

**ESTIMATED ENVIRONMENTAL RISKS OF  
ENGINEERED NANOMATERIALS IN  
GAUTENG**

*By*

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## DECLARATION

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## ABSTRACT

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Nanotechnology-based products, referred here as nanoproducts, have increased dramatically on the global markets over the past few years due to the benefits incurred to both consumers and manufacturers. This has resulted in substantially non-quantified releases of engineered nanomaterials (ENMs) into different environmental media, viz., water, air, and soil. Previous studies have quantified the amounts of ENMs into different environmental media using modelling techniques, due to lack of metrology to quantify the releases. These releases have been estimated to pose risk to various environments because the risk quotient (RQ) values calculated were largely greater than 1. However, all these studies have shown that only nano-TiO<sub>2</sub> (nTiO<sub>2</sub>) is the ENM of concern in the environment currently.

The predicted environmental concentration (PEC) values were calculated in this study on the aquatic and terrestrial environments for ENMs: nTiO<sub>2</sub>, nanosilver (nAg), carbon nanotubes (CNT), and fullerenes (nC<sub>60</sub>) under the minimum, probable and maximum scenarios using modelling techniques as these are among the most used nanoscale materials in numerous consumer nanoproducts and industrial applications. The PEC values were calculated under high and low efficiency regimes of wastewater treatment plants of the Gauteng Province in South Africa (SA) – where the probable scenario values were deemed as most realistic estimates. The reported toxicological data of these ENMs in published scientific literature were applied in evaluating the predicted no-effect concentrations (PNEC). Risk posed to both environmental media considered in this study was calculated as the ratio of PEC/PNEC for each ENM.

Findings from this study showed that the PEC<sub>water</sub> values of ENMs under high and low efficiency plants ranged from 5.22 x 10<sup>-7</sup> to 9.69 µg/L. The values were obtained when the dilution factor ( $D_k$ ) values were taken as 0.75, 1 or 3; given Gauteng is a water scarce region. The calculated PEC values in the terrestrial environment (PEC<sub>soil</sub>) were very low, ranging from 3.97 x 10<sup>-9</sup> to 3.31 x 10<sup>-2</sup> µg/kg. On predicting risks of ENMs to the aquatic environment, the RQ<sub>water</sub> values ranged from 5.58 x 10<sup>-8</sup> to 57.22 for high efficiency plants; whereas under low efficiency plants the RQ<sub>water</sub> values were found to be much higher ranging from 7.74 x 10<sup>-8</sup> to 96.93 under the minimum, probable and maximum scenarios. The RQ<sub>soil</sub> values for nAg, CNT and nC<sub>60</sub> were determined for the first time in this study, where nAg had the highest RQ value of 2.846 x 10<sup>-2</sup> at maximum scenario implying that the concentration of nAg in Gauteng are so low at present to raise any possible risk concerns.

This study estimated for the first time that nAg poses risk to the aquatic environment because the calculated  $RQ_{\text{water}}$  values were found  $\gg 1$  for probable and maximum scenarios (both under low and high efficiency regimes). This implies that nAg is of concern and measures towards reducing its potential risks in Gauteng merits consideration to avoid unintended long-term adverse effects. Moreover, this study calculated for the first time  $RQ_{\text{water}}$  values  $> 10$  for both nTiO<sub>2</sub> and nAg under the probable and maximum scenarios for both high and low efficiency plants. This means that these ENMs are of immediate concern to the aquatic environment and more data to validate the results is needed. And finally, it appears from these results that even countries such as South Africa which have limited fabrication and manufacturing capabilities of nanotechnology-based consumer products may be impacted by the ENMs from imported products. This calls for the consideration of ENMs risks at this early phase of nanotechnology application to avoid unintended long-term effects.

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## OPSOMMING

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Nanotegnologie-gebaseerde produkte, hierna verwys as nanoprodukte, het dramaties toegeneem op globale market oor die laaste aantal jare, as gevolg van die voordele wat dit inhou vir verbruikers en vervaardigers. Dit het gelei tot aansienlike nie-gekwantifiseerde vrylating van ingenieursnanomateriale (INMs) in verskillende omgewings, te wete water, lug en grond. Vorige outeurs wat die hoeveelhede INMs wat in die verskillende omgewings vrygelaat is, gekwantifiseer het, het so gedoen deur gebruik van modelle, a.g.v. die gebrek aan gemete data om die vrylatings te kwantifiseer. Na beraaming, plaas hierdie vrylatings verskeie omgewings op risiko, aangesien die risikokoëffisiënte wat met verskillende modelle bereken is, hoofsaaklik groter as 1 is. Desnieteenstaande, het al hierdie studies aangetoon dat slegs nano-titaandioksied ( $n\text{TiO}_2$ ) huidig 'n potensieel problematiese INM is wat die omgewing betref.

Voorspelde omgewingskonsentrasies (VOKs) is in die studie beraam vir akwatiese en terrestriële omgewings vir die volgende ENMs:  $n\text{TiO}_2$ , nanosilwer (nAg), koolstofnanobuis (CNT) en fullerene ( $n\text{C}_{60}$ ) onder die minimum, waarskynlike en maksimum scenarios, deur gebruik te maak van modelle, aangesien hierdie INMs onder die mees algemenes is wat in talle nanoprodukte en nywerheidstoepassings voorkom. Die VOKs is beraam onder hoë en lae doeltreffendheidsregimes op afvalwaterverwerkingsaanlegte in die Gauteng provinsie in Suid-Afrika – waar die waarskynlikste scenarios as die mees realistiese gesien kon word. Die toksikologiese data van die ENMs gerapporteer in die wetenskaplike literatuur is toegepas om die voorspelde geen-effek konsentrasies (VGEK) te evalueer. Die risiko vir beide omgewings wat in die studie oorweeg is, is bereken as die verhouding van VOK/VGEK vir elke INM.

Bevindinge van die studie het aangetoon dat die  $\text{VOK}_{\text{water}}$  waardes vir die hoë en lae doeltreffendheidsaanlegte in die bereik van  $5.22 \times 10^{-7}$  tot  $9.69 \mu\text{g/L}$  was, afhangende van die betrokke INM. Die waardes is bepaal met waardes vir die verdunningsfaktor van 0.75, 1 of 3, gegewe dat Gauteng gekenmerk word deur waterskaarste. Die berekende VOKs in die terrestriële omgewing ( $\text{VOK}_{\text{grond}}$ ) was baie laag en het gewissel van  $3.97 \times 10^{-9}$  tot  $3.31 \times 10^{-2} \mu\text{g/kg}$ , afhangende van die betrokke INM. Betreffende die voorspelling van risiko vir die akwatiese omgewings, het die risikokoëffisiënte vir water gewissel van  $5.58 \times 10^{-8}$  tot 57.22 vir hoëdoeltreffendheidsaanlegte. Vir lae doeltreffendheidsaanlegte was die risikokoëffisiënte hoër en het gewissel van  $7.74 \times 10^{-8}$  tot 96.93 onder die minimum, waarskynlike en maksimum scenarios. Die risikokoëffisiënte vir grond vir nAg, koolstofnanobuis en  $n\text{C}_{60}$  is vir die eerste keer bepaal in hierdie studie. nAg het die hoogste waarde van  $2.846 \times 10^{-2}$  gehad onder die

maksimum scenario, wat beteken dat die konsentrasie van nAg in Gauteng so laag is op die oomblik, dat dit nie beduidende risiko verteenwoordig nie.

Die studie het ook vir die eerste keer beraam dat nAg 'n risiko inhou vir die akwatiese omgewing, angesien die berekende risikokoëffisiënte baie groter was as een vir die waarskynlik en maksimum scenarios (beide onder hoë en lae doeltreffendheidsregimes). Dit beteken dat nAg van belang is en dat maatreëls om blootstelling daaraan in Gauteng te verminder, oorweging behoort te geniet, ten einde nadelige langtermyn-effekte daarvan te vermy. Verder is in die studie vir die eerste keer bereken dat die risikokoëffisiënte van nTiO<sub>2</sub> en nAg in water > 10 onder beide die waarskynlike en maksimum scenarios vir beide hoë en lae doeltreffendheidsaanlegte. Dit beteken die INM moet onmiddellik aandag geniet t.o.v. die akwatiese omgewing en meer data word benodig om die risiko te bevestig. Laastens wil dit ook voorkom asof lande soos Suid-Afrika, wat beperkte vervaardigings- en nywerheidskapasiteit t.o.v. nanomateriale het, wel geïmpakteer kan word deur INMs in ingevoerde produkte. Dit sou dus raadsaam wees om die risiko's rondom INMs reeds in hierdie vroeë fase te oorweeg, om onbedoelde langtermyn-effekte te vermy.

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## GLOSSARY

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AW – Artificial Wastewater

BSI – British Standards Institution

BTSE – Biologically Treated Sewage Effluent

Caltech – California Institute of Technology

*cf* – Correction Factor

CNT – Carbon Nanotubes

$D_k$  – Dilution Factor

DOC – Dissolved Organic Carbon

DST – Department of Science and Technology

DWCNT – Double Wall Carbon Nanotubes

EC<sub>50</sub> – Effective Concentration (50% casualties)

ECB – European Chemicals Bureau

ENM(s) – Engineered Nanomaterial(s)

ENP(s) – Engineered Nanoparticle(s)

GDP – Gross Domestic Product

GMO(s) – Genetically Modified Organism(s)

GP – Gauteng Province

HE – High Exposure (scenario)

ICP-OES – Inductively Coupled Plasma Optical Emission Spectroscopy

ISO – International Standardisation Organisation

JHB – Johannesburg city

LC<sub>50</sub> – Lethal Concentration (50% casualties)

LD<sub>10</sub> – Lethal Dosage (10% casualties)

LFS – Liquid Flame Spray

Max – Maximum (scenario)

Min – Minimum (scenario)

MWCNT – Multi Wall Carbon Nanotubes

nAg – nanoscale Silver

nC<sub>60</sub> – Fullerene at the nanoscale

NIS – National Initiative Strategy

NNI – National Nanotechnology Initiative

NOEC – No Observed Effect Concentration

NP – Nanoparticle

NPM – NanoParticulate Material

nTiO<sub>2</sub> – nanoscale Titanium Dioxide

OECD – Organisation for Economic Co-operation and Development

PEC – Predicted Environmental Concentration

PEN – Project on Emerging Nanotechnologies

PGPR – Plant Growth Promoting Rhizobacteria

PNEC – Predicted No Effect Concentration

POP – Population

Prob – Probable (scenario)

R & D – Research and Development

RE – Realistic Exposure (scenario)

REACH – Registration Evaluation Authorisation and restriction of Chemicals

ROS – Reactive Oxygen Species

RQ – Risk Quotient

RS and RAE – Royal Society and the Royal Academy of Engineering

SA – South Africa

SDS – Sodium Dodecyl Sulphate

SW – Sea Water

SW – Switzerland

SWCNT – Single Wall Carbon Nanotubes

THF – Tetrahydrofuran

UK – United Kingdom

WWTP – Wastewater Treatment Plant

WWTW – Wastewater Treatment Works

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# Chapter 1 - INTRODUCTION

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## 1.1 GENERAL BACKGROUND

The repetitive examples of past technologies that left a legacy of damage under their names have awakened nanotechnology to take precautionary measures for safe use, prior to its wide distribution, so as to achieve the full potential and sustainability. Any new technology, despite the benefits, has potential risks that are unknown at inception - nanotechnology is no exception. In the recent past, there has been general consensus on the usage of several proposed definitions to describe various processes of the nanotechnology industry. The theoretical capability to build things from bottom up with atomic precision was envisioned by the renowned physicist Richard Feynman, back in 1959. This was during his talk at the annual meeting of the American Physical Society at the California Institute of Technology (Caltech), though the term nanotechnology was not explicitly mentioned then (Feynman, 1960).

The term 'nanotechnology' was then coined in 1974, by a Professor from the Tokyo Science University when describing the semiconductor processes in the order of nanometre: he described nanotechnology to mainly consist of the processing of separation, consolidation and deformation of materials by one atom or one molecule (Taniguchi, 1983). Ultimately, during the eighties and nineties, researchers like Eric Drexler promoted the technological significance of the nanoscale phenomena through publishing books; 'Engines of Creations: The Coming era of nanotechnology,' in 1986, and the 'Nanosystems: Molecular Machinery, Manufacturing and Computation' was also published thereafter (Drexler, 1991).

Recently, nanotechnology has been defined by the Royal Society and the Royal Academy of Engineering: as the design, characterisation, production and application of structures, devices and systems by controlling shape and size at the nanometre level (RS and RAE, 2004). Definition by the United States National Nanotechnology Initiative (NNI) is the most cited, and it defines nanotechnology as the understanding and control of matter at dimensions of 1 to 100 nm where unique phenomena enable novel applications (NNI, 2008). However, nanotechnology is often described as a cross disciplinary technology since it covers a wide range of scientific fields including physics, chemistry, biology, materials science and electronics.

Concurrently, nanoscience has been defined as the study of phenomena and manipulation of materials at atomic, molecular and macromolecular scales, where properties differ significantly

from those at a larger scale (RS and RAE, 2004). Nanoparticles are defined as particles with sizes between about 1 and 100 nm, which exhibit properties that are not found in bulk samples of the same material, whereas for nanomaterials; the material should have some structural characteristics of at least one dimension in the approximate range of 1-100 nm, and the nanostructure must give system properties differing from the bulk properties (RS and RAE, 2004).

Recently, the European Network on the Health and Environmental Impacts of Nanomaterials (NanoImpactNet - NIN) has published the first version of International Standardisation Organisation (ISO) approved nomenclature for definition of terms in the field of nanotechnology (Clift et al., 2009). To provide consistency and clarity; the Organisation for Economic Co-operation and Development (OECD), International Standards Organisation (ISO), the British Standards Institution (BSI), and the Registration Evaluation Authorisation and Restriction of Chemicals (REACH) have approved definitions on nanotechnology nomenclature, as listed in Appendix 1.

Nanotechnology has evolved into four generations of development: namely; passive nanostructures, active nanostructures, systems of nanosystems and molecular nanosystems, as illustrated in Figure 1.1 (CRN, 2009; Roco, 2004).

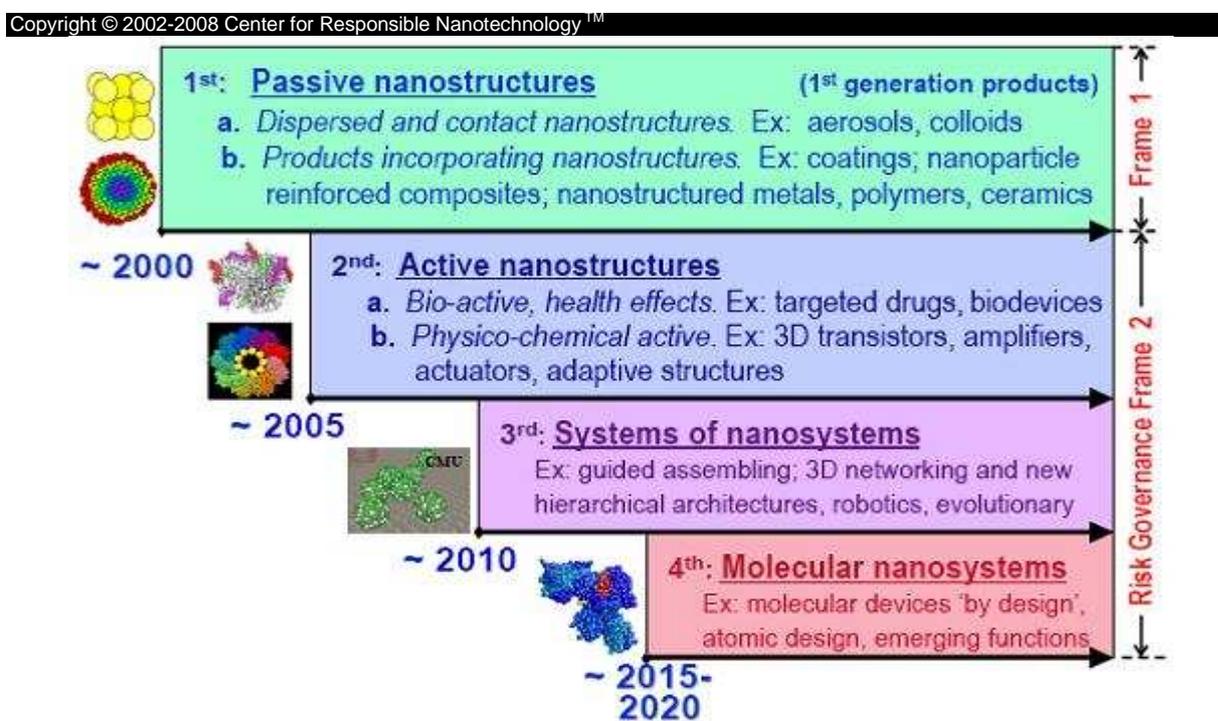


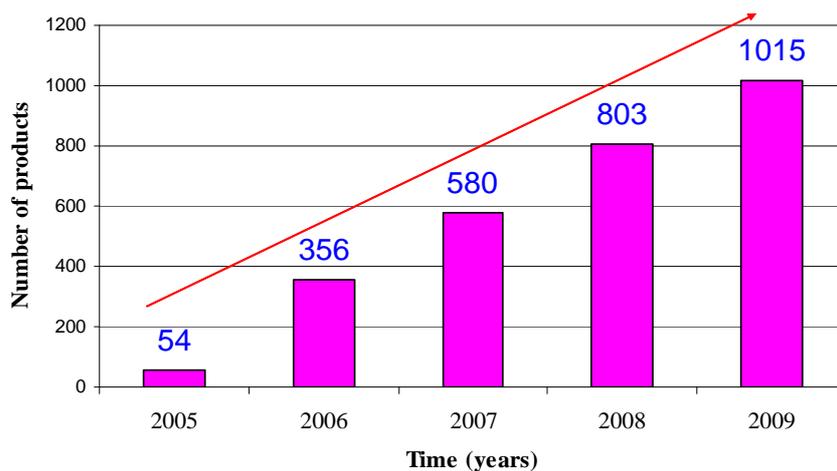
Figure 1.1: Four generations of nanotechnology development (CRN, 2009).

Ex: example

Essentially, nanotechnology forms the basis for most technological innovations in the 21<sup>st</sup> century (Meyer, 2007). Surfaces and interfaces of particles are particularly important components of nanoscale materials because, as the particle size decreases, the proportion of atoms found at the surface is magnified relative to the proportion inside its volume (Warheit et al., 2008). This results in products and systems that are more reactive, and thus generating much effective catalysts in a variety of applications. For instance, new medical treatments are emerging for treating fatal diseases such as Tuberculosis (TB), Human Immune Virus (HIV), brain tumours, and Alzheimer's disease (Gelperina et al., 2005; Haes et al., 2005; McCarthy et al., 2005; Amiji et al., 2006; Swai, 2006; Ahmad et al., 2007; Nazem, and Mansoori, 2008; Swai et al., 2009).

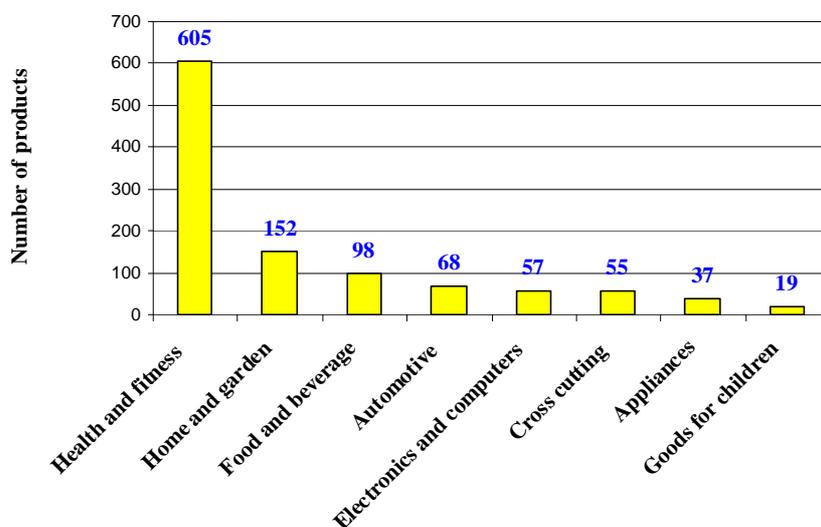
Nanotechnology is already playing an important role in the development of new methods for water purification and environmental remediation, using novel nanomaterials (Sun et al., 2004; Theron et al., 2008). Computers are built with nanoscale components that improve their performance, yet shrinking the dimensions further (Tejada et al., 2001; Dwyer et al., 2004; Horcas et al., 2007). Finally, electricity generation is expected to improve through the development of efficient light sources and better performing batteries (Poizot et al., 2000; Taberna et al., 2006; Chan et al., 2007).

In addition, nanotechnology-based consumer products (referred hereafter as nanoproducts) are already in the market globally, even though industries still have difficulties in claiming 'nano' because of limitations in regulations (Maynard, 2006). The Project on Emerging Nanotechnologies (PEN) gives an inventory of company declared nanoproducts that are commercially available in the market. This inventory is not comprehensive, since it depends on companies submitting their product profiles; however, it gives insights on the rapid growth of manufacturer identified nanoproducts from 2005 as shown in Figure 1.2 (PEN, 2010).

