Recycling and Disposal of Carbon Nanotubes

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ABSTRACTS AT CONFERENCES ARISING FROM THIS STUDY

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ABSTRACT

Nanoparticles may be released throughout the lifecycle of products. Information on the handling, treatment, toxicity and mobility of carbon nanotubes (CNTs) is currently minimal. The objectives of the study were to determine the practices employed by nano-organisations for recycling and disposal of CNTs, identify quality management tools to aid responsible development and observe the behaviour, toxicity and leaching potential of CNTs. These objectives would support the development of a strategy for managing the potential environmental risks associated with the recycling and disposal of CNT.

A mixed method tactic was adopted for this study. In addition to validated protocols based on OECD guidelines for validity and reliability in experimental work, the validity of the review was achieved by obtaining expert knowledge through scholarly articles and the internal consistency. While the reliability of the review was achieved by monitoring the repetitive information from literature sources and Cronbach alpha.

Respondents participating in the survey indicated that there are differing practices for the recycling and disposal of engineered materials (ENMs). The toxicity studies revealed that while the nano-clay had a lethal response to earthworms during the pilot toxicity study, the main toxicity study showed that carbon nanotubes did not have a lethal response to earthworms in soil. However, worms were observed to be highly sensitised to increased concentrations of carbon nanotubes. The leaching in a soil column test showed that the movement of carbon nanotubes was inhibited with the largest portion of carbon nanotubes remaining on the surface and in the topmost layers of the soil.

Results pointed to contradicting views with regards to environmental risks amongst people working in the field of nanotechnology. For this reason a continued precautionary approach is suggested until standardised legislation has been enforced for nanotechnology.

Future work requires a more detailed understanding of the fate, behaviour, uptake and distribution of CNT in the environment. This can be achieved through the sharing of knowledge between nano-facilities.

Key words: Nanotechnology, nanomaterials, carbon nanotubes, toxicity, leaching

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ACRONYMS AND ABBREVIATIONS

AIDS	Acute Immune Deficiency Syndrome
BSI	British Standard Institution
CCl_2	Calcium Chloride
CNTs	Carbon Nanotubes
COA	Certificate of Assurance
DDT	Organic Chlorine Pesticide
DUT	Durban University of Technology
EC	European Commission
ENMs	Engineered nanomaterials
EPA	Environmental Protection Agency
EU	European Union
FDA	Food and Drug Agency/Administration
FIFRA	Federal Insecticide and Fungicide and Rodenticide Act
GHS	Global harmonised system
GLP	Good Laboratory Practices
HIV	Human Immunodeficiency Virus
MDGs	Millennium Development Goals
MSDS	Material Safety Data Sheet
MWCNT	Multi-walled Carbon Nanotube
Nano TiO_2	Nano titanium
NIOSH	National Institute for Occupational Safety and Health
NNI	National Nanotechnology Initiative
nZVi	Nano-sized zero- valent iron
OECD	Organization for Economic Cooperation and Development
PAS	Publicly Available Specification

PDCA	Plan-Do-Check-Act
PELS	Permissible Exposure Limits
PPE	Personal Protective Equipment
PVC	Poly Vinyl Chloride
RCRA	Resource Conservation and Recovery Act
RELS	Recommended Exposure Limits
SWCNT	Single-walled Carbon Nanotube
TOC	Total Organic Carbon
TQM	Total Quality Management
TSCA	Toxic Substances Control Act
UK	United Kingdom
US	United States of America
WHS	Work Health and Safety Regulations
ZnO	Zinc Oxide nanoparticles

CHAPTER ONE: INTRODUCTION

1.1 Overview of the study

The versatility and wide scope of the applicability of nanotechnology allow it to be beneficial in a number of sectors with the increased possibility of scientific and technological advancements. Van Dooren (2011) suggests that this is due to the technology's unique properties being used to enhance specific functions of products. Manzetti and Andersen (2013) pronounced that carbon nanotube (CNT) products are being introduced into various sectors at an alarming rate. As the extensive uses of these ENMs continue to grow at a substantial rate, the potential for exposure to humans and the environment becomes a reality.

Bystrzejewska-Piotrowska, Golimowski and Urban (2009) acknowledged that with the many benefits of nanotechnology there are also side effects as their novel characteristics can be harmful. One such effect is that this technology creates a new type of waste and it is, currently still unclear what types of handling and treatment methods will be effective for nano-waste management. This is concerning as nanoparticles may be released throughout the lifecycle of the manufacturing processes, during the clean-up of spills, consumer use of nano-products and end-of-life treatment.

The following chapter introduces the conceptual foundation for this study. The headings under which this chapter will be guided are the background to the study, statement of problem, aim, objectives, scope and delimitations of the study.

1.2 Background to the study

Cozzens *et al*. (2012) asserts that in 2000, the United Nations at the Millennium Summit established eight international Millennium Development Goals (MDGs) to be achieved by 2015. Against this backdrop, nanotechnology was quickly considered advantageous to the achievement of some of these MDGs particularly by addressing global grand challenges and solving the problems of "developing countries".

Drawing on Breggin and Pendergrass (2007), nanotechnology is identified to have particles in the 1 to 100 nano-meter range. Moreover, they declared that nanoparticles do have core assets that differ from their bulk/macro counterparts. McIntyre (2012) conveys that nanotechnology is a diverse concept that offers scientific and technological advancements which could help to revolutionise the world. In particular, nanotechnology affords the opportunity to engineer and improve on bulk/macro material structures at a molecular level to create nanomaterials having very specific properties to be stronger, more lightweight, flexible, durable, enhance reactivity or to exhibit low noise levels. Engineered nanomaterials (ENMs) may be nano-scale materials or materials that have nanostructures present internally or on its surface. It is speculated by Marquardt (2013) that nanoparticles may be combined with conventional materials to produce a hybrid material having specific properties. Carbon nanotubes (CNTs) are a typical example of a hybrid material (Recyctube 2012). Marquardt (2013) concedes that a CNT is an example of an ENM. As depicted in Figure 1.1 Marquardt (2013) supposes that a CNT may be single-walled (SWCNT) or multi-walled (MWCNT). He further informs that they can be non-functionalised in their virgin form meaning that they will be characteristically repellent to water making them insoluble. These non-functionalised CNTs can be processed to make them more soluble by altering their surfaces. This allows nanotubes to be more adaptable to use in various applications, for example, in the medical sector.

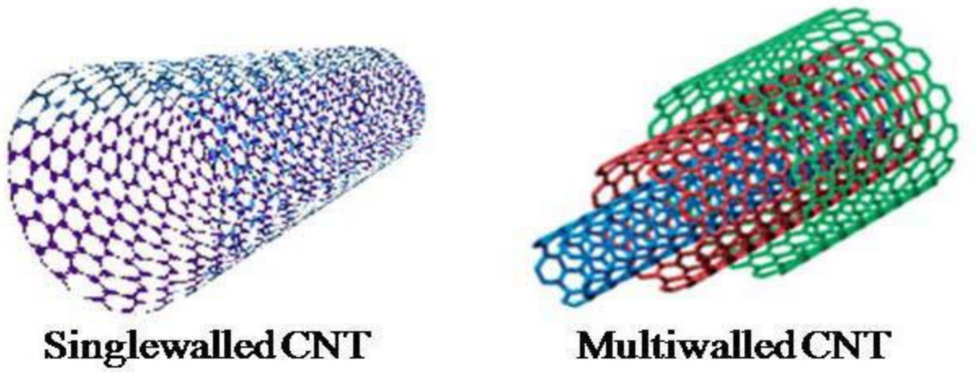

Figure 1.1 The structures of single-walled and multi-walled carbon nanotubes.
(adapted from Singh and Agrawal 2013)

The application of nanotechnology is multifaceted. Manoharan (2011), states that nanotechnology is currently being used in areas of healthcare to fight diseases and in agriculture to increase food production. For example, in healthcare, gold nano-shells concentrate heat from an infrared light to help destroy cancer cells with little damage to the surrounding healthy cells and in agriculture, nanotubes enhance agricultural growth when used in soil by increasing the water retention of plants and crops. Other applications of nanotechnology are used to provide clean, cheap water and energy, for example, nano-fibre membranes and nano-biocides are being used in the improvement of water filtration membranes to ensure clean water while prototype solar panels incorporating nanotechnology can convert sunlight into energy, inexpensively. Moreover, nano-batteries are quicker charging, more lightweight and hold an electrical charge for longer (National Nanotechnology Initiative 2012). The preceding examples highlight the advancement that nanotechnology has already made in the medicine, water, energy, agriculture and food sectors. Advancement in these sectors will have an immense impact globally especially in the upliftment of developing countries.

Carbon nanotubes (CNTs) also are used significantly in various applications and in many industrial sectors proving beneficial to global innovation. Resonating from De Volder *et al*. (2013) the integration of small amounts of CNTs with metals, polymers and resin composites increase the tensile strength and modulus of the metal composite thus making it suitable for aerospace and automotive applications and more lightweight and stronger resin composites for wind turbines.

In spite of these positive aspects of nanotechnology, by contrast, there is also risk implications associated with the use of ENMs.

As suggested in the 2012 report, Environmental Risks of Nanoparticles, the effects of nanotechnology may be vast. Of concern, they may react directly with other species in the environment, may break down and act as a catalyst or even prevent key reactions from taking place. Moreover, in the case of human health, nanomaterials are capable of entering the body and exhibiting a biological activity that is associated with their nanostructure. Acknowledging these sentiments, suitable management of recycling and disposal of ENMs may be helpful in reducing or eliminating some of the foregoing risks.

There are lessons which can be learned from products such as lead and lead compounds in paint and polyvinyl chloride that have entered the market to revolutionise consumer products. These products were later found to release toxic chemicals that were harmful to human health and the environment (Workgroup for Safe Markets 2017). Similarly, CNTs are suspected to have negative impacts on human health and the environment similar to that of asbestos. Thus, the ensuing section will deliberate on a comparison and the similarities between CNTs and asbestos. Gustavsson, Hedmer and Rissler (2011) propose that the chemical, physical and bioactive characteristic of a CNT enables it to provide unprecedented technological advancement. However, the effects of exposure to CNTs are being compared to that of asbestos. This may indicate that CNTs could be potentially dangerous to the environment and human health. According to Luus (2007), due to its thermal properties, asbestos had initially been identified as a novel manufactured product and was used widely in building materials such as flooring and roofing. However, asbestos was later found to have disastrous effects on human health and the environment. With its microscopic fibres, asbestos has been known to cause pleural changes in humans and animals. Similarly, a 2012 article, Human Health Hazard Assessment and Classification of Carbon nanotubes, confirms that CNTs have been found to cause negative pulmonary effects on rats. This may be indicative of the negative effect that this material can have on humans and the environment. It is, therefore, necessary that consideration to similar materials be taken in order to minimise its potential effects on humans and the environment. Lam *et al*. (2006) affirm that regardless of how CNTs are synthesised or constituted they can potentially cause inflammation, fibrosis and biochemical/toxicological changes in the lungs.

The Greenfacts (2016) report on the Risks of Nanoparticles and Nanomaterials created the impetus to cast consideration to the end-of-life of products using CNTs. It mooted that with the increased popularity of nanotechnology throughout the world and the benefits associated with its use, a significant quantity of man-made nanoparticles will enter the atmosphere, soil and water environments thus increasing the risk of exposure to humans and the environment. This progression has illicited a need for concern for this infant technology as risks and latent risk effects remain inconclusive and under-explored.

Khalid *et al*. (2016) recently accentuated that a persistent concern is that the toxicity of nanoparticles has yet to be investigated fully as there are indications that certain types of CNTs may be hazardous to human health and the environment. Beaudrie

(2010) maintains that risk to human health is enhanced through exposure by either direct contact, ingestion or inhalation of the toxic nanoparticles. Although potential risks surrounding the use of CNTs have been identified, information is still very limited and specific characteristics of concern have yet to be identified. This brings to the fore that suitable management of recycling and disposal of nanomaterials may assist in reducing or eliminating the above mentioned risks.

Saugeron (2012), also cautions that CNT toxicity has not yet been fully investigated. Therefore, a precautionary approach should be employed with regard to all aspects of the lifecycle of CNTs. The 2012 report, Good Practice Handling of Carbon nanotubes, highlighted the urgency for toxicity investigation by stating that although there are some indications that CNT exposure may be detrimental to workers there are no specific regulations that apply to the nano-waste. Acknowledging this, there is a threat of micro-organisms becoming toxic as a result of nano-waste. These toxic micro-organisms may then be ingested by other animals. Similarly, plants and crops are also at risk of becoming toxic and will also have a follow-on effect on the food chain. In-lieu of potential harm that could be caused from the use of CNTs and with nanomaterial being used in products so diversely, it is prudent to review current legislation for air, water, waste management and emission value limits. This has been addressed in Chapter Two of this study.

The view that the manufacturing process of nanomaterials will have a detrimental effect more than 100 times greater than conventional materials on the depletion of the ozone layer, global warming, as well as environmental and human toxicity was maintained in the report (International POPs Elimination Network (IPEN) Nanotechnology Working Group 2012). Of particular importance, Senjen (2009) cautions that due to their unique shape and large surface area, CNTs are more active than their bulk/macro counterparts and is released at various stages from production to use and end-of-life processes.

Beaudrie (2010) reiterates that the Resource Conservation and Recovery Act (RCRA) of 1970 authorises the regulation of key areas such as disposal in the lifecycle of hazardous chemicals. This act ensures that the procedures for the disposal of hazardous waste materials are controlled so that the risk to humans and the environment are limited. Standardised regulations such as the RCRA will allow for nanotechnology to be used in applications while ensuring that manufacturers implement steps to control the potentially harmful waste. Thus, for nanotechnology, the tenet would be to assume the worst case scenario and to treat nano-waste as hazardous chemical waste.

1.3 Statement of problem:

Kolodziejczyk (2016) reveals that although the electronic digital revolution has been beneficial to the world, electronic waste continues to be a problem. Similarly nanotechnology introduces a new type of waste for which intervention is required so that end-of-life treatment can be addressed.

While guidelines and regulations are in place for bulk/macro materials in terms of the Waste Act no. 59 of 2008, information directly related to the recycling and disposal of ENMs and, in particular, CNTs is limited. Musee (2011) reports that there is a lack of scientific publications addressing the management of nano-waste streams. Allan *et al*. (2009) posit that the management of nano-waste generated by researchers and manufacturers has not yet prompted standardised legislative amendments among regulators even internationally. This is due to the ongoing deliberation on nanosafety and whether there is a true need for nanotechnology to be legislated.

This may mean that in the current form, recycling and disposal processes could contribute to the contamination of waste streams and pollution of air, as well as contribute to the negative impact on human health through the unintended release of unbound nano-fibres as a result of inadequate handling and treatment measures. Therefore, it makes sense to embark on a study for a suitable strategy to ensure the safe management of the recycling and disposal processes.

Van Dooren (2011) identifies another issue as the lack of knowledge in the area of nanotechnology and stresses that specifically, a gap of knowledge exists on the effects on human health and the environment. Breggin and Pendergrass (2007) confirm that environmental regulation and health and safety implications are still under- explored. McKenna (2008) confirms that silver-nano is being added to socks due to its antibacterial properties. It is believed that bacteria that causes foot odour will be eliminated. However, these nanoparticles have been observed to release during the laundry process. Silver nanoparticles are washed down the drain and enter the waste water stream. With a lack of standardised methods governing end-of-life handling and treatment there is little to no control on the consequent effects.

The concern is further highlighted by the Environment Policy Committee in their 2015 report, Landfilling of Waste Containing Nanomaterials and Nano-waste, where it is revealed that the key source of ENMs that end up in municipal landfill originates from consumer products that contain nanoparticles. CNT was further identified as one of the main nanomaterials that is used in plastic composites. With no nano-regulatory framework in place for landfill sites as well as a lack of understanding of the associated risks, ENMs will accumulate in the environment with possible chronic and latent effects. The possibility also exists that these nanoparticles may bond with other pollutants which may enhance toxicity.

This study will use quality management, environmental management, quality engineering and other related concepts, together with a better understanding of the toxicity effects of CNTs to help guide their use in recycling and disposal processes.

1.6 Ethical considerations

Participation in the questionnaire will be voluntary and the anonymity and confidentiality of the participants will be respected throughout the study. Anonymity and confidentiality are important in ensuring that responses from participants are unbiased. Individual identities will not be revealed as data will be reported in aggregate. Completed questionnaires will not be copied or distributed to any unauthorised external parties. Participants will have the option of withdrawing from the study at any stage with no adverse consequences.

Animal testing will be undertaken in this experimental design. All considerations from the relevant ethical committee and the OECD guideline will be adopted. Protection from all known harmful effects using the materials in this experiment will be undertaken in accordance with the literature reviewed.

1.4 Aim

To develop a suitable strategy for the recycling and disposal of CNTs.

1.5 Objectives of the study

- To establish, through review of literature in Chapter Two, a base-line practice for recycling and disposal of ENMs and CNTs.
- To determine the practices which are employed by nano-organisations for recycling and disposal of ENMs and CNTs through the use of a questionnaire.
- To observe through experimental work the behaviour, toxicity and leaching potential of CNTs in soil through the use of the Organisation for Economic Co-operation and Development (OECD) Test Methods Test No. 207– Earthworm Acute Toxicity Tests, as well as Test No. 312– Leaching in Soil Columns.
- To develop suitable strategies for the recycling and disposal of CNTs using the objectives above and selected quality tools.

1.7 Rationale for this study

Due to the novel characteristics portrayed by nanotechnology, there is an ever growing interest in their potential applications. As the popularity of nanotechnology continues to rapidly spread throughout the world, the many benefits that arise from the technology are evident. Liu (2006) states that the ENMs cover industries such as electrical, engineering, agriculture, food and medicine. These industries are vital to the growth and development of global communities.

However, with the growth of industry there is an increase in the volume of nanoparticles used and consequently contaminating the atmosphere, soil and water environments. Furthermore, the possible effects on human health also remains a concern as more people become exposed to ENMs. In the view that there exists a gap in knowledge on the toxicity effects of nanoparticles on human health and the environment, this study will bridge the gap in knowledge by developing a strategy for the recycling and disposal of CNTs with the intention of allowing manufacturers to use these materials more responsibly.

1.8 Delimitations

Globally, the work in this field is only in its infancy. Carbon nanotubes (CNTs) are very expensive and the number of samples analysed in this study will depend on the availability of the CNT. Every effort will be made to ensure that the validity and reliability of the results are established to prove credibility of the study.

The OECD guidelines are typically recommended to assess the potential effects of chemicals on humans and the environment. For this research study, OECD Test No. 207 – earthworm acute toxicity tests and OECD Test No. 312 – leaching in soil columns will be used. According to the report, Nanotechnology Regulation and the OECD (2015), the use of the test guidelines is not specifically intended for nanomaterials. However, according to OECD (2016) report on Nanotechnology Measurements and Regulation it is discussed that the appropriateness of the current test guidelines to nanomaterials is being evaluated. The report proposes that the current OECD tests may need to be adapted to suit nanomaterials.

1.9 Scope

This study is confined to the effects of CNT in soil. In particular, the research will focus on the toxicity effects of CNT to *Eisenia foetida* earthworms, as well as CNTs leaching ability in soil. Additional research is needed to investigate the toxicity and leaching ability of other types of nano-waste via different waste streams.

1.10 Outline of research methodology and research design

This study was stimulated by inductive reasoning. With the use of literature sources, the study will investigate the adequacy of current strategies and propose new strategies for the recycling and disposal of CNTs. The study will adopt a strategy that is both theoretical and empirical. A qualitative and quantitative research approach will be used for the study. In addition, to the consultation of literature sources, data collection will involve a submission of questionnaires to active researchers. The information gathered from the questionnaires and toxicology experiment will be analysed and utilised fully, in order to achieve the research aim and objectives. The research will be validated through repetition of information until saturation is achieved. Data will be collected from literature, respondents participating in the questionnaire, and experiments. For this study, the questionnaire will be distributed amongst participants who are active researchers from various laboratories working with CNTs in research and development facilities or as part of their individual research studies. Three experiments will be carried out. These tests will be discussed in detail in Chapter Three. The OECD Test No. 207 – earthworm acute toxicity tests will include a preliminary study using filter paper while the main study will make use of artificial soil.

The OCED Test No. 312 – leaching in soil columns will be used to gather data on the behaviour of CNTs in soil, as well as its leaching potential in soil.

1.11 Outline of chapters

The study is made up of six chapters. These chapters have been described briefly below.

Chapter 1 – Introduction	This chapter will provide the background to the research topic. The benefits and associated risks of carbon nanotubes will be highlighted along with the scope and delimitations of the research area. The problem, aim and objectives, as well as the rationale for the study, will also be presented.
Chapter 2 –Literature Review	This chapter will highlight the theory that already exists in the area of the research study. Technical reports and regulatory guidelines will be used to highlight the lifecycle, in particular, the recycling and disposal of carbon nanotubes. Journal articles will be used to show the risks associated with inefficient recycling and disposal of carbon nanotubes. Technical reports will be used to highlight prescribed methods for handling, recycling and disposal of carbon nanotubes.
Chapter 3 – Research Design and Methodology	The methods, strategy and techniques selected for research will be identified and discussed in this chapter. The research design will consider the information from literature, as well as the observations and experience of the researchers. The life cycle covering the recycling, waste disposal, will also be incorporated into the study. An empirical, as well as a theoretical strategy, will be adopted for the study. Data collection will be in the form of literature (preliminary work), questionnaires (active researchers) and observation (experimental work).
Chapter 4 and Chapter 5– Results and Discussion	The results of the data collected and interpreted. This data will be used to conclude the adequacy of recycling and disposal of carbon nanotubes.
Chapter 6 – Conclusions and Recommendations	The strengths, weaknesses and deductions from the previous chapters will be summarised. Recommendations for future work based on outcome of the study.

1.12 Summary

Chapter One provided the background information on the research topic. It can be gleaned that nanotechnology can be advantageous to the development and progress of many different sectors across the globe. However, as little information is available on the potential risks, the same characteristics and properties that make this technology unique with unlimited potential could also have detrimental consequences on human health and the environment and thus need to be managed responsibly. The ensuing study will undertake to contribute information in the area of toxicity and leaching potential of CNT as well as bring forth the current practises utilised in research facilities for the handling and treatment of nanomaterials and nano-waste.

The following chapter entails an appraisal of different literature sources in order to become familiar with the facts that are available in the area of the research study. In particular, the factual content of interest will be with reference to the recycling and disposal of ENMs and CNTs, as well as the leaching potential, behaviour and toxicity of CNTs.

CHAPTER TWO: LITERATURE REVIEW

2.1 Overview of Chapter Two

Chapter Two outlines the theory that exists in the area of the study by presenting sources of pre-existing literature and prior scientific data. The greater part of this chapter will comprise literature pertinent to the study under the following headings: terminology and definitions used in the nanotechnology field, benefits and risks, current practices for recycling and disposal, regulation, toxicity, leaching potential and quality tools. Chapter Two will bring clarity and focus to the study by first making prominent the terminology used in the study and then highlighting the risks and concerns that have instigated the study.

2.2 Terminology and Definitions in the Nanotechnology field

Terminology is a group of terms applied to a specific field. For this study the following terminology and definitions have been applied:

2.2.1 Nano-scale/nano-sized:

Publicly available specification (PAS) 136, which identifies terminology for nanomaterials and publicly available specification (PAS) 134, which lists terminology for carbon nanostructures agree that the size range is from approximately 1 nano-meter to 100 nano-meter.

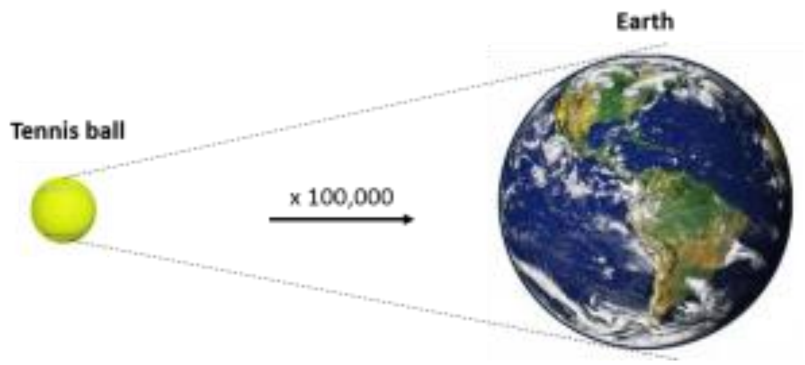

Figure 2.1 Comparison of nano-scale

(Adapted from Amanor 2016). One nano-meter is to the tennis ball what the tennis ball is to the earth.

