University of the Western Cape		In vivo tests using cell lines
University of Pretoria		In vivo tests using cell lines
NRE (CSIR)	Algal (chronic test) using standard and indigenous species, *Daphnia* (acute and chronic),	Enzymatic and non-enzymatic biomarkers.
Rand Water	Bacteria, Algal, *Daphnia* (acute and chronic), Fish (acute and growth) using standard test organisms	
Johannesburg Water	Bacteria (*Vibrio*)	
George Municipality	Bacteria (*Vibrio*)	
eThekwini Metro	Bacteria (*Vibrio*)	
Umgeni Water	Bacteria, Algal, *Daphnia* (acute and chronic), Fish (acute and growth) using standard test organisms	
Golder Research Laboratory (Pty) Ltd	Bacteria, Algal, *Daphnia* (acute and chronic), Fish (acute and growth) using standard test organisms. Freshwater sediment toxicity testing.	
Sasol Technology	Bacteria (*Vibrio*), Algae, *Daphnia* (acute), Fish (acute and growth) using standard test organisms.	
Renaissance Environmental Hub	Bacteria, Algal, *Daphnia* (acute and chronic), Fish (acute and growth) using standard test organisms. Freshwater sediment toxicity testing. Soil tests (PHYTOTOX).	
Cleanstream Biological Services	Bacteria, Algal, *Daphnia* (acute and chronic), Fish (acute and growth) using standard test organisms. Freshwater sediment toxicity testing. Soil tests (PHYTOTOX).	
Resource Quality Services, Department of Water Affairs	*Daphnia* (acute and chronic), Fish (acute and growth) using standard test organisms.	
Buckman	Bacteria (*Vibrio*)	
Improchem	Bacteria (*Vibrio*)	

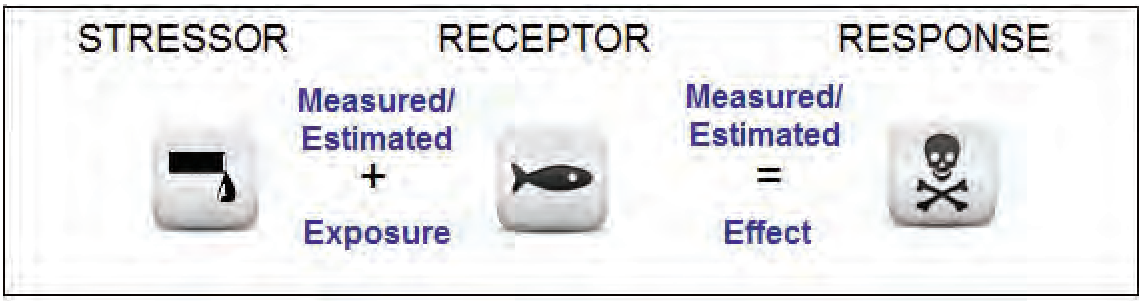

Figure 3. The causal relationship between exposure and effect that form the backbone of ecological risk assessment.

4.2 Generic framework to structure research priorities for ecological risk assessment of ENMs in South African waters

According to Suter *et al*. (2003) risk assessment has been reliant on the linking of physical and chemical behaviour to effects on organisms, and the environment and ultimately humans. However, Klaine *et al*. (2012) attributes the inability to develop quantitative risk assessments for ENMs with acceptable levels of uncertainty stems from the inherent limitations of current approaches applied for estimating exposure and toxicity. These authors identify the challenges related to environmental nano-research as: detecting ENMs in biological and environmental matrices; predicting the environmental fate of ENMs; assessing the hazard of these materials; and based on the aforementioned results being able to develop quantitative risk assessment strategies. It is well documented that the behaviour of ENMs in the environment and the resulting bioavailability with ensuing organism exposure is vastly different from dissolved chemicals (Klaine *et al*., 2012; Nowack *et al*., 2012). While these limitations in relation to risk assessment of ENMs are acknowledged, decades of knowledge developed to address chemical exposure and biological responses provide an invaluable basis to approach the identified research challenges. This approach can be illustrated using the traditional chemical effects assessment framework (Connell *et al*., 1999) indicating the cascade from source through to individuals and the ecosystem (Figure 4). The research priorities required to understand environmental exposures, environmental dose and bioavailability and effects following internal exposure to ENMs are summarized from Klaine *et al*. (2012) and NNI (2011), viz. Table 2.