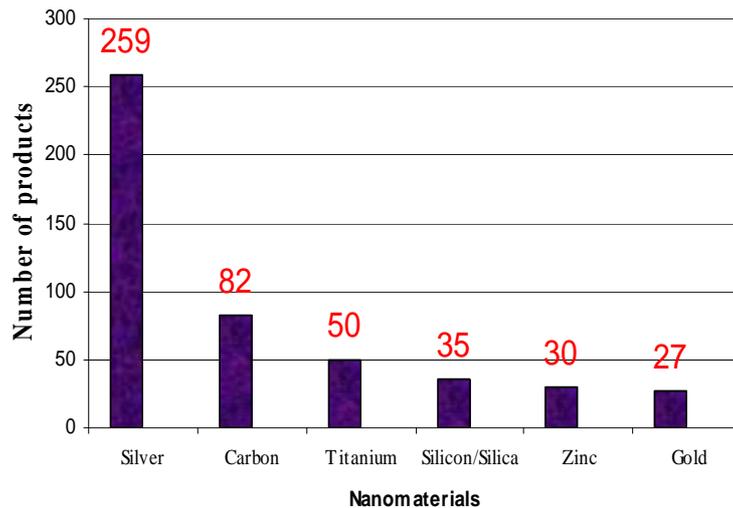


**Figure 1.2: Number of nanoproducts modified from the PEN inventory (PEN, 2010).**

Nanoproducts listed in the inventory are further distributed per category, based on the publicly available consumer product classification systems (PEN, 2010) as illustrated in Figure 1.3. Each category is associated with a number of sub-categories that allow for further organisation of products, though some products have relevance to multiple categories – these are accounted for in each category of relevance. The health and fitness category has the largest number of products, and its sub-categories include cosmetics, clothing, personal care, sporting goods, sunscreens and filtration products (PEN, 2010). The most significant materials (referred hereafter as engineered nanomaterials – ENMs), associated with nanoproducts in the PEN inventory are shown in Figure 1.4. ENMs of carbon constitute carbon nanotubes, fullerenes, carbon black, amongst others.

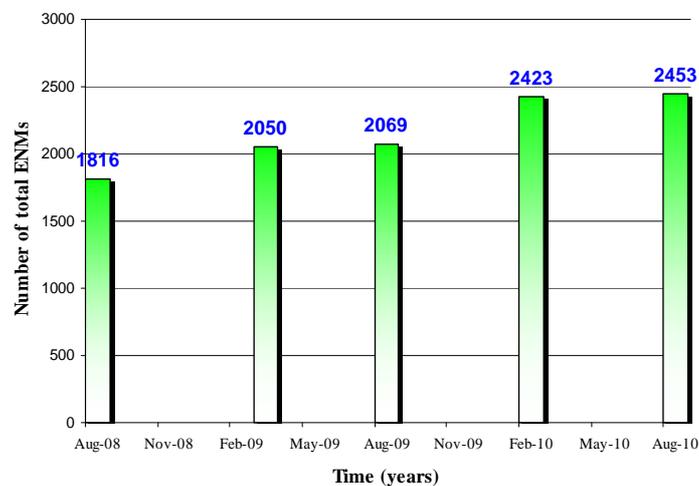


**Figure 1.3: Number of nanoproducts per category (PEN, 2010).**



**Figure 1.4: Number of nanoproducts associated with specific materials (PEN, 2010).**

Concurrently, the Nanowerk database contained 2092 nanotechnology commercial organisations, 1875 research communities, and over 2400 ENMs in nine different categories (Nanowerk, 2010). The trend in ENMs increase on the Nanowerk inventory from August 2008 is illustrated in Figure 1.5. Both inventories suggest a significant global increase of ENMs use and applications under different forms of commercial nanoproducts.



**Figure 1.5: ENMs growth trend in the Nanowerk inventory (Nanowerk, 2010).**

The increasing numbers of nanoproducts and ENMs in the market clearly revokes the need for undertaking their risk assessment, in order to safeguard human health and protect potential environmental harm. With all the benefits that nanotechnology offers, the introduction of ENMs to various environmental ecosystems is set to inevitably increase as the number of applications

and nanoproducts increases. When considering the potential health implications, the most reactive groups are likely to influence the biological (potentially toxicological) effects on surfaces of particles when compared to nonreactive surfaces or surface coatings (Warheit et al., 2008).

A report on economic research in New York predicted the annual turnover of goods, based on nanotechnologies, to exceed US\$ 2.5 trillion by 2014 (Lux Research, 2004). The research further projected that 4% of general manufactured goods, 50% of electronics and IT products, and 16% of goods in healthcare and life sciences, by revenue, will incorporate emerging nanotechnology (Lux Research, 2004).

As the field of nanotechnology undergoes a dramatic expansion, most research efforts have focused on the synthesis and characterisation of ENMs geared towards real world applications. However, research on the potential health and environmental effects is still at an infancy phase. It remains to be seen how these ENMs will be chemically characterised from the view point of reactivity and bioavailability (Handy and Shaw, 2007). Yet, the fate of ENMs in the environment, whether through intentional or unintentional releases poses human and environmental health concerns. Nonetheless, little is currently known about the pathways of ENMs in the environment and their resultant toxicity from environmental exposures (O'Brien and Cummins, 2008).

ENMs of particular and immediate concern to the public and environmental health have yet to be fully identified, since assumptions cannot be made regarding toxicity of the same materials at larger macro scale (Maynard, 2006). This is because of the alterations encountered as the physical and chemical properties, such as particle size, decrease to the nanoscale range. Hence, there are current growing concerns on the health and safety of ENMs as a result of potential exposures to both consumer and the environment (Helland et al., 2007). The currently identified sources and pathways of ENMs (O'Brien and Cummins, 2008) include:

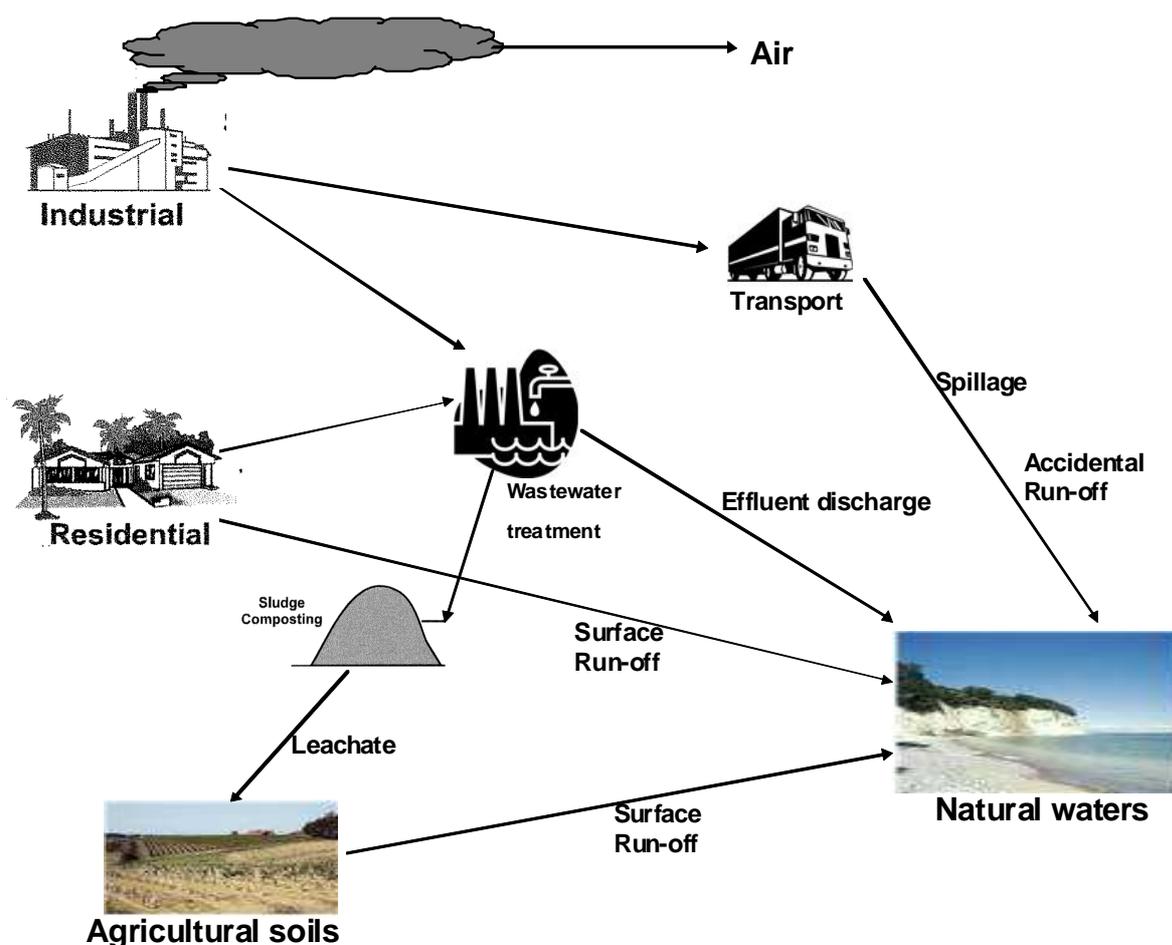
- Occupational exposure in the workplace;
- Exposure from “unintentional” environmental releases, for example, industrial and domestic waste streams – generally regarded as nanowastes (Musee, 2010a);
- Exposure from consumer products, such as cosmetics; and
- Exposure from medical products, including drugs, treatments, and devices.

The exposure to ENMs because of the widespread use of nanoproducts can be considered as the most challenging. This is because a large population is likely to be exposed as opposed to fewer numbers under the occupational settings. The workplace exposure can be managed effectively,

for example, by applying stringent house-keeping rules and use of engineering controls. Yet, this is unlikely for the release of ENMs into the environment. Figure 1.6 illustrates the possible pathways of ENM releases into the aquatic and terrestrial environmental media.

Consequently, the assessment of potential health risks from exposures to ENMs is an emerging research area in the field of toxicology (Oberdorster et al., 2005). The developments of toxicity data sets, as well as methodologies for facilitating exposure assessments for various nanoparticle-types, are emerging as new ENMs are developed (Warheit et al., 2008).

As such, there is lack of data on the quantities of ENMs that have already been released into different environmental media. This limits the ability to assess the potential risks of such nanoscale materials in the environment. Therefore, in this research, modelling techniques are applied to estimate the quantities of different types of ENMs into the environment and the consequent potential risks they pose to aquatic and terrestrial environments.



**Figure 1.6: Schematic illustration of potential ENM pathways into the environment**

## 1.2 STUDY MOTIVATION

As nanotechnology develops into full large-scale commercial production, globally, the introduction of ENMs into environmental media is inevitably increasing. Since this is an emerging technology, at present, there is lack of metrology to detect and quantify the behaviour of these ENMs in various ecosystems (Lead and Wilkinson, 2006; Maynard, 2006). Thus, the concentrations of ENMs in the environment are unknown, and therefore, one way of quantifying the expected environmental concentrations is by use of modelling techniques (Muller, 2007; Muller and Nowack, 2008; Musee, 2010b), as applied in this study.

Also, many research centres and study institutions in South Africa are currently conducting studies, mainly to determine the potential positive impacts of ENMs in enhancing human life (Pouris, 2007). However, studies on the potential safety and environmental effects of these ENMs are not well understood (Musee et al., 2010c). Hence, this study seeks to address part of this knowledge gap.

Moreover, companies on a global scale are introducing nanoproducts into the market, to enhance their profit, though their risks have not been well investigated and defined (Helland et al., 2007). Clearly, these practices indicate that ENMs are uncontrollably being released into the public market, and eventually into the environment before their potential harmful effects have been thoroughly investigated (Musee, 2010a).

The scientific community and policy makers are keen to avoid a confrontation scenario that developed in the recent past – concerning genetically modified organisms (GMOs): in which public boycott and lack of confidence on the technology's products partly stemmed, as a result of the scientific community's failure to communicate risk issues at early stages of the technology's development (Handy and Shaw, 2007). Thus, the inadequacy of past approaches to chemicals and technology management has been a lesson to develop preliminary risk evaluations for any new materials to promote safe, responsible and sustainable technology development (Handy et al., 2008; Hansen et al, 2008).

As a precautionary, research towards risk assessment of nanotechnology has demonstrated that, it is instructive to characterize risk in a particular context because of the complexity and lack of experimental data on most ENMs (Hansen, 2009). Thus, due to lack of nano-specific regulation and official Best Practices in the nanotechnology industry, companies must self regulate and forge best practices in order to gain consumer confidence, and pre-empt harsher governmental

regulation (O'Brien and Cummins, 2008). It is for this reason that a nano-risk framework, that has a potential for wide use by companies and other organisations, has been developed to establish a process of ensuring responsible development of ENMs (DuPont, 2007). This framework seeks to promote responsible production, application, and end-of-life disposal or recycling of ENMs throughout their life cycle (DuPont, 2007).

Similarly, the South African Government, through Department of Science and Technology (DST), initiated the Nanotechnology Initiative Strategy (NIS) in its effort to develop a sustainable technology (DST, 2007). The strategy includes responsible development of nanotechnology, by incorporating health and safety concerns whilst the technology is in its initial phase of research and development as well as the introduction of nanoproducts into the market. Meanwhile, a study on nanoscale research in South Africa concluded that nanotechnology activities are mainly at research level in the country (Pouris, 2007).

A noted aspect for the South African nanotechnology's development is the lack of reported data on risk assessment of ENMs in ecological systems. Hence, this study seeks to address part of this knowledge gap through use of modelling tools in quantifying the risk levels as a result of releases of ENMs into the South African environment. The study focused in evaluating the risk profiles of nanoTitanium Dioxide ( $n\text{TiO}_2$ ), nanoSilver ( $n\text{Ag}$ ), Carbon Nanotubes (CNT) and Fullerenes ( $\text{C}_{60}$ ) into the aquatic and terrestrial environments. The geographical region considered was the Gauteng Province.

### **1.3 STUDY OBJECTIVES AND THESIS OUTLINE**

The study has mainly two-fold objectives, these include:

- (i) To estimate the environmental concentrations of  $n\text{TiO}_2$ ,  $n\text{Ag}$ , CNT and  $\text{C}_{60}$  in aquatic and terrestrial environments of the Gauteng Province, South Africa; and
- (ii) To estimate the potential risks of these ENMs in both environmental media through risk characterisation.

Computational methods to estimate the abundance of ENMs in the environment have been utilised elsewhere in the literature (Blaser et al., 2008; Muller and Nowack, 2008; Gottschalk et al., 2009a and 2009b; Musee, 2010b). In addition, a number of toxicity studies on ENMs in ecological organisms have been reported (Oberdorster et al., 2006; Yoon et al., 2007; Baun et al., 2008; Hongcheng Li et al., 2008; Aruoja et al. 2009; Cattaneo et al., 2009; Kahru and

Dubourguier, 2009; Gladisa et al., 2010). In chapter 2, ecotoxicity studies of ENMs of nTiO<sub>2</sub>, nAg, CNT and C<sub>60</sub> are reviewed to provide basis for the evaluation of predicted no-effect concentrations (PNEC). Also, computational methods for estimating ENMs in the environment are discussed. Chapter 3 will provide a summary of the methodology used for estimating ENM quantities and concentrations in the aquatic and terrestrial environments of Gauteng Province. Results will be analysed and discussed in Chapter 4, and Chapter 5 provides a summary of the main findings for this study.

## 1.4 DELINEATIONS AND LIMITATIONS

This study focused on ENMs since naturally occurring nanoparticles in the environment exist in small quantities (Muller, 2007). Specifically, ENMs released ‘unintentionally’ to the environment through industrial and domestic wastewater streams will be examined. Furthermore, the modelling risk tools will be applied for the aquatic and terrestrial environments as these are the most significant potential final sinks of ENMs after use as well as via incidental releases.

Research will be conducted and limited on the following ENMs: nTiO<sub>2</sub>, nAg, CNT and nC<sub>60</sub>, because there is available data on their global production volumes (Muller, 2007; Muller and Nowack, 2008; Gottschalk et al. 2009a) which can be extrapolated to the South African scenario. Also, there is no accessible data from the South African industries which utilise nanotechnology. Moreover, the selected ENMs are amongst the most abundant materials in reported nanoproducts and nanomaterials inventories (PEN and Nanowerk inventories), and have a wide variety of applications in most industries operating internationally and in South Africa as well. Therefore, they are likely to be found in substantial quantities in South African environmental systems. Products and goods in South Africa that incorporate ENMs of nTiO<sub>2</sub>, nAg, CNT, and C<sub>60</sub> include: cosmetics, plastics, textiles, paints, coatings and cleaning agents, metal products, consumer electronics, as well as in research and development, and composites. Many companies that manufacture these products operate globally; hence, they are likely to introduce the same products in South Africa with the same ENM concentrations as of the products traded elsewhere worldwide.

The model is limited to the estimated published data on worldwide ENM production volumes (model input parameter), because there are few companies that have declared the use and amounts to date (Muller and Nowack, 2008; Schmid and Riediker, 2008). There is high uncertainty on the published values, thus, the range of available data is very broad. Gottschalk describes uncertainty of knowledge as ‘the measure of knowledge incompleteness about a

quantity whose true value could be measured if suitable measuring methods were available' (Gottschalk et. al., 2009a).

The modelled predicted no effect concentration (PNEC) values are derived from published ecotoxicological data on experimental results for ecological organisms – though these values are yet to be universally standardised. The biodegradation and chemical transformation of ENMs during their life-cycle stages is not considered in the model because the mechanisms which account for biodegradation of ENPs are still not fully understood (Hristozov and Malsch, 2009).

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## Chapter 2 - **LITERATURE REVIEW**

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The toxicity of ENMs in various environmental ecosystems has been scientifically reported in the recent past (Hund-Rinke and Simon, 2006; Navarro et al., 2008; Scott-Fordsmand et al., 2008; Zhu et al., 2009; Kwok et al., 2010). The reported toxicological effects of some ENMs have been shown to be dose-response dependent in different organs of living organisms (Adams et al., 2006; Lovern and Klaper, 2006; Lapied et al., 2010). Also, the environmental exposure to ENMs has been estimated, however, because of metrology limitations, the development of models to quantify the potential exposure levels and thereby estimate the risk posed to various environmental media has been proposed as an alternative approach (Muller and Nowack, 2007; Gottschalck et al., 2009a; Musee, 2010a). The ecotoxicology and developed modelling studies for ENMs in the aquatic and terrestrial environmental media are the focus of this study and are briefly reviewed in the following sections.

### **2.1 ECOTOXICITY OF ENGINEERED NANOMATERIALS**

The physicochemical properties of ENMs have been found to provide surfaces that may bind and transport co-existing toxic chemical pollutants in the environment, as well as being toxic on their own (Farre et al., 2009). These interactions may cause toxicity of specific chemicals towards aquatic invertebrates (Baun et al., 2008). In the aquatic environment, the stability of ENMs plays a vital role in determining their environmental implication and potential health risks (Zhang et al., 2008). It is these special properties that make ENMs useful, but may also cause toxic effects to humans and the environment, under certain conditions.

An examination of industrial manufacture and application of nanocomponents revealed that: up to 95% of ENMs used in cosmetics, paints, coatings and cleaners are most likely to end up in water treatment plants (Meyer et al., 2009). If these materials are not removed during waste treatment (like wastewater treatment), they will ultimately be released into the environment where they can undergo bioaccumulation (Luo, 2007). This potential exposure is the impetus for the great concern regarding ENMs ecotoxicity.

The major identified sources and pathways of ENMs to the environment are production facilities and processes, wastewater treatment plants, and accidents during transport (Batley and

McLaughlin, 2010). ENMs may form aggregates when in the environment; which are less mobile and can be eliminated through sedimentation or can undergo uptake by sediment-dwelling organisms, resulting in possible biomagnification in the food chain (Farre et al., 2009).

Ecotoxicological studies of ENMs are currently increasing, with more focus on aquatic organisms (Hund-Rinke and Simon, 2006; Lovern and Klaper, 2006; Smith et al., 2007; Blaise et al., 2008; Cattaneo et al., 2009; Zhu et al., 2010). Yet, there are limited similar studies for the terrestrial environment (Jemec et al., 2008; Wang et al., 2009; Hu et al., 2010; Musee et al., 2010d).

Guidance from the European Chemicals Bureau (ECB) document recommends the use of long-term NOEC (no observed effect concentration) values from peer-reviewed literature as the reliable toxicity values to be used during effects assessments of new substances. In cases where there are no NOEC values reported, the short-term L(E)C50 or EC50 (lowest effect concentration), LC10 or EC10 and LD10 (lethal or effective concentration) values can also be used (ECB, 2003). NOEC is defined as the highest concentration tested at which the measured parameter shows no significant inhibition; and L(E)C50 and EC50 in a survival experiment indicate the concentration where 50 % of the test animals would die in that experiment; while LC10, EC10 and LD10 indicate concentrations where 10 % of the test organisms would die (ECB, 2003). A literature search done for the toxicity end-point parameters on ENMs of focus in this study is covered in the following sub-section.

### 2.1.1 Titanium Dioxide (nTiO<sub>2</sub>)

An earlier study tested nTiO<sub>2</sub> photocatalytic activity effects on Algae and Daphnids, using standardized test systems originally developed for the characterization of chemicals (Hund-Rinke and Simon, 2006). Suspensions of two different sized nTiO<sub>2</sub> (25 nm and 100 nm) were illuminated and tested for growth inhibition on green alga (*Desmodesmus subspicatus*) and *Daphnia magna*. Results showed a NOEC value of < 12.5 mg/l on algae during potential shading (Hund-Rinke and Simon, 2006).

Filtered and sonicated nTiO<sub>2</sub> solutions were prepared and tested on *D. magna* to detect the mortality rates (Lovern and Klaper, 2006). The filtered solution was prepared with tetrahydrofuran (THF) and sonicated solutions were varied from 100-500 nTiO<sub>2</sub> in a 48hour immobilisation test. A dose-dependent increase in mortality of *D. magna* with the NOEC value of 1 ppm (1 mg/l) was observed for the filtered nTiO<sub>2</sub> (Lovern and Klaper, 2006). Adams et al (2006) investigated the ecotoxicity of nTiO<sub>2</sub> on bacterial activity using gram-positive *Bacillus*

*subtilis* and gram-negative *Escherichia coli*. Results from the study indicated a dose-dependent increased antibacterial activity with the presence of light, due to the generation of reactive oxygen species (ROS) (Adams et al., 2006).