2.2.2 Nanotechnology:

Publicly available specification (PAS) 130 is a guidance document on the labelling of manufactured nanoparticles and products containing manufactured nanoparticles. This document defines nanotechnology as an enabling technology that looks at promoting the novel characteristics of nano-sized materials and products. Nano-scale/nano-size is depicted in Figure 2.1. While the National Nanotechnology Initiative (NNI) describes nanotechnology as the understanding and control of matter at dimensions between approximately 1 and 100nm. Dimensions between 1 and 100nm are known as the nano-scale. The NNI further establishes that unusual physical, chemical and biological properties can emerge in materials at this scale. These properties may differ in important ways from the properties of bulk/macro materials and single atoms or molecules (Kreyling, Semmler-Behnke and Chaudhry 2010).

2.2.3 Nanoparticle:

According to Publicly available specification (PAS) 136, which identifies terminology for nanomaterials, nanoparticles are nano-objects otherwise known as nano-fibres with all three external dimensions in the nano-scale (ranging from 1 nano-meter (nm) to 100nm) while the Publicly available specification (PAS) 130 guidance document on the labelling of manufactured nanoparticles and products containing manufactured nanoparticles, builds on this definition to describe a manufactured nanoparticle as solid entities having a size from approximately 1nm to 100nm in at least two dimensions that have been produced by a manufacturing process.

This sentiment is closely linked to the explanation provided by Kreyling *et al.* (2010) which states that ENMs is any material that is deliberately created such that it is composed of discrete functional and structural parts, either internally or at the surface, many of which will have one or more dimensions of the order of 100nm or less.

2.2.4 Nanotubes:

According to Publicly available specification (PAS) 136, which identifies terminology for nanomaterials, states that nanotubes are hollow nano-objects also known as nano-fibres with two similar external dimensions in the nano-scale (1nm to 100nm) and the third dimension is significantly larger than the other two external dimensions.

The definition is further expanded to include CNTs which are nanotubes consisting of carbon. These nanotubes either single-walled or multi-walled. Publicly available specification (PAS) 134 lists terminology for carbon nanostructures and identifies a SWCNT as a carbon nanotube consisting of a single cylindrical graphene layer while a MWCNT is described as CNTs with nested, concentric or near - concentric graphene sheets.

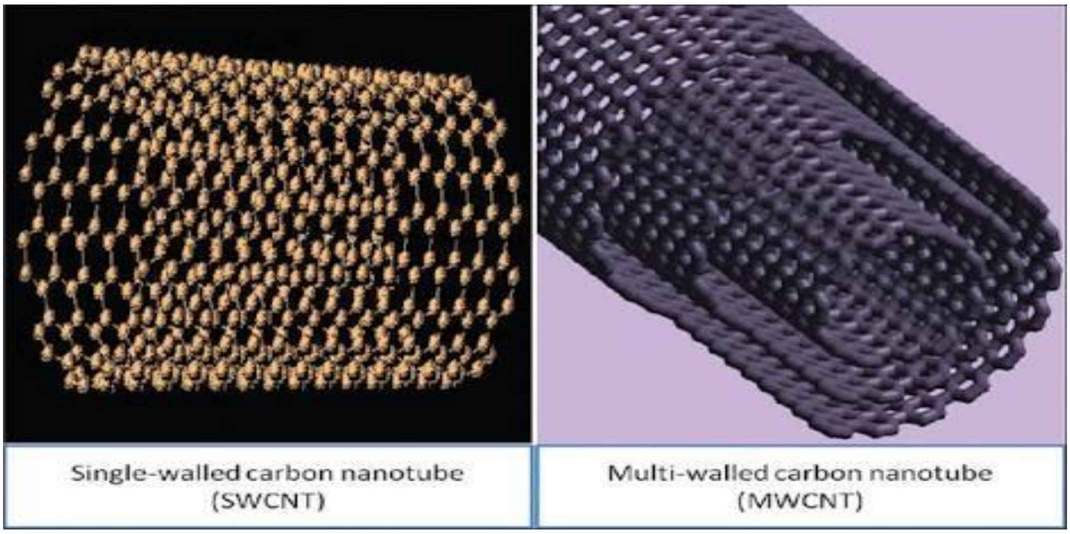

Figure 2.2 Structural difference between a single-walled carbon nanotube and a multi-walled carbon nanotube
(Adapted from Dineshkhumar *et al*. 2015)

2.2.5 Waste:

With regards to bulk/macro waste, Taylor and Allen (2006) define waste as any material that is discarded because it cannot be used further. Waste may be categorized as a gas, liquid or solid. Depending on the composition and laws that apply to specific waste, it may be treated or remain untreated. Waste in the gas form can be vented into the atmosphere, liquid waste can enter rivers or sewers and solid waste is primarily disposed of via landfill sites. Disposal via landfill sites is popular in less developed countries and is the main route to addressing solid waste because it is cheap and simple. This has been expounded by CEN ISO/TS 27687:2008 which states that nano-waste contains nano-objects.

2.3 The Benefits, Risks and Impacts of Nanotechnology

Feitshans (2013) describes nanotechnology as being a science, engineering and technology at the nano-level. Some of the benefits of nanotechnology include use in the energy sector for longer lasting batteries and fuel cells, smaller solar cells which are still effective as they are able to hold more charge. In the medical field, one of the uses includes a carrier for drug delivery. Nonetheless, many disadvantages are also pointed out by the authors. These include latent behaviour of ENMs, loss of jobs in traditional farming and manufacturing sectors through the use of nanotechnology equipped materials, possible adverse effects due to the health implications from this new field, the lack of personal protective equipment and the gap in knowledge regarding handling, recycling and disposal in this field.

Although Beaudrie (2010) lauded that there are numerous benefits of nanotechnology. These include nanomaterials that are able to target and destroy cancer cells, improve solar collection, water-treatment and consumer products with specific requirements. For instance, products are lighter, stronger, fresher or longer lasting. Beaudrie (2010) also brings to the fore that some of the novel characteristics could be a ‘double edged sword’ and may also present themselves as risks. He uses the example of nano-aluminium and bulk/macro aluminium (Al). In the bulk/macro form, Al is stable, strong and inert making it ideal for use as a beverage container while in the nano-scale Al can be used as rocket propellant. Sauser (2009) further explains that in the nano-scale Al properties cause an increase in combustion temperature and maintains the burn rate of the fuel. The discernible difference in properties of bulk/macro aluminium and nano-scale aluminium highlights the need for full characterisation of nanoparticles. Results from this study can inform the characterisation of CNT in terms of toxicity and leaching potential in soil.

Schulte *et al*. (2014) posit that responsible development in the field of nanotechnology is important in order to prevent harm to humans and the environment. They reason that the uncertainty of regulation and lack of standardisation that surrounds nanotechnology throughout its lifecycle is one of the main reasons that humans and the environment will be negatively impacted by this technology. They postulate that exposure of workers, consumers and the environment to nanotechnology must be limited until such time as the risks of exposure are understood and can be addressed. Furthermore, they are of the opinion that occupational health and safety for the responsible development for nanotechnology can be accomplished by addressing each of the five criterion in Figure 2.3 below.

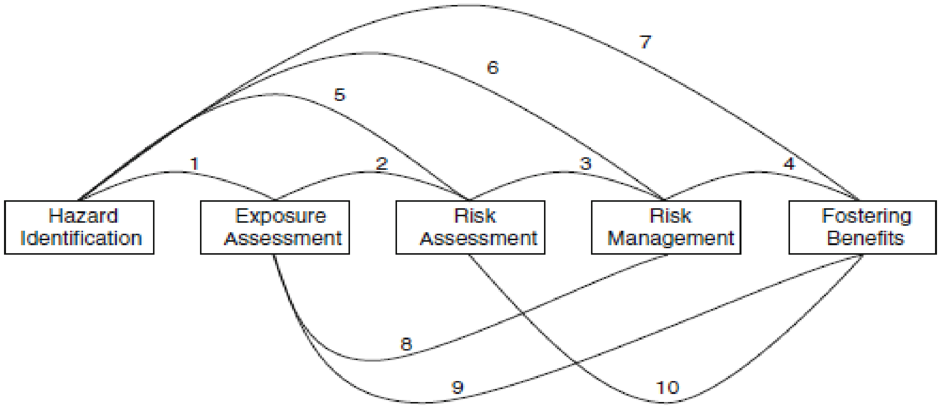

Figure 2.3 Five criterion for responsible development of nanotechnology and their interaction scenarios

(Adapted from Schulte *et al*. 2014)

Schulte *et al.* (2014) appraise Figure 2.3 according to each criterion in terms of "Hazard Identification" at the workplace. This appraisal allows for nano-industries to plan for, recognise and monitor potentially harmful nanomaterials. "Exposure assessment" looks specifically at gaging the amount of contact experienced by workers in the environment exposed to nanoparticles. "Risk assessment" considers the possible effects of nanotechnology on workers and makes the workers aware of these effects. This leads to "Risk management" which involves taking the necessary steps at the workplace to control the effects of the nanoparticles. Management of the potential risks leads to the last criterion identified as "Fostering benefits". Nanotechnology has shown to be beneficial in most industries and is seeping into everyday life more rapidly through various products and by-products. Thus, they suggest that the safe development of nanotechnology must be prioritised and nurtured so that these benefits can be enjoyed fully. While it is apparent that attention must be given to the safe development of nanotechnology there is also a vital need to concurrently develop safe end-of-life processes. It is hoped that this will minimise unintended exposure to humans and the environment.

Wijnhoven *et al.* (2009) surmise that material at nano-scale may differ to bulk/macro materials due to its high surface reactivity. This can be supported by considering the use of bulk/macro-silver and nano-silver. Wijnhoven *et al.* (2009) use silver as an example. They share the sentiment that while bulk/macro silver may not cause harm to human health and the environment its counterpart, nano-silver, may present cause for concern. This is due to the specific properties of nano-silver. The risks of nanomaterials have not been finalised due to the great gaps in factual content related to nanotechnology. However, in some limited instances, knowledge is being gathered from toxicity studies. In the case of nanoparticles, it was found that those materials containing nano-silver which released free silver ions more readily were more toxic than those nano-silver materials that did not release free silver ions. In summary, the more free silver ions released the greater the chance of toxicity. Because silver's strength lies in its antiseptic and sterile properties, nano-silver is being used throughout the food chain, in consumer products and in the medical field. Of concern, Wijnhoven *et al.* (2009) allude that studies involving zebrafish have shown that nano-silver affects the initial growth of fish embryos. It has also positively caused abnormalities and damage to the zebrafish genes, thus supporting the premise that nano-toxicity will have a negative effect on human health and the environment. Barbazak *et al*. (2000) elucidate that zebrafish is essential in research studies. This vertebrate has a significant likeness to human organs and human tissue. The results of zebrafish studies can therefore be inferred to humans.

Luoma (2013) indicates that research on the effects of nano-silver on rabbit reproduction has shown that studies on nano-silver have been mostly *in vitro* and that nanomaterial remains a poorly understood material. This has led both regulators and researchers to continue to be split on how to address regulation of this material. The uncertainty surrounding the regulation has created great concerns as nano-silver continues to be used in a diverse range of consumer products. The concerns raised for nano-silver may also be applicable to other nanomaterials. It is, therefore, prudent that the regulation and standardisation of practice for nanomaterials remain a primacy.

Fuldo, Weber-Bruls and Werth (2014) supported the statements of Beaudrie (2010) and Sauser (2009) by mooting that nanomaterials have a large definite area with precise physiochemical properties that are different to the bulk/macro form of the same material. Of great concern, this may mean that the current legislation for bulk/macro materials may not be suitable. The need for this study is therefore strengthened as results from the study could minimise the void that exists for nano-regulation by providing toxicity and mobility data for CNT.

Gustavsson, Hedmer and Rissler (2011) proposed that the chemical, physical and bioactive characteristic of CNTs enable it to be used in almost every aspect of our technological advancement. However, they support Beaudrie (2010) in the notion that the novel properties of CNT could potentially be dangerous. To further support this notion, the physical features of CNT has been compared against that of asbestos. The similarities between CNT and asbestos might be indicative of the same biological effects. This may indicate that CNTs could be potentially dangerous to the environment and human health.

Luus (2007) cautions that asbestos has had disastrous effects on human health. This, he largely attributes to its fibrous nature. Asbestos fibres may either be long and curly or short and straight. These fibres have been found to be comparable to the fibres of CNT. Furthermore, Luus (2007) found that the exposure pathways were also common between asbestos and CNT. The prevalent exposure routes remain via ingestion (oral), contact with skin (transdermal) and inhalation (pulmonary). The intravenous route is another example of an exposure route although it is also the least likely route. Yang *et al*. (2008), recorded the bio-distribution of SWCNTs administered intravenously in mice. The intravenous route can also be administered as part of preclinical trial studies. Anselmo and Mitragotri (2016) declare that intravenous administration of nanoparticles for the purpose of drug delivery in cancer treatment has been approved by the Food and drug Administration (FDA). This is distressing because animal studies have already implied that CNT does negatively impact health.

Luus's (2007) beliefs are commensurate with Gustavsson *et al.* (2011), that it is necessary to study and classify CNT in terms of its toxicity so that it may be handled correctly. This is in light of findings proclaiming that handling dry CNT material is the foremost route for occupational exposure. They also put forward that the fibre size of CNT does impact the effect of CNT. In addition, Gustavsson *et al.* (2011) promulgated that studies have shown that longer CNT fibres would be more persistent and difficult to expel than the shorter fibres.

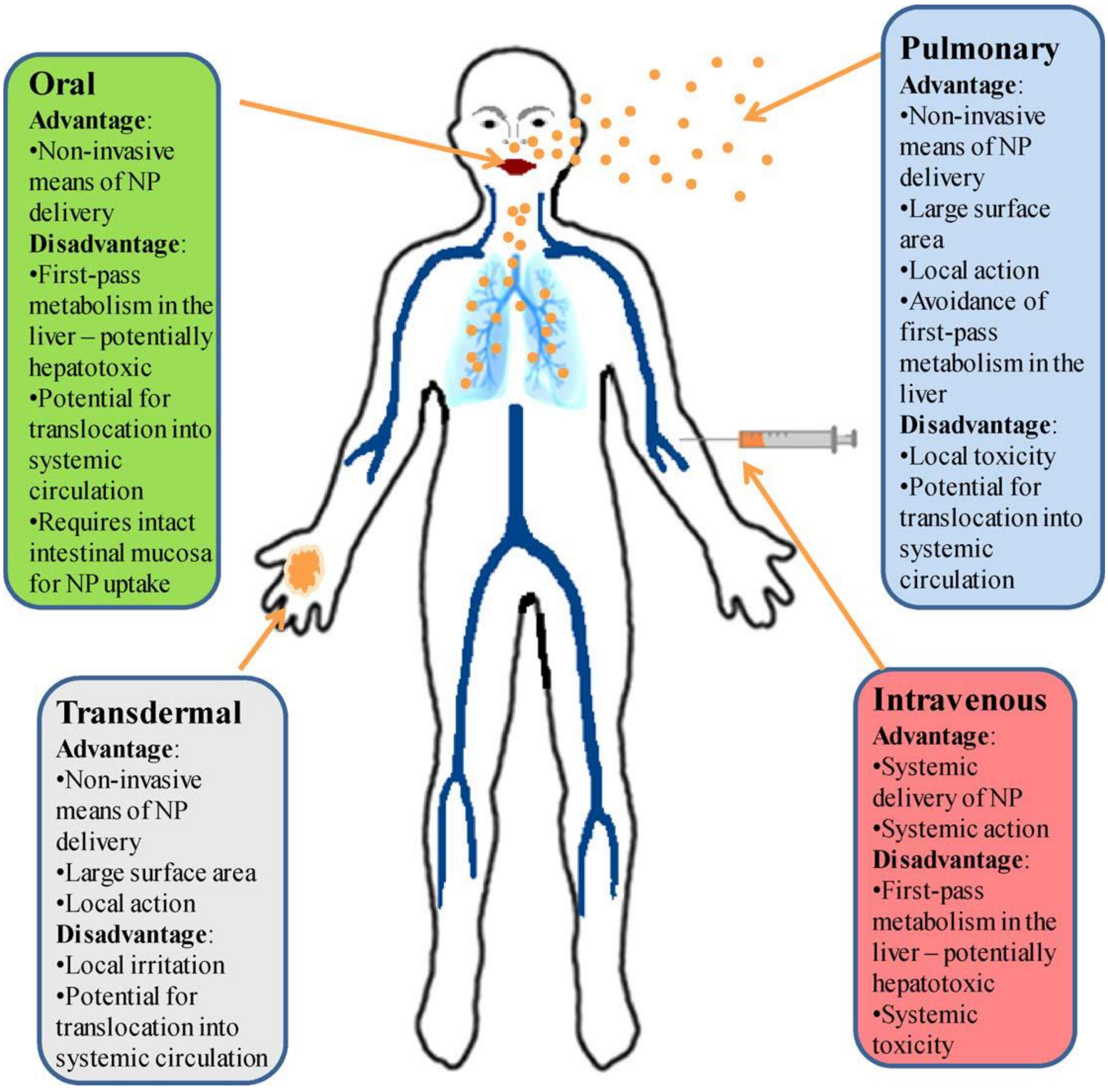

Figure 2.4 Exposure pathways for human health
(Adapted from Yildirimer *et al*. 2011)

Invernizzi (2013) alleges that the small size promotes the transference of particles through the respiratory route. Size once again allows travel through blood barriers and to the brain. Their slow bio-degradation means that they could lie dormant and persist in future generations. Invernizzi (2013) clarifies that while many studies have taken place these have mostly been in *in vitro* lab studies or animal testing and the effects on humans still remain unclear. However, there has been sporadic feedback on actual incidents involving humans. For example, Lyn (2009) reported that China experienced an incident where two employees died following their stay in a hospital with several other co-workers for pulmonary trouble. It was found that the lungs of the victims contained nanoparticles of acrylic resin. The victims, who all worked in a paint factory had been exposed to nanoparticles due to the lack of proper personal protective equipment. The exposure to nanoparticles caused fibrosis and granulomas on the lung.

Invernizzi (2013) identifies workers as the first to be and most easily affected by nanoparticles. Similarly, Luus (2007) and Invernizzi (2013) advise that because the exposure routes, as represented in Figure 2.4, are through inhalation, skin and direct ingestion during manufacture, there should be complete sanitation of the work area. Consumers of the products may then also be at risk through the same exposure pathways. Industrial debris and production waste, as well as disposal of products, can lead to risk of the environment. The studies above confirm that nanoparticles are biopersistent and also owing to their small size can be present in the air for long periods of time. Translocation of these fibres could be possible by wind carrying the fibres further and by rain washing down fibres to ground level.

The Biomedical Research Ethics Committee (2017) reveals that as CNT toxicity has not yet been fully investigated, and thus a precautionary approach should be employed with regard to all aspects of the lifecycle of CNTs. However, Haywood (2012) implores that although there are some indications that CNT exposure may be detrimental to workers there are as yet no specific regulations that apply to the nano-waste. This report implores users of CNT to treat CNT and its structures as hazardous until such time there is evidence to the contrary. With the gap in knowledge for nanotechnology as well as no available nano-specific legislation, adopting a precautionary approach may provide some safeguard against risks.

According to Ray, Yu and Fu (2009), there is a threat of micro-organisms becoming toxic as a result of being exposed to nano-waste. These toxic micro-organisms may then be ingested by other animals. Similarly, plants and crops are also at risk of becoming toxic, which will result in a follow-on effect in the food chain. With the potential for toxicity to spread rapidly, they suggest that is important to ensure that proper recycling and disposal methods for nano-waste are available.

Allan *et al.* (2009) support the belief that nano-waste can be generated by different resources such as synthesis of nano-objects, nano-manufacturing, use in consumer products and during the lifecycle of nano-products. The BSI Guide PD6699-2 identifies four main nano-waste streams namely, pure nanomaterials, contaminated nanomaterials, and liquid and suspensions containing any amount of nanomaterials.

There is little to no nano-waste practices utilised at institutions/organisations operating in the field of nanotechnology. Allan *et al.* (2009), ascertain that some of the reasons for the latter as being due to lack of toxicity information to make an informed decision, lack of standards and codes to direct a uniform practice, as well as competitiveness amongst nano-institutions/organisations, limit knowledge transfer. The results from this study's questionnaire uncover the practises in research facilities on the subject of handling and disposal of CNT.

Jackson *et al*. (2013), describe the uses of CNT to be ever growing. Some of the uses are reinforced composites, conductive material and drug delivery vessels. With CNT being more widely used in industry and consumer products, there is an increase in waste containing nanomaterials and this intensifies the risk to the environment. Jackson *et al.* (2013) confirm that CNT may enter the environment inadvertently as waste from several processes. Examples of these processes include treatment of sewage waste, waste incinerators and landfill sites. It is imperative that these processes be considered for governance in order to minimise potential contamination of the environment.

Allan *et al.* (2009), state that the royal society and the royal academy of engineering have collectively concurred that a precautionary approach should be used which would be to treat nano-waste as hazardous waste. Due to nanomaterials being used in products so diversely, there is a need for the review of legislation for air, water, waste management and emission value limits. However, for the interim, the general consensus is that a precautionary approach be applied when handling nano-waste. While this new technology hints at endless benefits the possibility of risk does exist. It is, therefore, very necessary to explore the potential impacts of this emergent technology.

2.4 Current Practices for Recycling and Disposal

In South Africa, there is no specific legislation that regulates and guides the fate of ENMs such as CNTs from production to end-of-life. Musee, Brent and Ashton (2008) pronounced that South Africa has not yet developed a national research strategy to understand the environmental, health and safety risks of nanotechnology. While the general legislation that applies to bulk/macro materials is currently being applied to nano-sized materials, this practice raises questions whether bulk/macro material legislation can adequately cater to its nanomaterial counterpart requirements. Research has highlighted the differences between the behaviour of nano-sized materials and their bulk/macro counterparts. According to (Arora, Rajwade and Paknikar 2011; Musee, Brent and Ashton 2008), the properties and functions of ENMs differ from those of their macroscale counterpart due to their size, shape, surface charge, surface area and other physicochemical properties, which means that the current management tools for macroscale material could most likely be inadequate for ENMs. Some of the advantageous and unique properties associated with nanoparticles could also be responsible for their potential toxicity. According to Arora *et al.* (2011), there are already a great number of nano-products available commercially. Of these products, some are designed to have direct contact with the surrounding environment. An example of direct contact is the disposal of ENMs via landfill sites where an interface is created between the soil and ENMs. An example of an indirect route is the uptake of ENMs via contaminated water by plants. As mentioned earlier, there is no legislation controlling the disposal of ENMs, which implies that there is no uniform and standardised manner to handle the disposal of ENMs. According to Niemeyer (2011), a material flow analysis of CNTs was conducted on available products and the results suggested that most CNTs would end up being incinerated or disposed of via landfill sites. This notion has been supported by the Environment Policy Committee in the report, Landfilling of Waste Containing Nanomaterials and Nano-waste (2015) which states that disposal by landfilling and dumping are the most favoured methods of disposal globally. The report further stresses that ENMs have been found in varying waste leachates. This brings to the fore a need for intervention for end-of-life handling and treatment of nano-waste.

Wild (2013) supports the notions of Lozano and Berge (2012) by stating that nano-products are mostly imported into South Africa, in the form of textiles (nano-fibres), paints and cosmetics. This triggers the need for nano-specific regulations as, currently, in South Africa there is no legislation to regulate the production, handling, recycling and disposal of ENMs. Nowack *et al* (2013) state that there may be a notable release of nanoparticles in the recycling process. The authors advised that in countries such as Africa and Asia there is a lack of health and safety measures for recycling operations. This is due to the operations being done in unregulated areas and by nonprofessionals. Nowack *et al* (2013) further put forth that the exposure to nonprofessional workers will depend on the type of recycling process being used. Kruger, Ndebele and Horn (2014) warn that the African continent could become exposed to oppressive research. Africa currently does not have a regulatory framework in place for nanotechnology. This makes Africa enticing to researchers who want to escape stringent frameworks in their own countries.

Kolodziejczyk (2016) outlines that nano-waste is comprised of man-made nanomaterials. He proposes that nano-waste is challenging to monitor due to the size of the nanoparticles contained in the waste. The author pronounces that the effects of ENMs on human health and the environment are not well understood and that not all nanomaterials are harmful. Kolodziejczyk (2016) agrees that various factors influence the potential risk of nanomaterials. Some of these factors are, its potential to be soluble, dimension, form, its ability to cluster, as well as other physiochemical factors. The author is also of the opinion that a solitary procedure for end-of-life treatment would suffice for nanomaterials as a whole. However, care must be forefront at all times during the process in order to minimise the unintended release of these materials. He supports the notion that more information must be gathered on the properties of these materials so that together with pre-existing guidelines an end-of-life practice that works can be created.

Kolodziejczyk (2016) identified several measures that can be considered for the monitoring, recycling and disposal of ENMs. These measures include:

- government-implemented directives, as well as assessments for developers of nanomaterials and nano-products;
- obligatory risk studies for any new nanomaterial;
- availability of funding for nano-research and
- driving awareness campaigns to educate researchers, manufacturers and consumers about the responsible use of nanotechnology, as well as the use of prevailing control measures for risk management.