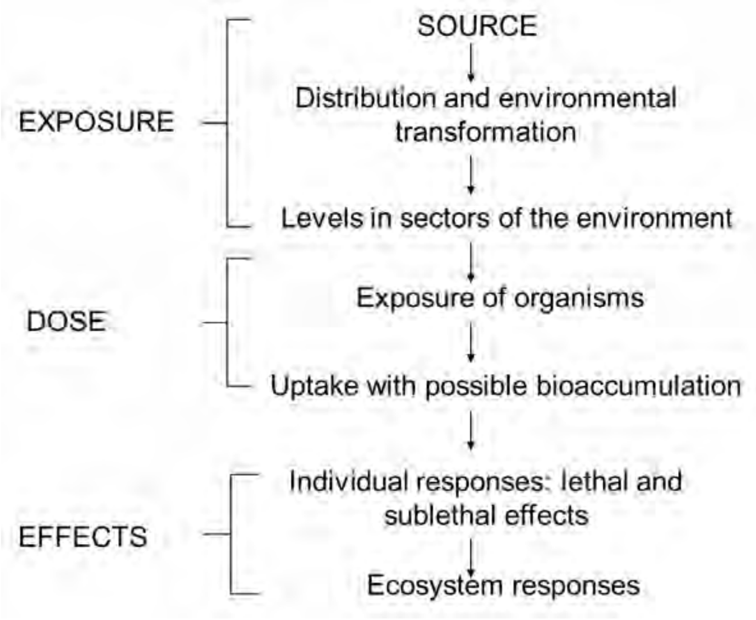

Figure 4. The cascade effect of chemicals from the source to the individual and ecosystems (adapted from Connell *et al*., 1999).

4.2.1. Exposure

The ENMs sources are both point and diffuse, and potentially can be released into the environment at any point in their product life cycle (Nowack *et al*., 2012). It is therefore essential that the physico-chemical properties of different ENMs are well characterized as this is critical in understanding the movement, deposition, accumulation, adsorption, aggregation, absorption, and environmental transformation in a changing environment (NNI, 2011). Biological and photochemical transformations, coupled to contact with a different environmental matrix, e.g. water, will alter the persistence of ENM and influence its exposure concentration (Nowack *et al*., 2012). These exposure concentrations together with other modifying factors such as water hardness, dissolved organic carbon, etc. would determine the ultimate exposure (NNI, 2011).

Research priority 1. Environmental exposure
Identify principal sources of exposure and exposure routes
• Understand manufacturing processes and product incorporation. • Evaluate product lifecycle and potential exposure subsequent to the manufacturing of the product. • Develop analytical approaches to measure newly manufactured and aged ENMS properties throughout their life cycle. • Apply existing models to estimate and quantity ENMs releases into the environment but importantly validate the applicability of the models to account for factors such as aging and the type of recipient environmental matrix. • Identify the relevant environmental factors that control (e.g. pH, ionic strength, natural organic matter, etc.) biological exposure assessment.

The transportation of ENMs through the environment is governed by the prevailing physical and chemical properties however these are not stable and could rather be considered to populations of variables over time (NNI, 2011). According to Klaine *et al*. (2012) the activity and transport of particles are determined by size, i.e. agglomeration and aggregation processes that are not transported by water entirely but both hydrological as well as aerial movement.

Research priority 2. Environmental fate and transport
• Determine the dominant physico-chemical properties (e.g. surface chemistry, size, functionalisation, zeta potential, etc.) that affect environmental transportation of ENMs. • Determine key transport and fate process particular to the environment under consideration. • Adapt existing and develop new modelling tools that take into consideration the unique properties of ENMs to predict the fate and transport in the environment.