The effects of diluted nTiO<sub>2</sub> have been tested on bacteria, algae, crustaceans and soil enzymes. Velzeboer et al. (2008) tested the ecotoxic effects of nTiO<sub>2</sub> when diluted in ultrapure water (milli-Q) and in natural water (pond) on *Vibrio fischeri*, *Pseudokirchneriella subcapitata* and *Chydorus sphaericus* species. The dilution of nanoparticle suspensions led to the formation of larger particles, and no appreciable effects were observed at nominal concentrations of up to 100 mg/l (Velzeboer et al., 2008). The study concluded that the absence of ecotoxicity was the result of low concentrations of free nanoparticles in the tests, and suggested that colloid instability is of primary importance in explaining ecotoxic effects of nanoparticles in the natural environment (Velzeboer et al., 2008).

Ecotoxicity of nTiO<sub>2</sub> (prepared from flocculation of artificial wastewater, biologically treated sewage effluent and sea water using TiCl<sub>4</sub> as a coagulant), was tested on *D. magna* and *V. fischeri* (Lee et al., 2009). The nTiO<sub>2</sub> that was produced from artificial wastewater (AW), biologically treated sewage effluent (BTSE) and sea water (SW) flocculation was found to have low toxicity, with a NOEC value of 80mg/l (Lee et al., 2009). However, the nTiO<sub>2</sub> was more toxic when filtered with a 0.22µm filter and a NOEC of 1mg/l was observed (Lee et al., 2009).

Potential shading of light was also tested if it affects the toxicity of nanoparticles in algal growth, on *P.subcapitata* (Aruoja et al, 2009). The results showed that the shading effect by nanoparticles was negligible. Meanwhile, the bulk TiO<sub>2</sub> was found less toxic than nTiO<sub>2</sub> as EC50 values of 35.9 mg Ti/L and 5.83 mg Ti/L were derived, respectively; whereas, the NOEC value for nTiO<sub>2</sub> was 0.98 mg Ti/L (Aruoja et al., 2009).

The ecotoxicity tests were also done in water suspensions of nTiO<sub>2</sub> on *Daphnia*, and results showed that nanoparticles were more toxic than bulk materials with EC50 value of 35.306 mg/L and LC50 of 143.387 mg/L nTiO<sub>2</sub> (Zhu et al., 2009). The accumulation analysis of nTiO<sub>2</sub> was also done for acute (72 hours) and chronic (21 days) tests on *D. magna*, and the results showed an increased mortality rate due to high bioaccumulation with a NOEC value of < 0.1 mg/L after 72 hour exposure (Zhu et al. 2010).

In the terrestrial environment, short-term dietary exposures of terrestrial isopods (*Porcellio scaber*) were studied after nTiO<sub>2</sub> ingestion (Jemec et al., 2008). Results after 3 days showed that

the activities of antioxidant enzymes in digestive glands were affected in a dose-independent manner, but higher-level endpoints like the weight change, feeding rate, food assimilation efficiency and survival were not affected (Jemec et al., 2008).

**Table 2.1: Summary of the ecotoxicity end-points for nTiO<sub>2</sub> in aquatic and terrestrial ecosystems**

Exposure method	Media	Test organism	Toxicity value	Source
48 hr immobilisation, filtered with THF	Water	<i>D. magna</i>	NOEC = 1 ppm ~ 1 mg/L	Lovern and Klaper (2006)
Illuminated under potential shading		<i>D. subspicatus</i> <i>D. magna</i>	NOEC < 12.5 mg/L EC <sub>50</sub> = 14 mg/L	Hund-Rinke and Simon (2006)
Dilution in ultrapure (milli-Q) water or in natural (pond) water		<i>V. fischeri</i> <i>P. subcapitata</i> <i>C. sphaericus</i>	EC <sub>50</sub> > 100 mg/L EC <sub>50</sub> > 100 mg/L LC <sub>50</sub> > 100 mg/L	Velzeboer et al. (2008)
Flocculation Filtered with 0.22µm filter		<i>D. magna</i>	NOEC = 80 mg/L NOEC = 1 mg/L	Lee et al. (2009)
Potential shading of light		<i>P. subcapitata</i>	NOEC = 0.98 mg Ti/L EC <sub>50</sub> = 5.83 mg Ti/L	Aruoja et al. (2009)
In water suspensions		<i>D. magna</i>	EC <sub>50</sub> = 35.31 mg/L LC <sub>50</sub> = 143.387 mg/L	Zhu et al. (2009)
48hr in water suspension. 72hr water suspension		<i>D. magna</i> <i>D. magna</i>	NOEC < 50 mg/L NOEC < 0.1 mg/L EC <sub>50</sub> = 1.62 mg/L LC <sub>50</sub> = 2.02 mg/L	Zhu et al. (2010)
14day dietary exposure on catalase and glutathione-S-transferase	Soil	<i>P. scaber</i>	NOEC > 1000 µg/g	Drobne et al. (2009)
With <i>E. coli</i> as food		<i>C. elegans</i>	LC <sub>50</sub> = 80 mg/L	Wang et al. (2009)

On testing the lethality for nTiO<sub>2</sub> in the nematode *Caenorhabditis elegans*, no measurable effects at lower doses of 10-50 mg/L were detected; whereas, at higher levels of 100-250 mg/L, significant effects were observed – resulting in LC<sub>50</sub> values of 80 mg/L (Wang et al., 2009). Similar observations, of no effects at low dosages of nTiO<sub>2</sub>, were observed when evaluating

toxicities to earthworm *Eisenia fetida* (Hu et al., 2010). The same consistency, of no effects in earthworms was observed in acute filter paper tests (Beibei Qi, 2009).

Drobne et al. (2009) tested the effects of nTiO<sub>2</sub> on terrestrial arthropod (*Porcellio scaber*) over 14 days and the observations gave a NOEC value for soil. The intensity of the response was dependent on the duration of exposure and not the consumed quantity of nTiO<sub>2</sub> or the exposure concentration, as observed in previous tests (Jemec et al., 2008; Beibei Qi, 2009; Hu et al., 2010) on other terrestrial organisms. The response was described as threshold-like, and the results yielded an observed NOEC value of >1000 µg/g (Drobne et al., 2009). Table 2.1 summarises the observed toxicity end-points for nTiO<sub>2</sub> tests in both aquatic and terrestrial ecosystems.

### 2.1.2 Silver (nAg)

Silver nanoparticles have been studied for their antimicrobial properties and applications in medical devices and water treatment filters, however, the susceptibility of bacterial membranes lead to cell death (Yoon et al., 2007). This is the impetus for the great concern in ecotoxicity of nAg.

A study on nAg ecotoxicity showed a concentration-dependent mortality increase and hatching delay in embryos of zebrafish (*Danio rerio*) (Asharani et al., 2008). The LC<sub>50</sub> for the embryos was dependent on the growth stage of the embryos exposed to nanoparticle treatment, and varied from 25–50 µg/ml (Asharani et al., 2008). Acute toxicity of nAg and ionic silver (Ag<sup>+</sup>) was examined in the photosynthesis of *Chlamydomonas reinhardtii* using fluorescence (Navarro et al., 2008). The EC<sub>50</sub> values of nAg (as a function of total Ag) ranged from 3300 nM after 1 hour to 829 nM after 5 hours; whereas, with nAg as a function of free Ag<sup>+</sup>, the EC<sub>50</sub> values ranged from 33 nM after 1 hour to 8 nM after 5 hours; meanwhile, when 5 µM nAg was mixed with cysteine, the recorded EC<sub>50</sub> value was 57 nM after 1 hour (Navarro et al., 2008).

The environmental impact of nAg was investigated by studying changes in the expression of stress-related genes on Japanese medaka (*Oryzias latipes*) and compared with medaka exposed to soluble silver ions, to elucidate if the observed toxicity was due to dissolved metal species. Results suggested that nAg led to cellular and DNA damage, carcinogenic and oxidative stresses, genes related with metal detoxification/metabolism regulation and radical scavenging action were also induced. On the other hand, the ionic silver led to the induction of inflammatory response and metallic detoxification processes in the liver of the exposed fish, but resulted to lower overall stress response when compared with the nAg (Chae et al., 2009). The LC<sub>50</sub> values

for nAg were  $34.6 \pm 0.9 \mu\text{g/L}$  and  $36.5 \pm 1.8 \mu\text{g/L}$  for  $\text{AgNO}_3$ , after a 96 hour exposure time (Chae et al., 2009).

The embryos of *Pimephales promelas* were exposed to the commercially available nAg products (NanoAmor and Sigma) under varying concentrations of either sonicated or stirred solutions for 96 hours (Laban et al., 2010). Dissolved silver released from nAg had effects that were found to be three times less toxic when compared to  $\text{AgNO}_3$  and the percentage of dissolved Ag released was inversely proportional to the concentration of nAg, while the percentage release was similar regardless if concentrations were stirred or sonicated (Laban et al., 2010). The LC50 values for NanoAmor and Sigma nAg were 9.4 and 10.6 mg/L for stirred as well as 1.25 and 1.36 mg/L for sonicated ENPs, respectively (Laban et al., 2010).

A recent ecotoxicity study using *Daphnia magna* as test organisms showed that the toxicity was caused by  $\text{Ag}^+$  ions and not the nAg (Kim et al., 2010). In the study,  $\text{Ag}^+$  was adsorbed onto the synthesized sorbents packed in a column and *D. magna* was exposed to the column-passed-300 nm nAg suspensions, and there was no acute toxicity with the nAg suspensions which did not have  $\text{Ag}^+$  ions (Kim et al., 2010). The observed EC50 values after 96 hour exposure were 1.0  $\mu\text{g Ag/L}$  for the 60 nm nAg suspension and 1.4  $\mu\text{g Ag/L}$  for the 300 nm suspension (Kim et al., 2010).

The potential for the engineered nanoparticles to form reactive oxygen species (ROS) was tested using a set of recombinant luminescent bacterial strains (*Escherichia coli*), and the role of solubilised metal ions was evaluated in an ecotoxicity profiling study. The metal sensor bacteria showed that ROS formation in the tested metals was due to metal ions, whereas in the case of silver, the nAg also had an effect (Ivask et al., 2010). Toxicity tests after 2 hour exposure showed the EC50 values of 46 mg Ag/L and 3.1 mg Ag/L for nAg toxicity to the triple sod mutant than the wild type bacteria, respectively (Ivask et al., 2010).

Cytotoxicity of nAg was assessed on rainbow trout (*Oncorhynchus mykiss*) hepatocytes, with and without dissolved organic carbon (DOC) as well as  $\text{AgNO}_3$  as the ionic solution. Results showed that nAg caused a significant reduction in membrane integrity and cellular metabolic activity in a concentration-dependent manner, and the addition of DOC did not alter the nanoparticles potency of cytotoxicity or ROS induction capacity (Farkas et al., 2010). The EC50 values calculated from a regression curve were in terms of inhibition of cellular metabolic activity 2.5 mg/L, 1.1 mg/L and 2.6 mg/L for nAg,  $\text{Ag}^+$  and nAg with DOC, respectively. When using

membrane stability as an endpoint the EC50 values were determined as 4.9 mg/L, 13.9 mg/L and 3.5 mg/L for nAg, Ag<sup>+</sup> and nAg with DOC, respectively (Farkas et al., 2010).

In the terrestrial environment, only a few ecotoxicological studies have been published for nAg. The positive or negative potential impacts of metal nanoparticles (including nAg) have been evaluated in a study done on plant growth-promoting rhizobacteria (PGPR). Results from the study suggested that metal nanoparticles could significantly produce ecotoxicity and killing of phytostimulatory soil bacteria – like the PGPR – that are responsible for maintaining soil health, ecosystem functions and crop productivity (Mishra and Kumar, 2010).

Another study investigated the ecotoxicity of nAg on the soil nematode (*Caenorhabditis elegans*) using survival, growth, and reproduction as toxicity endpoints as well as stress response gene expression (Roh et al., 2009). nAg exerted considerable toxicity in *C. elegans*, as the reproduction potential decreased, and increased expression of the superoxide dismutases-3 (*sod-3*) and abnormal dauer formation protein (*daf-12*) genes with 0.1 and 0.5 mg/L of nAg exposures occurred concurrently with significant decreases in reproduction ability (Roh et al., 2009). Overall results of functional genomic studies using mutant analyses suggested that the *sod-3* and *daf-12* gene expressions may have been related to the nAg-induced reproductive failure in *C. elegans* (Roh et al., 2009). The study suggested that oxidative stress may have been an important mechanism in nAg toxicity.

There has been lack of reported ecotoxicity values for nAg in the terrestrial environment thus far, but a recent study exposed the earthworm (*Lumbricus terrestris*) to nAg and measured the impact on apoptosis in different tissues (Lapied et al., 2010). The study demonstrated that apoptosis can be the sensitive parameter to be used as an ecotoxicological endpoint in earthworms; also nAg has been shown to have significant negative effects in earthworms exposed to low concentrations in soil (Lapied et al., 2010).

The smaller nanoparticles were proven to be more toxic than larger nanoparticles of the same composition, and no bioaccumulation was observed in earthworms (Lapied et al., 2010). Response to apoptotic activity was dose-dependent indicating that this parameter is a valid measure of sublethal toxicity. Detection of enhanced apoptotic activity at concentrations of as low as 4 mg/kg soil is a sign of adverse effects on earthworms at concentrations more than two orders of magnitude lower than previously reported effects on earthworms (Lapied et al., 2010). A dramatic increase in apoptotic activity after 24 hour exposure was observed on worms, surviving nAg concentrations both above and below an estimated LD50 of 12 mg/ml (Lapied et

al., 2010). The overall observed ecotoxicity endpoints for nAg in aquatic and terrestrial organisms are summarised in Table 2.2.

**Table 2.2: Observed ecotoxicity end-points for nAg to the aquatic and terrestrial organisms.**

Exposure method	Media	Test organism	Toxicity value	Source
nAg synthesised using starch & BSA	Water	<i>D. rerio</i>	LC50 = 25 µg/ml LC50 = 50 µg/ml	Asharani et al. (2010)
Fluorometry as a function of total Ag		<i>C. reinhardtii</i>	EC50 (1h) = 3300 nM, 2h = 1049 nM, 3h = 879 nM, 4h = 801 nM, 5h = 829 nM	Navarro et al. (2008)
nAg as a function of free Ag <sup>+</sup>		<i>C. reinhardtii</i>	EC50 (1h) = 33 nM 2h = 10 nM, 3h = 9 nM, 4h = 8 nM, 5h = 8 nM	
5 µM nAg + cysteine		<i>C. reinhardtii</i>	EC50 = 57 nM	
10 µM nAg + cysteine		<i>C. reinhardtii</i>	EC50 = 61 nM	
nAg prepared using RT-PCR analysis	Water	<i>O. latipes</i>	LC50 (96h) = 34.6 ± 0.9 µg/L	Chae et al. (2010)
Stirred NanoAmor nAg		<i>P. promelas</i>	LC50 = 9.4 mg/L	Laban et al. (2010)
Stirred Sigma nAg		<i>P. promelas</i>	LC50 = 10.6 mg/L	Laban et al. (2010)
Sonicated NanoAmor nAg		<i>P. promelas</i>	LC50 = 1.25 mg/L	
Sonicated Sigma nAg		<i>P. promelas</i>	LC50 = 1.36 mg/L	
Suspension for 60 nm nAg		<i>D. magna</i>	EC50 (96h) = 1.0 µg Ag/L	Kim et al. (2010)
Suspension for 300 nm nAg		<i>D. magna</i>	EC50 (96h) = 1.4 µg Ag/L	
Metal sensor bacteria (Tripple <i>Sod</i> mutant)		<i>E. coli</i>	EC50 (2h) = 46 mg Ag/L	Ivask et al. (2010)
Metal sensor		<i>E. coli</i>	EC50 (2h) = 3.1 mg	

bacteria (Wild type)			Ag/L	
nAg with DOC (inhibition of cellular activity)		<i>O. mykiss</i>	EC50 = 2.6 mg/L	Farkas et al. (2010)
nAg (inhibition of cellular activity)		<i>O. mykiss</i>	EC50 = 2.5 mg/L	
nAg with DOC (using membrane stability)		<i>O. mykiss</i>	EC50 = 3.5 mg/L	
nAg (using membrane stability)		<i>O. mykiss</i>	EC50 = 4.9 mg/L	
Exposure at concentrations of 4mg/kg soil	Soil	<i>L. terrestris</i>	LD50 (24h) = 12 mg/ml	Lapied et al. (2010)

### 2.1.3 Carbon nanotubes (CNT)

Acute toxicity and genotoxicity of CNT was analysed in a 12 day exposure of amphibian larvae (*Ambystoma mexicanum*) under laboratory conditions. The genotoxic effects were analysed by scoring the micronucleated erythrocytes in the circulating blood of the larvae, and results demonstrated that CNTs were neither acutely toxic nor genotoxic to larvae regardless of the CNTs concentration in the water – though black masses of CNTs were observed in the guts (Mouchet et al., 2007). The concentrations of CNTs were increased (from 10 – 500 mg/L) and acute toxicity of double-walled CNTs (DWCNTs) was evaluated according to the mortality and growth of larvae *Xenopus laevis* (Mouchet et al., 2008). Results showed no genotoxicity in erythrocytes of larvae exposed to DWCNTs in water, but acute toxicity at every concentration was observed – related to physical blockage of the gills and/or digestive tract (Mouchet et al., 2008).

The first detailed report on the toxicity of single-walled CNTs (SWCNTs) to rainbow trout (*Onchorhynchus mykiss*) using a body systems approach was described by Smith et al. (2007). Stock solutions of dispersed SWCNTs were prepared using a combination of solvent (sodium dodecyl sulphate, SDS) and sonication, and the rainbow trout were exposed for 10 days (Smith et al., 2007). A dose-dependent rise in ventilation rate, gill pathologies (oedema, altered mucocytes, hyperplasia), and mucus secretion with SWCNTs precipitation on the gill mucus was observed (Smith et al., 2007). Liver cells exposed to SWCNTs showed condensed nuclear bodies

(apoptotic bodies) and cells in abnormal nuclear division, and ingested water containing SWCNTs during exposure (presumably stress-induced drinking) resulted in precipitated SWCNTs in the gut lumen and intestinal pathology (Smith et al., 2007). Aggressive behaviour and fin nipping caused some mortality at the end of the experiment, and were considered to be associated with the gill irritation and brain injury, although the solvent may have also partly contributed to aggression (Smith et al., 2007).

When acute toxicities of manufactured nanomaterial suspensions in water were evaluated on *D. magna* using immobilisation and mortality as toxicity endpoints, increase in concentrations of SWCNTs and MWCNTs (multi-walled CNTs) caused increased toxicities (Zhu et al., 2009). The 48 hour exposure experiment resulted in EC50 and LC50 values for MWCNTs of 8.723 mg/L and 22.751 mg/l, respectively (Zhu et al., 2009). However, the SWCNTs demonstrated lower EC50 and LC50 values of 1.306 mg/L and 2.425 mg/L, respectively (Zhu et al., 2009). These results indicated that SWCNTs suspensions were more toxic to *D. magna* than the MWCNTs in both immobilisation and mortality toxicity endpoints.

Chronic toxicity of DWCNTs was investigated on the diatom *Thalassiosira pseudonana*, copepod *Tigriopus japonicus* and medaka *Oryzias melastigma*, with exposure solutions prepared using sonication (so-DWCNTs) and stirring (st-DWCNTs) methods (Kwok et al., 2010). Exposure to DWCNTs led to growth inhibition of *T. pseudonana* with EC50 values of 1.86 and 22.7 mg/l for so- and st-DWCNTs, respectively (Kwok et al., 2010). The population growth of *T. japonicus* was reduced to 0.1 mg/l for so-DWCNTs and 10 mg/l for st-DWCNTs. Growth inhibition in *O. melastigma* was observed at 10 mg/l for so-DWCNTs but not for st-DWCNTs (Kwok et al., 2010). Findings suggested that the preparation method in these studies had a significant contribution to the toxicity where sonicated DWCNTs exhibited higher toxicity than stirred DWCNTs.

The biological uptake of CNTs in the terrestrial environment was investigated and results revealed no bioaccumulation in earthworms (Petersen et al., 2008). However, it was unclear if the lack of uptake stemmed from the strong sorption of the CNTs to soil/sediment particles, or whether the CNTs were minimally absorbed across intestinal or dermal tissues, thus limiting systemic circulation. Conversely, a distinctively different uptake and depuration behaviour was reported for *D. magna* (Petersen et al., 2009). A 48 hour exposure of *D. magna* to a 0.4 µg/mL solution of dispersed nanotubes, the CNTs comprised  $6.3 \pm 1.5$  % of the residual organism dry mass; moreover, the *D. magna* were unable to excrete CNTs to either clean artificial freshwater or filtered water after 24 hour depuration periods (Petersen et al., 2009).

A pilot study on the effects of functionalized and non-functionalized SWCNTs on root elongation of six crop species (cabbage, carrot, cucumber, lettuce, onion, and tomato) routinely used in phytotoxicity testing have been reported (Canas et al., 2008). The non-functionalized nanotubes affected root length more than the functionalized nanotubes. In addition, non-functionalized nanotubes inhibited root elongation in tomato and enhanced root elongation in onion and cucumber; however, the functionalized nanotubes inhibited root elongation in lettuce (Canas et al., 2008). Cabbage and carrots were not affected by both forms of nanotubes, and microscopy images showed the presence of nanotube sheets on the root surfaces - but no visible uptake was observed (Canas et al., 2008).

The transport behaviour of carboxyl-functionalized SWCNTs in columns packed in a natural soil was investigated. The study findings showed that the deposition (filtration) rate increased with increasing solution ionic strength and the divalent cations ( $\text{Ca}^{2+}$ ) being more effective in increasing SWCNT retention than monovalent cations ( $\text{K}^+$ ). However, SWCNT deposition rate occurred over a very wide range of monovalent and divalent cation concentrations (0.03 to 100 mM) was relatively high and changed only slightly above 0.3 mM KCl or 0.1 mM  $\text{CaCl}_2$  (Jaisi and Elimelech, 2009). Observations made suggested that physical straining governs SWCNT filtration and transport under all the solution chemistries; hence, it was proposed that SWCNT shape and structure, particularly the very large aspect ratio and aggregated state in aqueous solutions as well as the heterogeneity of soil particle size, porosity, and permeability collectively contributed in straining the in flow through soil media (Jaisi and Elimelech, 2009).