Schauerman *et al* (2012) identifies one of the limited recycling options for products containing nanomaterials as using a mixture of acids before thermal treatments. This process has been positive in restoring SWCNT to its pure state with additional coulombic efficiencies. Schauerman *et al* (2013) are confident that the cost of raw material could be lowered as the SWCNT could be used more than a single life cycle. However, Zhang *et al* (2017) found that rather than achieving a pure state of raw material there was a decline in the melt viscosity and chemical structure in the nanocomposites.

The British standard PD6699-2 (2007) suggests that if risks cannot be prevented, then they must be controlled. Action can be taken in the form of control measures for dermal and inhalation risks. The recommended hierarchy of control measures are illustrated in Figure 2.5. The control measures are temporary measures until a permanent solution is identified. The measures should be used as listed from most effective (top of the inverted pyramid) to the least effective (bottom of the inverted pyramid).

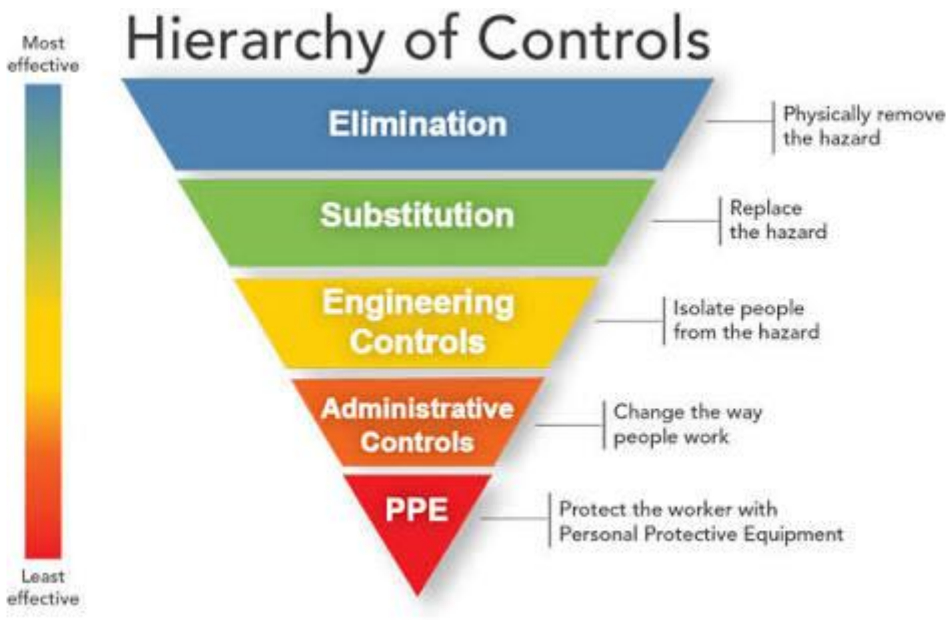

Figure 2.5: Hierarchy of control measures.
(Source: http://www.cdc.gov/niosh/topics/hierarchy)

Hester (2006), declares that there has been a boom in the progression of nanotechnology. They envisage that while nanomaterial may have novel properties, the same could be expected for nano-waste.

Lowry *et al*. (2010) confirm that ENMs will be released throughout its lifecycle. The authors identify some release scenarios as being from the nanoparticles used in the manufacturing process of nanomaterials during the use of consumer products containing ENMs, during the recycling process of products containing ENMs, and similarly during disposal of ENM products. In addition, Lowry *et al.* (2010) found that various factors influenced the effects of ENMs. For instance, surface coatings modify the transport properties, reactivity and toxicity of ENMs.

The thoughts of Part *et al*. (2015) are synchronized with Lowry *et al.* (2010). Part *et al.* (2015) contend that ENMs can be released during waste treatment. For example, during incineration thermally stable ENMs were found to collate in the bottom ash. In addition, they were also released during recycling in the form of quantum dots from LEDs in electronic equipment. Part *et al.* (2015) also stressed that various environmental factors influence the behaviour and fate of ENMs in landfill sites. These factors include pH value, ion strength, total organic content, humic acid concentration and colloidal stability of ENMs.

Marquardt (2013) supported Part *et al.* (2015) by stating that CNT behaviour is dependent on its physiochemical properties. Examples of these properties are particle size, shape, surface area and surface chemistry. The above sentiments draws awareness for the need of governance throughout the lifecycle of nanomaterials. This action will help to control nano-waste at each step of its lifecycle in addition to the end-of-life handling and treatment.

Amoabediny *et al*. (2009) attest that most organisations employ conventional chemical safety methods through the life cycle of nanomaterials. They characterise nano-waste as potentially hazardous and use regulation specific to their organisation including Recycling and Disposal methods in order to manage their waste. Amoabediny *et al.* (2009) further grant that the Health and safety executive (2013) classifies CNT waste as hazardous waste for which the preferred method of disposal has been identified as high temperature ignition. However, Connett (2013) disparages this notion claiming that there are concerns about nanoparticle emissions through incineration. The British standard PD6699-2 (2007) which guides organisations on the safe handling and disposal of manufactured nanomaterials agrees that all nano-waste should be classified as hazardous waste and treated for disposal accordingly.

According to the Environment Agency interim advice (2008), an alternate method is to have the waste chemically treated so that the toxicity is eliminated. An alternate method could minimise some of the risks posed by recommended methods of recycling and disposal. Integration of existing quality management systems and the use of standard practices can help to guide the recycling and disposal processes of ENMs. It will also help organisations to integrate these recycling and disposal practices with their existing management systems.

Holder *et al*. (2013), prophesised that due to the widespread use of ENMs, there is an increase of nanomaterials entering different waste streams and, thereby the environment. The authors are concerned that during incineration there may be changes to the properties of the nanomaterial. This may influence its transference to its surroundings and the impact on that environment. Holder *et al*. (2013) stress that there is no regulation directly related to the field of nanotechnology. However, in the United States of America, there is a possibility that nanotechnology may fall under the existing regulation of the RCRA. The debate that exists for the EPA is whether nanoparticles present in waste form is actually toxic or not. The debate remains open due to lack of knowledge that exists with regards to nano-toxicology. No classification of nano-waste also means that household waste containing nanomaterials is entering landfill sites without incineration or being treated as hazardous waste. This notion was supported by Curtis (2013) who found that recycling CNTs was more difficult than ordinary plastic. This is attributed to the lack of nano-waste classification which makes it difficult to collect and separate CNTs for any remedial process. However, Curtis (2013) also disagrees with Holder *et al* (2013) that there could be changes in properties during the incineration process. Curtis (2013) believes that CNTs are mechanically and thermally stable to the point that their main functional properties will not be altered by recycling processes.

Todea *et al*. (2003), conducted an experiment to investigate if municipal incinerators could remove additives from flue gases or solid deposit. The results indicated that it was not likely that CNT would be released from municipal incinerators. This was established using two tube furnaces connected in series with a quartz glass tube. No traces of airborne free CNT were perceived.

Vejerano *et al*. (2014), comment that the ability of nanomaterials to separate between the bottom ash and particulate matter decides the outcome after incineration as well as the extent of exposure. Vejerano *et al.* (2014) put forward that during the incineration process a bulk/macro amount of nanomaterials is retained in the bottom ash. These nanomaterials end up in landfill sites. This may be due to incomplete incineration owing to burn temperature or even the composition of the nanomaterial itself. Current practices for bulk/macro waste is being utilised for the handling and treatment of nano-waste. However, it has not yet been determined which practice, if any, is most suitable to the task of recycling and disposal of ENMs. It should be noted that nano-waste may be difficult to observe as fibres may be too small to be seen with the naked eye. Information gathered from this study could inform this gap in knowledge.

2.5 Regulation for nanotechnology

Regulation is lacking for nanotechnology throughout its lifecycle. This includes the end-of-life treatment of nano-waste which is not yet regulated by the government. The lack of standardised practices for handling, recycling and disposal of nanomaterials may result in the unintended release of nanomaterials into the environment. In addition, there is minimal regulation available for the handling, recycling and disposal of ENMs, no regulation has been released to control the toxicity of ENMs. Regulation is paramount in identifying the classification and toxicity of chemicals.

Owen (2005), as well as Theodore and Kunz (2005), showed concern at the lack of legislation for nanotechnology. Owen (2005), in particular, drew attention to the lack of legislation in the United Kingdom (UK). With minimal information available on toxicity and exposure, regulatory bodies have decided to adopt a precautionary approach. The UK Government called for a voluntary cessation of the release of ENMs into the environment until the environmental, health and safety risks were well understood. Similarly, Mc Alpine (2010) states that even though the United States (US) does not have regulations on the handling and disposal of ENMs, the UK and the US have established voluntary programmes to gather data from manufacturers of ENMs. The author advises that in the US, the Nanotechnology Safety Act of 2010 was established to ensure that the health and safety concerns of nanotechnology were investigated and aligned to develop guidelines for manufacturers. In the US, the Resource Conservation and Recovery Act (RCRA) of 1976 authorises the regulation of key areas such as disposal in the lifecycle of hazardous chemicals. It suggests the practise to assume the worst case scenario and to treat nano-waste as hazardous chemical waste. Ireland and Morris (2013) report that in Australia, there is no regulation for nano-waste.

However, the Work Health and Safety (WHS) Regulations, as well as general waste handling regulations, are applicable to the handling of nano-waste. Almeida (2013) reports that although Brazil has food and drugs containing nanomaterial available commercially, the Brazil Congress rejected a bill to regulate the labelling of these products. The Brazil Senate reported that the move to regulate the labelling of products would be alarming to the public and may be seen as a warning when there was still no scientific proof that warning was necessary.

Amoabediny *et al.* (2009) explained that exposure control methods could be beneficial to reduce the risk of exposure to ENMs. Nanoparticle safety and health guidelines (2010) explained that exposure control methods are a set of tools or strategies that can be used to reduce or eliminate worker exposure. Control methods are identified as elimination, substitution, engineering, warnings, administrative and personal protective equipment (PPE). Castillo (2013) thinks that nano-workers, health will go unprotected until such time that regulations have been established and implemented. As disposal of nano-waste is identified as an activity that leads to nanoparticle exposure, the control methods could assist in minimising the risk to workers at incineration and disposal facilities.

According to Nanomaterials in Waste (2011), Netherlands has an action plan for the handling, recycling and disposal of nano-waste. The action plan consists of several steps which include prevention through waste prevention, prevention by smart product design, recovery through product reuse, recycling, recovery as fuel, removal by incineration or removal by dumping. Manufacturers utilising any of the steps mentioned early in the action plan will ensure that the ultimate problem is minimised. This will reduce the need for the latter steps in the action plan such as incineration and dumping. For example, in step one, the use of potentially hazardous materials such as CNTs are eliminated or avoided, step two makes use of substitution by changing the material or process to help reduce the hazard. This could be done by handling CNTs in liquid. Step three advises isolating areas of potential release of CNTs by enclosing these areas or operations, or limiting access. Step four employs engineering solutions such as the use of - ventilation extractors, fume hoods and exhaust extractions. Step five looks at the standardisation practice through the use of administrative procedures for the handling and treatment of hazards. The last step looks at the use of necessary personal protective equipment. As mostly conventional PPE is used, light coloured PPE and work surfaces would make it easier for contamination to be identified.

Furthermore, it is recommended that the hierarchy of control is utilised as part of risk management. It is stressed that the control measures higher on the hierarchy are more effective and should first be utilised fully. The Administrative and PPE control measures are identified as the least effective measures as they bank on human judgement which differs from one person to another.

Falkner and Jaspers (2012) argue that directive on nanotechnology regulation is trailing behind its advancement. The type of risk that exists is often uncertain. The testing procedures that could apply and which test procedures still need to be established, must be accounted for per product and in their lifecycle. Hindering of international trade is mentioned as one of the consequences. The authors believe that the stigma surrounding novel technology with regards to potential hazardous effects is due to a lack of tangible facts. This forces political influence on regulation. However, political ordinance differs from place to place which could hamper global trade. There exists added stress in the nano-field as it is not proven that pre-existing rules and regulations sufficiently address nanotechnology in its entirety. Faulkner and Jaspers (2012) suggest three ways to harmonise regulation. The first is to improve the intellectual background to ensure sufficient evidence for decision making. Next, Faulkner and Jaspers (2012) advocate linking risk assessment and risk management. The authors' final proposal is to get input from developing countries. Faulkner and Jaspers (2012) found that there are mixed reactions to regulation. While some rule makers wait for enough factual evidence before implementing rules, others adopt a precautionary stance very early. An example of this was the differing position taken by the EU which adopted a precautionary approach to Genetically Modified Food while the US was pushing for trade limitations to be based on factual proof.

Falkner and Jaspers (2012) elaborate on regulation in the US admitting that there are many federal agencies responsible for specific sections of nanotechnology and nanomaterials. Nevertheless, the National Nanotechnology Initiative (NNI) directs nano-studies, nano-growth and nano-strategies. The Environment Protection Agency (EPA) is one federal agency that investigates original threats. This was affirmed when CNT was categorized as a new chemical with harsher regulation under the Toxic

Substances Control Act (TSCA). It is still under deliberation if existing frameworks address nanotechnology adequately. While some agencies feel that more evidence must be gathered to show the cause for alarm surrounding nanotechnology other agencies such as the Federal Drug Administration (FDA) are convinced that there is need to worry over the health concerns and suspicions of risk. The authors compare the United States against other countries such as Europe confiding that Europe faces the same knowledge gaps and uncertainties as the United States. Due to the concern over new technologies, the European Union (EU) has been pre-emptive in putting together a plan of action for nanotechnology. The European Commission (EC) promotes a precautionary approach. Additionally, in Europe a new chemical substances regulation Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) is being implemented to address the rules and regulations concerning nanotechnology. One area that will see REACH take effect is the manufacturing of nanomaterials. Creators of nanomaterials will be obligated to consider and generate information on chemicals for safe use and to add such information to the knowledge pool. Falkner and Jasper (2012) expound that the EC recommends that the legislation could be edited as new evidence is added to the current gaps of knowledge for nanotechnology. Other countries such as Australia, Canada, Korea and New Zealand have applied existing Environmental, Health and Safety requirements. In addition, Japan has implemented guidelines on the safe handling of nanomaterials. South Africa, along with China, India, Russia and Brazil, have allocated funds for nanotechnology exploration in relation to unambiguous and practical research. The authors surmise that while the US and EU are the front-runners in nanotechnology development, international governance has been stumped by indecisions in regulation and scientific advancement. The US prefers to base trade restrictions on thorough science while the EU supports the practice of a precautionary approach. This may mean that international regulation could be set without scientific proof. The chance exists that laws could possibly be either too lax or too restrictive for the new-fangled technology.

Marchant, Sylvester and Abbott (2009) identify nanotechnology as an empowering technology that is quickly occupying many different industrial fields. The authors are of the opinion that regulation will be perplexing because of the greater chance for both risk and benefits. Currently, it appears that the rate of technology is advancing more rapidly than regulation implementation. Marchant *et al.* (2009) proposed that a lot can be learned from history with regards to technology regulation. Marchant *et al.* (2009) put forward five specific lessons from the past.

Lesson A focuses on “Central importance of public confidence and trust”. It is discussed that it can take just one incident to lose public trust and this trust is difficult and sometimes impossible to rebuild. For example, the “Starlink” incident where the human food supply was tainted with genetically altered corn which had only been sanctioned for animal feed. In addition, false declarations of safety should be avoided as regulators can suffer long-term damage from such claims. An example of this is where the British government gave the public reassurances that “mad cow disease” could not be transferred to humans. It was later discovered that eating contaminated beef products did indeed cause a version of the disease in humans (Lava 2016). It is, therefore, prudent that government base proposed laws on factual evidence. This stresses at the need for toxicity investigation of ENMs. The questionnaire used for this study will advise on the knowledge of the researchers on the handling and waste management of ENMs.

Lesson B highlights the need to “Level the playing field”. Avoid implementing stringent regulations because of discriminatory and stigmatised public view, as well as avoid information about the actual risks. An example of this was the European Union (EU) applying more rigid regulation for genetically modified food even though the EU’s own research showed that scrutiny around genetically modified food probably made it safer. Similarly, there is no concrete evidence that nano-products would be more perilous than products without nanomaterials. The toxicological studies carried out for this study are important in ensuring that regulation is based on factual evidence to ensure regulation is appropriate and fair.

Lesson C addresses “Adaptive regulatory approaches”. The authors discuss that due to new technology intensifying rapidly, frameworks must be adaptable to ensure that there is suitably appropriate regulation as the technology develops. Examples of new technology are internet privacy and embryonic stem cell research.

Marchant *et al.* (2009) identify Lesson D as “Address social and moral concerns” and mention that, in addition, to environmental, health and safety concerns, social and ethical concerns should be considered. However, the authors do agree that it is difficult to reach a census on ethical and moral concerns.

Lesson E “International harmonization” is the last lesson identified. While regulation has habitually been addressed for each country in isolation, this method has not been problem free. Problems resulted from disorganisation and clashes due to irregularities among national approaches. For example, trade of GM Foods between EU and the US have encountered difficulties because of how regulation has been addressed in these countries. Marchant *et al.* (2009) concur that there needs to be global harmonisation through the standardisation of national regulations. The benefits of standardisation of practice have been described as being minimal disruptions to international trade, effective protection of human health and the environment, consistency for companies operating in multiple countries and countries do not become targeted as ‘risk havens’ due to their lack of regulation.

Arts *et al*. (2014) propose that nanotechnology should be investigated for threats, vulnerabilities and biokinetics from nanomaterial exposure. Although globally there are strategies being implemented on the manufacture, management and use of ENMs, these are restricted per country and relevant officialdom. It is evident that global consistency is required in the nanotechnology field. World-wide uniformity in legislation will be advantageous as standardisation of governance promotes understanding of requirements, clarity of individual expectations and awareness of the consequences of abuse.

Invernizzi (2013) suggests that there are differing views on the regulation of ENMs by different industrial areas. For example, some repel any form of regulation, while others opt for voluntary systems. There are others that are partial to minimal regulation. Reasons for these missed sentiments, he believes, are due to the mixed and multiple findings on the risk of nanomaterials in the public domain. Particularly, because ENMs are known to exhibit fresh and unfamiliar physiochemical and biological properties. Some have also shown to be toxic when analysed *in vitro* and through animal testing.

Preston *et al*. (2010) reveal that the United States Environmental Protection Agency (EPA) is attempting to use the existing Toxic Substances Control Act (TCSA) (1976). This Act gives the EPA a rudimentary starting point for regulation, as well as controlling the substances manufactured and distributed. It is unclear if the public is aware of the risk. The properties may differ significantly to the properties of the bulk/macro materials. Nanomaterials might be regulated by TCSA under one of the following categories: size only, size plus one and significant new use.

Preston *et al.* (2010) explain that these categories have been identified in order to ensure the correct regulation of nanotechnology to avoid the conjecture that nanomaterials are a threat to human health and the environment. The size only category takes into account the actual size of the material. Through existing research, it has been shown that a large number of unique characteristics can emerge at the smaller dimension. For example, gold - which is a well-known precious metal popular for its non-reactive nature, becomes useful as a catalytic agent when its scale is reduced. In other cases, nanomaterial toxicity has been linked closely to its size. The size plus one category essential looks at the size, as well as the shape of the material. A relationship was found between the novel and useful properties from nanomaterials and related health issues. For example, CNTs with a span ranging from 0.4nm to 100nm have an affinity to clump up and have, therefore, been found to be toxic for living cells as it may not be easily expelled. The significant “New use” category has been introduced and makes use of prevailing TCSA decree. It affords the EPA the prospect to monitor manufacturers in new technology the same way they would monitor a manufacturer of a new chemical. Preston *et al.* (2010) surmise that while the benefit of using these categories would be the fair implementation of nanotechnology regulation there is still much more information required on the toxicity of nanomaterials. Toxicity information for nanomaterials is still minimal. The earthworm toxicity experiment could be valuable in adding to the knowledge of potential toxicity.

Kolodziejczyk (2016) comments that the nanotechnology field is in need of unambiguous and effectual strategies or guidelines related to monitoring of nano-products through their lifecycle.

Petratos (2015) believes that a well-designed legislation can aid the commercial acceptance of products containing nanomaterials. For instance, the FDA cannot sufficiently monitor medical products containing nanoparticles. This impedes the FDA’s ability to support modernization. Nano-regulations are specifically difficult for the FDA due to the fragile state of the scientific knowledge surrounding the nano-field. This means that nano-products cannot be easily slotted into existing FDA regulations.

Petratos (2015) continues his observations that the FDA will focus on the end product and have released guidance documents accordingly, however, these guidelines may also focus on end products that fall outside of the nano-range. Nevertheless, the EPA has actually claimed dominion over nanotechnology under the TSCA which may mean mixed decisions on approval of nano-products from the FDA and EPA. This will cause delays in marketing nano-products. The author directs us on the way forward which includes ensuring that the definition of nanotechnology has been agreed upon and understood by all involved parties as this removes ambiguity on what falls under regulation, supports a co-dependent relationship among regulatory organisations, promotes knowledge sharing on best practice by including the private sector in decisions on regulation and finally, launches nano-specific requirements for measuring risks, cost and benefits. The inclusion of all relevant parties will allow for informed decision-making on the way forward for regulating nanotechnology. In addition, the sharing and contribution of knowledge and information from parties involved in different aspects of the ENM lifecycle will give regulatory requirements a chance to advance at the rate that the new technology is advancing.

Haywood (2012) states that the National Institute for Occupational Safety and Health (NIOSH) concedes that animal studies indicate an asbestos like reaction when animals are exposed to longer, straighter styled CNTs. The Occupational Health and Safety Act (Act No.85 of 1993) is in place for Asbestos Regulation. It is interesting to note that even with the similarities between CNTs and asbestos being increasingly highlighted regulation still does not exist for CNTs.

Allan *et al.* (2009), supposes that the effects of asbestos have made the world at large cautionary to new technology. In particular, wariness exists for revolutionary technology that is not yet governed by legislation. Current regulators have not made inclusions for waste generated from nano-industries so there remains vagueness for the treatment of airborne, effluent and solid nano-waste.

Breggin and Pendergrass (2007), point out that there are two federal laws that are specific for waste and concerns that arise from end-of-life handling. One of these regulators is the Resource Conservation and Recovery Act (RCRA) whose purpose is to control the handling, re-use, recycling, storage, treatment and disposal of solid wastes including hazardous waste. The authors confirm that nanomaterials have special physical, chemical and biological properties. This has increased its use in a variety of products and in most industries in some form or other. With its current use including consumer products, they conclude that while the RCRA does cover nano-waste, it may not be appropriate for nano-waste because consumer products including those containing nanoparticles are mostly identified as household waste which is excluded from hazardous waste treatment.

As a general guideline, nano-waste is supposed to be handled and treated as hazardous waste (Soler, Aires and Morales 2015). Hester (2006) explains that the RCRA provides the EPA with the authority to regulate the management of hazardous waste. However, nano-waste may have original risks that may not have been deliberated by the EPA.

Murashov *et al*. (2011) compared the regulatory approaches to worker protection between different countries. Their findings showed that France employs measures of strict containment of nano-waste in order to safeguard their workers while Germany has aligned their ACT to the European Directive. The European community has implemented minimum requirements and fundamental principles for health and safety issues. Employers in the EU are required to conduct hazard assessments and to implement measures from the hierarchy of control. Risk management approaches can then be implemented. The report Control of Substances Hazardous to Health (2013) also advises that a precautionary approach be followed on nano-waste due to the uncertainty of CNT risks. This view is supported by Theodore and Kunz (2005) who allege that although nano-regulations are not yet available, organisations, in general, are taking a precautionary approach in relation to the handling, recycling and disposal of their nanoproducts. This stems largely from a fear of litigation in the event of their nanoproducts negatively impacting on humans and the environment.

Murashov *et al.* (2011), further add that in the United States, the Occupational Safety and Health Act of 1970 (OSHAct), Toxic Substances Control Act (TSCA) and the Federal Insecticide and Fungacide and Rodenticide Act (FIFRA) work together to protect worker health. In addition, the National Institute for Occupational Safety and Health (NIOSH) is responsible for investigating incipient technologies and then making recommendations for worker health and safety. While there are no health and safety standards for ENMs, recommended exposure limits (RELs) for titanium oxide and

CNTs are being investigated. This would be, in addition, to the permissible exposure limits (PELs) set for carbon black ($3.5mg/m^3$) and synthetic graphite ($15mg/m^3$). However, the limits set for these materials were adopted prior to the knowledge that these were ENMs. Crowley *et al*. (2003) identify workers exposed to nanoparticles at the workplace as one lot of people that could be at risk. Landfill site disposal can lead to gas and leachate contaminated with nanoparticles. Similarly, the incineration process releases various volatile and gaseous emissions. There is a need to regulate aerosol limits to minimise exposure of workers at end-of-life facilities.