4.2.2 Dose

The exposure concentration to which organisms are exposed are influenced by biological / and or photochemical transformation processes (Auffan *et al*., 2010; Nowack *et al*. 2012). The transformation processes can be benign or malign and also either increase or decrease the persistence of the ENMs (NNI 2011). For instance, ENMs may agglomerate to micrometer scale which will result to diminished or insignificant bioavailability. In the presence of other contaminants the agglomerates may bind to the former and either increase or decrease the toxicity owing to Trojan horse effects (Chen *et al*., 2004; Yang *et al*., 2006; Limbach *et al*., 2007).

Research priority 3. Bioavailability of ENMs
Understand transformation under different environments
• Identify and evaluate properties and transformation process (es) that influences persistence, toxicity and formation of by-products of ENMs. • Determine the rate of aggregation and the long-term stability of the aggregates/agglomerates in different environmental matrixes. • Develop tools with capability to model the transformation and degradability of ENMs under different environmental conditions

4.2.3 Effects

Most research on the effects of ENMs has focused on individual species using standardized protocols of assessing endpoints that range from molecular to whole organism responses such as mortality (Handy *et al*. 2011; NNI 2011). The applicability of these tests and test conditions (see section 3.2) to assess the toxicity of ENMs has not been validated (Handy *et al*., 2012). Furthermore the mortality-based results indicate that ENMs are generally less acutely toxic that their chemical equivalents. This motivates the use of sub-lethal endpoints during chronic (long-term) exposure periods such as the “omics” and oxidative stress biomarkers be developed further within the context of toxicants with nanoscale dimensions (Handy *et al*. 2012; NNI 2011). According to the NNI (2011) the validation of high throughput standardized tests are important to punctually identify initiating events in toxic pathways and early developmental stages (Thomas *et al*., 2011).

Research priority 4. Individual responses
Applicability of effects tests on individual species
• Evaluate existing protocols to test the effects of ENMs on standardized test species. • Understand the dose-response characteristics of ENMs. • Understand the uptake/elimination kinetics and tissue/organ distribution of ENMs in model species. • Study the mechanism of toxic action and develop predictive tools. • Develop tiered testing schemes to provide the best estimation **of** ENMs hazard to aquatic organisms. • Ensure environmental realism through exposures not limited to pristine-ENMs.

Undertaking of environmental realistic studies is required to understand the fate and behaviour of ENMs in the aquatic environment (NNI, 2011). The studies should comprise of micro and microcosm exposures that indicate effects on populations and communities as reflected in the responses of individuals. In addition, the interactions between population and community responses should also be evaluated (see Liu *et al*., 2011 for the appropriate references). According to NNI (2011) these studies are more environmentally realistic providing insight into factors such as receptor exposure in different media, relative impacts of different ENMs in the same media, etc.

Research priority 5. Ecosystem responses
• Evaluate effects at population level. • Evaluate effects at community level. • Study ecosystem function reactions to ENMs exposure. • Study the mechanism of toxic action and develop predictive tools. • Develop predictive tools for population, community and ecosystem responses to ENMs.

5. CONCLUSIONS

Data needs for risk assessment of ENMs in many respects will deviate from those of traditional counterpart parent chemicals. In the preceding sections the current priority research needs that were identified. The relationship between the information requirements at exposure, dose and effect level; and the risk assessment requirements for exposure and effects characterization at different product life stages is presented in Figure 5. Notably, enhancing our ability to detect and characterize ENMs and establish their linkages to behaviour and effects in laboratory-based bioassays as well as natural systems will result to improved robustness of the risk assessment process (Klaine *et al*., 2012). This will substantively reduce uncertainty to allow for more informed decisions about managing ENMs. The key to understanding and development of nanotechnology is in interdisciplinary collaboration (Klaine *et al*., 2012). Therefore it is essential to train a new generation of environmental scientists that have technical backgrounds in physics and material sciences in addition to biology and chemistry. Furthermore greater international collaboration is required to make optimum use of limited resources and to make best use of the data generated (Klaine *et al*., 2012).