The few studies on ecological toxicity of CNTs in the terrestrial environment lacked the lethal and sublethal toxicity values in soil organisms. A study that investigated these parameters reported that the DWCNTs used were unlikely to have toxicity effects due to co-contaminants. This was because of the low ash content of DWCNTs indicated by the supplier (Scott-Fordsmand et al., 2008). The effects concentrations observed when *Eisenia veneta* was exposed to DWCNT-spiked food were; 94 ( $\pm$  45) mg/kg for growth, 37 ( $\pm$  73) mg/kg for reproduction, >500 mg/kg hatchability indicating the EC10 values and >50 mg/kg for growth, 176 ( $\pm$  150) mg/kg for reproduction, and >500 mg/kg hatchability indicating the EC50 values (Scott-Fordsmand et al., 2008). These results were surprising when considering the previously reported lack of bioaccumulation of CNTs in earthworms (Petersen et al., 2008). Table 2.3 summarises the observed toxicity endpoints for CNTs.

**Table 2.3: Observed ecotoxicity end-points for CNTs to aquatic and terrestrial organisms**

Exposure method	Media	Test organism	Toxicity value	Source
MWCNT suspensions in water	Water	<i>D. magna</i>	EC50 = 8.723 mg/L LC50 = 22.751 mg/L	Zhu et al. (2009)
SWCNT suspensions in water		<i>D. magna</i>	EC50 = 1.306 mg/L LC50 = 2.425 mg/L	
DWCNT sonicated		<i>T. pseudomona</i>	EC50 = 1.86 mg/L	Kwok et al. (2010)
	<i>T. japonicus</i>	EC50 = 0.1 mg/L		
	<i>O. melastigma</i>	EC50 = 10 mg/L		
DWCNT stirred	Water	<i>T. pseudomona</i>	EC50 = 22.7 mg/L	Kwok et al. (2010)
		<i>T. japonicus</i>	EC50 = 10 mg/L	
DWCNT with 99.5 % purity and a metal content of < 0.4 %	Soil	<i>E. veneta</i>	Growth (mg/kg): EC10 = 94 (± 45) EC50 > 50	Scott-Fordsmand et al. (2008)
		<i>E. veneta</i>	Reproduction (mg/kg): EC10 = 37 (± 73) EC50 = 176 (± 150)	
		<i>E. veneta</i>	Hatchability (mg/kg): EC10 > 500 EC50 > 500	

#### 2.1.4 Fullerenes (nC<sub>60</sub>)

Lovern and Klaper (2006) reported the toxicity of nC<sub>60</sub> on *D. magna* where the exposure media was prepared using THF and sonication. Exposure to filtered nC<sub>60</sub> caused elevated mortality as the concentration increased. The nC<sub>60</sub> showed higher levels of toxicity at lower concentrations; whereas, exposure to the sonicated solutions caused varied mortality (Lovern and Klaper, 2006). Observed LC50 values for THF-filtered nC<sub>60</sub> were 460 ppb and a NOEC of 180ppb; while sonicated nC<sub>60</sub> gave LC50 values of 7.9 ppm and NOEC of 0.18 ppm was obtained (Lovern and Klaper, 2006).

Effect of preparing nC<sub>60</sub> by stirring was tested as prior studies (Lovern and Klaper, 2006) found that sonication and use of THF increased the toxicity of nC<sub>60</sub> (Oberdorster et al., 2006). Acute

toxicity of nC<sub>60</sub> was tested on freshwater crustaceans *D. magna*, *Hyalella azteca* (a marine harpacticoid copepod) and two fish species (fathead minnow *Pimephales promelas* and Japanese medaka *Oryzias latipes*) (Oberdorster et al., 2006). The maximum concentrations tested were 35 ppm for freshwater and 22.5 ppm for full-strength (35 ppt) seawater because at higher concentrations the nC<sub>60</sub> precipitated out of solution; and for all the tested species, LC50 values could not be obtained in the study of Oberdorster et al. (2006). Hence preparing nC<sub>60</sub> by stirring did not result in acute toxicity on the tested organisms at 48 or 96 hour exposure.

The effects of preparation method for nC<sub>60</sub> were evaluated in a study where THF-solubilised nC<sub>60</sub> was compared with water-stirred nC<sub>60</sub> tested on *D. magna* and *P. promelas* (Zhu et al., 2006). The daphnia 48 hour LC50 for THF-nC<sub>60</sub> was at least one order of magnitude less (0.8 ppm) than that for water-stirred nC<sub>60</sub> (> 35 ppm), and there was 100% mortality in the THF-nC<sub>60</sub> exposed fish between 6 and 18 hour, while the water-stirred nC<sub>60</sub>-exposed fish showed no obvious physical effects after 48 hour (Zhu et al., 2006). These results suggested that the high toxicity of THF-prepared nC<sub>60</sub> as opposed to preparation by stirring in water was due to the preparation techniques applied in the aquatic toxicity testing.

Ecotoxicity studies done when nanoparticles were prepared by suspension in water showed that the Microtox experiments did not induce measurable effects for *V. fischeri* with a concentration of 100 mg/L, except for SWCNTs and fullerene (EC50 > 1 mg/L for Microtox in fullerenes) (Velzeboer et al., 2008). A few noticeable observations were made during the test; first, the 100 mg/L suspensions were not transparent and had a white turbidity, which can affect the Microtox and PAM measurements because of quenching (absorbing light); second, the 100 mg/L suspensions were unstable and tended to precipitate after some time. In comparison with the results of Lovern and Klaper (2006), no appreciable differences were determined in the study. The absence of ecotoxic effects in the present experiments were explained, at least in part, by low concentrations of small particles in suspension; and the actual concentrations of nano-sized materials to which the test organisms were exposed probably were far lower than the nominally added 100 mg/L (Velzeboer et al., 2008).

The acute toxicity of water suspension for nC<sub>60</sub>, on the other hand, tested using *D. magna* and the species were found to ingest nanomaterials from the test solutions through feeding behaviours; which suggested that the potential ecotoxicities and environmental health effects cannot be neglected (Zhu et al., 2009). The EC50 of immobilization and LC50 of mortality for nC<sub>60</sub> were found to be 9.344 mg/L and 10.515 mg/L, respectively; meanwhile, carbon black EC50 and LC50

values were 37.563 mg/L and 61.547 mg/L, correspondingly (Zhu et al., 2009). These results indicated that nanoparticles were more toxic than their bulk counterparts.

In soil-dwelling organisms (earthworm *E. veneta*) the effects of nC<sub>60</sub> were also tested. The most sensitive toxicological parameter tested was reproduction (cocoon production), however, no effect on hatchability, survival or mortality up to 1000 mg nC<sub>60</sub>/kg were detected (Scott-Fordsmand et al., 2008). In aquatic studies it has been observed that the toxicity levels depended on the exposure procedure, particularly on how the nanoparticle (NP) was dissolved in the exposure media. In a pilot experiment to the study, three methods of addition were tested: (i) stirring NP in water for 3 weeks, (ii) stirring NP in water using sonication and (iii) direct NP addition to the test media. The addition by stirring (i) was quickly abandoned because very little NP disaggregated in the solution and most precipitated out, making it difficult to transfer to the soil media. For the other two methods, (ii) and (iii) pilot toxicity experiments indicated that there was little difference in toxicity (Scott-Fordsmand et al., 2008). A summary of toxicity endpoint parameters observed for nC<sub>60</sub> is shown in Table 2.4.

**Table 2.4: Observed toxicity end-points for nC<sub>60</sub> in aquatic and terrestrial organisms**

Exposure method	Media	Test organism	Toxicity value	Source
THF solubilised nC <sub>60</sub>	Water	<i>D. magna</i>	LC50 = 0.8 ppm	Zhu et al. (2006)
Water-stirred nC <sub>60</sub>		<i>D. magna</i>	LC50 > 35 ppm	
THF filtration		<i>D. magna</i>	LC50 = 460 ppb NOEC = 180 ppb	Lovern and Klaper (2006)
Sonication		<i>D. magna</i>	LC50 = 7.9 ppm NOEC = 0.18 ppm	
Suspension by Microtox		<i>V. fischeri</i>	EC50 > 1mg/l	Velzeboer et al. (2008)
nC <sub>60</sub> suspension		<i>D. magna</i>	EC50 = 9.34 mg/L LC50 = 10.51 mg/L	Zhu et al. (2009)
nC <sub>60</sub> with 99.5% purity and a metal content of < 0.4 %	Soil	<i>E. veneta</i>	NOEC = 1000 mg C60/kg	Scott-Fordsmand et al. (2008)

The reviewed ecotoxicological data in this sub-section were used to compute the predicted no-effect concentrations (PNEC) values in preceding chapters, and only the most sensitive values

(more toxic) were used in the calculations in order to offer maximum protection for the environment. A review of studies with direct evidence on the detection of ENMs in the environment is presented in the following sub-section.

## 2.2 DIRECT EVIDENCE OF ENM RELEASE INTO THE ENVIRONMENT

In environmental risk assessment of ENMs, both the exposure and the hazard need to be taken into account. However, as highlighted in several reviews (Wiesner et al., 2006; Nowack and Bucheli, 2007; Baun et al., 2008; Meyer et al., 2009; O'Brien and Cummins, 2010), data on the release of ENMs into the environment is largely lacking. A few case studies have reported direct evidence on the release of ENMs into the environment. Our review in this section focuses on ENMs of interest to this study.

Kaegi et al. (2008) investigated nTiO<sub>2</sub> because they are used in large quantities in exterior paints as whitening pigments and to a certain extent are present in the nano range. During paint weathering containing white pigments of nTiO<sub>2</sub>, oxidation occurs at the surface layer of the material which eventually erodes away leaving the nanoscale particles exposed at the surface.

In their study, the sampling site was a facade runoff from a building painted in May 2006 (referred to as aged facade), and the runoff was collected using an aluminum gutter (1.0 m length) mounted at the bottom of the facades' drain rail; the runoff drained into a plastic bottle. After each runoff event, samples were directly taken to the laboratory and analysed within a few hours.

In addition, runoff from a new facade (an experimental building area, 1.3 m<sup>2</sup>) painted with the same product was studied. Near the existing building, surface runoff from a greater urban catchment was sampled right before its discharge into the receiving creek. The catchment was characterized by residential and business buildings, paved surfaces around the buildings and pavements on sidewalks and roads. Thus, all particulate and dissolved substances in storm water runoff entered inevitably into the surface water and water samples were collected from complete runoff events (Kaegi et al., 2008).

A centrifugation based sample preparation recovered nTiO<sub>2</sub> with sizes ranging between roughly 20 and 300 nm. A combination of analytical electron microscopy and bulk chemical methods were used to measure the amounts of nTiO<sub>2</sub> in runoff. Analytical tests from the study revealed that nTiO<sub>2</sub> were detached from new and aged facade paints by natural weather conditions, and

transported by facade runoff and eventually discharged into natural receiving waters (Kaegi et al., 2008). The concentrations of Titanium (Ti) in the runoff of the new façade were as high as 600 µg/L, and half of the particulate load consisted of larger nTiO<sub>2</sub> or agglomerates with > 300 nm diameter estimated to have originated from other sources such as road paints or other surface coatings. The study findings showed for the first time that nTiO<sub>2</sub> were released in significant amounts to the aquatic environment from nanoproducts such as paints used in buildings.

Due to various uses of nTiO<sub>2</sub> in consumer products and even in foods (PEN, 2010), humans would excrete the ingested nTiO<sub>2</sub> and thereafter transported into sewage wastewater treatment plants (WWTPs). However, there are limited publications reported on the removal of ENMs from WWTPs. In addition to excretion from humans, nTiO<sub>2</sub> used in sunscreens and paints are likely to be washed or disposed of into the sewage systems.

Kiser et al. (2009) investigated Ti concentrations in a full-scale municipal WWTP; the morphology and composition of Ti-based solids were characterized in consumer products, wastewater effluents and biosolids. The Ti concentrations were quantified in lab-scale treatment reactors and the sorption capacity of wastewater biomass for nTiO<sub>2</sub> was recorded. This research was conducted at a wastewater reclamation facility in central Arizona (U.S.A.) that uses an activated sludge process and tertiary filtration treatment at four different times (7:00, 11:00, 15:00, and 18:00) daily during the month of June 2008. The effluent samples from each unit process, activated sludge from the aeration basins (except at 7:00), wasted solids from the primary and secondary clarifiers, as well as finished biosolids were collected from the facility. For seasonal variations, effluent samples were also collected from each unit process at the same facility, once around noon (during peak flow) on a day in January 2009; and for quality control, field and trip blanks of ultrapure water (< 1.5 µS/cm) were included in the sampling (Kiser et al., 2009).

For laboratory-scale sampling: nTiO<sub>2</sub> suspension was prepared by adding TiO<sub>2</sub> to ultrapure water, sonicating for 1 hour (200 W/L), and centrifuging at  $F \pm 1000$  G for 30 minutes; following centrifugation, the supernatant containing suspended TiO<sub>2</sub> was removed and used as stock solution. Liquid and solid samples were acid digested using the HNO<sub>3</sub>/H<sub>2</sub>SO<sub>4</sub> digestion method for Ti, and the digested samples were analyzed by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) (Kiser et al., 2009).

Approximately 91% of the Ti that entered into the treatment plant was removed from wastewater. The removed Ti accumulated in the primary solids, activated sludge biomass, and secondary solids. In addition, samples collected at other times of the day exhibited similar removal and solid-accumulation trends across the WWTP. Results from the lab-scale experiments indicated that  $n\text{TiO}_2$  have an affinity for solids, and the majority of  $\text{TiO}_2$  in water (in the order of 70-85 %) were removed by wastewater biomass concentrations of around 2000-3000 mg/L. Hence, the study showed that  $n\text{TiO}_2$  were evidently released from the WWTP to the aquatic environment.

The use of commercial products containing  $n\text{Ag}$  potentially can compromise the ecological health, for example, household washing of clothing containing  $n\text{Ag}$  may release both silver ions and nanoparticles into sewer systems. If  $n\text{Ag}$  proves to be difficult to remove in a wastewater treatment system,  $n\text{Ag}$  remaining in the treated effluent stream may enter surface water environments, potentially disrupting numerous biological ecosystems (Benn and Westerhoff, 2008). Therefore, the quantification and characterization of the  $\text{Ag}$  released from nano- $\text{Ag}$ -products is an important parameter required to predict the effect of  $n\text{Ag}$  into organisms at different environmental media (for example water and soil).

The  $n\text{Ag}$  release from commercial clothing (specifically, socks) into water as well as the form of this silver ( $\text{Ag}$ ) and the adsorption characteristics that determine its fate in WWTPs was investigated. Six brands of commercially available socks were purchased based on the manufacturer's claims that the socks contained  $n\text{Ag}$ , and a modified digestion method was used to quantify the amount of  $\text{Ag}$  in the socks. An air-dry mass (100- 500 mg-dry) of each sock was submerged in a solution of 5 mL of an ultrapure reagent grade nitric acid and 5 mL of deionised water,  $\text{Ag}$  was quantified using ICP-OES (Benn and Westerhoff, 2008). Socks were placed in 1 L amber glass bottles with 500 mL of ultrapure water, the bottles were agitated for either 24- or 1-hour contact times on an orbital shaker table at approximately 50 rpm. The 24-hour contact time was chosen to allow sufficient opportunity for the socks to leach  $\text{Ag}$ .

Three approaches were employed to separate the  $n\text{Ag}$  from ionic silver ( $\text{Ag}^+$ ) and to quantify both forms. The scanning electron microscopy and transmission electron microscopy were used to confirm the presence of  $n\text{Ag}$  in the sock material and in the washwater samples, respectively. Batch adsorption isotherms were used to quantify the potential removal of  $n\text{Ag}$  and  $\text{Ag}^+$  from the washwater by wastewater treatment system biomass (activated sludge), and isotherm studies were conducted on two types of  $\text{Ag}$ -containing water solutions (the sock washwaters and a reagent  $\text{Ag}^+$  solution). Acid digestion of the sock material indicated that 5 of the 6 types of socks

contained detectable levels of Ag ranging from 2 to 1360  $\mu\text{g-Ag/g}$  sock, three of the six sock sample types detectably leached Ag into the ultrapure washwater (1- or 24-hour contact time). Based upon the existing water quality criteria for Ag, the model suggested that WWTP were capable of removing a much higher Ag load from a wastewater stream than should be reasonably expected from an increased number of consumer products containing nAg (Benn and Westerhoff, 2008).

Another study investigated the amount and the form of Ag released during washing from nine fabrics with different ways of incorporating Ag into or onto the fibers. One sample was a commercially available conventional antibacterial sock, a second one was commercially available nanosock, six fabrics were obtained directly from two companies, and the last sample was sourced from an ongoing research project (PLASMA-NP). To determine the total Ag-content, three replicates of each textile were digested with 3.5 mL of  $\text{HNO}_3$  (65%) and 1 mL of  $\text{H}_2\text{O}_2$  (30%), and the Ag was quantified by ICP-OES (Geranio et al., 2009).

The release of Ag from textiles was studied at pH 10, buffered by 0.005 M  $\text{Na}_2\text{CO}_3$  with SDS (Sodium Dodecyl Sulfate) as a surfactant (0.1 g/L). The washing procedure was conducted following an ISO-method for washtests using a Washtec-P Roaches washing machine with a motor speed of  $40 \pm 2$  rpm, equipped with steel vessels of 550 mL capacity. The detergent/container volume ratio (0.279) was kept the same and the detergent was prepared by dispersing 4 g/L ECE98-standard washing powder in distilled water, the textiles were washed for 30 min at 40 °C. Preliminary tests were performed to assess any possible losses or contaminations of Ag during washing and in blank washings (no textile), but during filtering and ultrasonication, the Ag signal was below the detection limit of ICP-OES. To account for the possible absorption on the steel balls, a similar test was performed spiking the detergent water with 0.9  $\mu\text{M}$  Ag and measuring the concentration before and after a cycle – the recovery of Ag was 85% (Geranio et al., 2009).

During the first phase: without added oxidants, a slight increase of the dissolved  $\text{Ag}^+$  was measured over time, and the presence of SDS decreased the concentration of released  $\text{Ag}^+$ . No influence of SDS or LAS was observed on the dissolution of the carbonate-coated nAg. After addition of  $\text{H}_2\text{O}_2$  a very rapid dissolution was observed with similar reactivity for both nAg; the final  $\text{Ag}^+/\text{H}_2\text{O}_2$  ratio was 1.00 (NP-powder, no surfactant), 1.72 (NP-powder, SDS), 0.96 (NP-suspension, no surfactant), 1.23 (NP-suspension, SDS), and 1.82 (NP-suspension, LAS). The addition of the same amount of  $\text{H}_2\text{O}_2$  resulted in a  $\text{Ag}^+$  concentration being 100-times lower; and under all conditions tested, the concentration of  $\text{Ag}^+$  decreased after the addition of  $\text{H}_2\text{O}_2$ .

Four out of the nine fabrics did not release any detectable Ag<sup>+</sup> (<0.08 μM). The other five samples exhibited very different behaviour: (sock-types) AgKilBact released almost immediately a high concentration of Ag<sup>+</sup> (about 25% of the total Ag), followed by a plateau and the PAA addition did not yield big changes, also X-STATIC and NP-PES-SURF released relatively rapidly Ag<sup>+</sup> - followed by a more gradual increase, PLASMA-NP released little Ag<sup>+</sup> without oxidants but showed a rapid increase after both additions of PAA, the sample AgCl only gave a Ag<sup>+</sup> concentration above the detection limit after the second addition of PAA. The concentration at the detection limit was converted for each fabric into the maximum Ag fraction that could have been leached. Results showed that the total released Ag varied from 1.3 to 35% of the total Ag in the fabric; meanwhile, the textiles with Ag incorporated into the fiber matrix released only very small amounts of Ag, and for all the fabrics used the Ag release decreased in the second wash (Geranio et al., 2009).

The amount of Ag leached from the socks found in the study (0.3-377 μg/g) was higher than the range reported by Benn and Westerhoff (1-68 μg/g). This may be due to the fact that the latter experiment only used distilled water and gentle agitation. However, the learning from these studies is that Ag in the particulate fraction > 450 nm is probably the predominant form of Ag released into the washing liquor and subsequently to wastewater. The release of metallic nAg was again investigated from paints used for outdoor applications, and a facade panel was mounted on a model house which was exposed to ambient weather conditions over a period of one year. Strong leaching of the nAg was observed during the initial runoff events with a maximum concentration of 145 mg Ag/L; after a period of one year, more than 30% of the nAg were released to the environment with particles < 15 nm and were released as composite colloids attached to the organic binders of the paint (Kaegi et al., 2010).

A recent study suggested that screening-level life cycle assessment (LCA) can provide a quick tool to identify the life cycle hot spots and focus research efforts to help minimize the burdens of a technology while maximising its benefits. The study examined the environmental impact of including silver nanoparticles in commercially available socks using screening-level LCA as the previously reported results suggested that washing during the use phase contributes substantially more than the manufacturing phase to the product life cycle impacts (Meyer et al., 2010). In the study, comparison of nanoparticles prepared by either chemical reduction, liquid flame spray (LFS), or plasma arc demonstrated how the type of manufacturing process used for the nanoscale silver can change the resulting life cycle impact of the sock product.

Results from the study suggested that the magnitude of this impact depended on the type of process used to manufacture the nanoscale silver, with LFS having the most impact because of the need for large quantities of hydrogen and oxygen (Meyer et al., 2010). Although the increased impacts for single ENMs may be relatively small, the added environmental load can actually be a significant quantity when considered at the regional or global production level. The next sub-section reviews models that have been developed to quantify these releases either at regional or global level.