Castillo (2013) puts forward that in Europe, key bodies such as the European Commission, European Parliament and member states cannot agree on the regulation of nanomaterials. He advises that it is clear that regulation is moving slowly in comparison to the development of the field. Castillo (2013) goes on to state that the differing views on regulations are evident amongst all stakeholders. Some require strict regulation, most others prefer a cautionary approach with strict regulation and a few support little to no regulation. The European Commission is under the impression that the current legislation covers the potential risks since they are similar to chemical substances. A lack of standardised regulation may fast-track the effects on the environment and human health as nano-waste may not be contained, recycled or disposed of appropriately.

Fuldo *et al*. (2014) confirm that Europe has always adopted a cautionary approach for any new technology. Europe's approach has also remained pre-emptive. The EU has introduced regulation for nanotechnology due to the continued expansion of the nano-field into commercial, research and industrial markets. Fuldo *et al.* (2014) clarify that Europe has a team of legislation makers. The rules and legal requirements developed are dependent on the type of product requiring legislation. They established that the end product rather than the process of nanotechnology is being legislated in Europe. For example, in the cosmetic industry, products are not identified as harmful if they contain nanoparticles which could potentially influence their sales and use. Rather legislation requires that products containing any amount of nanoparticles must be identified as such so that the consumer is making an informed decision when purchasing such items.

Based on the aforementioned information it is apparent that regulation for nanomaterials is severely lacking. This is due to the limited information on toxicity and exposure risks. It is, therefore, critical that toxicity studies and leaching potential of ENMs be concluded in order to inform developing regulation.

2.6 Behaviour, Toxicity and Leaching Potential of CNTs in Water and Soil Waste Streams

According to Taylor and Allen (2006), waste is mostly disposed of via landfill sites. This may be because the method of disposal is simple, cheap and cost effective. In order to address the safety concerns associated with inefficient recycling and disposal of nano-waste, the behaviour, potential toxicity and leaching ability of CNTs must be understood. According to the 2004 OECD Guidance document for neurotoxicity testing manufactured chemicals such as CNTs may reach the soil via the direct route from agrochemicals or the indirect route from the movement of substance deeper into soil layers eventually reaching groundwater. It is, therefore, prudent that the mobility of CNT through soil be investigated in order to establish the extent to which leaching may contaminate the environmental surrounds.

Falkner and Jaspers (2012) concede that there are many possible release scenarios from production to use and finally disposal. They too advise that MWCNTs have been likened to asbestos and animal studies have shown that it can cause mesothelioma in the lung coating. The release into wastewater can cause contamination of the surrounding environment. The authors point out an incident in China in 2009 where several workers exposed to nanoparticles over a period of a few months fell ill resulting in two deaths and leaving several other workers disabled. While the toxicological claims remain contested, it was concluded that the conventional techniques used for recycling and disposal of bulk/macro materials may not be suitable for nanomaterials. Fanatical based regulation could hinder the growth in technology so a step-by-step approach to regulation implementation is advised so that informed modifications can be made to the potential regulatory framework.

Terekhova and Gladkova (2013) criticise researchers as having different opinions about the toxicity effects of ENMs on soil and living organisms. This is due to there being no clear identification of the hazards associated with nanomaterials and a lack of knowledge in this area. Terekhova and Gladkova (2013) found no correlation between the concentration of nanomaterials and its effects on living organisms. A suspension of nano-titanium added to mustard seedlings at various concentrations showed that growth was inhibited at all concentrations. The effect of nano-titanium on a protozoan culture showed that a low concentration of nano-titanium stimulated a low growth response. The growth response increased as the concentration of nano-titanium was increased. However, a low concentration of nano-titanium suppressed the brightness of luminescent bacteria. The luminescence was stimulated with an increase in concentration of nano-titanium. In the same manner that nano-titanium negatively impacts the bacteria ENMs could have a similar effect on environmental quality. The environmental quality could be compromised during the recycling and disposal stages which have been identified as a contributor to environmental contamination. The deficiency of conclusive evidence impedes the advancement of this technology. This may be due to the lack of standardised test methods specific to nanotechnology. Standardised test methods could support the gain of useful and reliable data.

As the potential use of CNT appears to be infinite, Hoang and O'Malley (2013), fear that the limited information on nanomaterials does not allow for an informed decision for laws and regulation that can govern nanomaterials throughout its lifecycle. Hoang and O'Malley (2013) reiterate the concern about the similarity of the biopersistence and fibrous nature of CNT to asbestos. The concern is strengthened by the size of the fibres as this allows for easy entry into the body. While the shorter MWCNT could be tweaked so that they are released from the body, larger MWCNT may not be so easily expelled. This will mean accumulation of CNT which may lead to similar diseases as those shown by asbestos. Hoang and O' Malley (2013), illustrate their beliefs through the example of studies conducted at NASA's Johnson Space Centre in the United States of America which showed that CNTs caused lacerations in the lungs of rodents. The wounds became eviler over time indicating the toxicity of CNT. The results from the animal studies may be indicative of the potential effects on human health and the environment.

Petersen and Henry (2012), emphasise that particles of CNTs must be characterised for ecotoxicity to be understood and addressed. They elaborate by stating that the purity of CNT is unreliable and CNT could, therefore, contain toxic chemicals which would make them harmful. In addition, to the characteristics of the CNT before, during and after testing, the authors recommend that the raw starting materials are also considered for toxicity contribution. Murthy, Kishore and Surekha (2011) revealed that there was no mortality recorded for acute toxicity testing using Wistar rats. The testing was administered both orally and dermally. Haywood (2012) conveys that the OECD test guidelines which used MWCNTs were found to be of low acute oral and dermal toxicity. Although it appears that CNT does not have a toxic effect on animal health and may be suggestive of no toxic effect on human health and the environment, the latent effects of CNT are yet to be determined.

Heckmann et al. (2011), state that there are nano-ecotoxicological studies which confirm that there is a dose-response relationship for nanoparticles as with increased concentration there is an increase in mortality. However, they bemoan that apart from nano-related toxicity, consideration must be given to the toxicity contribution from materials combined with nanoparticles to form nanomaterials. They concluded that it is impulsive to make recommendations on nanoparticle impact.

To augment the gap of knowledge in order to standardise ecotoxicity testing, Petersen and Henry (2012) highlight the need for research laboratories to share and compare results achieved as this will facilitate interpretation of toxicity results and standardisation of practice. The sharing of knowledge may also allow nanotechnology to advance quicker as it will minimise duplication in information gathering studies.

2.7 Quality Tools

Total quality management is the management of activities to produce quality products or service. The concept of quality management can be expanded to Good Laboratory or Manufacturing Practice (Matsoso and Benedict 2015). For example, Engineering Controls and Lean Manufacturing could help to control, reduce or eliminate nanoparticle exposure to workers and the environment. Anvari, Ismail and Hojjati (2011) state that Total Quality Management is made up of various practices. These practices include a systematic approach to continuous improvement, problem solving for improvement and benchmarking. Total Quality Management could be used to aid in the responsible development of nanotechnology.

Anvari, Ismail and Hojjati (2011) divulge that Lean Manufacturing is a management philosophy that has helped in the success of the Toyota Production System. Lean Manufacturing is concentrated on optimising processes by reducing non-value adding activities. Anvari, Ismail and Hojjati (2011) write that the main objective is to eliminate waste through the optimisation of processes.

Soutter (2012) explains that limiting the waste generated during production processes manufacturers can reduce the burden on the environment. This means corresponding benefits for people and animals as less nano-waste will need to be recycled and disposed of through landfill sites.

The teachings from Quality Gurus will be used to aid the formulation of the strategy to be used by nano-organisations for the recycling and disposal of nano-waste. The Gurus have concepts and approaches to quality that have a significant and lasting impact on organisations. One such guru, W Edward Deming's, Plan - Do- Check- Act (PDCA) cycle is a systematic approach to problem solving and improvement. The use of the PDCA cycle promotes process learning and ongoing improvement (Austenfeld 2001) which from the foregoing commentary is needed by the nanotechnology fraternity.

Haywood (2012) recommends that a risk management process is used for the handling of CNTs. This risk management process should look at collecting information to identify risks, implementing controls or preventative measures to minimise risk occurrence and to check that the measures taken remain suitable. The Risk Management process suggested in this report could also be likened to Deming's PDCA cycle which is portrayed in Figure 2.6. The PDCA cycle is a four step quality management method for continual improvement. The steps to the cycle can be adapted to nanotechnology.

(i) Plan: Consider the material and the application thereof. Material properties, risks, hazards and exposure must be identified and controls selected. Establish policies and procedures of handling and disposal of ENMs. Conduct Safety, Health and Environmental Risk Assessments. Establish medical requirements for workers at landfill sites and incineration facilities. For example, the use of titanium dioxide nanoparticles in hair styling tools. Nano-waste from the production process will enter the waste stream. Shah, Shah, Hussain and Khan (2017) point out that nano-titanium can have genotoxic, carcinogenic and phototoxic effects. Nano-titanium will enter the environment during the disposal process and there is a risk for negative impact on soil, plants and the surrounds.

(ii) Do: Implementation of controls. Implement training programmes for workers that are part of the production and end-of-life treatment of nano-waste. Develop policies and procedures to standardise the regulation of nano-waste with regards to end-of-life handling and treatment at nano-facilities and disposal facilities.

(iii) Check: Check the verification of adherence to implemented policies and procedures to determine where gaps exist. Check that hazards are reduced, the adequacy of engineering and administrative controls and PPE appropriateness through staff medical screenings. Nano-facilities could be monitored for adherence to the policies and procedures when disposing of nano-waste. Air, water and soil monitoring could take place at disposal facilities. Workers that are part of the end-of-life process could have regular medical screenings.

(iv) Act: Review and adapt based on results. Incentives and fines could be used to have nano facilities conform to the regulations for disposal. Filters found to be inadequate for trapping nanoparticles from incinerators could be changed.

Figure 2.6 Plan-Do-Check-Act (PDCA) cycle
(Adapted from Ong and Sheriff 2009)

Malsch (2014) confides that the NNI has put the training of staff involved in nanotechnology on the International Policy and Scholarly agendas while the EU has its own Nanotechnology Action Plan for promoting nano-science and nanotechnology education. Further to that as mentioned earlier, the European Commission promotes education and has united nanotechnology education into its policies. It is preferable that the educational material is specific to the natural dialect. This minimises or eliminates the need for translation of prevailing information or creating fresh material which can become very expensive.

Malsch (2014) lists the ways that training can be carried out. These include attending specific courses and on the job training or through networking within or outside of the organisation. A quality tool referred to as benchmarking can be used for networking. Benchmarking is a tool used for gap analysis to encourage breakthrough within an organisation. Helgason (1997) identified comparison as a motivator for performance. He stated that there are two methods to benchmarking which aims to identify and implement best practice. The top-down method is when an outside party is driving benchmarking within an organisation while a bottom-up method is when an organisation drives benchmarking from within. Helgason (1997) breaks down the benchmarking process into five steps namely:

- Step one is to find an organisation that offers the best service in your field.
- Step two is to determine how they succeed in achieving the best result.
- Step three looks at putting together an action plan for implementation of best practice in your organisation.
- Step four is to put the plan into action within your organisation.
- The last step is to assess the results to determine if the practice has been implemented successfully.

In the nano-field, benchmarking could be beneficial for countries such as South Africa which could benchmark against countries such as the United States or Europe.

Malsch (2014) stresses that there are four key interested parties that contribute to risk decision-making. These are political, scientific, business and civil society. Quality tools can help with the decision making process. Watson (2004) confirms that Dr Kaoru Ishikawa was the quality guru who had conceptualised the Ishikawa diagram described in Figure 2.7 otherwise known as the fishbone or cause and effect diagram. This diagram was used for problem solving and improvement. The diagram identifies all causes to problems on the left and the problem on the right. This enables the user to analyse the problem taking the possible causes into consideration in order to identify root causes of problems and implement appropriate corrective action.

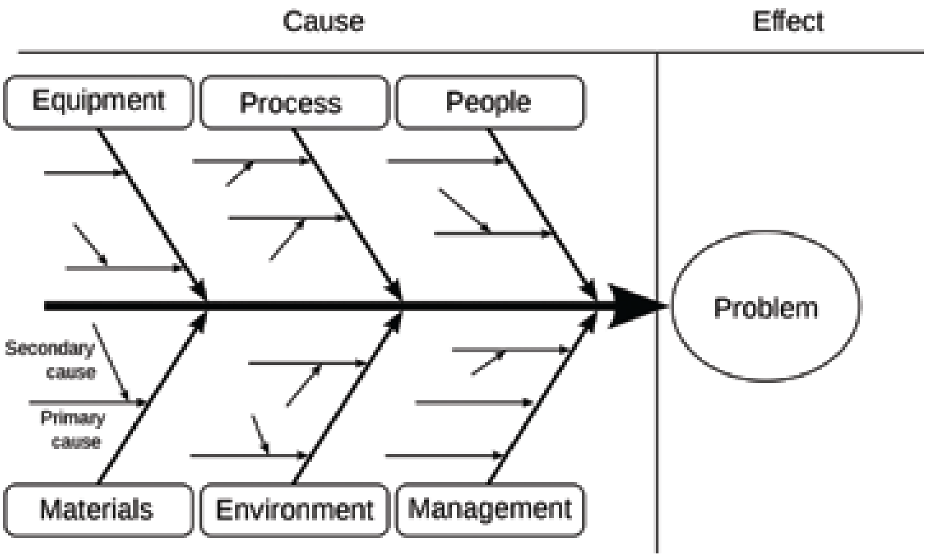

Figure 2.7 Ishikawa (Fishbone / Cause and Effect) Diagram
(Adapted from Clary and Wandersee 2010)

As Malsch (2014) elaborates that the four key interested parties (political, scientific, business and civil society) assist in defining the problem and then generate solutions which are evaluated for suitability so that an agreed upon solution can be implemented. It was, therefore, prudent to have feedback from researchers about their knowledge on handling, treatment, recycling and disposal of ENMs in order to develop solutions that could assist researchers with regards to end-of-life treatment of ENMs.

2.8 Summary

This chapter has highlighted the many ways that nanotechnology can be progressive. Conversely, it also brings attention to the evidence that its distinctive characteristics have similarities to asbestos, lack of nano-specific personal protective equipment and potential latent behaviour could have a negative impact on human health and the environment. There is a lack of evidence indicating that current legislation for bulk/macro materials can effectively be applied to nanotechnology. A global strategy is lacking for the standardisation of nanomaterials. While options for disposal do exist these methods have not been proven suitable for nano-waste. This is a concern as the evidence indicates that there is release of the nanoparticles throughout its lifecycle including end-of-life treatment. A precautionary stance remains the first line of defence.

The following chapter will look at the research plan and techniques that will be used for the research study.

CHAPTER THREE: RESEARCH DESIGN AND METHODOLOGY

3.1 Overview of Chapter Three

This chapter outlines the research design and research methodology used for this study with reference to data collection, sampling, data analysis, validity, reliability and ethical considerations. A description of the pilot and main study is presented for a series of toxicity tests, leaching potential testing of CNTs and a survey in the form of a questionnaire.

3.2 Introduction and background of research design and methodology

Mugo (2002), terms research as being the process to look for or to examine thoroughly or to collect data on a particular subject. This is reiterated by Rajasekar, Philominathan and Chinnathambi (2013) who stress that research is a logical process of obtaining sought after knowledge. The authors proffered that through research people have been made aware that heroin is addictive, cigarette smoking is dangerous to health and that Acute Immune Deficiency Syndrome (AIDS) is due to the Human Immunodeficiency Virus (HIV).

As the popular proverb goes "knowledge is power" and the knowledge gained from this research study may inform the action to be taken for mitigating risk implications and improvement of processes for effective recycling and disposal of nanomaterials.

In the efforts of gathering the necessary information for strategic development, this research study comprised of a review of literature sources, a questionnaire, as well as experimental work as a means of collecting data.

A questionnaire will be used to achieve objective 2. This objective is to determine the practices used for recycling and disposal of ENMs within a research organisation. The questionnaire will also be indicative of the knowledge base of researchers with regards to the end-of-life handling and potential risks of nanomaterials.

Experimental work will be conducted to achieve objective 3 of this study which is to observe the behaviour, the potential toxicity and the leaching potential of CNTs using the Organisation for Economic Co-operation and Development (OECD) Test guidelines. The results from the experimental work could inform better management of landfill sites in terms of leachate seepage to groundwater and effects on fauna, flora and related ecosystems. It will also provide a better understanding of the behaviour of nanomaterials in the environment and hence facilitate ease of management.

As Suarez (1992) divulges, the Plan-Do-Check-Act (PDCA) model is a four-step model that can be used for problem solving. The PDCA model is, therefore, thought to be ideal for addressing the research problem. The research study can be sectioned into the different stages of the PDCA model to aid problem solving.

Stage one will use Chapter One Introduction and Chapter Two Literature review to ensure that factually based plans are developed. The Strategy and Approach of the study will be planned based on the aims and objectives of the research study.

Stage two advances to the implementation of the plan. In this stage, data is sampled and collected. Methods for data collection include the completion of questionnaires and experimental test work.

Stage three allows for the completion of data analysis and the evaluation of the results. Data may be compared to literature that has been reviewed to form part of discussion.

Stage four describes the action taken based on the results from data analysis. For this study, this will be the development of a strategy for the recycling and disposal of CNTs.

3.3 Methodological blueprint of the study

The methodology as applied to this study has been stated in the subsequent sections.

3.3.1 Conceptualising this study

Borm and Berube (2008) claim that nanomaterials are being presented into the world on the foundation of potential benefits. This study was stimulated by inductive reasoning stemming from this innovative technology not only offering potential benefits but also presenting uncertainty and risk. The inductive approach reflects shared repetitive information obtained through content analysis in order to condense data and make valid inferences (Thomas 2003). Stemler (2001) purports that content analysis sanctions inferences which can then be substantiated by using other data collection techniques. Critical thinking was also instigated through content analysis and involved the review of texts such as technical reports, journals, prescribed test guidelines and other literature sources, as well as graphics. Experimental observation was a contributor to deductive reasoning and comprised of toxicity and leachability testing of CNTs, as well as the circulation of a questionnaire related to the researcher's knowledge on the recycling of and disposal practices of CNTs.

3.3.2 Strategy adopted for this study

Both a theoretical and an empirical strategy was used in this study in order to collect necessary information. Kumar (2011) defines the theoretical and interpretivist strategy as one that relies on the accumulated knowledge gained from all literature sources having a direct or indirect influence on the study. He suggests that the knowledge gained must be reviewed to sort out the critical information and further explains that an empirical and positivist strategy stems from primary evidence obtained through observation of scientific experiments and observations such as responses from a questionnaire. In this study, the theoretical strategy has been applied in Chapter Two. The empirical strategy has been applied in Chapter Three to address the experimental work and questionnaire.

3.3.3 The Approach of this study

A mixed methods research approach was adopted in this study as the tactic was based on content analysis, observation of experimental work and non-participant observation using a questionnaire. Creswell (2014) concludes that the mixed method approach makes use of both the qualitative and quantitative approaches. Due to the novelty of this area of research, the qualitative approach entailed the review of all sources of methodology and this information was used to inform the research design and questionnaire. The quantitative aspect of the study looked at the measurement of variables for experimental work undertaken, as well as the evaluation of responses from the questionnaire using the Likert scale.

3.4 Materials

The subsequent materials were key resources utilised for this study.

3.4.1 Test Species used for the pilot and main toxicity testing

Eisenia foetida (Michaelsen earthworm) species is used in vermicomposting of both domestic and industrial waste streams (Gallessich, 2001). The *Eisenia foetida* species was used for the toxicity tests in this study. Worms were purchased from a well- known breeder, Eco Worm Farms. Worms used in the experimental work had to be adult worms, at least two months old. Worms having clitellum was also a prerequisite. The clitellum is a thickened, glandular, non-segmented portion near the head of the worm as shown in Figure 3.1. The eggs of the earthworm are deposited here. Each worm had to weigh approximately between 300mg to 600mg.

The worms were stored in a specially designed worm bin that mimics the conditions required for the earthworms. The worm bin was housed in a controlled environment in the Animal Laboratory located on the Durban University of Technology, Steve Biko Campus. The lid of the bin remained closed as the worms are sensitive to bright light. Kim (2016) conveys that the *Eisenia foetida* earthworm can tolerate a wide range of temperatures, varied moisture conditions and variation in soil pH. These worms live on the surface of the soil or in the topsoil layer. They will, therefore, migrate away from the area of soil that has an unsuitable temperature. For the study, the temperature was considered by conducting the filter paper toxicity test at two temperatures. The moisture of the soil was considered for the main toxicity testing. The moisture content of the soil was monitored at the start, during and at the end of the main toxicity study. Kim (2016) further explains that these worms flourish in putrefying organic matter. Therefore, the worms were fed organic food for the duration of the study.

3.4.1.1 Preparation of test species for the toxicity study

Prior to, testing the worms were placed on moist filter paper so that they could void their gut contents. They were washed with tap water, blot dried using a paper towel and weighed. Tap water was used as it did not affect the integument of the earthworms. Although earthworms can survive in water as long as there is sufficient oxygen, the earthworms were not submerged into the water for long periods and had only been rinsed quickly with minimal water. Earthworms used for the control test were selected at random after the washing process (OECD Test 2017 1984).

Figure 3.1 Clitellated adult *Eisenia foetida* earthworm
(Source: https://wormfarmingequipment.com/red-worms)

3.4.2 Test substances

3.4.2.1 Pilot toxicity study: nano-clay

Nano-clay was the test substance used for the pilot work. The test substance was obtained from a CNT supplier to the Mechanical Engineering Department of DUT. Although it had originally been planned to use CNTs for the pilot work, as well as the main study, the number of CNTs required for both studies would have cost an exorbitant. For this reason, a decision was made to use nano-clay for the pilot work.

3.4.2.2 Main toxicity study and leaching in soil columns test: carbon nanotubes

Multi-walled carbon nanotubes (MWCNTs) structure as shown in Figure 2.2 were used for the main toxicity test, as well as the leaching in soil columns test. These CNTs appear in the form of a black powder and do not contain an odour. The test samples were obtained from Nanoshel LLC via the Mechanical Engineering Department of DUT. The test substance was kept isolated from the worm bin for the duration of the study to avoid contamination of the breed stock.

3.4.3 Reference Substances

3.4.3.1 Toxicity testing

2-Chloracetamide is a crystalline pale white to yellow chlorinated organic compound that is used as a preservative and herbicide (Liebert 1991). This compound has been recommended by OECD Test 207 guideline (1984) as the reference substance for toxicity testing as it is known to be toxic to earthworms. The reference substance was included in both the pilot work, as well as the main study. According to the findings of (Liebert 1991), the lethal dose (LD50) that kills up to half of the test species is dependent on the animal used for the testing. Navjot, Ramash and Kumar (2014) state that the lethal concentration (LC50) for chloracetamide that kills up to half of the earthworms is approximately between 20 to 80 mg/kg in dry soil. As there is no set lethal dose that has been established 0.01% was used as the lethal dose. For the study 0.015g of chloracetamide was added to every 150g of artificial soil.

3.4.3.2 Leaching in soil column test

Atrazine was recommended by the OECD Test 312 guideline (2004) for the testing. Kersante *et al*. (2006) explain that atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) is an herbicide with moderate to high mobility in soil.

3.4.4 Artificial soil used for main toxicity study and leaching in soil column test

The artificial soil was made up as per the OECD Test 207 (1984). Sphagnum peat and kaolin clay were purchased individually from retail suppliers Grovidas and Kamel Pottery respectively. According to OECD Test 207 (1984), the peat must have a pH as close to 5.5 to 6 as possible. The pH of the peat was determined on a 1:10 slurry. The leachate was read on a pH meter using a pH electrode. The pH could have been adjusted with calcium carbonate if the pH was not within range. The pH fell within the acceptable range and did not require adjustment prior to testing. Industrial sand was obtained from Spec Silica Sands. The industrial sand obtained was fine, dry, untreated and more than 50% of the particles passed between 50 and 200 microns. The soil test substrate was made up by combining 10% sphagnum peat, 20% kaolin clay and 70%

industrial sand. After combining 0.45kg sphagnum peat, 0.9kg kaolin clay and 3.15kg industrial sand, by manual mixing, the moisture content of the soil was determined by weight difference following oven drying at 105ºC for 1 hour. The overall moisture content was then adjusted using deionized water to give a moisture content of approximately 35% of the dry weight. The moisture content was calculated using the formulae below.

% moisture = [(net wet weight in grams -net dry weight in grams) ÷ net dry weight in grams] x 100

3.4.5 Artificial rain used for the leaching in soil column test

0.01 molar solution of calcium chloride ($CaCl_2$) was used as artificial rain. 7.3505 grams of $CaCl_2$ was weighed out into a beaker using a 4 place analytical balance. The salt was transferred quantitatively to a 5-litre volumetric flask. Sufficient deionized water was added to completely dissolve the $CaCl_2$ before the solution was diluted to volume with deionized water (OECD Test 312 2004).