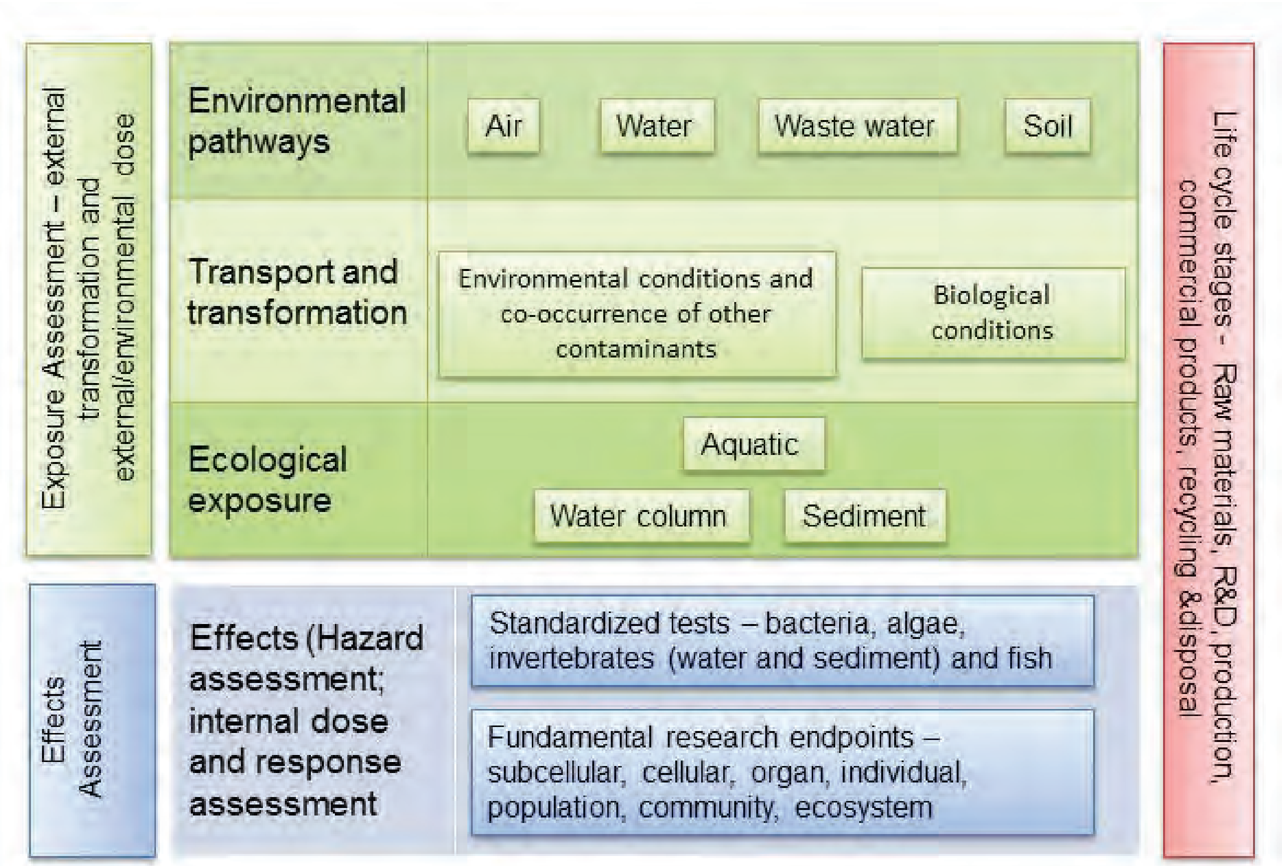

Figure 5. The integrated exposure and effects assessment framework that will contribute to ecological risk assessment of ENMs throughout all life cycle stages (adapted from NNI, 2011).

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