## **2.3 DEVELOPED MODELS ON ENMS RISK ESTIMATION**

The lack of knowledge and data on the hazards and exposure of nano-scale materials, because of metrology limitations, poses a challenge in quantifying these novel materials into different environmental media. This has led to the development of models to quantify the potential exposure levels and thereby estimate the risk levels posed to various organisms in different ecosystems.

Already established models have highlighted that, risk estimation of new compounds has to integrate the uncertainty about accuracy of the calculated modelling parameters and the natural variability of these parameters (Blaser, 2006; Muller, 2007; Gottschalk et al., 2009a). The lack of empirical data cannot be treated only statistically, because we often need predictions on parameters that are yet to be measured. As a result, some technically informed judgements or predictions in the form of probability or frequency distributions are required (Gottschalk et al. 2009b). Results from the developed models for the estimation of ENMs in the environment are discussed in the following sub-sections.

### **2.3.1 Predicted environmental exposure to ENMs in UK**

The first study to report risk estimation of ENMs predicted the current and future environmental exposure to engineered nanoparticles (ENPs) in the United Kingdom (UK). This was achieved by identifying the ENP potential releases and reviewing their fate in environmental systems (Boxal et al., 2007). The study concluded that the route of exposure to the environment is primarily dependent on the end use of nanoproducts.

In the study, a framework of simplistic models and algorithms to estimate the likely concentrations of ENPs in water, soil and air for a range of usage scenarios was developed.

Environmental exposure arising from existing production, use and disposal of ENPs and prediction of likely future exposures of ENPs under development was evaluated. This was addressed by identifying the current ENPs produced in the UK, with their actual and predicted usage volumes. New developments in the field of ENPs were identified and potential environmental contamination arising from them was assessed; the potential for exposure for ENPs from production, use, disposal and recycling phases of the life-cycle was determined (Boxal et al., 2007). Product types that were identified to have the greatest potential for release of ENPs into the environment were: cosmetics and personal care products, paints and coatings, catalysts and lubricants, water treatment, food and food packaging, human and veterinary medicines as well as the plant protection products - like pesticides. The fate and environmental behaviour of ENPs was described for the water systems, air, suspended solids, sediments and soils. Releases to water and air from the available data suggested that ENPs will form aggregates, the degree of aggregation and size will depend on the characteristics and concentrations of particles as well as the characteristics of the environmental systems they are released to.

Modelling approaches applied in the study estimated the concentrations of ENPs in water, soil and air environmental systems. Assuming a 10% market penetration in the UK, the predicted concentrations of silver, aluminium, and fullerenes (C60) were found to be in the nano-gram per litre range (ng/l); whereas titanium dioxide, zinc oxide, nanolatex and hydroxyapatite were predicted to be in the micro-gram per litre ( $\mu\text{g/l}$ ) range in water systems. The predicted concentrations in soil ranged from less than 0.01 (cerium dioxide) to around 4.3 mg/kg (nanolatex). Only a few concentrations could be calculated for the air; cerium oxide was calculated to be  $6 \times 10^{-7}$  mg/m<sup>3</sup> and titanium dioxide at 7 mg/m<sup>3</sup>. Future predictions showed that; if all of the product types investigated contained ENPs, then concentrations in water could range from less than 1ng/l (cerium dioxide) to 1mg/l (nanolatex) (Boxal., et al., 2007). Table 2.5 gives a summary of calculated concentrations in the water, soil and air environmental systems.

**Table 2.5: Summary of estimated concentrations for ENPs in the UK (Boxal et al., 2007)**

	Water ( $\mu\text{g/l}$ )	Soil ( $\mu\text{g/kg}$ )	Air ( $\text{mg/m}^3$ )
Ag	0.010	0.43	-
AlO <sub>3</sub>	0.0002	0.01	-
Au	0.14	5.99	-
CeO <sub>2</sub>	<0.0001	<0.01	6 x 10 <sup>-7</sup>
Fullerenes	0.31	13.1	-
Hydroxyapatite	10.1	422	-
Latex	103	4307	-
Organo-silica	0.0005	0.02	-
SiO <sub>2</sub>	0.0007	0.03	-
TiO <sub>2</sub>	24.5	1030	7
ZnO	76	3194	-

The study provided a benchmark in identifying potential environmental exposure arising from a range of key ENP types. It also raised research questions to establish detailed knowledge on the content and use of products containing ENPs. Following the study, industry surveys were conducted and a review of these is covered in the next sub-section.

#### 2.3.1.1 Industry surveys

One of the research questions raised from the previously reviewed study (Boxal et al., 2007) was addressed, and a survey on how industry approaches issues of nanoparticulate material (NPM) risk and safety was conducted. A survey was done in industries in Germany and Switzerland for the study: the study concluded that 65% of companies in the survey indicated that they did not perform any risk assessment of NPM, and 32.5% performed risk assessment sometimes or always (Helland et al., 2007). Results from the study also revealed that the fate of NPM in the use and disposal stages received very little attention by industry, and the majority of companies did not foresee unintentional release of NPM throughout the life cycle. The study was limited to the responses given by surveyed companies, and as stated in their discussion, large companies declined the request to participate. Hence, the study did not give a true reflection of ENMs exposure in the workplace.

Another survey was conducted by Schmid and Riediker, who targeted their industrial survey on estimating the types and quantities of industrially used manufactured nanoparticles and the

level of exposure in the Swiss industry. The study revealed that nanoparticles are used in many industrial sectors; not only by companies in the new field of nanotechnology, but also in more traditional sectors like paints. The survey revealed that 21.8% of companies declared use or production of nanoparticles, and 5.6% traded with prepacked goods that contain nanoparticles (Schmid and Riediker, 2008). Nanoparticles that were found to be used in considerable amounts (>1000 kg/year per company) were Ag, Al-ox, Fe-ox, SiO<sub>2</sub>, TiO<sub>2</sub>, and ZnO; these were used in the production of cosmetics, food, paints, powders and treatment of surface industries. The respondents in this survey had many questions on best practices and guidelines for usage of products with engineered nanoparticles. Also, the nanomaterial production quantities published from the study aided quantification of concentrations in different environmental ecosystem in Switzerland.

### 2.3.2 Environmental exposure modelling of nanoparticles in Switzerland

Due to data limitations from the surveys done in industries, environmental exposure modelling studies were conducted. A life cycle perspective to model the quantities of ENMs released into the environment was implemented. The quantification was based on the substance flow analysis of ENMs of nAg, nTiO<sub>2</sub> and CNT from nanoproducts to three environmental media (air, soil and water) in Switzerland (Muller, 2007). The environmental concentrations of nanoparticles were estimated as PEC (predicted environmental concentration) and were compared to PNEC (predicted no-effect concentration) derived from ecotoxicity values of these nanomaterials from the literature. Risk of each nanoparticle in the environment was characterised by calculating the ratio of PEC/PNEC. Results showed that nTiO<sub>2</sub> had a risk quotient (RQ)  $RQ > 1$  in water for the modelled high emission (HE) scenario, meaning that further risk assessment needed to be done for this ENM since it poses risk to the environment. A paper was also published in a peer-reviewed journal on these results (Muller and Nowack, 2008). A summary of the calculated PEC and RQ values for nTiO<sub>2</sub>, nAg and CNT is shown in Table 2.6.

The study constituted the first quantitative risk assessment of ENMs, and has made it possible to model risk in any other geographical region. However, there was a variety of limitations in the used data, which led to huge uncertainties in the results derived. Moreover, the model could not calculate RQ values for the soil compartment in all three modelled ENMs.

**Table 2.6: PEC and RQ values for the three modelled ENMs in water, air and soil (Muller and Nowack, 2007)**

	Unit (PEC <sup>a</sup> )	nAg		nTiO <sub>2</sub>		CNT	
		RE	HE	RE	HE	RE	HE
Air	µg m <sup>-3</sup>	1.7 x 10 <sup>-3</sup>	4.4 x 10 <sup>-3</sup>	1.5 x 10 <sup>-3</sup>	4.2 x 10 <sup>-2</sup>	1.5 x 10 <sup>-3</sup>	2.3 x 10 <sup>-3</sup>
Water	µg L <sup>-1</sup>	0.03	0.08	0.7	16	0.0005	0.0008
Soil	µg kg <sup>-1</sup>	0.02	0.1	0.4	4.8	0.01	0.02
	RQ <sup>b</sup>						
Air		nd	nd	0.0015	0.004	1.5 x 10 <sup>-5</sup>	2.3 x 10 <sup>-5</sup>
Water		0.0008	0.002	> 0.7	> 16	0.005	0.008
Soil		nd	nd	nd	nd	nd	nd

<sup>a</sup>RE: realistic scenario; HE: high emission scenario; <sup>b</sup>nd: not determined

Another study was done to estimate the aquatic exposure and risk due to silver from plastics and textiles. Blaser et al. (2008) contextualise their study and analysed risk to fresh water ecosystems due to silver released from nanoparticles incorporated into plastics and textiles (Blaser et al., 2008). The study focused on the Rhine River case study, which is also in Switzerland. Conclusions were that, biocidal plastics and textiles were predicted to account for up to 15% of the total silver released into water in the European Union (Blaser et al., 2008). It was found that the majority of silver released into wastewater was incorporated into the sewage sludge which might be spread on agricultural fields, and the amount of silver reaching natural waters depends on the fraction of wastewater that is effectively treated (Blaser et al., 2008).

The study had limitations because of lack of data on toxicity of environmentally relevant silver species; hence, a complete risk characterisation was not possible at the time. Thus, no risk was predicted for microbial communities in sewage treatment plants for the study.

### 2.3.2.1 Probabilistic modelling studies

The limitations encountered during environmental exposure modelling were then applied with statistical methods of evaluation through probabilistic modelling studies. A similar study to environmental modelling was done, but in different regions and with addition of other ENMs. Though the flow analysis from a life cycle perspective approach as used in previous exposure modelling study (Muller and Nowack, 2008) was used, the environmental concentrations were calculated as probabilistic density functions and compared to data from ecotoxicological studies.

Gottschalk et al. (2009a) modelled environmental concentrations of nTiO<sub>2</sub>, nZnO, nAg, CNT and fullerenes for the U.S., Europe and Switzerland. Results from the study indicated that risks to aquatic organisms may currently emanate from nTiO<sub>2</sub>, nZnO and nAg in sewage treatment effluents for all studied regions and for nAg in surface waters. The calculation of environmental concentrations of ENMs as probabilistic density functions from Gottschalk's first study (Gottschalk et al., 2009a), resulted to a study on probabilistic material flow modelling that used statistical simulations. Gottschalk et al. (2009b) published another paper in the environmental modelling and software journal, which presented a probabilistic method to compute distributions of PECs by means of a stochastic stationary substance/material flow modelling.

The model incurred uncertainties concerning the estimation of the model parameters and the exposure casual mechanisms by combining sensitivity and uncertainty analysis, Monte Carlo simulation and Markov Chain Monte Carlo modelling (Gottschalk et al., 2009b). Results from the study showed that the combination of the two methods is appropriate to calculate realistic PECs when facing a lack of data. However, all the reviewed modelling studies were done in highly industrialised and developed countries where nanotechnology is already at its full operational phases (UK, Europe, US and Switzerland). Yet, developing countries are still at initial phases with regards to nanotechnology implementation, and the geographical settings differ from developed countries (Musee, 2010b).

The next chapter presents the methodology taken to estimate ENM quantities in the Gauteng Province environmental media in a developing country setting (like South Africa), using exposure modelling techniques applied in previous studies (Muller and Nowack, 2008; Musee, 2010b).

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## Chapter 3 - **STUDY METHODOLOGY**

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Presently there is lack of data to quantify ENM releases into the environment. This can be attributed to unavailability of analytical methods (metrology limitations) to detect such emissions. Modelling approaches have been applied to estimate the ENM releases into different environmental media (Boxal et al., 2007; Muller and Nowack, 2008; Gottschalk et al., 2009; Musee, 2010b).

The abundance of ENMs in the environment is yet to be determined for South Africa (SA). This study determined the concentration of selected ENMs and characterised their risks in the Gauteng water resources and terrestrial environment through use of modelling approaches. These methods have been contextualised for the developing countries like South Africa and a specific region (Gauteng) used as the case study to illustrate potential risks of ENMs.

This study initially focused on soliciting information to determine the quantities of ENMs in SA through a survey. Several institutions and industries were identified from a baseline study conducted by the Department of Science and Technology (DST) (DST, 2004). These organisations were approached, and relevant contacts established. Questionnaires were distributed to a total of 76 contacts from 41 organisations comprising of university institutions, science research councils, research laboratories and companies.

The response rate was very poor since only 25% of the total questionnaires were completed, all of which were from the academic institutions – universities and research organisations. Findings from the survey indicated that there was limited production of ENMs in SA, and these were mainly material synthesis practices with no risk assessment performed. Uneasiness to provide data from companies was also highlighted. Based on these limitations, there was no baseline data to quantify the ENMs potentially released into the environment from different sources – like consumer products and industrial applications. This led to the inability to estimate the potential risks of ENMs in different ecological systems in SA. An alternative method, using modelling techniques, was applied to quantify the potential risks. Modelling was applied in the region of Gauteng, which was used as a case study to illustrate the functionality of the proposed methodology.

### 3.1 PROBLEM BOUNDARY

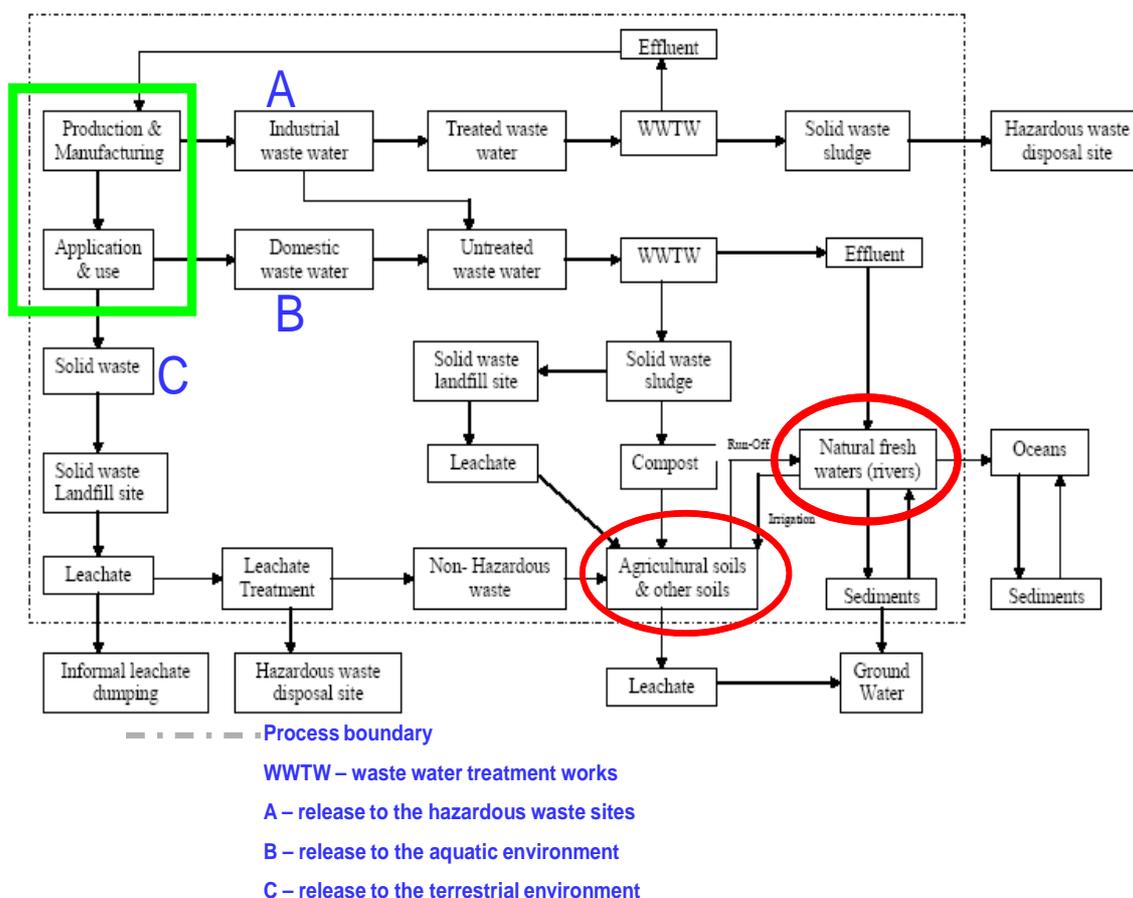
The Gauteng Province was selected as the region of study for SA as it contributes the highest gross domestic product (GDP) per capita among nine provinces in the country (Statistics SA, 2009). Though the Gauteng Province is the smallest province in SA, occupying about 17 010km<sup>2</sup> (1.4% of the total land area of SA), it comprises 21.4% of the total SA population with 10.53 million inhabitants. The working age population constituted approximately 4.747 million inhabitants that were economically active and only 2.247 million are economically inactive (Stats SA, 2009). Thus, Gauteng is considered as the ‘economic powerhouse’ of SA, because it constitutes 33% of the country’s GDP per capita (Stats SA, 2009). Because of the high income per capita, Gauteng is likely to experience the highest use and application of ENMs from consumer products and industrial activities in SA. Hence, most industrial and domestic environmental releases of ENMs in SA will most likely occur in the province.

Though the Gauteng Province is likely to have most industrial activities involving ENMs, releases from production and manufacturing are not considered for the model since data to quantify them were inaccessible. Environmental releases considered were from application and use of ENMs, through domestic waste streams due to consumer nanoproducts - like cosmetics, paints, textiles, coatings and cleaning agents to mention a few.

### 3.2 MATERIAL FLOWS

In some of the consumer applications, ENMs are either in a fixed or bound form and hence pose minimal risks to the environment (Boxal et al., 2007). This study focused on applications that pose a relatively higher risk of exposure to the environment, these include airborne ENMs or nanoproducts that are likely to be disposed of in wastewaters and eventually to the soils.

Applications and product categories that were considered in this study comprise of; cosmetics, paints, coatings or cleaning agents, textiles, plastics, composites, energy production or storage, metals, as well as research and development. The use of ENMs in these applications is likely to result in the domestic waste streams that are treated in wastewater treatment plants, which would consequently lead to their release into the aquatic and terrestrial environments. In the SA scenario, the possible pathways of ENMs release into the Gauteng environment are illustrated in Figure 3.1.



**Figure 3.1: Schematic flow of ENMs into the aquatic and terrestrial environments in Gauteng.**

The model considered flows through route B and C, since releases through route A could not be quantified due to data limitations. Thus, production and manufacturing of ENMs could not be quantified since there was no data available from companies that manufacture ENMs in SA. It should be noted that this challenge is not unique to SA but rather a global phenomenon in the nanotechnology field.

### 3.3 MODEL ASSUMPTIONS

Due to limited data published on quantities of ENMs for each product category, the model assumed that the published quantities for nTiO<sub>2</sub> and nAg in Switzerland (Muller, 2007) can be modified by use of empirical correction factors into the Gauteng environment. The model also assumed that quantities of nC<sub>60</sub> are equally distributed to the estimated product categories of cosmetics, composites, and research and development. This was due to the lack of company

declared global production volumes under this ENM. Moreover, an equal distribution was assumed for quantities of CNT to plastics and consumer electronics categories. The weighting factors applied for product categories in Switzerland (Muller and Nowack, 2008) was assumed to be the same for SA, since companies that manufacture these products also trade the same products in SA with the same concentrations – particularly because of the global presence of companies.

An even distribution of ENMs has been assumed in all environmental media (aquatic and terrestrial), and each product category contributes significantly to the final releases of ENMs. Upon reaching the environmental media, ENMs have been assumed to maintain the nanoscale properties – biodegradability has been neglected because the fate and behaviour of ENMs in the environment is not well defined (Hristov and Malsch, 2009).

The published ecotoxicological data for ENMs of nTiO<sub>2</sub>, nAg, CNT and nC<sub>60</sub> reported in the literature on organisms in the aquatic and terrestrial environments is assumed to give the best potential estimate for organisms found in the same environmental media in SA (regardless of the different climatic conditions).

### 3.4 MODEL SCENARIOS

Due to uncertainties and variability of available data used as inputs for the model, three emission scenarios were considered. The used production volumes of ENMs from published data introduced a lot of uncertainty to the model because the published values differ significantly. Also, the potential market penetration of products containing ENMs in the Gauteng, because of income variability, was considered for the population of Gauteng. The emission scenarios considered were the minimum (min), probable (prob) and maximum (max) scenarios.

The minimum scenario represented the lowest releases of ENMs to the environment, with the least estimated market penetration of products containing ENMs for the population of Gauteng. Meanwhile, the probable scenario was considered as the realistic estimate of market penetration for products with ENMs in Gauteng population that led to the realistic estimates for the emission of ENMs to the environment. On the other hand, the maximum scenario signified the worst-possible case observable for the emissions.

### 3.5 RISK ESTIMATION

The process followed when new chemical substances need to be authorised involves risk assessment, which should be performed as described in the technical guidance document on risk assessment (ECB, 2003). The assessment is based on representative measured data; however, in the absence of sufficiently detailed data, the document states that the most realistic information available should be given preference to determine exposure assessments. The exposure is measured by the predicted environmental concentration (PEC), and the expected toxicity or hazard is estimated by the predicted no-effect concentrations (PNEC). The general principle for assessing risks of substances involve estimation of the PEC, this is compared to the concentration below which unacceptable effects on organisms will most likely not occur - the PNEC (ECB, 2003). Risk is then estimated by calculating the ratio of PEC/PNEC, and this is the risk quotient (RQ).

As there are no measurements of ENMs in the environment, and because of new characteristics displayed by nanoparticles, an exposure model based on the estimated substance flow of ENMs was developed to calculate the PEC values. The available ecotoxicological values like the no-observed effect concentration (NOEC) were used to estimate the PNEC values of ENMs in various organisms in the aquatic and terrestrial environments. According to the ECB document, an extrapolation factor of 1000 from the toxicity values to the PNEC was applied to obtain the maximum environmental protection from ENMs (ECB, 2003). Risk was estimated from the ratio of PEC to PNEC, where if the quotient is  $< 1$  the substance is of no immediate concern and can be reconsidered when new information is available. Whereas if the ratio is  $> 1$ , the substance is of concern and additional data is required to verify the assessment. On the other hand, if the ratio is  $> 10$ , the substance is of immediate concern and more detailed information is required to validate the results (ECB, 2003).