3.4.6 Soil columns used for leaching in soil column test

Figure 3.2 Setup for leaching in soil columns test
(Adapted from OECD Test 312 2004)

OECD Test 312 (2004) requires columns to be made from an inert material, for example glass, stainless steel, Teflon and PVC. Inert columns are required so that the column itself does not react chemically during testing. For this study, glass columns were used. Approximately 100 grams of soil was transferred in small portions into the column using a spatula. The columns were gently tapped in order to settle the soil before a glass rod was used as a plunger to compact the soil. The sand was pre-wetted with the artificial rain solution.

3.4.7 Preparation of glass vials for pilot toxicity testing

Glass vials of approximately 7.5 to 8cm in length and approximately 2.5 to 3cm in diameter were used. The vials were lined with medium grade filter paper allowing for minimal overlap. The filter paper was then moistened with 1ml deionised water for the Control, 1ml of chloracetamide for the reference and 1ml treatment solution for the test samples (OECD Test 207 guideline 1984). 1ml of each treatment level was added to the filter paper using a graduated pipette. The test substance was allowed to dry. As per (OECD Test 207 guideline 1984), for test substances that are relatively insoluble in either deionised water or solvent, a further 1ml of the relevant treatment solution can be added to the filter paper and dried in order to obtain a larger deposit of the test substance. The worms were then placed into the vials. Each vial contained one worm.

3.4.8 Test Chambers

All test chambers were adapted from OECD Test 207 guideline (1984) and OECD Test 312 guideline (2004). For the pilot toxicity study, the tests were done in glass vials. For the main toxicity study, 500ml glass honey jars were used.

The leaching soil column test made use of a glass ion exchange column.

3.4.9 Environmental conditions for experimental testing

Environmental conditions were monitored during the experimental studies. These conditions have been described below.

OECD Test 207 guideline (1984) recommend that the filter paper toxicity test (pilot test) is done in the dark at a temperature of approximately 20ºC ± 2ºC.

In contrast, the same guideline recommends the main toxicity test is done in continuous light also at a temperature of approximately 20ºC ± 2ºC.

OECD Test 312 guideline (2004) gives the required test temperature range as between 18ºC - 25ºC. The soil columns were kept in the dark for the duration of the 48 hour testing period.

3.4.10 Dose/treatment levels

The dose/treatment levels for the experimental testing are described below.

3.4.10.1 Dose/treatment levels used for the toxicity studies

The OECD Test 207 (1984), which is suitable for bulk /macro materials, suggests that 0.1, 1.0, 10, 100 and 1000 mg/kg/ parts per million (ppm) be used. The dose/treatment levels were adjusted for the testing of the nano-clay used in the pilot toxicity study and the CNT used in the main toxicity study. The concentrations were adjusted bearing in mind the cost of the nanomaterials which meant that as little as possible was used. For the toxicity studies, a six range dose/treatment levels were used as suggested by the OECD Test 207 (1984). However, in an effort not to overlook the lethal concentration (LC_{50} or LC_{100}) of the nanomaterial, an additional concentration was added. The final dose/treatment levels were in the range 2ppm to 10 000ppm. Baskar, Sudha and Tamilselvan (2016) attest that establishing the lethal dose of a substance can indicate the safety of that chemical. This was proven by determining the lethal dose of chloracetamide to animals and human beings. Chloracetamide was ruled to be unsafe as a component of cosmetics.

For the pilot toxicity study the test substance (nano-clay) was not soluble in deionised water. A solvent (acetone) was used but the test substance still remained insoluble. A decision was taken to use the deionised water suspension. The test substance was weighed out on Balance 22 Adam Scale Model PGW 603e (DUT Serial 221388). A weighing boat was zeroed on the scale and approximately 7 grams of the test substance was weighed into the boat. This was transferred into a beaker and 300ml of deionised water was added with a measuring cylinder. The suspension was mixed thoroughly by swirling, using a clean spatula to break down any lumps. This solution was identified as solution 5 (largest concentration) with an expected value of approximately 20 000ppm. This solution was used to determine the subsequent treatment levels by serial dilution. This solution was diluted by mixing and transferring it directly into a 10ml measuring cylinder. The solution was transferred into a 100ml-volumetric flask and diluted to volume with deionised water to give solution 4 with an expected value of approximately 2 000ppm. Solution 4 was mixed and transferred into a 10ml measuring cylinder. The solution was transferred into a 100ml-volumetric flask and diluted to volume with deionised water to give solution 3 with an expected value of approximately 200ppm. The above process was repeated to make up solution 2 with an expected value of approximately 20ppm and solution 1 (lowest concentration) with an expected value of approximately 2.0ppm.

For the main toxicity study the concentration values were the same as used for the pilot toxicity testing. However, for the main toxicity testing, CNT was used as the test substance. As this test required the use of soil as the test medium and the CNT is not soluble in deionised water, the test substance was added directly to the soil in powder form. The amount of test substance added was sufficient to give the final concentration required per test jar. A weight of 150g of artificial soil was used per jar. Table 3.1 shows the weight of the CNTs used to achieve the desired concentration in 150g of soil.

Table 3.1 The weight of CNT required per 150g of artificial soil

Dose/Treatment level required (ppm)	**Weight of CNT per 150g soil (g)**
2	0.0003
20	0.003
200	0.03
2000	0.3
20000	3

The CNT was weighed into a tared weighing boat before being added directly to the test jar containing the soil medium. The CNT was mixed thoroughly into the soil using a clean glass rod.

3.4.10.2 Leaching in soil column test

The pilot toxicity testing informed the selection of the concentrations used for the leaching in soil column test. The LC_{50} concentration was estimated at 200 ppm and the LC_{100} concentration was estimated to be 20000 ppm were used for the study. As per OECD Test 312 guideline (2004), poorly soluble test substance should be added directly to the surface of the soil. The weight of CNT used was as established in Table 3.1.

3.5 Sampling for the questionnaire and experimental work

Mugo (2002) and Alvi (2016) are in agreement that sampling is the process of removing a portion (sample) of a population in order to make deductions about the whole population. For this study, purposeful sampling was employed in order to accumulate exhaustive knowledge on nanotechnology.

3.5.1 Sampling for the questionnaire

Alvi (2016) defines probability sampling as sampling with a definite target population. For the questionnaire, the population was limited to researchers working with nanomaterials in research and development facilities or as part of their individual research studies.

3.5.2 Sampling for the toxicity studies and leaching in soil columns test

The requirements of OECD Test 207 (1984) and OECD Test 312 (2004) were followed as closely as possible.

3.5.3 Sample size

Mugo (2002) advises that in order to determine the sample size consideration must first be given to the population. Various other constraints are also considered. For example, cost, availability, required precision and the tests required.

The sample size requirements for the questionnaire and experimental work are stipulated below.

For the pilot work, the questionnaires were distributed to five researchers from the Durban University of Technology (DUT) Composite Research Group. Feedback from the pilot study on the design of the questionnaire was used to finalise the questionnaire before distribution for the main study.

For the Main Study, the questionnaire was distributed to thirty participants from DUT and DUT collaborating research facilities. Participants were researchers from various institutions working with CNTs in research and development facilities or as part of their individual research studies.

For the pilot toxicity study the sample size requirements had been stipulated in the OECD Test 207 guideline (1984). Ten replicates were to be used for each concentration. One worm was required per replicate. Ten control samples were also tested simultaneously. The control/uncontaminated sample was treated with 1ml of deionised water and was absent of any of the test substance.

For the main toxicity study the sample size requirements had been stipulated in the OECD Test 207 guideline (1984). However, adjustments were made to reduce the number of CNTs required for testing. This was due to the high cost and availability of CNTs. As the contamination level of the test substance is determined on the quantity of soil used, the soil content was reduced in order to reduce the quantity of CNT required. This meant that the number of worms used had to be decreased in accordance with the amount of soil used.

For the main study, two worms were required per replicate containing 150g of contaminated soil. Duplicate analyses were tested for each treatment level. Two control samples were also tested simultaneously. This involved the exposure of two earthworms to uncontaminated soil.

3.5.3.2.3 Sample size for leaching in soil column test

For leaching in the soil column test, each treatment level was tested in duplicate. Duplicates were done due to the high cost of CNT.

3.6 Data Collection techniques used in the study

Purposeful probability sampling was adopted for this study. The questionnaire was submitted to a specific group of researchers. Researchers had to be working in the focus area of nanotechnology. The experimental work was done using the OECD test guideline 312 (2004).

Data were collected from several means for this study. These means included information obtained from literature sources, pilot and main study test work and feedback from researchers in the nanotechnology field through the use of a questionnaire. The questionnaire was verified through face validity by an expert in the field of the research study. Post-administration Cronbach alpha was used to determine reliability.

Content analysis was assessed through the use of corroboration of literature sources by evaluating alternative explanations and searching for trends and patterns or lack thereof amongst the available literature (Thomas 2003).

Data were analysed using descriptive as well as inferential statistics. Experimental observations were evaluated through the use of Cronbach's alpha and Excel Data Analysis, for example, the T-test which forms part of the Analysis of Variance (ANOVA).

3.7 Method applicable to the study

The following section describes the process followed to administer the questionnaire. In addition, the test procedures used to gather data on the toxicity testing and leaching in soil column test have been detailed.

3.7.1 Method for administering questionnaire

Kumar (2011) defines a questionnaire as a list of written questions to which participants respond. This sentiment is shared by Mcleod (2018) who likens a questionnaire to a written interview. In order to obtain quantitative data closed ended questions were responded to a rating scale. Qualitative data was obtained by giving respondents an opportunity to share their viewpoint through the use of open-ended questions.

The questionnaire involved the analysis of processes within research institutions with regard to recycling and disposal of ENMs. Three main themes were considered for the questionnaire namely the availability and suitability of material safety data sheets for ENMs and certificate of assurance for ENMs, availability and suitability of procedures for recycling and disposal at the organisation and researcher’s knowledge on safety measures and end-of-life treatment of ENMs. The questionnaire was submitted to researchers working in the field of nanotechnology.

Questionnaires were either printed and handed to participants where possible, or emailed to remote participants. Participation was voluntary and the anonymity and confidentiality of the participants have been respected throughout the study. Anonymity and confidentiality were important in ensuring that responses were unbiased. Participants had the option of withdrawing from the study at any time with the knowledge that there would be no adverse consequences.

The Likert scale was used to consolidate responses from questionnaires. Bertram (2013), reports that the Likert scale is a technique developed by Dr Rensis Likert. This scale can be used to make a qualitative questionnaire adaptable for data analysis. The feedback is then totalled and analysed. For this reason, the questionnaire was evaluated using a five-point Likert scale. Willits, Theodori and Luloff (2016) expand on this by stating that participants can be posed with questions to gauge the magnitude to which they agree or disagree. Quantitative values were assigned to responses to indicate the degree of agreement from participants.

The information obtained from the responses to the questionnaire helped to establish the gaps in the researcher’s knowledge of the risk and safety associated with nanomaterials. Appropriate corrective measures can then be put into place.

3.7.1.1 Feedback from pilot questionnaire

William (2003) advises that the questions must not be ambiguous and the range of responses must be suitable to the questions posed. Feedback from the pilot study revealed that the questions were considered to be straight forward with respondents being able to navigate through the questions easily. Furthermore, the questionnaire was not considered too time consuming.

Feedback on the availability and suitability of material safety data sheets for ENMs and certificate of assurance for ENMs were as follows:

100% of respondents were in agreement that it was necessary to have the Material Safety Data Sheet (MSDS) and Certificate of Assurance (COA) available for ENMs. However, 60% of the respondents were neutral in thinking that the information contained in these documents provided adequate information for ENMs with 80% of the respondents were of the notion that the information directly related to ENMs was lacking. However, majority (60%) could not decide if the information was more suited for bulk materials. Results indicate that the researchers were unsure if the information is suitable for ENMs while they do agree that the overall information specific to ENMs is lacking. This view supports the need for the experimental work conducted for this study.

Feedback on the availability and suitability of procedures for recycling and disposal at the organisation were as follows:

Only 40% agreed that there was a procedure for waste removal at their organisation while only 20% agreed that the procedure used internally had been based on a validated guideline or standard practice. Respondents were split (40% agreeing and 40% disagreeing) when asked if the organisation allows for recycling and disposal of ENMs and if the organisation has considered the effects of ENMs. 80% of the researchers involved in the pilot study did not agree that a reputable waste removal company was used and while 60% remained neutral as to whether the waste disposal certificate identified waste as nano-waste. The aforementioned feedback indicates that there is a need to develop a suitable strategy for the recycling and disposal of ENMs.

Feedback on respondent’s knowledge of safety measures and end-of-life treatment of ENMs were as follows:

60% of respondents were in agreement as having knowledge on the current waste removal process for their organisation. 80% also agreed that they were familiar with the Waste Manifest related to their work but only 60% admitted to having access to the Waste Manifest. The bulk number of respondents (60%) agreed that they make use of conventional PPE. This indicates that respondents are aware that there are potential risks associated with nanotechnology and they are taking a cautionary stance by using conventional PPE. The feedback highlights the necessity for toxicity studies in order to establish if there is a need for a cautionary approach towards ENMs.

Feedback indicated that the questionnaire was considered suitable to proceed to the main study application.

3.7.2 Experimental Work

Manzetti and Anderson (2013) pronounced that CNT products are being introduced into various sectors at an alarming rate. This is concerning as the potential risks of these materials have yet to be fully determined. It is reasoned from Nanotechnology Regulation and the OECD (2015) that the OECD makes its contribution to the advancement of technology by ensuring that potential risks to the environment as a result of social or economic advancement is considered at the forefront. Test guidelines introduced for bulk/macro chemicals by the OECD have been internationally supported. These test guidelines are used to determine the safety of new chemicals. It should be noted that the applicability of the OECD test guidelines to nanotechnology has not yet been determined.

Sentiments on Nanosafety at the OECD (2011) are that while preliminary exercises have shown that OECD guidelines are suitable to nanomaterial testing, there are indications that modification of OECD guidelines may be required. This is due to the current guidelines being suited to bulk/macro chemical testing and the absence of other appropriate test methods for nanotechnology.

Perry (2008) proposed that the type and extent of experiments played an important role in the welfare of animals in research. He advises that there are factors that must be considered for an experiment with animals. These include breeding, transport, housing, handling, restraint and signs of distress. The deliberation is ongoing as to whether data from animal research can be effective when related to humans. This is largely due to the differences in biology between animals and humans.

Perry (2008) supports Balls (1994) in the notion that the three ‘R’ principles as described in Table 3.2 must be endorsed for animal research. The principle is defined as reduction, refinement and replacement. Researchers must consider each of the three Rs in relation to their animal studies.

Table 3.2 List of the ‘R’ principles used to ensure ethical animal testing

(Adapted from Russell, Burch and Hume 1992)

Three R Principles	
Reduction:	Any strategy that will result in fewer animals being used in research
Refinement:	Modification of experimental procedures to minimise pain
Replacement:	Methods which avoid or replace the use of animals in research

Balls (1994) initially reported that researchers may consider “Reducing” or limiting the number of animals in the study without compromising the validity. “Refinement” may be achieved by identifying toxic materials prior to animal testing so that the animals have a mild effect to exposure thus curtailing suffering and maintaining the well-being of the animal for as long as possible. Finally, researchers should try “Replacement” which may mean using an alternate scientific method, with the ideal method being one that does not require the use of animals.

3.7.2.1 Method for pilot toxicity study

Stockmann-Juvala *et al.* (2014) emphasised that while tests are being developed that are more suitable for nanomaterials, OECD test guidelines are being utilised. OECD Test 207: Earthworm, Acute Toxicity Test recommends that the filter paper contact toxicity test be carried out to determine those substances that are likely to be toxic to earthworms before the more in-depth soil test is conducted.

The purpose of the experimental work in this study was to try to determine if the nano-clay was harmful to the earthworms. This will indicate if there is a potential for the test substance to cause harm to human health and the environment surrounds. This will feed into the development of appropriate regulatory action.

The pre-screening filter paper test was adapted from OECD Test 207 guideline (1984). The results informed the leaching in soil column test in terms of the treatment levels that were used. In addition, the pre-screening gave an initial indication as to whether the nano-clay could be toxic to the earthworm. For this exercise, OECD Test 207 guideline (1984) was used. The treatment levels had been adjusted based on consideration for the cost and availability of nanomaterials. The toxicity of the test substance was determined by exposing earthworms to moist filter paper containing various concentrations of the test substance. The treatment levels had been prepared as per 3.4.10.1.2 dose/treatment levels used for the main toxicity study

The worms and glass vials were prepared as per Figure 3.3 below.

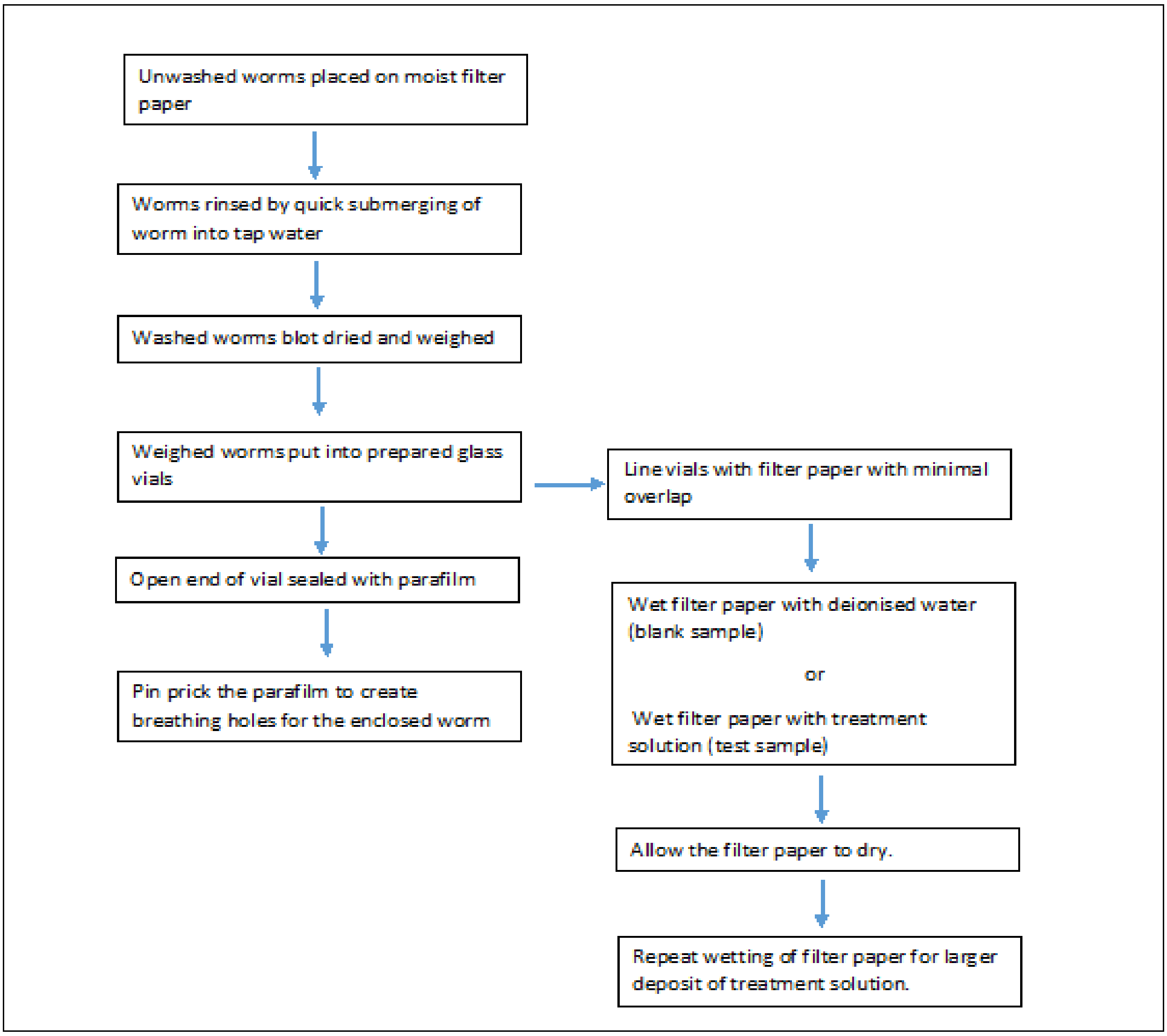

Figure 3.3 Schematic representation of preparation of worms as per 3.4.1.1 and glass vials as per 3.4.7.

The vials were placed on their sides within a cardboard box and kept in a dark room at a test temperature of $20^0C \pm 2^0C$ for the duration of the test. Simultaneously, a second test set was run at a slightly elevated test temperature. This was done to determine if a slight change in temperature would impact on the mortality of the earthworms. This would be indicative of the effect of the test substance in landfill sites conditions where temperatures exceeding ambient temperature is the norm. The room temperature was maintained using air conditioning. After 48 and 72 hours, the worms were assessed for mortality, appearance, behaviour and pathological changes. Worms were classified as dead if there had been no response to gentle mechanical stimulus by means of a gentle touch with a small glass rod at the front end of the body.

3.7.2.1.1 Results from pilot toxicity study

Results indicated that the nano-clay did have an effect on the mortality of the earthworms. Deaths were noted at treatment levels other than the reference substance.

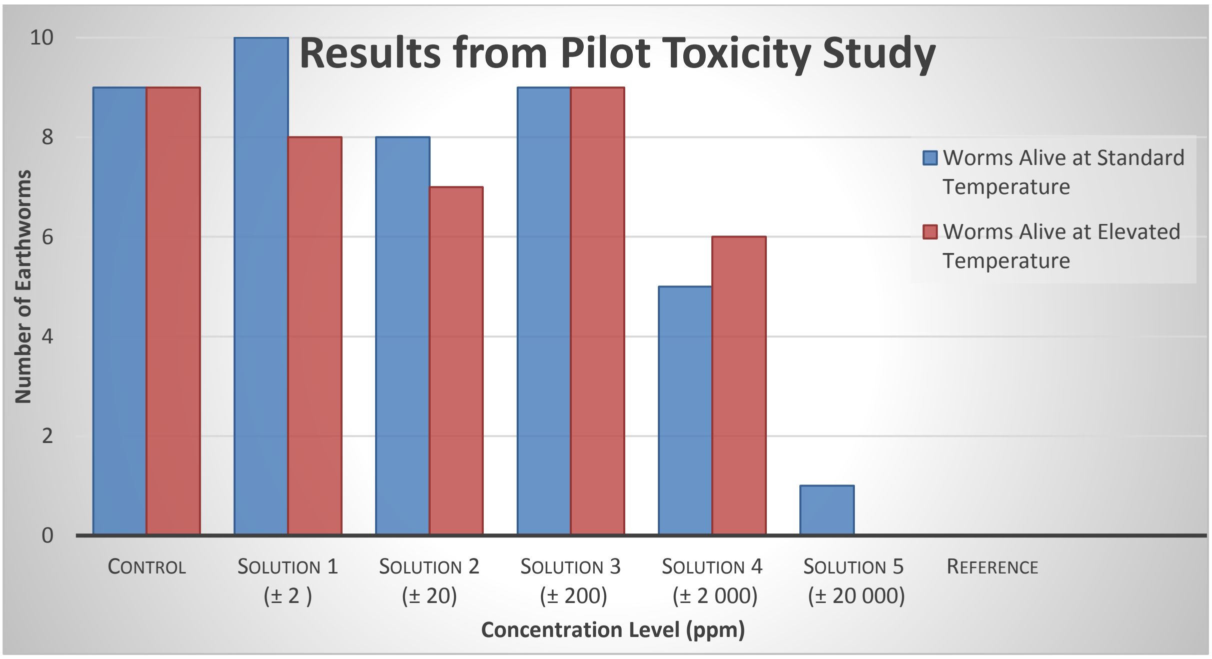

Figure 3.4 Number of earthworms that were alive at the end of the pilot toxicity study

Figure 3.4 shows the number of earthworms that were alive after 48 hours exposure at the standard test temperature as well as the slightly elevated test temperature. The standard test temperature showed an increase in deaths with increased concentration. The standard test temperature also showed that the lower concentrations had less of an effect on the worms than at the increased concentrations in terms of the number of haemorrhages recorded, changes in movement and the presence of odour. This is evident from the observations recorded in Table 3.3. For the control samples, no haemorrhages were observed and movement of the worms remained normal. At the lower treatment levels no haemorrhages were observed for the worms and the movement of the worms remained normal to slow. However, at treatment levels with much higher concentrations of the test substance, worms experienced blood loss and their movement became slow and sluggish. This is indicative that the test substance did have a toxicological effect on the worms. Gallessich (2001) apprises that when *Eisenia foetida* become stressed they exude a pungent malodourous liquid. The author further explains that the worms may excrete build-up of contaminants from their tissues. The excretion of the nanoparticles may have been the catalyst for the bleeding and fragmenting of the worms.