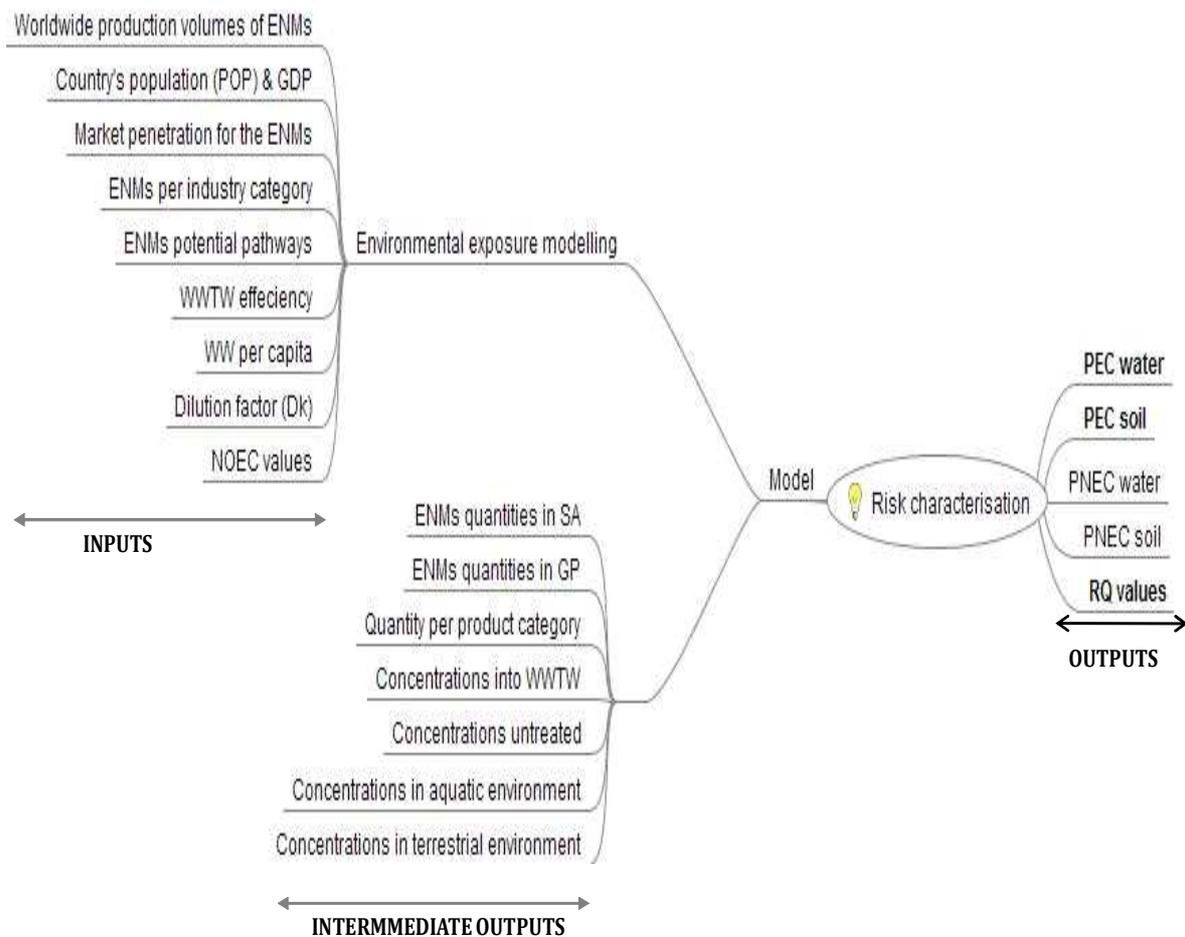
In this study, the available data was used to determine the exposure assessment of ENMs and thereby characterise risks they may pose to the aquatic and terrestrial environments. The methodologies implemented were based on identifying the emission sources in order to identify where exposures should be minimised. The PEC values were derived from available measured data and/or model calculations; while the PNEC values were determined on the basis of results from single species laboratory tests or established effect and/or no-effect concentrations from model ecosystem tests, taking into account adequate assessment factors (ECB, 2003). No measured environmental concentrations will normally be available for new substances; therefore, concentrations of a substance in the environment must be estimated (ECB, 2003).

Hence this study modelled the PEC and PNEC values from available data and thereafter quantified the RQ values.

Based on the model flows of ENMs in the SA environment (shown in figure 7), the available data was used to calculate the PEC values for Gauteng. Data was obtained from the wastewater treatment works (WWTW) of Gauteng; also the demographic data for Switzerland (SW), SA and Gauteng was used. The identified input and output parameters for the model are illustrated in Figure 3.2. Table 3.1 lists the model input values for the minimum, probable and maximum values for the worldwide production volumes of ENMs in 2008, with corresponding references.

**Table 3.1: Model input values - Global ENM production volumes in 2008**

<b>ENM</b>	<b>Min (t/a)</b>	<b>Reference</b>	<b>Prob (t/a)</b>	<b>Reference</b>	<b>Max (t/a)</b>	<b>Reference</b>
<b>nTiO<sub>2</sub></b>	3000	Gottschalck et al. (2009a)	5000	UNEP (2007)	60926	Schmid and Riediker (2008)
<b>nAg</b>	5	Gottschalck et al. (2009a)	434	Schmid and Riediker (2008)	563	Blaser et al. (2008)
<b>CNT</b>	140	Schmid and Riediker (2008)	295	Koehler et al. (2008)	426	Cientifica (2008)
<b>nC<sub>60</sub></b>	0.15	Plasmacarb (2002)	5	Gottschalck et al. (2009a)	10	Fortner et al. (2005)



**Figure 3.2: Schematic representation of identified input and output parameters to characterise risk of ENMs environmental exposure in Gauteng**

The model output parameters ( $PEC_{water}$ ,  $PEC_{soil}$ ,  $PNEC_{water}$  and  $PNEC_{soil}$ ) required quantification of the indicated intermediate output parameters from Figure 3.2. The quantities of ENMs in SA were allocated from the quantities calculated for SW (allocated from the global production volumes of each ENM), which aided quantification of ENMs in Gauteng. The calculated ENM quantities for Gauteng were broken down into product categories for each ENM and the quantities in each product category were calculated as intermediate outputs. The wastewater efficiency values obtained from data on the WWTW of Gauteng were used to quantify the concentrations of ENMs in the aquatic and terrestrial environmental media. All the calculated intermediate output parameters for the model were necessary to calculate the output parameters. The model output parameters were then calculated to characterise risk as RQ values. The RQ values were calculated as the ratio of  $PEC/PNEC$  for both water and soil environmental media.

## **3.6 METHODS OF DATA COLLECTION**

Due to lack of data to quantify the risks of ENMs in SA, data was collected from the WWTW in Gauteng. This data aided in calculating the WWTW efficiency values that were used inputs for the the model. Through communication with experts in the field of water research, a method of data collection was implemented. Several municipality contacts were developed where wastewater treatment data could be acquired. Then contacts from WWTW and municipalities were established, thereafter separate questionnaires were developed to collect the data.

### **3.6.1 Municipal wastewater treatment and services questionnaire**

To acquire data from municipalities, a questionnaire was developed and distributed to three major metropolitan municipalities responsible for the WWTW in Gauteng. However, it was established that these municipalities did not operate the treatment plants, but either had separate bodies (wastewater treatment plants – WWTP) responsible for their wastewater treatment or had delegated smaller municipalities at district and local municipality levels. This led to the development of two separate questionnaires, one for the municipalities and the other for WWTP. The municipal wastewater treatment questionnaire solicited information on their waste treatment and services; details of questions asked in the questionnaire are shown in Appendix B. Several municipalities had their own waste treatment plants, for example, the local municipalities operated their own plants.

### **3.6.2 Wastewater treatment works questionnaire**

A questionnaire was developed for distribution to the wastewater treatment works or plants operated by the municipality and those that were not. This questionnaire aimed at collecting data on the following aspects (Appendix B gives detailed information):

- The location of treatment works including areas served.
- The type of waste, either domestic or industrial waste treated.
- The capacity and actual flows of the treatment works.
- The technology used to treat the waste.
- The nature of sludge generated and ways of sludge disposal.
- The downstream water users, among other information and data enquired.

The collected data was used to calculate the unknown values in the model formulation, like the  $f_{WWTP}$  values.

### 3.7 MODEL FORMULATION

The model was computed using available published data and the data collected from the wastewater treatment plants in Gauteng. The formulation methods used for the model are discussed in the next sub-sections.

#### 3.7.1 Allocation of ENM quantities to Gauteng

The worldwide production volumes of ENMs have been considered as the crucial input parameter for determination of ENMs in the environment (Muller, 2007; Muller and Nowack, 2008; Gottschalk et al, 2009a; Musee, 2010b). The published production volumes of ENMs during the year 2008 were allocated to SW by Gottschalk et al (2009a) based on a comparison of the total population of developed countries to SW population. A similar approach has been used to allocate production volumes of ENMs into Gauteng from SW data and the published global production values in 2008 was used and allocated to Gauteng using the expression:

$$GP_{ENM} = SW_{ENM} \cdot cf_1 \cdot cf_2 \cdot cf_3 \cdot \frac{GDP_{GP}}{GDP_{SA}} \quad (1)$$

where  $GP_{ENM}$  is the quantity of ENMs in the Gauteng Province environment,  $SW_{ENM}$  is the quantity of ENMs in Switzerland,  $cf$  is the correction factor.  $cf_1 = \frac{(1-f_p) \cdot POP_{SA}}{POP_{SW}}$  where  $f_p$  is the

economically inactive percentage of SA population (estimated at 0.35, 0.45 and 0.55 for maximum, probable and minimum scenarios, respectively), POP represents the population of SA and SW during the year 2008;  $cf_2 = \frac{GDP/capita_{SA}}{GDP/capita_{SW}}$  where GDP/capita is the gross domestic product

per capita for both countries in 2008; and  $cf_3$  = market penetration, representing the market penetration of ENMs in Gauteng. The market penetration values were estimated as 0.05, 0.20 and 0.35 for the minimum, probable and maximum scenarios, respectively. The averaged lower income per capita as compared to Johannesburg (JHB) city (Musee, 2010b) translates to lower purchasing power for luxury goods in Gauteng, hence, estimated lower values for the market penetration in Gauteng than JHB city. A value of 0.05 market penetration signifies that 5% of the population of Gauteng will potentially use products containing ENMs, 0.20 represents 20% and 0.35 for 35% of the Gauteng population.

### 3.7.2 Estimating the quantities of ENMs used per industry category

The absolute number of ENMs used per industry category is not known; however, an approach to estimate these quantities using weighting factors (relative co-efficient) has been used in the literature (Muller, 2007). These were calculated using the expression:

$$ENM_{total} = ENM_{cat1} + ENM_{cat2} + ENM_{cat3} + \dots + ENM_{catx} \quad (2)$$

where  $ENM_{total}$  is the total amount of ENMs used,  $ENM_{catx}$  is the amount of ENMs used in each product category.  $ENM_{catx}$  is calculated using the expression:

$$ENM_{catx} = ENM_{total} \cdot WF_{weight} \cdot WF_{article} \quad (3)$$

where  $ENM_{total}$  is the quantity of ENMs in Gauteng,  $WF$  is the weighting factor.  $WF_{weight}$  rectifies the outcome measurement error of unequal amounts of ENMs in the product category, and  $WF_{article}$  corrects the unequal amounts of articles sold per product category (Muller, 2007). Product categories that were considered for the calculated ENMs include cosmetics, paints, coatings or cleaning agents, textiles, plastics, composites, energy production or storage, metals, as well as research and development.

### 3.7.3 Calculating ENMs entering the aquatic environment

ENMs into the aquatic environment were calculated from the domestic wastewater entering the wastewater treatment plants (WWTP) or the wastewater treatment works (WWTW). The expression used to compute these quantities included the amounts reaching the aquatic environment without passing through the WWTP as well as the run-off untreated in the WWTP, using the expression:

$$ENM_{water, input(i)} = ENM_{ww, total(i)} \cdot (1 - f_{WWTP(i)}) + ENM_{ww, total(i)} \cdot (f_{WWTP(i)} - f_{WWTP(i)} \cdot f_{removal(i)}) \quad (4)$$

where  $ENM_{water, input(i)}$  is the total ENMs of type  $(i)$  reaching the natural waters, calculated in kg/a.  $ENM_{ww, total(i)}$  is the total ENMs of type  $(i)$  released into the WWTP,  $f_{WWTP(i)}$  is the fraction of wastewater treated in WWTP from ENMs of type  $(i)$ ,  $f_{removal(i)}$  is the fraction of ENMs of type  $(i)$

removed in the WWTP. The first part of equation (4) calculates ENMs reaching the aquatic environment without passing through the WWTP, while the second part refers to ENMs that passed the WWTP untreated because of the systems inefficiency. Equation (4) was simplified to obtain the expression:

$$ENM_{water,input(i)} = ENM_{WW,total(i)} \left( 1 - f_{WWTP(i)} \cdot f_{removal(i)} \right) \quad (5)$$

The fraction of wastewater treated in WWTP ( $f_{WWTP}$ ) was quantified from the data collected from WWTW plants in Gauteng. These values were taken as the fraction of WWTW efficiencies of plants in the Gauteng Province and uniform distribution (amounts averaged) was applied in WWTW under the same water treatment body (for example; City of Johannesburg (COJ) WWTW). The WWTW efficiencies were calculated as the fraction of the actual flow volumes of influent to the total treated wastewater for each treatment works. The average of these values for all treatment works in Gauteng was taken as the probable value. To obtain the minimum and maximum values, a 5% variation was assumed. Hence, values used for  $f_{WWTP}$  were 0.96, 0.91 and 0.86 for minimum, probable and maximum efficiencies, respectively. These were considered as values for the high efficiency plants. The low efficiency plant values were considered to operate at 50% less efficiency than the high efficiency plants. Thus,  $f_{WWTP}$  for low efficiency plants were 0.46, 0.41 and 0.36 for minimum, probable and maximum efficiency values, respectively.

The fraction of removal ( $f_{removal}$ ) differs for each ENM as evident by the published values in the literature on removal efficiencies of WWTW during water treatment process. The published removal efficiencies for nTiO<sub>2</sub> range from 90 – 99.5% (Westerhoff et al., 2008; Limbach et al., 2008). For nAg, the reported removal efficiency is 90.6% (Limbach et al., 2008). CNTs have been reported to have a removal efficiency range of 96.3 – 99.7% based on its aggregation in high concentrations of dissolved organic matter (Hyung et al., 2007; Kennedy et al., 2008). The removal efficiency for nC<sub>60</sub> has been reported to be between 0 – 95%; based on the fraction remaining after coagulation, sedimentation and filtration (Westerhoff et al., 2008). Meanwhile, the mean ENM removal efficiency predicted for different treatment stages in Irish WWTP was 59.8 – 70.2% (O'Brien and Cummins, 2010). Thus, due to low operational efficiencies of WWTP in developing countries like South Africa (Musee, 2010b), the published removal efficiency ranges were averaged and reduced by 20 – 30% for all calculated ENMs in this study. Hence, the values used for  $f_{removal}$  in high efficiency plants were less by 30, 25 and 20% for minimum, probable and maximum of the averaged published values. On the other hand, the  $f_{removal}$  values for low efficiency plants were estimated to be 5% less than the high efficiency plants. The calculation of

ENMs entering the aquatic environment led to the quantification of concentrations into the WWTPs, as discussed in the next sub-subsection.

### 3.7.4 Concentrations of ENMs in the wastewater treatment plants (WWTP)

Quantifying ENMs in the WWTP provided information on risk posed to the organisms in WWTP, which might affect the efficiency - leading to the determination of quality of the released effluent into the environment. Concentrations of ENMs in the WWTP ( $C_{WWTP}$ ) were calculated as an equivalent of concentrations in the wastewater ( $C_{WW}$ ) using the expression:

$$C_{WW(i)} = C_{WWTP(i)} = \frac{ENM_{(i)WW,WWTP} \times 10^9}{WW_{percapita} \cdot f_{WWTP} \cdot POP} \quad (6)$$

where  $C_{WW(i)}$  is the concentration of ENM type  $i$  in wastewater, calculated in  $\mu\text{g/L}$ ,  $C_{WWTP(i)}$  is the concentration of ENM type  $i$  in the WWTP (in  $\mu\text{g/L}$ ),  $ENM_{(i)WW,WWTP}$  is the amount of ENM type  $i$  entering the WWTP (in kg/a),  $WW_{percapita}$  is the wastewater generated per person per year (in  $\text{m}^3\text{p}^{-1}\text{a}^{-1}$ ),  $f_{WWTP}$  is the fraction of wastewater treated in WWTP, POP is the estimated economically active population of Gauteng in 2008. The amount used for  $WW_{percapita}$  in Gauteng is  $57.5 \text{ m}^3\text{p}^{-1}\text{a}^{-1}$  acquired from personal communication. The calculation of concentrations in WWTP and ENM into the aquatic environment yielded the predicted environmental concentrations (PEC) for each ENM quantified, as discussed in the next section.

### 3.7.5 Calculation of predicted environmental concentrations in aquatic environment ( $PEC_{water}$ )

The concentrations of ENMs predicted in the aquatic environment were quantified using the expression:

$$PEC_{water(i)} = \frac{ENM_{(i)water} \times 10^9}{POP \cdot WW_{percapita} \cdot D_k} \quad (7)$$

where  $PEC_{water(i)}$  is the predicted concentration in the aquatic environment for ENM type ( $i$ ), calculated in  $\mu\text{g/L}$ ,  $D_k$  is the dilution factor. By dividing equation (7) with equation (6) and simplifying yielded:

$$PEC_{water(i)} = C_{WWTP(i)} \cdot \frac{ENM_{(i)water}}{ENM_{(i)WW,WWTP}} \cdot \frac{f_{WWTP}}{D_k} \quad (8)$$

The dilution factor ( $D_k$ ) values used were 0.75, 1, and 3; due to water scarcity in SA (Musee, 2010b). A value of 0.75 represented dry seasons in the country, while the dilution factor of 1 provides a more realistic estimation because generally Gauteng has prolonged dry periods.

### 3.7.6 Calculation of predicted environmental concentrations in the terrestrial environment ( $PEC_{soil}$ )

The concentrations of ENMs were also calculated in the terrestrial environment using the expression:

$$ENM_{soil,input(i)} = ENM_{WW,total(i)} \left\{ 1 - f_{WWTP} \cdot \left( 1 - f_{removal(i)} \right) \right\} \quad (9)$$

where  $ENM_{soil,input(i)}$  is the amount of ENM type ( $i$ ) found in the soil compartment, as contributed by releases from wastewater treatment into the sludge as a result of agricultural activities. The concentrations of each ENM in the terrestrial environment were quantified using the expression:

$$PEC_{soil} = \frac{A}{RHO_{soil} \cdot D_{soil}} \quad (10)$$

where  $A$  is the total applied sludge per year into the terrestrial environment calculated from the  $ENM_{soil,input(i)}$  values,  $RHO_{soil}$  is the bulk soil density, and  $D_{soil}$  is the agricultural mixing depth. The values used for  $RHO_{soil}$  were 1700 kg/m<sup>3</sup> and a  $D_{soil}$  value of 0.2m (ECB, 2003). Agricultural mixing percentage used was 97.4 % with the sludge application rate of 10 t/hectare/annum, which equates to 1 kgm<sup>-2</sup>a<sup>-1</sup> (Musee, 2010b).

### 3.7.7 Determination of the predicted no effect concentrations (PNEC)

The lowest toxicity value published for each ENM was used to quantify the PNEC values, using the highest risk assessment factor of 1000, as recommended by the ECB guidelines when the short-term toxicity values are used (ECB, 2003). To determine the toxicity value to be used, the preparation method during testing was taken into consideration.

In the aquatic environment, the PNEC value used for nTiO<sub>2</sub> was 0.1 µg/L derived from the published NOEC value of 0.1 mg/l for *D. magna* (Zhu et al., 2010). The value was more realistic since it represented tests performed using nTiO<sub>2</sub> in water suspension, which is a more representative of the real environment for aquatic organisms. A PNEC value of 1.0 ng/L was

used for nAg derived from the EC50 value of 1.0 µg/L for *D. magna* (Kim et al., 2010). Meanwhile, a PNEC value of 1.3 µg/L was used for CNT derived from an EC50 value of 1.306 mg/L for SWCNT on *D. magna* (Zhu et al., 2010). This value was selected as for our calculations since the lowest available EC50 value of 0.1 mg/L for DWCNT on *T. japonicus* (Kwok et al., 2010) cannot be used since ENMs were prepared using sonication process - which is not representative of the actual environmental conditions where organisms live. Also, a PNEC value of 9.34 µg/L was used for nC<sub>60</sub> derived from the EC50 of 9.34 mg/L for *D. magna* (Zhu et al., 2009). This value was selected instead of the lowest available NOEC value of 0.18 ppm for *D. magna* (Lovern and Klaper, 2006), as the latter was obtained from where the suspensions were achieved during the experiment using sonication.

On the other hand, PNEC values in the terrestrial environment were considered as PNEC<sub>soil</sub>. The used PNEC<sub>soil</sub> value for nTiO<sub>2</sub> was 80 µg/L derived from the lowest observed LC50 value of 80 mg/L on *C. elegans* shown in Table 2.1 (Wang et al., 2009). This value was selected as realistic since the test organisms were fed on the *E. coli*, which provides a normal environment. A PNEC<sub>soil</sub> value of 0.012 µg/L was used for nAg as derived from the recently published LD50 value of 12 mg/ml for *L. terrestris* shown in Table 2.2 (Lapied et al., 2010). This is the only published ecotoxicity value for nAg in the terrestrial environment. The used PNEC<sub>soil</sub> value for CNT was 37 µg/kg derived from the published EC10 value of 37 mg/kg for DWCNT observed on *E. veneta* as shown in Table 2.3 (Scott-Fordsmand et al., 2008). Meanwhile, the PNEC<sub>soil</sub> value of 1000 µg/kg was used for nC<sub>60</sub> derived from the NOEC value of 1000 mg C<sub>60</sub>/kg on the earthworm *E. veneta* shown in Table 2.4 (Scott-Fordsmand et al., 2008).

### 3.7.8 Risk quantification in aquatic environment (RQ<sub>water</sub>)

To quantify the overall risk that each ENM poses to the aquatic environment, the RQ value was calculated as a ratio of PEC to the PNEC expressed as:

$$RQ_{water} = \frac{PEC_{water}}{PNEC_{water}} \quad (11)$$

where RQ<sub>water</sub> is the risk quotient in water, PNEC<sub>water</sub> is the predicted no effect concentration quantified from the ecotoxicity values (NOEC, EC50 or LC50 values) published in the literature for each ENM on aquatic organisms and discussed in section 2.1 of chapter 2.

### 3.7.9 Risk quantification in the terrestrial environment ( $RQ_{soil}$ )

Risk posed to the terrestrial environment was then quantified as the ratio of  $PEC_{soil}$  to  $PNEC_{soil}$ , using the expression:

$$RQ_{soil} = \frac{PEC_{soil}}{PNEC_{soil}} \quad (12)$$

where  $RQ_{soil}$  is the risk quotient in soil,  $PNEC_{soil}$  is the predicted no effect concentration calculated from published ecotoxicological values of each ENMs on terrestrial organisms. To obtain the  $PNEC_{soil}$  values, the rule of available lowest toxicity values was implemented - as done for the aquatic environment, using the assessment factor of 1000.

#### 3.7.10 Interpretation of PEC/PNEC (RQ)

The ratio of PEC/PNEC for any given material is used as an indicator of risk and is called the risk quotient (ECB, 2003). Values obtained for the risk quotient (RQ) are interpreted as follows:

**RQ < 1:** no immediate concern for the material and has to be reconsidered only when new information is available.

**RQ > 1 but < 10:** The material is of concern and additional data to verify the assessment is required.

**RQ > 10:** Immediate attention needed for the material and more detailed information is required to validate the results.

**RQ > 1** and there is no additional data available, the material is of concern and immediate measures need to be taken to reduce risk.

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## Chapter 4 - **RESULTS AND DISCUSSION**

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Results derived for the model are discussed in this chapter, and ways to get the output parameters are shown successively from the calculated intermediate output parameters, as depicted in the schematic representation (Figure 8) of model parameters. The calculated output parameters yielded the estimated risks of ENMs in both aquatic and terrestrial environmental media. New developments on modelled environmental risks of ENMs have been observed in this study as opposed to the previous studies (Muller and Nowack, 2008; Gottschalck et al., 2009a; Musee, 2010b), this is because more data has been developed on ecotoxicity studies of ENMs and the increase in ENM production volumes.

### **4.1 INTERMEDIATE OUTPUT PARAMETERS**

The available global data on ENMs was allocated to the Gauteng through intermediate outputs because there was no available data for Gauteng. The input parameters represented in Figure 3.2 were used to calculate intermediate outputs for the model.

#### **4.1.1 Estimated ENM quantities for Gauteng**

The published worldwide production volumes of ENMs for the year 2008 were used as input parameters to estimate ENM quantities in Gauteng. The calculated quantities of nTiO<sub>2</sub> under the minimum, probable and maximum scenarios were 0.021, 0.201 and 5.847 t/a, respectively. These values were calculated from the global production volumes of 3000, 5000 and 60926 t/a (refer Table 3.1) which were taken as minimum, probable and maximum production values using equation 1 of the model equations.

The same input values were used to calculate quantities for the Johannesburg (JHB) city by Musee (2010b), but the output values for Gauteng were much higher than the JHB values (0.007, 0.048 and 1.289 t/a). This was because the JHB city values were calculated only for the cosmetics application while the Gauteng Province values incorporated more applications (like plastics, paints, coatings or cleaning agent, to mention just a few). In contrast, the nTiO<sub>2</sub> quantities modelled by Muller (2007) for Switzerland (35 and 400 t/a) were higher than the modelled amounts for Gauteng though the same number of product applications were considered and the same global input (probable scenario) was used. This observation is due to

the fact that the three studies compared (SW, Gauteng and JHB) are at different geographic levels; the SW study is at a country level, while the study for Gauteng is at a provincial level, and the JHB study is at the city level. Hence, comparing results observed from these studies will be difficult and misrepresenting the realistic situations represented by each study.

The calculated quantities of nAg for Gauteng were  $3.49 \times 10^{-5}$ , 0.017 and 0.054 t/a under minimum, probable and scenarios, respectively. These quantities were calculated using equation 1 of the model equations and the input global production quantities of 5, 434 and 563 t/a nAg as shown in Table 3.1. Meanwhile, CNT quantities for Gauteng were  $9.791 \times 10^{-4}$ , 0.012 and 0.041 t/a under the modelled scenarios using equation (1). On the other hand, the amounts for nC<sub>60</sub> calculated using equation 1 were  $1.049 \times 10^{-6}$ ,  $2.015 \times 10^{-4}$  and  $9.597 \times 10^{-4}$  t/a under the respective modelled scenarios. The calculated amounts of ENMs in Gauteng using equation 1 are summarised in Table 4.1.

**Table 4.1 Calculated ENM quantities for Gauteng**

<b>ENMs</b>	<b>Gauteng Min (t/a)</b>	<b>Gauteng Prob (t/a)</b>	<b>Gauteng Max (t/a)</b>
<b>nTiO<sub>2</sub></b>	0.021	0.201	5.847
<b>nAg</b>	3.497E-05	0.017	0.054
<b>CNT</b>	9.791E-04	0.012	0.041
<b>nC<sub>60</sub></b>	1.049E-06	2.015E-04	9.597E-04

The quantities for nTiO<sub>2</sub> in Gauteng were observed to be the highest among the calculated ENMs, followed by nAg, whilst nC<sub>60</sub> had the lowest quantified amounts. This trend agrees with the published quantities for SW by Gottschalk et al. (2009a). However, the survey done by Schmid and Riedeker (2008) on companies that use these ENMs contradicted this observation. Carbon black - also known as nC<sub>60</sub> - was the most abundantly used ENM that had the highest quantities (1 365 001 kg/a), followed by nTiO<sub>2</sub> (435 466 kg/a) (Schmid and Riedeker, 2008).

This introduces a high degree of variation in the worldwide published quantities of ENMs, which needs to be incurred when reporting these values. Contrary to that, it raises questions on whether the values given by companies during surveys were reliable or not. With this information upfront, the quantities of ENMs can only be reliable when declared in products and mandated by regulatory bodies to be included in the ingredient listings of products, as done with other types of products currently being traded globally.

#### 4.1.2 Quantities per product category

The calculated quantities in Gauteng were allocated to the product categories for each ENM and the amounts in each product application/category were quantified. This was based on the approach adopted by Muller (2007) and calculated using equations 2 and 3 of the model equations.

Amounts of nTiO<sub>2</sub> in Gauteng under the cosmetics, paints, coatings or cleaning agents, energy production or storage, plastics and metals applications ranged from 7.308 x 10<sup>-5</sup> to 3.530 t/a nTiO<sub>2</sub> under the calculated minimum, probable and maximum scenarios. The metals and plastics applications had the lowest amounts of nTiO<sub>2</sub> compared to other categories, with quantities ranging from 7.308 x 10<sup>-5</sup> to 0.116 t/a in the calculated scenarios. The total quantities of nTiO<sub>2</sub> in the calculated product categories were 0.021, 0.201 and 5.847 t/a nTiO<sub>2</sub> under minimum, probable and maximum scenarios, respectively (shown in Table 4.2).

The calculated quantities of nAg under the paints, cosmetics, coatings or cleaning agents, plastics, textiles, metals applications ranged from 9.398 x 10<sup>-7</sup> to 0.017 t/a nAg amounts (see Table 4.2). The paints category had the highest amounts of nAg with quantities ranging from 1.106 x 10<sup>-5</sup> to 0.017 t/a nAg, followed by the cosmetics application. The total quantities of nAg in the calculated categories were 3.497 x 10<sup>-5</sup>, 0.017 and 0.054 t/a nAg for minimum, probable and maximum scenarios, respectively.

The quantities for CNT ranged from 4.896 x 10<sup>-4</sup> to 0.020 t/a CNT in the plastics and consumer electronics applications. The total quantities calculated were 9.791 x 10<sup>-4</sup>, 1.189 x 10<sup>-2</sup> and 0.041 t/a CNT in the minimum, probable and maximum scenarios, respectively. While the amounts for nC<sub>60</sub> ranged from 3.497 x 10<sup>-7</sup> to 3.199 x 10<sup>-4</sup> t/a nC<sub>60</sub> for the calculated cosmetics, composites and research and development (R&D) applications. The total amounts of nC<sub>60</sub> were 1.049 x 10<sup>-6</sup>, 2.015 x 10<sup>-4</sup> and 9.597 x 10<sup>-4</sup> t/a in the calculated minimum, probable and maximum scenarios, respectively. Table 4.2 summarises the calculated quantities of ENMs using equation 3 under respective categories.

**Table 4.2: ENM quantities per product category in Gauteng**

<b>ENM</b>	<b>Product category</b>	<b>Min (t/a)</b>	<b>Prob (t/a)</b>	<b>Max (t/a)</b>
<b>nTiO<sub>2</sub></b>	Cosmetics	0.013	0.122	3.530
	Plastics	4.176E-04	4.010E-03	0.116
	Paints	4.942E-03	0.047	1.377
	Coatings/cleaning agents	4.454E-04	4.278E-03	0.124
	Energy production/storage	2.436E-03	0.023	0.679
	Metals	7.308E-05	7.018E-04	0.020
	<b>Totals</b>		<b>0.021</b>	<b>0.201</b>
<b>nAg</b>	Cosmetics	8.845E-06	4.424E-03	0.014
	Textiles	3.845E-06	1.923E-03	5.940E-03
	Plastics	4.201E-06	2.101E-03	6.492E-03
	Paints	1.106E-05	5.530E-03	0.017
	Coatings/cleaning agents	6.081E-06	3.041E-03	9.396E-03
	Metals	9.398E-07	4.700E-04	1.452E-03
	<b>Totals</b>		<b>3.497E-05</b>	<b>0.017</b>
<b>CNT</b>	Plastics	4.896E-04	5.944E-03	0.020
	Consumer electronics	4.896E-04	5.944E-03	0.020
	<b>Totals</b>	<b>9.791E-04</b>	<b>1.189E-02</b>	<b>0.041</b>
<b>nC<sub>60</sub></b>	Cosmetics	3.497E-07	6.716E-05	3.199E-04
	Composites	3.497E-07	6.716E-05	3.199E-04
	R&D	3.497E-07	6.716E-05	3.199E-04
	<b>Totals</b>	<b>1.049E-06</b>	<b>2.015E-04</b>	<b>9.597E-04</b>

### 4.1.3 Quantities of ENMs into the aquatic environment

The quantities of ENMs released into the aquatic environment were calculated using equation 5. The calculated ENM quantities in Gauteng were taken as the total volumes of each ENM that goes into wastewater from different nanoproducts, these were the  $ENM_{ww,total(i)}$  values in equation 5. Data was collected from WWTP to determine the treatment works efficiencies. The collected data from WWTP was aggregated according to treatment works that fall under three separate metropolitan municipalities in Gauteng – namely: City of Tshwane, City of Johannesburg and Ekurhuleni. The efficiencies of WWTP were quantified as the  $f_{WWTP}$  values (fraction of wastewater treated in WWTP) for equation 5. These were calculated as the fraction of the total treated volumes of influent, to the actual volumes into each plant per day. The totals for treatment works were averaged and varied by 5% to obtain the minimum, probable and maximum values of  $f_{WWTP}$  used for the model. The  $f_{WWTP}$  values calculated were 0.96, 0.91 and 0.86 for minimum, probable and maximum scenarios, respectively.

The fraction of removal for each ENM during water treatment was quantified from the published values in literature. These represented the  $f_{removal}$  values of high efficiency plants for equation 5 according to the process discussed in chapter 3. The values used for  $f_{removal}$  were 0.65, 0.70 and 0.75 under the respective minimum, probable and maximum scenarios for the removal of nTiO<sub>2</sub> in the WWTW of Gauteng. The  $f_{removal}$  values used for nAg were 0.61, 0.66 and 0.71; while for CNT and nC<sub>60</sub> the values were 0.68, 0.73, 0.78 and 0.18, 0.23, 0.28 under minimum, probable and maximum scenarios, respectively. The  $f_{removal}$  values for low efficiency plants were estimated to be 5% less than the high efficiency plants.

The quantities of ENMs entering the WWTP were calculated as the product of total ENMs into wastewater, and the fraction of wastewater treated in WWTP. While the quantities of ENMs removed as sludge were calculated as the product of total ENMs into wastewater, the fraction of wastewater treated in WWTP and the fraction of removal for each ENM. The quantities of ENMs released into effluent were calculated as ENMs entering the WWTP without the amounts that are removed as effluent. The quantities in untreated wastewater were calculated as the total ENMs into wastewater without the amounts entering the WWTP. Consequently, the quantities of ENMs reaching the aquatic environment were calculated as the sum of ENM amounts untreated but released in effluent and the amounts in untreated wastewater. The summary of these calculated values for each ENM is shown in Table 4.3.

**Table 4.3 Calculated quantities of nTiO<sub>2</sub>, nAg, CNT and nC<sub>60</sub> into the aquatic environment for the high efficiency plants in Gauteng**

ENM	Variable	Min	Prob	Max
<b>nTiO<sub>2</sub></b>	nTiO <sub>2 total</sub> : into waste water (WW in kg/a)	20.981	201.487	5847.077
	<i>f</i> <sub>WWTP</sub> : fraction of WW treated in WWTP	0.960	0.910	0.860
	<i>f</i> <sub>removal</sub> : fraction removed in WWTP	0.748	0.698	0.648
	nTiO <sub>2 WWTP</sub> : TiO <sub>2</sub> entering the WWTPs (kg/a)	20.142	183.353	5028.486
	nTiO <sub>2 WWTP,removed</sub> (sludge kg/a)	15.056	127.889	3255.945
	nTiO <sub>2 WWTP,unremoved</sub> (effluent kg/a)	5.086	55.464	1772.541
	nTiO <sub>2 untreated</sub> : in untreated WW (kg/a)	0.839	18.134	818.591
nTiO <sub>2 water</sub> : amount into aquatic environment (kg/a)	5.925	73.598	2591.132	
<b>nAg</b>	nAg <sub>total</sub> : into waste water (WW in kg/a)	0.035	17.489	54.031
	<i>f</i> <sub>WWTP</sub> : fraction of WW treated in WWTP	0.960	0.910	0.860
	<i>f</i> <sub>removal</sub> : fraction removed in WWTP	0.706	0.656	0.606
	nAg <sub>WWTP</sub> : TiO <sub>2</sub> entering the WWTPs (kg/a)	0.034	15.915	46.467
	nAg <sub>WWTP,removed</sub> (sludge kg/a)	0.024	10.440	28.159
	nAg <sub>WWTP,unremoved</sub> (effluent kg/a)	0.010	5.475	18.308
	nAg <sub>untreated</sub> : in untreated WW (kg/a)	0.001	1.574	7.564
nAg <sub>water</sub> : amount into aquatic environment (kg/a)	0.011	7.049	25.872	
<b>CNT</b>	CNT <sub>total</sub> : into waste water (WW in kg/a)	0.979	11.888	40.883
	<i>f</i> <sub>WWTP</sub> : fraction of WW treated in WWTP	0.960	0.910	0.860
	<i>f</i> <sub>removal</sub> : fraction removed in WWTP	0.780	0.730	0.680
	CNT <sub>WWTP</sub> : TiO <sub>2</sub> entering the WWTPs (kg/a)	0.940	10.818	35.160
	CNT <sub>WWTP,removed</sub> (sludge kg/a)	0.733	7.897	23.909
	CNT <sub>WWTP,unremoved</sub> (effluent kg/a)	0.207	2.921	11.251
	CNT <sub>untreated</sub> : in untreated WW (kg/a)	0.039	1.070	5.724
CNT <sub>water</sub> : amount into aquatic environment (kg/a)	0.246	3.991	16.975	
<b>nC<sub>60</sub></b>	nC <sub>60 total</sub> : into waste water (WW in kg/a)	1.049E-03	0.201	0.960
	<i>f</i> <sub>WWTP</sub> : fraction of WW treated in WWTP	0.960	0.910	0.860
	<i>f</i> <sub>removal</sub> : fraction removed in WWTP	0.175	0.225	0.175
	nC <sub>60 WWTP</sub> : TiO <sub>2</sub> entering the WWTPs (kg/a)	1.007E-03	0.183	0.175
	nC <sub>60 WWTP,removed</sub> (sludge kg/a)	1.762E-04	0.041	0.144
	nC <sub>60 WWTP,unremoved</sub> (effluent kg/a)	8.309E-04	0.142	0.031
	nC <sub>60 untreated</sub> : in untreated WW (kg/a)	4.196E-05	0.018	0.785
nC <sub>60 water</sub> : amount into aquatic environment (kg/a)	8.728E-04	0.160	0.815	

#### 4.1.4 Quantities of ENMs in wastewater treatment plants ( $C_{WWTP}$ )

The concentrations of ENMs in sewage treatment plants were calculated using equation 6. of the model. The concentration of each ENM in wastewater ( $C_{ww(i)}$ ) was equated to the concentration of each ENM in sewage treatment plant ( $C_{WWTP(i)}$ ). The wastewater per capita value ( $WW_{percapita}$ ) used acquired from personal communication was  $57.5 \text{ m}^3\text{p}^{-1}\text{a}^{-1}$ , calculated from a value of 150 L/day for Gauteng. The population of Gauteng (POP) in year 2008 was 10.4 million (StatsSA, 2009), this was taken as the probable scenario value. The previous year's population estimates (2007 population) were taken as the minimum and the preceding year's population estimate (2009 population) was taken as the maximum scenario.

The calculated concentrations of ENMs in WWTP for high efficiency plants ranged from  $1.622 \times 10^{-6}$  to  $5.001 \mu\text{g/L}$  under the minimum, probable and maximum scenarios. The concentrations in low efficiency plants were higher than those in high efficiency plants. Table 4.4 summarises the calculated ENM concentrations in WWTP for low and high efficiency plants.

**Table 4.4: Calculated concentrations of ENMs in WWTP ( $C_{WWTP}$ )**

ENM	High Efficiency Plants			Low Efficiency Plant		
	Min ( $\mu\text{g/L}$ )	Prob( $\mu\text{g/L}$ )	Max( $\mu\text{g/L}$ )	Min( $\mu\text{g/L}$ )	Prob( $\mu\text{g/L}$ )	Max( $\mu\text{g/L}$ )
nTiO <sub>2</sub>	0.011	0.133	5.001	0.034	0.289	8.472
nAg	2.094E-05	0.013	0.050	5.814E-05	0.026	0.080
CNT	4.571E-04	7.216E-03	0.033	1.547E-03	0.017	0.058
nC <sub>60</sub>	1.622E-06	2.897E-04	1.574E-03	2.247E-06	3.738E-04	1.72E-03

These results show that quantities of ENMs untreated in the treatment plants accumulated in the sludge as shown by the high values of  $C_{WWTP}$  in the low efficiency plants. Thus, if the WWTP are efficient for the treatment of ENMs there should be an effective treatment of the sludge as well so to minimise accumulation in the terrestrial environment.

## 4.2 MODEL OUTPUT PARAMETERS

The output parameters for the model were derived from the calculated intermediate output parameters. These constituted the predicted environmental concentration (PEC) values in both aquatic and terrestrial environments, and consequently the risk quotient (RQ) values were quantified.

#### 4.2.1 Predicted environmental concentrations (PEC) of ENMs

In the aquatic environment, the calculated PEC values were varied according to three dilution factor values ( $D_k$ ). Considering the water scarcity situation in SA, the PEC values in aquatic environment for Gauteng were calculated using  $D_k$  values of 0.75, 1 and 3. The dilution factor of 0.75 represented a situation where evaporation was considered in the environment. On the other hand, the dilution of  $D_k = 1$  was considered as the most realistic condition for the Gauteng and the dilution of  $D_k = 3$  represented rainy seasons of the province.