Spurgeon, Tomlin and Hopkin (1997), state that the variability of exposure conditions is necessary in order to determine the potential effects of pollutants to test specimens under natural conditions. The temperature was identified as one such exposure condition. They further elaborated that in the natural habitat earthworms are exposed to temperatures higher as well as lower than the specified test temperature of 20^{0}C. This was also acknowledged by Chang *et al*. (2012) who found that changes in pH induced a large increase in toxicity with similar effects being noted when changes were made to temperature.

The NYC Compost Project Tip Sheet (2013) posited that *Eisenia foetida* earthworms are able to tolerate a large variation in temperature. Although it was noted that the survival rate decreased for the standard test temperature as well as the elevated test temperature as the concentrations increased, there was no significant difference in mortality and behaviour between the survival rate at standard test temperature and the slightly elevated test temperature. Even though Solution 5 (highest concentration) at an elevated temperature was the only concentration to have all the worms die, the increased temperature showed no significant effect on the earthworms when compared to results of the test at a standard temperature at the same concentration. The results of the controls at elevated test temperature were the same to that of the controls at the standard test temperature. This could be attributed to the sturdiness of the *Eisenia foetida* earthworm to a wide range of temperatures, moisture conditions and pH (Kim 2016).

The number of dead worms remained constant between the standard test temperature and the elevated test temperature for solutions 3, 4 and 5. The filter paper test represents dermal uptake of the test substance by the earthworm only. The worm's dermal layer will be exposed to the test substance on the surface of the filter paper. Additional exposure pathways namely ingestion and pleural may intensify the reaction of the animal species to the test substance. Results of the experiment contended that an increased temperature did not appear to affect the dermal uptake of the test substance.

Table 3.3 Observation after 48 hour test period

Treatment Level	Observations at Standard Test Temperature	Observations at Elevated Test Temperature
Control	No haemorrhage observed. Normal movement. No odour. One worm escaped.	No haemorrhage observed. Normal movement. No odour. One worm escaped.
Solution 1 **(± 2 ppm)**	No haemorrhage observed. Normal to slow movement. No odour. No worms escaped.	No haemorrhage observed. Normal to slow movement. No odour. One worm escaped.
Solution 2 **(± 20 ppm)**	Haemorrhage observed for one worm. Slow movement. No odour. No worms escaped.	Haemorrhage observed for one worm. Slow movement. No odour. Two worms escaped.
Solution 3 **(± 200 ppm)**	No haemorrhage observed. Slow movement. No odour. No worms escaped.	No haemorrhage observed. Slow movement. No odour. No worms escaped.
Solution 4 **(± 2 000 ppm)**	Haemorrhage observed for two worms. Slow and sluggish movement. Slight odour in vials with worms that experienced blood loss. One worm escaped.	Haemorrhage observed for two worms. Slow and sluggish movement. Slight odour in vials with worms that experienced blood loss. One worm escaped.
Solution 5 **(± 20 000 ppm)**	Haemorrhage observed for nine worms. Slow and sluggish movement. Strong odour in vials with worms that experienced blood loss. No worms escaped.	Haemorrhage observed for nine worms. Slow and sluggish movement. Strong odour in vials with worms that experienced blood loss. One worm escaped.
Reference - Chloracetamide	Haemorrhage observed for five worms. Most worms were fragmented. Sluggish to no movement. Strong odour in vials. No worms escaped.	Difficult to detect any haemorrhage as most worms fragmented. No movement. Strong odour in vials. No worms escaped.

If worst case scenario is to be assumed, this would mean that the majority ENMs produced will end up at landfill sites. Landfill sites will be exposed to temperatures greatly exceeding the test temperature. The elevated test temperature showed an increase in deaths with increased concentration. The elevated test temperature also showed that the lower concentrations had less of an effect on the worms than at the increased concentrations. For the control samples, no haemorrhages were observed and movement of the worms remained normal. At the lower treatment levels, no haemorrhages were observed for the worms and the movement of the worms remained normal to slow. However, at treatment levels with much higher concentrations of the test substance, worms experienced blood loss and their movement became slow and sluggish. This is indicative that the test substance did have a toxicological effect on the worms. In addition, the results from the elevated temperature test support the results achieved at the standard test temperature in terms of mortality decreasing with an increase in test substance concentration

Table 3.3 summarises the observations at standard test temperature and elevated test temperature after gentle mechanical stimulus of the earthworms. The mechanical stimulus was initiated using a glass rod from the front end of the worm. Although some worms moved without the mechanical stimulus, movement was mostly slow and worms were sluggish. Movement could not be clearly ascertained for all the worms prior to mechanical stimulus.

Titanium oxide (TiO_2) and zinc oxide (ZnO) nanoparticles are some of the most widely used nanoparticles. Hu *et al*. (2010) are concerned that following exposure to TiO_2 and ZnO nanoparticles, earthworms showed signs of injury. Observations were made when dose exceeded 1 g/kg and injury to mitochondria was noticed when dose reached 5 g/kg. Their most controversial finding was that with increased nanoparticle dose the levels of elemental titanium and zinc increased proportionately in earthworm tissue. This find supports the concern of the risks of nanoparticles especially as these particles are already being used in commercial products such as sunscreen.

3.7.2.2 Method for main toxicity study

The purpose of the experimental work in this study was to try to determine if CNT was harmful to the earthworms. This will indicate if there is a potential for the test substance to affect the soil and by extension the surrounding environment. The impact from the soil contamination could have a detrimental effect on soil organisms and plants. It is especially worrying that disposal by landfill sites is utilised.

The main study is represented by a soil test adapted from OECD Test 207 guideline (1984) – Earthworm Acute Toxicity Tests. Results from the pilot study were used to inform the main study. The results from the pilot study indicated that nano-substances are harmful. The treatment levels established for the pilot study were used in the main study. The test substance was applied as indicated in 3.4.10.1.2 dose/treatment levels used for the main toxicity study.

The toxicity of the test substance was determined by exposing earthworms to various concentrations of contaminated artificial soil. The worms were prepared as per 3.4.1.1 preparation of test species for the toxicity study. The artificial soil was prepared as per 3.4.4 artificial soil used for main toxicity study and leaching in soil columns test. The test was done in duplicate with two worms per test jar. While the pilot study requires the test to be conducted in the dark, the main study had to be conducted in continuous light (OECD Test 207 guideline 1984).

The mortality of the worms had been assessed after 7 days and then again after 14 days. Worms were classified as dead if there had been no response to gentle mechanical stimulus by means of a gentle touch with a small glass rod at the front end of the worm. In addition, the appearance, behaviour and any pathological changes were observed.

The results of the main study indicated that there had been no harm to the earthworms. Death was only observed for the worms that were part of the reference testing.

3.7.3 Disposal of Earthworms

Earthworms were euthanized by freezing (Schopke 2017). They were then sent to the UKZN Animal Testing Facility for disposal.

3.7.4 Method for main leaching in soil column test

This test will provide information on the mobility effects of the test substance in soil and to determine if the movement of the test substance in soil will be sufficient to move into subterranean soil layers and eventually to groundwater. The necessary regulatory measures can then be implemented for control.

This study consisted of a main study only. The approximate median lethal concentration (LC 50) and approximate lethal concentration (LC 100) as determined in the pilot toxicity study was passed through the soil column (OECD Test 312 guideline 2004 – Leaching in soil columns).

The main soil column chromatography test was adapted from OECD guidelines (OECD Test 312 – Leaching in Soil Columns 2004). The setup for this experiment was demonstrated in Figure 3.2. For this study, glass extraction columns were packed as per 3.4.6 soil columns used for leaching in soil column test while the artificial soil was prepared as per 3.4.4 artificial soil used for main toxicity study and leaching in soil columns test. The test substance (CNT) was added to the surface of the soil. Artificial rain was then applied through the soil column opening to the surface of the soil. The artificial rain was prepared as per 3.4.5 artificial rain used for the leaching in soil columns test. The leachate was allowed to drain into a collection vessel. The soil was removed from the soil column and sectioned into top and middle segments. The soil segments and the leachate were analysed for the total organic carbon content at Talbot Laboratories and Umgeni Water respectively. In order to determine the presence of the reference substance in the soil and leachate testing was conducted by Arysta Life Science. The results of the test showed that the CNT movement through the soil was small to non-existent.

3.8 Validity

Bolarinwa (2018) surmises that validity is the degree to which a measurement measures what it is supposed to measure.

3.8.1 Validity of Questionnaire:

Specifically related to questionnaires, Bolarinwa (2018) defines validity as the amount of methodical built-in error in the questionnaire. The questionnaire was, therefore, validated by an expert using content validity. Gillham (2000) proposes that the use of open-ended questions can lead to better findings. The questionnaire did make use of both open-ended and closed-ended questions. As the students' anonymity has been maintained it allowed students the chance to be frank and sincere in their responses.

3.8.2 Validity of Experimental work:

The validity for the experimental work was established based on validated protocols from OECD test guidelines. Control samples were used to establish validity of the experimental phase of the study.

3.8.2.1 Validity of toxicity studies**:**

The validity was ensured by using OECD guidelines. For the filter paper toxicity testing, the guideline states that for testing to be valid, not more than 10% of the replicates for the control samples could die. This essentially translated to not more than one worm out of ten replicates from the control batch could succumb to death. For the main toxicity study, the same requirement was adopted. The validity guideline was achieved for both the pilot and main toxicity study.

3.8.2.2 Validity of leaching in soil column test:

OECD test guidelines recommended that validity is based on the recovery of the reference substance (atrazine). 3 grams of atrazine was used to mimic the maximum number of CNTs that were applied to the soil column. Approximately 100g of soil was used in the column. The recovery must lie between 70 to 110%. The recovery was calculated using the formula:

%recovery = (% atrazine found ÷ % atrazine applied to soil column) x 100

3.9 Reliability

Bolarinwa (2018) further explains that reliability is the extent to which the results obtained from a measurement can be achieved repeatedly. Reliability studies were necessary for the questionnaire as well as the experimental work.

3.9.1 Questionnaire reliability

Mckim (2017) explained that reliability for questionnaires can be shown by determining the Cronbach alpha value. The recommendation is that the alpha value should be 0.70 or higher. The Cronbach alpha value for this study was 0.828. This shows that the questions used and the applied Likert scale had acceptable reliability.

3.9.2 Experimental work reliability

The reliability for the experimental design was established based on validated protocols as recommended in the OECD test guidelines for the toxicity test and leaching in soil columns test.

3.9.2.1 Reliability of toxicity studies:

Reliability was assured through the use of replicate analyses. As per OECD test guidelines, ten replicates were done at each treatment level for the filter paper toxicity testing. For the main toxicity study, each treatment level was tested in duplicate with two worms per test jar. The replicates were reduced for the main toxicity study. This was due to the cost of the test substance.

3.9.2.2 Reliability of leaching in soil column test**:**

The reliability of the testing was governed by the use of the OECD Test guidelines. Testing was conducted in duplicate per treatment level.

3.10 Ethical considerations

Ethical considerations were necessary for the respondents of the questionnaire (humans) and for the animal toxicity testing (OECD Test 207). No ethical consideration was necessary for the leaching in columns testing (OECD Test 312).

3.10.1 Ethical consideration for Questionnaire

The questionnaire was necessary in order to acquire information on the current knowledge base of researchers in the field on nanotechnology. Participation in the questionnaire was completely voluntary. The anonymity and confidentiality of the participants were a forefront consideration throughout the study. Participants had the option of withdrawing from the study at any stage with no concern of penalties. Identities of the participants remained confidential even after the study as the collated data from the questionnaire was reported in aggregate. In addition, no identifying information was tracked for the study. The completed questionnaires will not be copied or distributed to any unauthorized external parties.

3.10.2 Ethical consideration for animal toxicity testing:

The use of animals for toxicity testing was necessary to determine if animals that inhabit soil will be affected by the presence of CNTs in the soil through waste disposal. The results will, therefore, help in managing landfill sites better in terms of leachate, seepage to groundwater and effects on fauna / flora and ecosystems. It will also provide a better understanding of the behaviour of nanomaterials in the environment and hence facilitate ease of management as well as help in understanding the possible effects on humans. The lowest number of animals was used and every effort was made to minimise the stress and discomfort of the animals. At the first indication of physical or behaviour changes the animals would be euthanized.

3.11 Summary

This chapter presented the mixed method approach as it involved collecting and assessing data that was both qualitative and quantitative. A purposeful sampling technique was applied for the administering of the questionnaire. The experimental strategy was also described with the pilot and main studies being detailed. The questionnaire provided feedback on the knowledge base of the researchers working in the nanotechnology field in terms of their familiarity with the risk management processes and end-of-life treatment of nanomaterials. The experimental work provided data on the toxicity effects of nano-clay on earthworms and the effects on CNT on earthworms in the soil environment. This was established by checking the mortality of the earthworms at specific intervals. Additionally, the movement of CNT through the soil was investigated. This was established visually as well as by testing sections of the soil and resulting leachate after the test period concluded.

The following chapter will evaluate the results and discuss the outcome of the questionnaire and experimental work.

CHAPTER FOUR: RESULTS AND DISCUSSION OF QUESTIONNAIRE

4.1 Overview of Chapter Four

Chapter Four summarises the information that was gathered from the responses to the main questionnaire. The information was obtained from people working in the field of nanotechnology. In addition to evaluation of the Cronbach alpha value, the responses were evaluated to determine the practices in place by nano-organisations for recycling and disposal of ENMs and CNTs.

4.2 Results and discussion from questionnaire

Surveys add to other forms of data collection by allowing researchers to accumulate information on the feelings and viewpoints of others (Williams 2003). Rickards, Magee and Artino (2012) define a questionnaire as a self-administered survey. They recommend that the questionnaire is set up in such a manner that the questions are understood in the same way by all participants and that participants are encouraged to put forth their responses with accuracy. Rickards, Magee and Artino (2012) caution that several factors can contribute to a questionnaire being unreliable. Some of these factors include poor phrasing of questions, poor layout of questions and poor response options for participants. Chapter Three described the pilot study for the questionnaire that helped to develop the main questionnaire. The main questionnaire made it possible to gather information from researchers working in the nanotechnology field.

4.2.1 Questionnaire: Analysis of responses for main study

The questionnaire for the main study consisted of fourteen questions which were designed in accordance with a Likert scale of one to five. The scoring assigned to responses has been summarised in Table 4.1.

Table 4.1 Ranking assigned to the Likert scale for responses to the questionnaire in the main study.

Scale	Response
5	Strongly Agree
4	Agree
3	Neutral
2	Disagree
1	Strongly Disagree

4.2.3.1 Cronbach alpha

The two most important aspects of precision in a quantitative study are reliability and validity (Mohajan 2017). Willits, Theodori and Luloff (2016) suggest that the reliability of a study improves with an increase in the number of replies to the questionnaire.

Tavakol and Dennick (2011) stress that to ensure validity of a study, the internal consistency should be determined prior to the distribution of the questionnaire. The magnitude to which a question posed in the questionnaire assesses the same view is termed internal consistency and is expressed using Cronbach alpha as a numeral between zero and one. Cronbach alpha is a popular tool used to measure reliability or the degree of measurement error in surveys and questionnaires. The internal consistency interpretation used in this study was defined in Table 4.2. The questionnaire for this study had an acceptable internal consistency indicating that the data from the questionnaire can be inferred with validity and accuracy.

Table 4.2 General guide for Cronbach alpha internal consistency interpretation (Adapted from Guilford 1956)

Cronbach alpha	Internal consistency
<0.20	Slight, negligible relationship. (Unacceptable)
0.2 – 0.4	Low correlation, definite but small relationship
0.4 – 0.7	moderate correlation, substantial relationship
0.7 – 0.9	high correlation, good relationship
>0.9	Very high correlation, very dependable relationship

Of interest, a reliability coefficient of 0.70 or higher is considered as being "Acceptable". Tavakol and Dennick (2011), explain that a low alpha value may be due to the too few items on the questionnaire, as well as a lack of sufficient relationship between the items. On the other hand, a too high alpha value which they identify as being greater than 0.90 may indicate redundancy in the items. The authors, therefore, recommend an alpha value not exceeding 0.90. Table 4.3 reflects the Cronbach alpha score for all the items that constituted the questionnaire in the main study.

Table 4.3 Survey scales and predictor variables in quantitative analysis

Section	**Number of Items**	**Cronbach Alpha**
Recycling and Disposal practices of Engineered Nanomaterials (ENMs) in research laboratories.	14 of 14	**0.828**

The reliability score (alpha value) of 0.828 exceeds the minimum recommended Cronbach alpha value of 0.700 and is indicative of acceptable reliability. The value of 0.828 is lower than the maximum recommended Cronbach alpha value of 0.90 and is, therefore, indicative of a "high correlation" and "good relationship" for data gathered from the respondents.

4.2.3.2 Questionnaire: Responses from main study

This section presents the scoring patterns of the respondents per variable. The results are first presented using summarised percentages for the variables that constitute each section. Results are then further analysed according to the importance of the statements. To determine whether the scoring patterns per statement were significantly different per option, one sample t-test was done. The null hypothesis claims that similar numbers of respondents scored across each option for each statement (one statement at a time). The alternate states that there is a significant difference between the levels of agreement and disagreement. The results are shown in Table 4.4 below. The highlighted significant values (p-values) are less than 0.05 (the level of significance), it implies that the distributions were not similar. That is, the differences between the way respondents scored namely “Strongly disagree”, “Disagree”, “Neutral”, “Agree” and Strongly agree” were significant.

The respondents rating on the recycling and disposal practices of ENMs in research laboratories are summarised in Table 4.4. As indicated by the level of significance, the t-test in Table 4.4 revealed that the respondents scoring patterns exhibited a statistically significant relationship. This implies that the responses had a high correlation and that the statistics are reliable. Reliability was also ensured by using a large number of respondents for the study.

Table 4.4 Respondents rating on recycling and disposal practices of (ENMs) in research laboratories

	No	Likert scale Responses					Mean	Std.	T-test Value	*P*-value
		SD	D	N	A	SA				
Q1	32	0.00%	0.00%	3.1%	28.1%	68.8%	4.66	0.55	48.301	0
Q2	32	0.00%	25.0%	15.6%	40.6%	18.8%	3.53	1.08	18.545	0
Q3	32	3.1%	21.9%	31.3%	37.5%	6.3%	3.13	0.97	18.675	0
Q4	32	0.00%	25.0%	46.9%	25.0%	3.1%	3.06	0.80	21.636	0
Q5	32	3.1%	43.8%	31.3%	18.8%	3.1%	2.75	0.96	16.986	0
Q6	32	0.00%	37.5%	37.5%	21.9%	3.1%	2.91	0.86	19.204	0
Q7	32	0.00%	59.4%	18.8%	18.8%	3.1%	2.66	0.90	16.660	0
Q8	32	0.00%	50.0%	37.5%	12.5%	0.00%	2.63	0.71	21.000	0
Q9	32	0.00%	18.8%	68.8%	12.5%	0.00%	2.94	0.56	29.442	0
Q10	32	0.00%	40.6%	25.0%	25.0%	9.4%	3.03	1.03	16.627	0
Q11	32	3.1%	62.5%	18.8%	15.6%	0.00%	2.47	0.80	17.400	0
Q12	32	0.00%	37.5%	25.0%	31.3%	6.3%	3.06	0.98	17.647	0
Q13	32	0.00%	37.5%	25.0%	34.4%	3.1%	3.03	0.93	18.384	0
Q14	32	0.00%	9.4%	18.8%	59.4%	12.5%	3.75	0.80	26.410	0

Table 4.4 surmises the responses to the main questionnaire. Three critical areas where addressed using the questionnaire namely the availability and suitability of material safety data sheets, as well as certificate of assurance for ENMs, availability and suitability of procedures for recycling and disposal at the organisation and finally, the respondents knowledge on safety measures at end-of-life treatment of ENMs. The responses are detailed below.

Q1: It is necessary for a certificate of assurance (COA) and/or material safety data sheet (MSDS) to be available for ENMs.

The majority of the respondents (96.9%) were in agreement (agree=28.1%; strongly agree=68.8%) that it is necessary for the certificate of assurance, as well as the MSDS to be available for ENMs. The response to this question is particularly important when considering that nanotechnology and its applications are still very much in their infancy. Viswanath and Kim (2016) stress that understanding the possible environmental and human health risks is crucial for responsible development and handling of the nanotechnology field. Responsible development can be ensured by having the information readily available to all people working with nanotechnology. The COA and MSDS should contain the information necessary for responsible handling.

Q2: The COA and/or MSDS contain adequate information for the Recycling and Disposal of ENMs.

Equally important to Q1 and Q2 showed that nearly half (40.6%) of the respondents agree that both the COA and MSDS contain adequate information for the recycling and disposal of ENMs. Raja, Huynh and Khabashesku (2015) are confident that nano-safety can be simple through the use of particular control measures. Organisations can ensure clear understanding amongst people in the nanotechnology field by providing adequate information and training about the properties of the ENMs. Raja, Huynh and Khabashesku (2015) strongly believe this is a positive step towards clearing up confusion with regard to handling of nanomaterials. Leung *et al*. (2017) caution that there is concern for public safety owing to the lack of regulation for ENM handling and disposal.

Q3: The information on the COA/MSDS directly related to ENMs is lacking. Q4: The information on the COA/MSDS directly related to ENMs is more suited to your bulk/macro materials/counterparts.

This question revealed that 37.6% of the respondents agreed that the information on the COA/MSDS relating specifically to ENMs was lacking. While some of the respondents felt that information contained in the COA and MSDS is lacking in content for disposal of ENMs, 46.9% of them were uncertain if the information on the COA/MSDS was directly related to ENMs instead of being more suited to bulk/macro materials. This feedback is consistent with the findings of Musee (2011) who agrees that the majority consensus amongst experts in the nanotechnology field is that sufficient information does not exist to adequately address spills and disposal of nanomaterials. He also highlights that this is true not only for industry but also for research and development facilities. Schulte *et al*. (2014) support the findings of Musee (2011) by highlighting the concern that the Global Harmonised System (GHS) of classification and labelling of chemicals is in effect for bulk/macro chemicals. The GHS does not define how the identification, classification and labelling of nanomaterials will be addressed. This may be true because of the minimal information available on toxicity and leaching ability. Further toxicity and leaching studies are necessary to inform legislation.

Q5: There are procedures in place for the Recycling and Disposal of ENMs in my organisation. Q6: The procedures in my organisation takes into account the accepted/validated guidelines/standard practices for the Recycling and Disposal of nanomaterials.

The responses revealed that 43.8% of the respondents “Disagree” that there are procedures in place for recycling and disposing of ENMs in their relevant organisations. More so, and in response to Question 6 “the procedures in my organisation takes into account the accepted/validated guidelines/standard practices for the recycling and disposal of nanomaterials”, 37.5% of the respondents “Disagree” while a similar number were uncertain if their organisation takes into consideration validated guidelines for the disposable and recycling of ENMs.

From an environmental perspective, the lack of adequate procedures for recycling and disposing of ENMs may be concerning, particularly from the health risks which have been associated with exposure to ENMs. Bystrzejewska-Piotrowska, Golimowski and Urban (2009) acknowledge that with the many benefits of nanotechnology there still exists possible side- effects. One such side-effect is that a new type of waste is being produced and it is, currently, unclear what types of handling and treatment methods will be effective for the management of these nano-wastes. The need for concern is further emphasised by Wang *et al*. (2012) who acknowledge that environmental, as well as health and safety effects of nanomaterials occur during and following the discharge of these materials into the environment. The impact can be as widespread as waste streams and human beings. Raja, Huynh and Khabashesku (2015) are of the notion that Good Laboratory Practices (GLPs) should be used in nanotechnology facilities as an interim measure until such time that nanotechnology-specific practices are developed.

Taking cognisance of the commentary from the foregoing section, the respondents' knowledge on nano-waste disposal used by their organisation was further evaluated.

Q7: I am knowledgeable about how nano-waste is currently removed from my organisation. Q8: The nano-waste generated by my organisation is removed using a reputable waste removal company.

The responses illustrated that 59.4% of the respondents disagreed on having knowledge about how nano-waste is currently removed from their relevant organisations. Similarly, half (50.0%) of the respondents disagree with the statement that nano-waste generated by their organisation is removed using a reputable waste removal company. These responses lean towards the feeling of unease as although guidelines and regulations are in place for bulk/macro materials in terms of the Waste Act No. 59 of 2008, the information directly related to recycling and disposal of ENMs is still very limited. Allan *et al.* (2009), postulate that a restricted number of nanotechnology facilities have procedures implemented for handling nano-waste. They attribute this to different reasons namely the lack of health and safety studies for nanomaterials, no standardised legislation and a lack of willingness amongst nanotechnology facilities to share information owing to competitiveness. This may mean that inefficiency of the current recycling and disposal processes could contribute to the contamination of waste streams, pollution of air, as well as contributing to the negative impact on human health.

Q9: The Waste Disposal Certificate/Waste Manifest does not list the ENMs waste as nano-waste.

68.8% of the respondents remain uncertain that the Waste Disposal Certificate does not list the ENMs waste as nano-waste, which suggests that organisations may not be providing sufficient training to educate and safeguard workers from ENMs exposure.