**Table 4.5: Calculated  $PEC_{\text{water}}$  values for ENMs under the dilution factors  $D_k = 0.75, 1$  and  $3$  in high and low efficiency plants**

Dilution factor	ENM	High Efficiency			Low Efficiency		
		Min( $\mu\text{g/L}$ )	Prob( $\mu\text{g/L}$ )	Max( $\mu\text{g/L}$ )	Min( $\mu\text{g/L}$ )	Prob( $\mu\text{g/L}$ )	Max( $\mu\text{g/L}$ )
$D_k = 0.75$	nTiO <sub>2</sub>	0.014	0.164	5.722	0.044	0.356	9.693
	nAg	2.694E-05	0.016	0.057	7.478E-05	0.032	0.091
	CNT	5.880E-04	0.009	0.037	1.990E-03	2.058E-02	6.667E-02
	nC <sub>60</sub>	2.087E-06	3.573E-04	1.800E-03	2.890E-06	4.609E-04	1.968E-03
$D_k = 1$	nTiO <sub>2</sub>	0.011	0.123	4.292	0.033	0.267	7.270
	nAg	2.020E-05	0.012	0.043	5.609E-05	0.024	0.069
	CNT	4.410E-04	6.673E-03	0.028	1.493E-03	0.015	0.050
	nC <sub>60</sub>	1.565E-06	2.679E-04	1.350E-03	2.168E-06	3.457E-04	1.476E-03
$D_k = 3$	nTiO <sub>2</sub>	3.541E-03	0.041	1.431	0.011	0.089	2.423
	nAg	6.734E-06	3.929E-03	0.014	1.870E-05	7.927E-03	0.023
	CNT	1.470E-04	2.224E-03	0.009	4.976E-04	5.146E-03	0.017
	nC <sub>60</sub>	5.216E-07	8.932E-05	4.501E-04	7.226E-07	1.152E-04	4.919E-04

The calculated PEC values of ENMs in aquatic environment ( $PEC_{\text{water}}$ ) under the  $D_k$  value of 0.75 ranged from  $2.087 \times 10^{-6}$  to  $5.722 \mu\text{g/L}$  under the calculated minimum, probable and maximum scenarios. These values represent the ENM concentrations in aquatic environment during dry seasons in Gauteng where there is estimated evaporation. Under the realistic dilution factor of  $D_k = 1$ , the calculated  $PEC_{\text{water}}$  values of ENMs ranged from  $1.565 \times 10^{-6}$  to  $4.292 \mu\text{g/L}$  under the calculated scenarios. Whereas, when considering the dilution factor of  $D_k = 3$ , the  $PEC_{\text{water}}$  values ranged from  $5.216 \times 10^{-7}$  to  $1.431 \mu\text{g/L}$  under the calculated minimum, probable and maximum scenarios. These were the values for high efficiency plants. A summary of  $PEC_{\text{water}(f)}$  values under the  $D_k$  values of 0.75, 1 and 3 in high and low efficiency plants is shown in Table 4.5.

The PEC values were also calculated for the terrestrial environment as  $PEC_{soil}$  using equation 11. The calculated values for  $PEC_{soil}$  were quantified from ENMs released into sludge and the amounts of run-offs were not considered. 97.4 % of the sludge is used for agricultural purposes applied at a rate of  $1 \text{ kgm}^{-2}\text{a}^{-1}$  were used for the ENM sludge amounts (Synman and Herselman, 2006). The calculated  $PEC_{soil}$  values ranged from  $3.967 \times 10^{-9}$  to  $0.033 \text{ }\mu\text{g}/\text{kg}$  under the calculated minimum, probable and maximum scenarios. It should be noted that values for the terrestrial environment did not have efficiency variations as the aquatic environment. Table 4.6 summarises the calculated  $PEC_{soil}$  values for each ENM.

**Table 4.6: Calculated  $PEC_{soil}$  values for ENMs in Gauteng (values in  $\mu\text{g}/\text{kg}$ )**

ENM	Min	Prob	Max
nTiO <sub>2</sub>	2.428E-05	6.286E-04	3.306E-02
NAg	4.712E-08	6.205E-05	3.415E-04
CNT	9.873E-07	3.310E-05	2.099E-04
nC <sub>60</sub>	3.967E-09	1.610E-06	1.270E-05

**Table 4.7: Comparison of the PEC values calculated for JHB, Gauteng and SW in the aquatic environment**

ENM	<u>JHB<sup>a</sup> (Musee, 2010b)</u>		<u>Gauteng<sup>a</sup> (this study)</u>		<u>SW<sup>b</sup> (Muller and Nowack, 2008)</u>	
	Prob ( $\mu\text{g}/\text{L}$ )	Max ( $\mu\text{g}/\text{L}$ )	Prob ( $\mu\text{g}/\text{L}$ )	Max ( $\mu\text{g}/\text{L}$ )	RE ( $\mu\text{g}/\text{L}$ )	HE ( $\mu\text{g}/\text{L}$ )
nTiO <sub>2</sub>	0.041	0.270	0.123	4.292	0.7	16
NAg	0.043	0.620	0.012	0.043	0.03	0.08
CNT	nc <sup>c</sup>	nc	0.007	0.028	0.0005	0.0008
nC <sub>60</sub>	nc	nc	2.679E-04	1.350E-03	nc	Nc

<sup>a</sup>Prob: probable scenario, Max: maximum scenario; <sup>b</sup>RE: realistic scenario, HE: high emission scenario; <sup>c</sup>nc: not calculated

The calculated PEC values in both aquatic and terrestrial environments for Gauteng were higher than the values calculated for JHB city (Musee, 2010b), since the latter were calculated for the cosmetics application only. Also, calculations for JHB were at city level compared to the Gauteng values at provincial level. Comparison of  $PEC_{water}$  values for Gauteng to the SW values (Muller

and Nowack, 2008) has been done for the realistic values in Gauteng (at  $D_k = 1$ ) under probable and maximum scenarios. The  $PEC_{\text{water}}$  values for nTiO<sub>2</sub> in SW were much higher (0.7 and 16 µg/L for realistic – RE and high emission – HE scenarios, respectively) than the values calculated for the high efficiency plants in Gauteng (0.123 and 4.292 µg/L for the probable and maximum scenarios, respectively). A comparison of the  $PEC_{\text{water}}$  values for JHB, Gauteng and SW is illustrated in Table 4.7.

#### 4.2.2 Estimated risk quotient (RQ) values

The risk quotient (RQ) values were calculated as the ratio of PEC/PNEC, with PNEC values quantified from published ecotoxicological values of ENMs tested on aquatic and terrestrial organisms. The process followed to calculate the PNEC values used for each ENM in both environmental media has been discussed in chapter 3.

In the aquatic environment, the recently published low ecotoxicological values of ENMs tested in organisms resulted in low PNEC values; this yielded high estimated RQ values. The toxicity values tested for nTiO<sub>2</sub> and nAg on *D. magna* (Kim et al., 2010; Zhu et al., 2010) resulted in the calculated PNEC values of 0.1 and 0.001 µg/L for these ENMs, respectively. These values were not used in previous estimations of RQ values (Muller, 2007; Gottschalck et al., 2009a, Musee, 2010b) as they were not yet published. Thus, the estimated RQ values in the aquatic environment ( $RQ_{\text{water}}$ ) for this study showed high risks for these ENMs.

The estimated  $RQ_{\text{water}}$  values for nTiO<sub>2</sub> under both high efficiency and low efficiency plants were mostly > 1. When considering dry seasons of the Gauteng (where the  $D_k$  value was estimated at 1 and 0.75), the  $RQ_{\text{water}}$  values were > 10 under the maximum scenario for both the high and low efficiency plants. This was also observed during the rainy seasons in the province (when  $D_k$  was estimated at 3). The estimated risks for nAg also showed  $RQ_{\text{water}}$  values that were > 1 for the probable and maximum scenarios. The RQ values that were observed to be > 1 mean that the ENMs are of concern and more data to verify the results is required, whereas the RQ values that are > 10 mean that the ENMs are of immediate concern and more detailed information is required to validate the assessment. Table 4.8 gives a summary of the estimated  $RQ_{\text{water}}$  values of ENMs for high and low efficiency plants.

The  $RQ_{\text{water}}$  values for low efficiency plants in Gauteng were observed to be higher than the high efficiency plants. This means that water treated at low efficiency operating plants is likely to be

at higher risk of ENMs than water treated at high efficiency plants. These results imply that potentially more environmental risk of ENMs will be in low efficiency operating plants.

**Table 4.8: Estimated risk quotient (RQ<sub>water</sub>) values for ENMs in Gauteng**

Dilution factor	ENM	<u>High Efficiency</u>			<u>Low Efficiency</u>		
		Min	Prob	Max	Min	Prob	Max
$D_k = 0.75$	nTiO <sub>2</sub>	0.14	1.64	57.22	0.44	3.56	96.93
	nAg	0.03	15.72	57.14	0.07	31.71	91.44
	CNT	6.30E-05	9.53E-04	4.01E-03	2.13E-04	2.20E-03	7.14E-03
	nC <sub>60</sub>	2.23E-07	3.83E-05	1.93E-04	3.09E-07	4.93E-05	2.11E-04
$D_k = 1$	nTiO <sub>2</sub>	0.11	1.23	42.92	0.33	2.67	72.70
	nAg	0.02	11.79	42.85	0.06	23.78	68.58
	CNT	3.39E-04	5.13E-03	0.02	1.15E-03	0.01	0.04
	nC <sub>60</sub>	1.68E-07	2.87E-05	1.45E-04	2.32E-07	3.70E-05	1.58E-04
$D_k = 3$	nTiO <sub>2</sub>	0.04	0.41	14.31	0.11	0.89	24.23
	nAg	6.73E-03	3.93	14.28	0.02	7.93	22.86
	CNT	1.13E-04	1.71E-03	7.21E-03	3.83E-04	3.96E-03	0.01
	nC <sub>60</sub>	5.58E-08	9.56E-06	4.82E-05	7.74E-08	1.23E-05	5.27E-05

To date, studies that have modelled risks of ENMs in the environment have not shown that nAg pose risk, according to the RQ values that have been calculated. This is the first study to calculate RQ values > 10 for ENMs, and the RQ values for nAg calculated as > 1. These observations are due to the increased ENM quantities from production and use, resulting in high concentrations of ENMs that are potentially released into the environment. Moreover, the ecotoxicological tests yield high toxicities of ENMs for organisms in the aquatic environment. A comparison of the calculated RQ<sub>water</sub> values for JHB city, Gauteng and SW is shown in Table 4.9. Results shown for Gauteng were obtained when considering the most realistic scenario under high efficiency plants.

**Table 4.9: Comparison of the RQ<sub>water</sub> values calculated for JHB, Gauteng and SW**

ENM	<u>JHB<sup>a</sup> (Musee, 2010b)</u>		<u>Gauteng<sup>a</sup> (this study)</u>		<u>SW<sup>b</sup> (Muller and Nowack, 2008)</u>	
	Prob	Max	Prob	Max	RE	HE
nTiO <sub>2</sub>	0.041	2.69	1.23	42.92	> 0.7	> 16
nAg	0.043	0.620	11.79	42.85	0.80E-03	0.20E-02
CNT	nc <sup>c</sup>	nc	5.13E-03	0.02	0.50E-02	0.80E-02
nC <sub>60</sub>	nc	nc	2.879E-05	1.450E-04	nc	nc

<sup>a</sup>Prob: probable scenario, Max: maximum scenario; <sup>b</sup>RE: realistic scenario, HE: high emission scenario; <sup>c</sup>nc: not calculated

**Table 4.10: Estimated RQ<sub>soil</sub> values for the ENMs in JHB, Gauteng and SW**

ENM	<u>JHB<sup>a</sup> (Musee, 2010b)</u>		<u>Gauteng<sup>a</sup> (this study)</u>		<u>SW<sup>b</sup> (Muller and Nowack, 2008)</u>	
	Prob	Max	Prob	Max	RE	HE
nTiO <sub>2</sub>	4.03E-08	2.72E-04	7.86E-06	4.13E-04	nc	nc
nAg	nc <sup>c</sup>	nc	5.17E-03	2.85E-02	nc	nc
CNT	nc	nc	8.95E-07	5.67E-06	nc	nc
nC <sub>60</sub>	nc	nc	1.61E-09	1.27E-08	nc	nc

<sup>a</sup>Prob: probable scenario, Max: maximum scenario; <sup>b</sup>RE: realistic scenario, HE: high emission scenario; <sup>c</sup>nc: not calculated

The calculated RQ values in the terrestrial environment (RQ<sub>soil</sub>) have not shown any risk concerns for all studied ENMs in this environmental media. This is because the calculated RQ<sub>soil</sub> values were all < 1. The previous studies that modelled the risk of ENMs did not determine the RQ<sub>soil</sub> values for nAg, CNT and nC<sub>60</sub>. This was due to the lack of ecotoxicity data for these ENMs in the terrestrial environment. The current study determined these RQ<sub>soil</sub> values for the first time, and the ENMs showed no risk concerns in this environmental median at present as the values were all < 1. Table 4.10 shows a comparison of estimated RQ<sub>soil</sub> values for JHB, Gauteng and SW.

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## Chapter 5 - CONCLUSIONS AND RECOMMENDATIONS

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The nanoproducts inventories have shown increasing trends in manufacturing, application and use of materials and products with novel nanoscale properties. Currently, the inventories show up to 1015 listed nanoproducts though the numbers are not comprehensive, and are deemed under estimated as many companies do not declare their products as containing ENMs. This has raised concerns on the releases of these novel products and materials thereof into the environment given their potential adverse effects on the ecosystems are largely unknown. A few studies have shown direct evidence of ENMs released into different environmental media. However, data to quantify their risks into these environments is lacking.

Several ecotoxicological studies have reported the toxicity of ENMs to various organisms in the aquatic and terrestrial environmental media. Recent studies on ecotoxicity show very high toxicities of ENMs in the aquatic environment. These have resulted in low PNEC values of ENMs as summarized in this study. The lowest ecotoxicity NOEC value published for nTiO<sub>2</sub> to date is 0.1 mg/L where *D. magna* was the test organism. This yielded the calculated PNEC value of 0.1 µg/L for nTiO<sub>2</sub> in the aquatic environment. Meanwhile, studies on toxicities of most ENMs in the terrestrial environment are still lacking in the scientific literature.

The risk posed to the environment has been modelled in the aquatic, terrestrial, air and sediments environments by calculating the predicted environmental concentration (PEC) values of ENMs. Among these studies, nTiO<sub>2</sub> has been shown to pose the highest degree of risk to the aquatic environment since the calculated risk quotient (RQ) values were mostly > 1. On the other hand, other ENMs have not been shown to pose any risk to the modelled environmental media as the estimated RQ values were << 1.

The calculated PEC values of ENMs in this study ranged from  $5.216 \times 10^{-7}$  to  $5.722 \mu\text{g/L}$ , these were for the aquatic environment under high efficiency operating plants. These values were calculated considering the dilution factor ( $D_k$ ) values of 0.75, 1 and 3 taking into consideration water scarcity conditions in South Africa. The dilution factor of  $D_k = 1$  was considered as more realistic, while the dilution of  $D_k = 3$  was estimated for rainy seasons in the country. The dry seasons were estimated to have a dilution factor of  $D_k = 0.75$  occasioned by high evaporation during the dry season. In the terrestrial environment, the calculated PEC values ranged from  $3.967 \times 10^{-9}$  to  $3.30 \times 10^{-2} \mu\text{g/kg}$ . The ratio of PEC to the PNEC values was used to estimate the risk (RQ) that ENMs are likely to pose to the environment. Because the recently published

ecotoxicity values for ENMs in the aquatic environment were very low, the estimated PNEC values in this study were low as well. This resulted to the calculated high  $RQ_{\text{water}}$  values which suggested high risk of ENMs in the aquatic environment. In summary, the study has shown how the risk posed by ENMs in the aquatic and terrestrial environment is depended on the quantities released into the environment, the efficiency of the treatment plants, the reported toxicity values, and the dilution factor given the variance of the precipitation in different regions.

This is the first study to show that nAg pose risk to the aquatic environment achieved by estimating the risk quotient value greater than 1 in the aquatic environment ( $RQ_{\text{water}} > 1$ ). The calculated RQ values under the probable and maximum scenarios were  $> 1$  when considering a realistic dilution factor of  $D_k = 1$ . During dry seasons, when the dilution was estimated at  $D_k = 0.75$  elevated risk levels were observed as the  $RQ_{\text{water}}$  values were  $> 10$ . The finding suggests that nAg should also be added to the list of ENMs which raises concern (based on previous results of Muller and Nowack (2008) and Musee, 2010b) with particular reference to the aquatic environment. It also reinforces the need for the development of measures of deriving more standardized ecotoxicological data for the ENMs to validate the findings of this study.

The current study also estimated, for the first time, the risk quotient values greater than 10 for nTiO<sub>2</sub> in the aquatic environment ( $RQ_{\text{water}} > 10$ ), under the modelled probable and maximum scenarios taking the realistic dilution factors in the Gauteng region. This means that the ENM is of immediate concern and more data to validate the results is needed. Hence, decisions on which additional data is needed for these ENMs are required to verify the assessment. Findings have predicted higher risks for regions with water scarcity, where there is little or no dilution of ENMs (with  $D_k$  values estimated at 0.75 and 1) after release into the environment.

The risks observed in this study could lead to the potential inefficiency of wastewater treatment facilities by inhibiting the bacteria responsible for waste treatment, given most of the ENMs exhibit high antibacterial properties. This could lead to poor quality of drinking water and harm to aquatic organisms.

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## RESEARCH OUTPUTS

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### Conference papers

Musee, N., **Nota, N.**, Prediction of engineered nanomaterials environmental exposure from cosmetic nanoproducts using simple empirical models. Paper presented at the Annual Conference hosted by Society of Cosmetic Chemists South Africa, Midrand, Johannesburg, South Africa, September 2–3, 2009 (*oral presentation*).

**Nota, N.**, Musee, N., Aldrich, C. Estimation of Carbon Nanotubes and Fullerenes environmental exposure risk in water: a case of Gauteng Province, South Africa. Nanosciences Young Researcher Symposium (NYRS)-Gauteng, University of Johannesburg-Doorfontein Campus, Johannesburg, South Africa, 07<sup>th</sup> May 2010 (*oral presentation*).

**Nota, N.**, Musee, N., Aldrich, C. Estimation of Titanium dioxide and Silver nanoparticles environmental exposure risks in water: a case of Gauteng Province. CSIR 2<sup>nd</sup> Conference, Pretoria, South Africa, 30-31, August 2010 (*poster presentation*).

**Nota, N.**, Musee, N., Aldrich, C. Estimated Risk Profiles of Titanium Dioxide and Carbon Nanotubes Engineered Nanomaterials in the Gauteng Province Environment. Nanosciences Young Researcher Symposium (NYRS)-Western Cape, University of Western Cape, Cape Town, South Africa, 17<sup>th</sup> September 2010 (*invited student presentation*).

### Journal articles

Musee, N., Thwala, M., **Nota, N.**, The antibacterial effects of engineered nanomaterials: implications to wastewater treatment plants, *Journal of Environmental Monitoring*, 2011 (*under review*)

**Nota, N.**, Musee, N., Aldrich, C. Modelled environmental risks of engineered nanomaterials in Gauteng. (*under preparation*)

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## APPENDIX A

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Current nomenclature as approved by ISO, OECD, REACH and BSI (Clift et al., 2009).

<b>Terminology</b>	<b>Abbreviation/Acronym</b>	<b>Definition</b>
Nano-	-	The term 'nano', is derived from the Greek prefix 'dwarf', referring to very small or microscopic. Used as a prefix it signifies $10^{-9}$
Nanotechnology	-	The application of scientific knowledge to control and utilize matter at the nanoscale, where size related properties and phenomena can emerge
Nanometre	nm	A measurement of size that is equal to one-billionth (0.000 000 001) of a metre ( $10^{-9}$ ).
Nanoscale	-	Size range from approximately 1nm to 100nm
Nano-structure	-	Possessing a structure comprising contiguous elements with one or more dimension in the nanoscale but excluding any primary atomic or molecular structure
Primary nano-structure	-	First level of ordered structuring of matter above disorder
Secondary nano-structure	-	Second level of ordered structuring of matter above disorder

Nanomaterial(s)	NM(s)	A material that has one or more external dimensions in the nanoscale or which is Nanostructured
Nano-effect	-	An effect that is caused via the use of a nanomaterial or nanostructure
Nano-object(s)	NO(s)	A material with one, two or three external dimensions in the nanoscale
Nanoparticle(s)	NP(s)	Nano-object with all three external dimensions in the nanoscale

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## APPENDIX B

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### Questionnaire details – Municipal Wastewater Treatment and Services

- Information regarding the location of the WWTW should include the
  - name of the facility,
  - the municipal area, area name,
  - GPS coordinates and
  - the catchment area code (according to DWAF)
  
- Information regarding the area served by this facility includes detailed description of
  - domestic and industrial areas that is equipped with formal waste collection infrastructure as well as
  - the catchment area in which the different housing and industrial areas reside (may not be the same as the WWTW)
  - known sources of informal waste contributions to this system
  - Information to help classify domestic areas in terms of the prevailing socio-economic situation
  
- The “type of waste” information must include a
  - Break-down of the domestic inflow volume and industrial inflow volumes
  - Include specific industries that release effluent into the specific system (if possible) and also
  - if these (or some of these) industries do any treatment of their own waste
  
- Capacity and actual flows (loads).
  - It is important to know the WWTW capacity and actual operating levels (actual volumes being treated) as this gives a good indication of the efficiency of the treatment system
  
- Technology – different WWTW use different types of technology to treat the waste water. The technology may affect the overall distribution of a particular pollutant in any specific treatment works
  - Primary treatment
    - Primary settling tanks or buffer (storage dams)
    - Oxidation ponds
    - Biological trickling filters
    - Activated sludge plants
  - Secondary treatment
    - Secondary settling tanks
    - Maturation ponds
  - Tertiary treatment and release
    - Chlorination
    - Wetland polishing
    - Release to surface water
  
- Cost of recent upgrades (since 1990) is important as it shows the steady increase in pollution for treatment in the WWTW.
  - Question the reason for upgrading

- Only to increase the capacity of the plant or
    - Change in technology required due to poor performance
  - Nature of sludge and disposal – It is very important to have detailed information of the sludge generation and disposal practises for a specific WWTW
    - Where does the primary sludge go?
    - Where does the secondary sludge (humus) go
    - Facility may use drying beds, anaerobic digestion or both
  - Final treated sludge must be disposed of
    - On site incineration?
    - Landfill?
    - Other
      - If land filled, provide more detail –
        - where,
        - method,
        - site location and name,
        - site is the responsibility of ?
        - site is constructed to handle leachates or not?
      - If leachate is captured – what treatment follows?
      - If other methods are used (like fertiliser production) provide full details
      - If incineration – provide full details
        - equipment,
        - procedures,
        - responsible entity
        - etc.
  - Treated water discharge – The treated water is generally discharged to a nearby river.
    - Full details regarding this river,
    - discharge point
    - discharge volumes
    - catchment area of discharge point
  - Downstream concerns (human contact through abstraction, recreational activities, direct use, irrigation of crops) must be identified to establish the potential risk of pollution to downstream water users,
    - Direct abstraction for potable water purification
    - Direct use by population
    - Direct contact by recreational users
- Indirect contact when water is used for agricultural irrigation