Q10: I am familiar with the Waste Manifest for the disposal of nano-waste generated through my research. Q11: I have access to the content of the Waste Manifest for the disposal of my nano-waste. Q12: My organisation has considered the effects of nano-engineered materials waste on the environment. Q13: My organisation allows for the recycling/reusing options of nano-engineered materials.

Of concern are the responses to the statements in (Q10-Q13) regarding the organisation's role in the disposal of ENMs. 40.6% of respondents "Disagree" that they are familiar with the Waste Manifest specific to their work. Respondents also scored 62.5% "Disagree" that they had access to the Waste Manifest. The findings of Musee (2011) also highlight a concern with regard to nano-waste categorisation. There still remains no concurrence on a nanotechnology framework for disposal and waste management in this field. 37.5% of the respondents believed that their organisation allowed for the recycling and disposal of ENMs while there was a split response (37.5% "Disagree" and 37.6% "Agree") that the organisation took into consideration the effects on the environment. Musee (2011) puts forth the thought that there remains uncontrolled release of nanomaterials into the environment and waste streams until the implementation of nanotechnology-specific legislation. While the development and implementation of legislation remain a work in progress it is prudent that researchers and workers in the field of nanotechnology are protected. This can be accomplished through the use of control measures namely through training and education of validated guidelines and maintaining a precautionary approach.

Q14: I use conventional Personal Protective Equipment (PPE) when handling nanomaterials.

Almost two-thirds of the respondents (59.4%) agree with using conventional PPE when handling nanomaterials. Gustavsson, Hedmer and Rissler (2011) point out that CNTs pose the most danger to employees working with nanotechnology when it is in its dry bulk form. The dry bulk form of CNT is mostly used in manufacturing processes. Gustavsson, Hedmer and Rissler (2011) reiterates that the structural similarities between CNTs and asbestos could manifest as similar risks. Raja, Huynh and Khabashesku (2015) strongly believe that man-made nanomaterials should be receiving intense attention as they may not be agreeable to their surroundings. The possibility exists that non-biodegradable nanomaterials will have latent consequences through accrual in plants, as well as the body parts of animals and humans. Toxicity studies are shown to be absolutely necessary in order to determine if there is cause for alarm. The outcome of the toxicity studies will help to map out the steps required to safeguard human life and the environment.

4.3 Summary

The aforesaid data indicates that the people working in the field of nanotechnology and their respective organisations have mostly adopted a precautionary stance for recycling and disposal of ENMs. However, it is also evident that there is a lack of standardised practices. Control measures and adopting best practice can enable the organisation and people in the nanotechnology field to employ more consistent protocols.

CHAPTER FIVE: RESULTS AND DISCUSSION OF MAIN TOXICITY STUDY AND LEACHING IN SOIL COLUMN TEST

5.1 Overview of Chapter Five

Chapter Five puts forth the information that has been gathered from the main toxicity study and the data from the leaching in soil column test. The data has been appraised to establish if there are potential toxicity effects of CNTs and if CNTs poses a risk in terms of its mobility through soil which could ultimately impact the environment. This is especially true if worst case scenario, disposal of ENMs via landfill sites, is to be assumed.

5.2 Results and discussion from experimental work

The following discussion is based on results for the main earthworm acute toxicity test and leaching in soil column test.

5.2.1 Results and discussion: Toxicity study

The OECD Test 207 guideline (1984) states that the purpose of this test is to measure the toxicity of chemicals to earthworms. While there are several methods available to measure the toxicity of chemicals, OECD Test 207 guideline (1984) recommends using the paper contact toxicity test as an initial screening test for toxicity to earthworms before moving onto an artificial soil test. The results of the initial screening test have been presented in Chapter Three.

5.2.2 OECD Test 207 – Main earthworm acute toxicity test background

Ukpabi *et al*. 2013) state that earthworms are good bio-indicators of soil organisms. Earthworms are common in soil and enhance soil characteristics. This makes them suitable as a test species for the investigation of the effects of a test substance in the soil environment. Lionetto Calisi and Schettino (2012) report that due to their interactions with soil and extremely permeable skin, earthworms are affected by pollutants. The main route for uptake of contaminants is the skin as it is in direct contact with the soil and water pathways. Ingestion is identified as the alternate route of pollutant uptake.

The OECD Test 207 guideline (1984) recommends the lighting to be between 400 to 800 lux. The range for artificial indoor light is 1000 lux and below (Lux, Lumens and Watts: Our Guide 2008). As landfill sites are exposed to a much stronger light energy, artificial indoor light was deemed suitable even though it was slightly higher than the range stipulated in the OECD guideline.

The initial pH for the artificial soil was 5.74 pH units based on a 1:10 slurry. The test guideline recommended that the pH of the artificial soil must be pH = 6.0 ±0.5 (OECD Test 207 guideline 1984). The pH reading of 5.74 was acceptable as it fell within the required range of 5.5 to 6.5.

The initial moisture content of the artificial soil was 5.09%. Moisture had been determined by drying a portion of the mixed bulk soil sample in an oven at 105 degrees Celsius for one hour. The test guideline advised that the moisture of the artificial soil must be 35% of the dry weight (OECD Test 207 guideline 1984). Based on the moisture result of 5.09% m/m that was obtained, water was added to the soil mixer in order to bring the soil moisture content to 35% dry weight. This meant adding 1350ml of deionized water to 4500 grams of artificial soil. This mixture was thoroughly homogenized.

The glass honey jars that housed the worms in the artificial soil for the test period were weighed on day one, day seven and day fourteen in order to determine if there had been significant moisture loss during the test period. The final moisture content was determined at the end of the test period by drying a portion of the mixed sample from each jar at 105 degrees Celsius for one hour. Table 5.1 shows the readings on the jars on the three days, as well as the final moisture content results. The three days were selected so that the moisture on the first day of the test was recorded and could be compared during and on conclusion of the test. The moisture was, therefore, monitored on the days that the mortality of the earthworms was checked.

Gallessich (2001) advise that the *Eisenia foetida* worm exhibits a social behavior. This means that they group together. On the days that the mortality was checked, if either of the experimental worms died then this worm would have been removed from the honey jar that also housed the living worm. This is done to prevent the social behavior of worms from allowing disease and the contaminant to spread from the dead worm to the living worm. Similarly in the contaminated environment worms can spread nanoparticles to uncontaminated worms and soil. Contamination higher up the food chain is possible with worms being eaten by insects and birds and the follow on effect in the food chain.

Table 5.1 Moisture observations for main study

Treatment Level	Weight of worm, grams (g)	Weight of Jar, grams (g) Day 1	Weight of Jar, grams (g) Day 7	Weight of Jar, grams (g) Day 14	Moisture content, % End of Test Period
Control A	0.52	352	352	351	32.42
Control B	0.55	355	355	355	32.93
± 2 mg/l A	0.49	354	354	353	33.19
± 2 mg/l B	0.52	353	353	352	32.88
± 20 mg/l A	0.59	346	347	346	34.08
± 20 mg/l B	0.35	356	356	355	33.03
± 200 mg/l A	0.48	352	352	352	33.70
± 200 mg/l B	0.50	352	352	351	30.42
± 2 000 mg/l A	0.36	351	351	351	31.61
± 2 000 mg/l B	0.42	355	355	355	34.59
± 20 000 mg/l A	0.58	354	354	354	30.36
± 20 000 mg/l B	0.50	364	363	363	33.56
Reference A	0.60	359	358	358	33.47
Reference B	0.58	357	357	357	34.73

The readings from Table 5.1 indicate that there was no appreciable moisture loss from the start of the experiment to the end of the test period. For this reason there was no need to make adjustments for the moisture of the soil during the test period. This indicates that there had been no significant change to the test environment that could have impacted on the outcome of the test results. The final moisture content results verify that the moisture content did not vary significantly from 35%.

Table 5.2 Mortality observations on day 7 and day 14 of main study

Treatment Level	Observations: Day 7	Observations: Day 14
Control A	Alive No haemorrhage Fast movement No odour	Alive No haemorrhage Fast very vigorous movement No odour
Control B	Alive No haemorrhage Fast movement No odour	Alive No haemorrhage Fast very vigorous movement No odour
± 2 mg/l A	Alive No haemorrhage Fast movement No odour	Alive No haemorrhage Slow movement with mechanical stimulus No odour
± 2 mg/l B	Alive No haemorrhage Fast movement No odour	Alive No haemorrhage Slow movement with mechanical stimulus No odour
± 20 mg/l A	Alive No haemorrhage Slow movement No odour	Alive No haemorrhage Slow movement with mechanical stimulus No odour
± 20 mg/l B	Alive No haemorrhage Slow movement No odour	Alive No haemorrhage Slow movement with mechanical stimulus No odour

± 200 mg/l A	Alive No haemorrhage Slow but faster movement with mechanical stimulus No odour	Alive No haemorrhage Slow movement with mechanical stimulus No odour
± 200 mg/l B	Alive No haemorrhage Slow but faster movement with mechanical stimulus No odour	Alive No haemorrhage Slow but faster movement with mechanical stimulus No odour
± 2 000 mg/l A	Alive No haemorrhage Slow but faster movement with mechanical stimulus No odour	Alive No haemorrhage 1 worm slow movement with mechanical stimulus, other worm barely moving with mechanical stimulus. No odour
± 2 000 mg/l B	Alive No haemorrhage Slow but faster movement with mechanical stimulus No odour	Alive No haemorrhage Slow movement with mechanical stimulus No odour
± 20 000 mg/l A	Alive No haemorrhage Slow to sluggish twitching with mechanical stimulus No odour	Alive No haemorrhage Slow movement with mechanical stimulus No odour
± 20 000 mg/l B	Alive No haemorrhage Slow to sluggish twitching with mechanical stimulus No odour	Alive No haemorrhage Slow movement with mechanical stimulus No odour
Reference A	Worms appear to have completely fragmented Odour Mould around fragmented worm	Worms appear to have completely fragmented Odour Mould around area where worm fragmented

Reference B	Worms appear to have completely fragmented Odour Mould around fragmented worm	Worms appear to have completely fragmented Odour Mould around area where worm fragmented
General Observations	The worms still exhibited the behaviour of clumping together. At the end of the test period the physical appearance of the worm remained the same. No discolouration, change in size or shape. There were tiny flying insects around the reference jars nearing the end of the test period. The worms appeared more sensitive to mechanical stimulus at the higher concentrations and nearing the end of the test period. There was no change to the worms that were part of the control set. These worms remained alive. The worms used in the reference set did not survive. These worms were greatly affected by the presence of atrazine.	

Several endpoints had been identified for the experiment. These included recording the number of dead worms and determining the dose-response curve for mortality. The results from the experiment have been surmised in Table 5.2. The general appearance and change in the appearance of the worms were also recorded which included, in addition to behavioural changes, pathological symptoms namely haemorrhages and lesions.

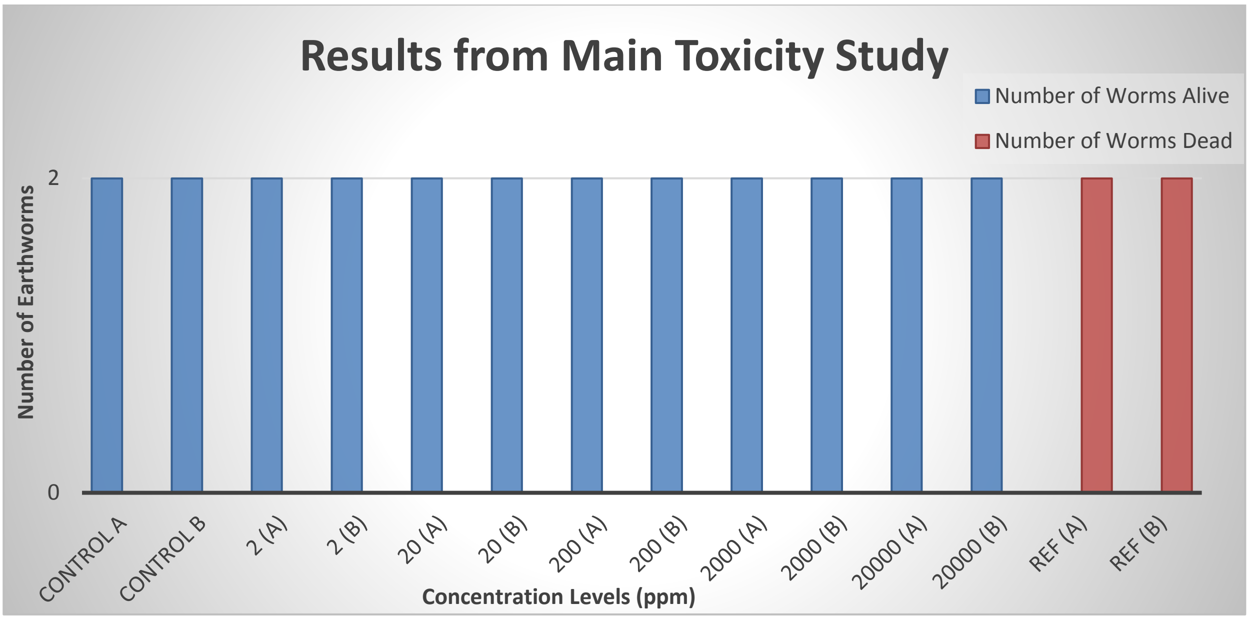

Figure 5.1 Number of earthworms that were alive and dead at the end of the main toxicity study

The condition for validity of the main test was based on the agreement that not more than one worm that was part of the control testing, could succumb to death (OECD Test 207 guideline 1984). This was achieved as none of the earthworms that were part of the control testing died as indicated in Figure 5.1. The reference solution resulted in complete fatalities. The median lethal concentration (LC50) of the test substance for the main test was not established. This was because none of the treatment levels containing the test substance had resulted in deaths of the earthworms by the end of the test period. The deaths in the reference set indicate that the test conditions were conducive for the experiment. The lack of deaths in all treatment levels including the control set clearly indicates that CNTs are not toxic to earthworms in their natural soil habitat at the treatment levels tested. This is good news if nano-waste is to end up at the landfill sites.

Jackson *et al*. (2013) found that while the risk of exposure of CNT could be heightened by increasing its mobility there were no deaths recorded for *Eisenia foetida* up to 3000 mg/kg soil. However, they advised that latent exposure has yet to be probed.

Ganzleben *et al*. (2011) found that different nanomaterials will pose different effects on the environment. They concur with Haywood (2012) that this is due to their unique chemical composition, shape and structure that influences ENMs behaviour in the environment. These findings were similar to the findings of Bystrzejewska-Piotrowska, Golimowski and Urban (2009) who also identified three causes for toxicity. The first was identified as being due to the chemical toxicity of the materials from which they are made while the next cause was attributed to the small size. The last cause identified was due to the shape. This could be the reason that while the worms succumbed to mortality during the pilot study indicating a toxic effect of nano-clay, the main toxicity study leaned toward little to no effect of CNT toxicity to earthworms.

Beaudrie (2010) suggests that exposure may be through inhalation, ingestion of particles and from wash off into household wastewater. The findings of Gustavsson, Hedmer and Rissler (2011) are in agreement with Beaudrie (2010) that possible exposure routes are inhalation, skin contact and ingestion. Gustavsson, Hedmer and Rissler (2011) believe that these exposure routes are possible owing to the ultrafine fibres of nanomaterials. The concern remains that people working in the field of nanotechnology may be exposed during the clean-up of spills prior to disposal and people involved in the end-of-life of life treatment of nano-waste may be unsuspecting of the risk that they are exposed to.

5.2.3 Results and Discussion: Leaching in soil columns

The OECD Test 312 guideline (2004) states that the purpose of this test is to measure the leaching potential of chemicals in soil under controlled laboratory conditions. While there are several methods available to measure the leaching potential of chemicals in soil, the soil column chromatography method has been selected by the OECD. The OECD has opted for this method as it is based on existing guidelines.

5.2.3.1 Leaching in soil columns: background

For this study, the worst case scenario, disposal via landfill sites, has been assumed. According to Taylor and Allen (2007), waste is mostly disposed of via landfill sites. This may be because the method of disposal is simple and cost effective. In order to address the safety concerns associated with inefficient recycling and disposal of nano-waste the behaviour, potential toxicity and leaching ability of CNTs must be investigated in order to be understood. It is proposed in OECD Test 312 guideline (2004) that manufactured chemicals such as CNTs may reach the soil via the direct route from agrochemicals or the indirect route from the movement of substance deeper into soil layers eventually reaching groundwater. This statement brought forth the importance of the leaching in soil column test. The results could support the need for standardised regulation for end-of-life of life treatment.

Multi-walled carbon nanotubes (MWCNTs) were used for the experiment. The test substance was obtained from Nanoshel LLC via the Mechanical Engineering Department of the Durban University of Technology. The CNTs were in the form of a black powder and did not contain any odour.

OECD Test 312 guideline (2004) recommends that where possible the physical and chemical properties of the test substance should be available. The information provided in Table 5.3 has been sourced from the Material Safety Data Sheet (MSDS) from Nanoshel LLC.

Table 5.3 Physical and Chemical Properties of the test substance

(Adapted from MSDS supplied by Nanoshel LLC)

Physical and Chemical Properties of Multi-walled Carbon Nanotubes	
Solubility in water	Not available
Solubility in organic solvents	Not available
Vapour pressure	Not available
Water partition coefficient	Not available
Adsorption coefficient	Not available
Hydrolysis	Not available
Dissociation constant	Not available
Aerobic and anaerobic transformation in soil	Not available
Additional Available information	
Description	Black powder
Length	3-10um
Diameter	4-12nm
Purity	>95% (MWCNT)
Special surface area	90-350*m2/g
Flashpoint	1800 Degree
Boiling point	2640 Degree
Melting point	3340 Degree
pH	7pH

Geckeler and Premkumar (2011) said that while CNTs have a number of positive properties such as chemical stability and large surface area there were also a number of negative properties namely extreme poor solubility in most of the common solvents due to their hydrophobicity. This notion is supported by the MSDS for the MWCNTs. The MSDS shows that there still exists a gap in knowledge on some physical and chemical properties of CNT. Khalid *et al*. (2016) highlight that the proposed use of CNT in consumer products heightens the need for investigation of the possible toxic properties of these nanoparticles. It is essential to explore the materials associated with the infant technology. This information could be critical for the correct end-of-life handling and disposal of CNTs.

5.2.3.2 Leaching in soil columns: analytical techniques to quantify nanoparticles

Petersen *et al*. (2016) recognise the need to quantify CNTs in environmental matrices that is to say water, soil, sediment and biological tissue. While the quantification of CNTs will help in addressing concerns on nanotechnology safety the authors point out that standardised procedures for quantification are not available. Petersen *et al.* (2016) have portrayed the availability of quantification techniques for CNT in Figure 5.2.

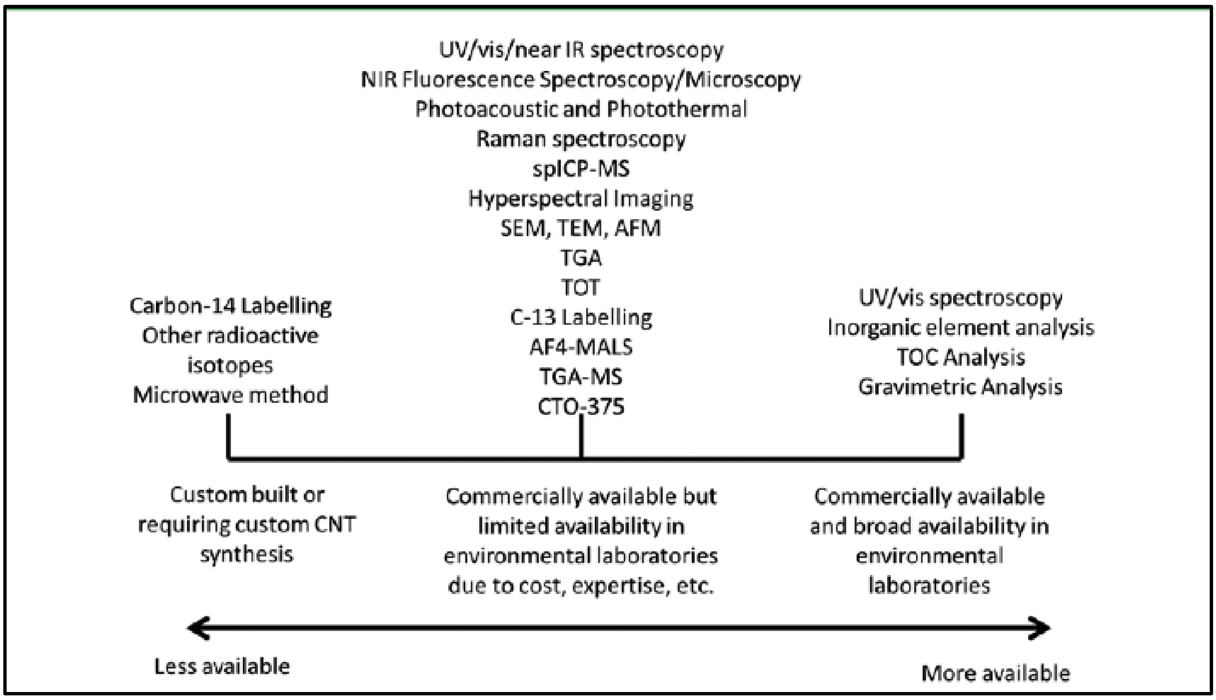

Figure 5.2 Quantification techniques available for CNT
(Adapted from Petersen *et al.* 2016).

Petersen *et al.* (2016) have also called attention to the fact that there are restrictions to the quantification methods that have been identified in Figure 5.2. As an example, the authors discuss that Transmission Electron Microscopy (TEM) may require single CNTs to be evenly spaced to avoid overlapping of particles. This is done in order to get a TEM image of sufficient clarity. Petersen *et al*. (2016) further point out that intricate and time consuming preparation techniques may be required prior to analysis. Therefore, there is a high possibility that the methods used will have to be modified for CNT quantification.

5.2.3.3 Analytical techniques to quantify nanoparticles

For this study, Total Organic Carbon (TOC) was determined in the soil segments and leachates. Florescu *et al*. (2013) explain that TOC is obtained by eliminating the inorganic carbon from total carbon. It is determined by acidifying a sample and then aerating the sample with pure gas namely hydrogen and air. This eradicates the inorganic carbon and the organic carbon can then be measured. Avramidis, Nikolaou and Bekiari (2015) convey that TOC is a key determinant in establishing environmental status specifically soil quality, source or organic matter and pollution indices. Determining the TOC content for the soil and leachate after CNT exposure will indicate if the organic matter has increased, thereby showing that CNT has seeped through the soil and to the leachate. This could be suggestive of a similar effect during landfill sites disposal.

5.3 Leaching in soil columns: results of the main study

Nickel *et al*. (2015) delineate OECD 312 as being used to estimate the leaching potential of an ENM test substance in soil. The method is grounded in soil column chromatography in order to establish if the soil will act as a sinker for the test substance creating pockets of trapped test substance within the soil or if the leaching potential allows for the mobility of the test substance through the soil to deeper soil layers and ultimately groundwater. The results in Table 5.4 indicate the total organic carbon (TOC) content following the leaching in soil column test.

Table 5.4 Total organic carbon (TOC) content in soil and leachate

Sample ID	TOC Result (%)	Duplicate TOC Result (%)
Soil Blank	5.45	6.27
200ppm Topsoil	6.58	5.69
20000ppm Topsoil	4.96	5.15
200ppm Middle Soil	4.76	5.22
20000ppm Middle Soil	4.54	4.92
Leachate Blank	0.00138	0.00138
200ppm Leachate	0.00140	0.00142
20000ppm Leachate	0.00131	0.00133

Table 5.4 reveals that the topsoil contained a slightly higher amount of TOC than the middle soil layer when comparing the same treatment level. The blank soil contains between 5-6% of TOC while the blank leachate contains 0.00138% TOC. These values are similar to the TOC response obtained per treatment level for the soil and leachate respectively. This indicates that the CNT has not contributed to the overall organic carbon content of the soil. This finding is supported by Lu, Yang and Lin (2014) who report that soil can act as a sink for CNT. This meant that the movement of CNT through a soil packed column was restrictive. Lozano and Berge (2012) posited that certain factors could influence the collection and retention of CNT. These factors include organic matter, electrolytic concentration and composition which speeds up the mobility. Nickel *et al.* (2015) add to this opinion by revealing a significant find that the type of soil and type of ENM contributed to the leaching potential of the ENM. Nickel *et al.* (2015) use the example of a particular nanoparticle namely titanium oxide that had a low mobility rate, therefore, making it unlikely that the test substance would reach groundwater. Based on the aforementioned findings from (Lu *et al*. 2014; Nickel *et al.* 2015 and Lozano and Berge 2012) there is hope that irrespective of the concentration of CNT the right type of soil with the correct soil content can inhibit the movement of CNT into the subterranean soil.

Nickel *et al.* (2015) support the belief of Lozano and Berge (2012) and Terekhova and Gladkova (2013) by stating that the soil texture plays a role in the transport and retention of CNT. For example, a soil high in clay and ionic strength content could act as a filter for nanoparticles while a high humic acid or surfactant content will aid the nanoparticle movement through the soil. The combination of soils that made up the artificial soil for this test restricted the movement of the CNT through the packed soil column. This is evident as seen in Figure 5.3 which shows the retention of CNT on the topmost layer of the soil during the test period.

Figure 5.3 Visual evidence of the restrictive movement of CNT through soil column

Kasel *et al*. (2013) found that CNT was retained in the top layer of soil close to the column inlet. However the authors do stress that heavy rain or a higher water content leaching solution could still lead to CNT sinking into subterranean soil layers. The findings of Kasel *et al.* (2013) can be supported by Florescu *et al*. (2013) who found that the occurrence of organic matter contamination into wastewater usually occurs during heavy rain. This raises the question as to whether disposal via landfill sites should even be an option for disposal of nanomaterials if the natural elements namely rain is to be considered. On a similar note Banks *et al*. (2010) investigated the movement of nano-silver and bulk silver through a soil column in an effort to determine if there was potential for leaching from the soil to soil organisms and surrounds. Banks *et al*. (2010) looked at the soil leachability of silver (Ag) nanoparticles using the column test. A comparison was carried out using various sized silver namely engineered nano Ag, micrometer Ag, Poly vinyl pyrrolidone coated nano Ag. The sizes of the Ag ranged from 35nm, 1.5-2.5µm and 20nm respectively. Banks *et al.* (2010) observed that the mobility of nano and bulk Ag was stronger when leached in nitric acid as compared to ultra-pure water. They also observed that the nano Ag spiked soil leached less total Ag than the soil spiked with bulk Ag soil. Overall the size of Ag was found to influence the migration of Ag in soil. Bulk Ag migrated faster and further than nano Ag migration. The slow mobility of nano Ag in soil, may be indicative that the release of nanomaterials into the soil environment could be contained for a period of time before it can spread and cause irreversible damage.

This cause for concern is further highlighted by Bystrzejewska-Piotrowska, Golimowski and Urban (2009) who warn that CNT is the least eco-friendly of all the ENMs due to its poor ability to biodegrade. Tortzen (1999) reports for the Danish Environmental Protection Agency that nanoparticles may leach from landfill sites. The report also states that landfill site conditions have an effect on nanoparticles such as reducing mobility. The aforementioned information makes it clear that there are many factors namely soil type, soil content, leaching solution type and size of the nanomaterial that contribute to the behaviour of CNT in soil.

The CNT used for this study was multi-walled. As is evident from the results, the MWCNTs have restrictive movement through the soil column. This supports the findings of Lu *et al*. (2014) who investigated the transport of MWCNT suspensions in columns packed with sized soil particles. They found that some of their fellow researchers, Jaisi and Elimelech (2009) had reported that the transport of SWCNTs was limited in soil. They attributed this to the irregular shape and large aspect ratio of the SWCNTs. The large variability in particle size of the soil, porosity and pore interconnectivity may also contribute to the limited mobility of SWCNT in soil. Furthermore, Lu, Yang and Lin (2014) reported that soils acted as a sink for MWCNTs with hardly any transport occurring in the vadose zone. The vadose zone is also known as the unsaturated zone and is the area between the ground surface and the water level. In this area the pore spaces of soil may be filled with air or water. The research of Lu *et al.* (2014) reported that the soil texture did indeed play a role in the transport and retention of CNT in soil. Humic acid, which is the main component of soil organic matter, facilitates dispersion and suspension of MWCNTs. This was confirmed by Wang *et al*. (2008) who found that humic acid enhances the transport of CNT by stabilising the CNT. They also state that the soil and surfactant type contributed to the mobility of MWCNTs in soil. Their research showed that CNT suspensions that were stabilised in anionic, non-ionic and cationic surfactants in columns containing fractioned soil had a different mobility pattern compared to the mobility of quartz sands. It was found that the soil texture and not the soil organic matter controlled the transport of CNT. This could be a positive finding that informs the treatment of nano-waste at landfill sites. Addressing disposal of nanomaterials via landfill sites could be as simple as using the type of soils that will act as a sinker for the nanomaterial.

Lozano and Berge (2012) investigated the behaviour of SWCNTs in representative mature leachate. They reported that a landfill leachate is made up of a mixture of dissolved organic matter, inorganics, heavy metals, xenobiotic organic compounds and other trace compounds. Their investigation showed that the properties of the leaching solution and the ENM used, influenced the soil's ability for adsorption. Hydrogen (pH) did not control the behaviour of ENMs in the waste environment. Nanoparticles are found to be stabilised by high molecular weight organics. Mobility is reduced by a high electrolytic concentration. The authors focussed their studies on the effects of the leaching ability of the solution used for leaching and did not study the effects of organic matter and ionic strength of the soil. Therefore, they could not confirm nor refute the findings of Lu *et al*. (2014) and Kasel *et al.* (2013). This finding could also possibly contribute to the handling of nano-waste at the landfill sites. The combination of the correct soils and ensuring that a high electrolytic concentration is achieved after combining these soils should stop the nano-waste from sinking deeper into the soil.

5.3.1 Leaching in soil columns: results of reference substance used in main study

The OECD Test 312 guideline (2004) recommends that atrazine is used as a reference substance for the test. The soil and leachate fractions were tested for atrazine content in order to establish if the atrazine had moved through the soil column. Table 5.5 shows the results obtained.

Table 5.5 Results for reference substance in soil and leachate

Sample ID	Atrazine content %	Recovery %
Topsoil	2.59	86
Topsoil duplicate	2.50	83
Middle soil	0.30	10
Middle soil duplicate	0.34	11
Leachate containing atrazine	0.00114	0.038
Blank leachate	0.00	0

The blank leachate was obtained after passing the artificial rain solution through the uncontaminated soil. Table 5.5 shows that the blank leachate contained no atrazine (o% recovered). The test further revealed that the top layer of soil retained most of the atrazine (average of 2.55%) after the 48 hour test period. While the middle soil layer does indicate that the atrazine did manage to leach through the topsoil layers in the soil column the average atrazine in the middle layer was significantly lower than in the top layer (0.32%). This corresponds to the results achieved using CNT. The total recovery of atrazine on sample 1 is 96% while on sample 2 it is 94%. Recoveries had to be between 70 to 110% (OECD Test 312 guideline 2004). The achieved recoveries indicate that the validity of the test has not been compromised.

Khan *et al*. (2013) looked at the SWNTs in mature and young leachates. Khan *et al.* (2013) reported that SWNTs have negative environmental impacts such as decreasing bacterial viability. They stated that municipal landfill sites and landfill site leachates are made up of constituents whose properties change with waste degradation and time. The mobility of SWNTs through mature and young leachates was observed. They used humic acid to represent the mature leachate and acetic acid to represent the young leachate. The concentration and composition of the leachate influence the stability of SWNTs. This was highlighted when the mobility of SWNT in acetic acid was suppressed indicating weak mobility in young leachates. By comparison the mobility of SWNT in mature leachates was very strong. This is due to the increase in humic acid concentration decreasing particle retention. This indicates that while the mobility of nanotubes may be slow when they initially enter the landfill sites, the mobility may increase over time due to the changes of other factors in the soil environment over a period of time. This corroborates the findings from the previously mentioned authors, Jaisi and Elimelech (2009) and Lu, Yang and Lin (2014) who found that the mobility of nanotubes through soil is influenced by various factors. Some of the factors they presented are pH, ionic strength and compactness. While it is necessary to try to pinpoint the correct combination of factors that impact on the behaviour of CNT in soil this can be challenging especially as landfill site conditions change considerably over time. It is crucial that the latent effects of CNT behaviour be investigated and understood as the 2010 Environment Agency report highlights that waste solidification is hampered by organic content.

5.3.2 Leaching in Soil Columns: Effects on the environment

Suppan (2013) states that international organisations including the United Nations Food and Agriculture Organisation (FAO) suggested that the use of nano-silver particles combined with pesticide will reduce the amount of pesticide that needs to be used by agricultural farmers. The combination will also be better able to destroy pests. The use of nano-silica can also increase the water adsorption rate by plants thus increasing crop growth. As such, it is a very real possibility that food derived from the use of ENMs will become increasingly available. While this affords us many positive opportunities, there is also a potential for negative effects due to the gap in knowledge surrounding the effects of ENMs. Furthermore, ENMs are being controlled mostly by the laws that already exist for bulk/macro materials. However, it is not clear if these laws are indeed effective for the nanotechnology discipline. Some of the properties that make the use of ENMs so popular, for example, their size and shape are also the properties that may be contributing to its toxicity. With findings that confirm the similarity in effects of CNT to that of asbestos, there are health and safety concerns as farmers making use of nanomaterials may develop symptoms of lung disease as observed in rats during toxicity investigations.

Terekhova and Gladkova (2013) state that researchers have different opinions about the toxicity effects of ENMs on soil and living organisms. This is due to there being no clear identification of the hazards associated with nanomaterials and a lack of knowledge in this area. Terekhova and Gladkova (2013) found no correlation between the concentration of nanomaterials and effects on living organisms. A suspension of nano-titanium added to mustard seedlings at various concentrations showed that growth was inhibited at all concentrations. The effect of nano-titanium on a protozoan culture showed that a low concentration of nano-titanium stimulated a low growth response. The growth response increased as the concentration of nano-titanium was increased. However, a low concentration of nano-titanium suppressed the brightness of luminescent bacteria. The luminescence was stimulated with an increase in concentration of nano-titanium. They reported that the properties of the soil may influence the effect of nanoparticles. Soil containing a high clay and high ionic strength content allow for nanoparticles to filter through the sand while the presence of humic acid or surfactants also increase the mobility of nanoparticles thus ensuring that nanoparticles can move freely through the soil. Terekhova and Gladkova (2013) confirm that researchers have different opinions about the toxicity effects of ENMs on soil and living organisms. This is due to there being no clear identification of the hazards associated with nanomaterials and a lack of knowledge in this area. They found no correlation between the concentration of nanomaterials and its effects on living organisms. However, it is also evident that the properties of the soil may influence the effect of nanoparticles in soil. A better understanding of the mobility of CNT through the soil will enable better handling of nano-waste. The information gleaned from the leaching in soil column test will aid in the management of landfill sites for the end-of-life of life treatment of nano-waste.

Dichlorodiphenyltrichloroethane (DDT) is an organic chlorine pesticide that has been widely used in agriculture throughout the world. El-Temsah, Oughton and Joner (2013) looked at the effects of nano-sized zero-valent iron (nZVI) on DDT in the hopes of finding a process to remediate soil containing DDT. The residual toxicity of DDT in soil was also observed in order to determine if the process would have any harmful effects on plants growing in the soil. Although the pesticide is used to kill agricultural pests, there is a possibility of DDTs uptake and concentration in food chains which can have a follow-on effect along the food chain. This could be potentially harmful to humans and animals. The experiment made use of the soil column test and is described in Figure 5.4.

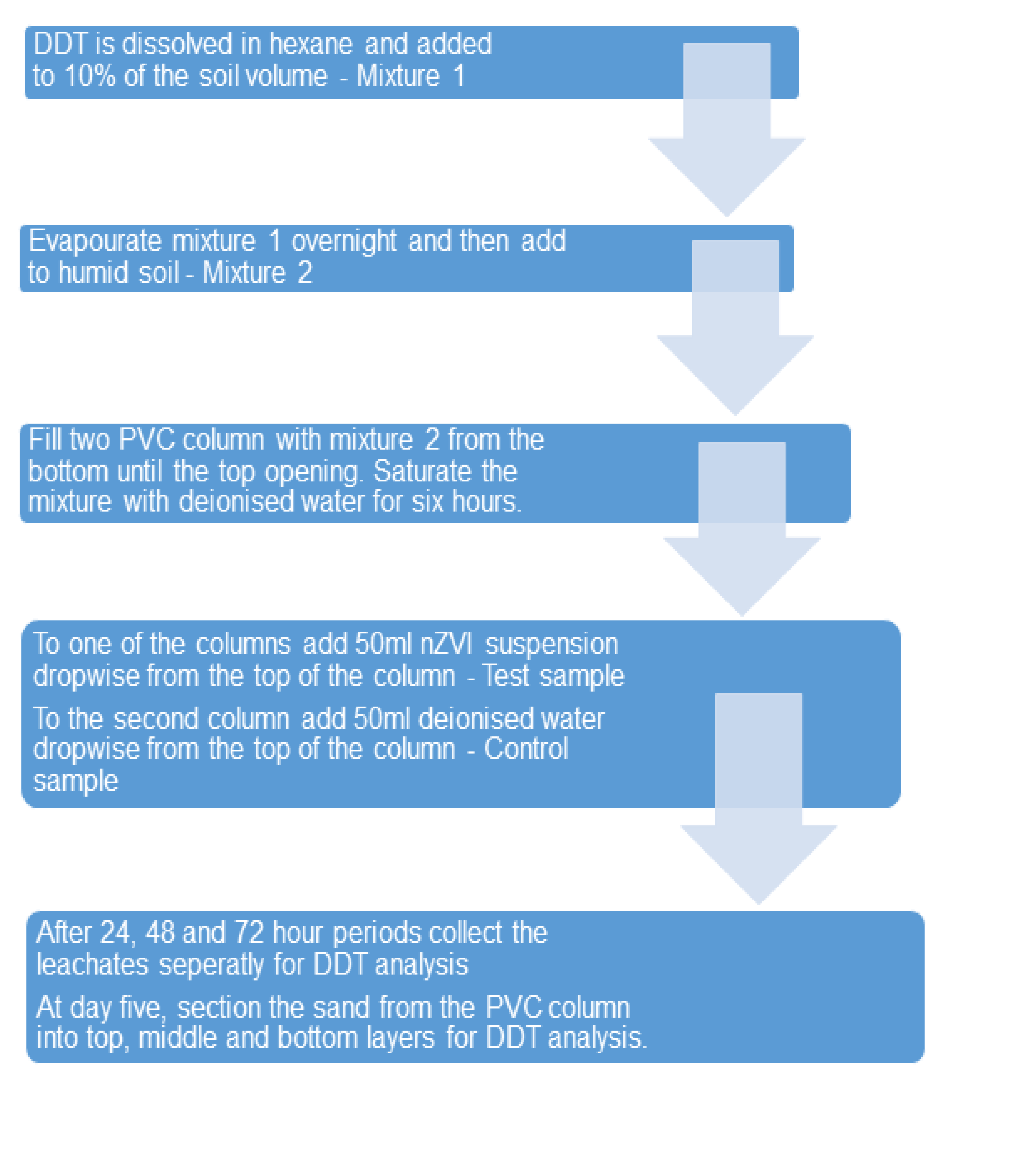

Figure 5.4 Flow chart of soil column testing process used by El-Temsah, Oughton and Joner (2013)

The remaining soil was used for seed germination. The seeds (for barley and flax) were first prepared by incubation in the dark at 25 degrees Celsius. The seeds were incubated on Whatman No.5 filter paper with 6ml of the remaining leached water (soil-less germination). The seeds in soil were then placed in a growth chamber and monitored over an 8 and 16-hour period.

El-Temsah, Oughton and Joner (2013) observed the effects of soil-less germination, soil germination and the nZVI suspension. The soil-less germination showed that the leachate from the control gave a higher germination rate than the first leachate from the nZVI columns which reduced germination of barley. However, the first leachate had no effect on the germination of flax seed. Negative effects on root and shoot development were also noted for soil-less germination. Seed germination in soil (that had been leached) showed that soil from all sections the nZVI column gave negative effects for root development. Shoot development was not affected for barley and only mildly affected for flax. By comparison the seed germination was completely inhibited when placed in nZVI contaminated soil that had not been leached. The undiluted nZVI aqueous phase suspension gave 90% reduction in seed development for soil-less germination while inhibition of germination was less pronounced in soil germination. El-Temsah, Oughton and Joner (2013) also confirmed previous findings by researchers (Lee *et al. (*2008*)*; Li *et al*. (2010) that oxidation of nZVI reduces its toxicity effects. Note with leaching, oxygen in water oxidises the nZVI. Leaching the contaminated soil helps to remove some of the contaminants from the soil, reducing the concentration of ENM (contaminant) in the soil. That is why the worst effects were noted on un-leached contaminated soil. The effects of nZVI indicate the effect that nano-sized particles can have on soil with a follow-on effect on plant life. With the widespread use of CNT in commercial products it is most likely that these products will end up at landfill sites. It is, therefore, necessary to investigate the mobility of CNT through soil.

Doshi *et al*. (2008) conducted experiments using two nano-sized aluminium particles in order to determine the mobility of the particles through sand columns, as well as the environmental effects on plants and soil communities. Aluminium oxide and carboxylate ligand coating aluminium were applied to soil columns containing California Red Kidney Bean Plants and Rye Grass. Doshi *et al*. (2008) report that the aluminium cation is very unfriendly to agriculture. The Al^{3+} ion damages root cells, interferes with root growth and hampers nutrient uptake in plants. Most aluminium compounds have a low solubility in water. The solubility can be improved by increasing the acidity of the water. For the experiment, the soil was fully characterised to determine if it was suitable for plant growth. The soil was packed into columns with silica sand and the nanoparticles were added to the top of the soil. Aluminium suspensions and 0.1M sodium chloride solutions were added to the columns and the leachate collected. Initially there was a large spread of aluminium through the soil and this was due to the mobility of aluminium being larger at a lower pH. However, the dissolved aluminium may combine with hydroxyl ions to produce aluminium hydroxide precipitate which will increase the pH of the soil. This is the cause for a large number of aluminium particles being retained in the top layer of the soil. This, in turn, meant a lower aluminium concentration in the leachate from the soil columns. Plant growth was monitored in the nano-contaminated soil. Controls were also in place and the plant growth was compared to the plant growth in nano-contaminated soil. The results of the experiment revealed that plants growing in the nano-contaminated soil grew faster and were stronger than the plants from the control. However, the aluminium concentration in the leaves and stems of the California Red Bean plants did not show a significant difference to the concentration of aluminium in the leaves and stems of the controls. This clearly indicates that mobility of nanoparticles through soil is dependent on various factors. In addition, the nanoparticle itself is crucial in determining the effects on soil and plants. These effects may not always be negative.

According to Miralles, Church and Harris (2012) nano-toxicology deals with understanding the impacts of ENMs in living organisms. Plants are a food source and largely form the basis of the food chain, therefore, understanding the effects ENMs have on plants may assist to follow the transfer throughout the natural food chain. Furthermore, plants are easy to manipulate and interact with all environmental spheres that are air, soil and water. Other sources of ENMs are waterborne and are purposefully used to fertilise the soil like wastewater sludge dumped in agricultural lands to make the soil fertile for plant growth but this may also have a negative effect on the environment.

Remedios, Rosario and Bastos (2012) looked at the environmental nanoparticle interactions with plants. They noted that differing results have been found concerning the effects of nanoparticles on seed germination, root elongation and biomass. Furthermore, based on investigations into the interaction of nanoparticles with plants results were found to be inconclusive. While some authors Zhang *et al*. (2012) found that nanoparticles such as carbon nanoparticles negatively affected seed germination rate, root elongation and germination index other studies showed Lee *et al.* (2010), that while germination rates were not affected, root length had been reduced when Ag, Cu, ZnO and Si multi-walled carbon nanotubes were used. Remedios, Rosario and Bastos (2012) therefore, concluded that germination and root elongation monitoring were not conducive to measuring nanoparticle toxicity.

5.4 Summary

This chapter has highlighted the need for toxicity testing and leaching in soil column test. The results of the main study for the toxicity test has indicated that CNT is not toxic to earthworms in their natural environment. However, it should be noted that the test was based on artificial soil only and the latent effects have not yet been established. The leaching in soil column test also indicates that the CNT is retained on the topmost soil layers. The CNT did not contribute to the TOC content of the soil and leachate. This is indicative of the CNT having a low mobility through the artificial soil. Similarly, it is anticipated that CNT will not have an effect on soil, soil organisms and water during recycling and disposal.

CHAPTER SIX: CONCLUSION AND RECOMMENDATION

6.1 Overview of Chapter Six

Based on the findings from Chapter Four and Five this final chapter is a summary of the conclusions that have been drawn from the study, as well as recommendations for further investigation. This study looked at the toxicity and leaching potential of CNT. A mixed method research strategy was adopted. Data was collected through the use of a questionnaire and experimental work.

6.2 Conclusion

Chapter Six concludes how each of the objectives presented in chapter 1 has been addressed.

The first objective was to establish through the review of literature in Chapter Two, a base-line practice for recycling and disposal of ENMs and CNTs. It has been established that the current regulatory requirements for end-of-life handling and treatment are essentially non-existent. South Africa has no legislation to address the fate of CNTs throughout its lifecycle. With the absence of legislative procedures specific for CNTs a precautionary approach has been adopted for this novel technology. This means that regulations and guidelines that are in place for bulk/macro materials are being adopted for almost every aspect of nanotechnology, including the recycling and disposal stages. Hence, currently, nano-waste is being handled as hazardous waste while clarity on the handling and treatment of nano-waste is still being sought.

High temperature incineration and disposal at landfill sites were prominent as the most popular end-of-life disposal methods. However, due to the novel properties of nanoparticles the effects of high temperature incineration of nanoparticles still remains a concern. It is suspected that the increase in temperature may cause the properties of the nanoparticles to be altered to make them toxic. Uncertainty on the safety of nanoparticle, emissions from high temperature incineration also exists. In addition the nanoparticles that accumulate in the bottom ash following incineration does end up at landfill sites. Landfill sites still lack governance on handling and treatment of CNTs. The need for defined regulation and guidelines is legitimate as literature points out that nanoparticles are more reactive than their bulk/macro counterparts and could, therefore, be more toxic than bulk/macro materials.

Although handling nano-waste as hazardous waste may be seen as a step in the right direction it is important to remember that it has not yet been established if the regulations and guidelines used for bulk/macro materials are suitable for ENMs which include CNTs. Current practices mean that CNT will end up in landfill sites because this method of disposal is cheap and simple. Good nano-waste management and procedures for the handling and treatment of nano-waste could minimise the risk to the environment and human health. For this to become a reality, it is recommended that intervention is needed from government level to drive implementation. It is critical that the most appropriate laws and regulation are applied to the field of nanotechnology especially as the use of nanomaterials in consumer products continue to increase.

The second objective was to determine the practices which are employed by nano-organisations for recycling and disposal of ENMs and CNTs through the use of a questionnaire. Information from the questionnaire revealed that overall persons working in the field of nanotechnology have adopted a precautionary stance for recycling and disposal of CNTs. The difference in scoring by respondents was significant. This indicates that there was no standardisation of processes and practices in the field of nanotechnology. Respondents were agreement that COA's and MSDS's are necessary and were also of the opinion that the information specific to nanotechnology was minimal.

The results of the questionnaire further pointed to there being a lack of procedures in place for responsible end-of-life handling of nanomaterials. While some respondents confirmed that they had knowledge on how nano-waste was handled and removed from their facility, other respondents did not believe that a reputable waste removal company was used. The difference in opinions was attributed to the insufficient accurate data available with regard to recycling and disposal of nanotechnology. With the lack of data on the fate and behaviour of CNTs, it is difficult to envisage how these particles will react in the environment, especially over time. It is, therefore, prudent that the characterisation of CNT in waste streams is adequately probed.

The third objective was to observe through experimental work the behaviour, toxicity and leaching potential of CNTs in soil through the use of the OECD Test Methods Test

No. 207– Earthworm Acute Toxicity Tests, as well as Test No. 312– Leaching in Soil Columns.Existing knowledge on toxicity and the results from experimental work pointed out that various factors namely the properties of the soil such as soil texture, soil combination, size of the CNT and the raw materials that are used in combination to produce nanomaterials, may influence the toxicity and mobility of CNTs. Due to the similarity between CNT and asbestos, CNT has been identified as a possible threat to the environment and human health. The results from experimental data were two sided. While nano-clay was found to be toxic to the earthworm at an increased concentration of CNT and temperature. Pure CNT presented as non-toxic to earthworms in soil once experiments that had been concluded. In addition, there were no adverse effects of CNT on the earthworms during the toxicity testing. The CNT had virtually no mobility through the soil column. A significant number of CNTs were retained on the uppermost level of the soil which could be seen visually and confirmed that little to no movement of CNT through the soil column had occurred. Thus, indicating that fears of seepage to groundwater level and possible contamination of that water source is not warranted for pure CNT.

While the results of this study revealed that pure CNT may not be considered harmful, literature has indicated that the toxicity of the bulk/macro materials would influence or enhance the toxicity of the nanomaterial. Landfill site regulation needs to be considerate of this notion. It must also be considered that earthworms are low level animals and that animals with more gene similarities to humans will provide more effective data from which inferences can be made about the toxicity to humans.

The final obective was to develop suitable strategies for the recycling and disposal of CNTs using the objectives above and selected quality tools.Throughout the study, strategies have been identified that could minimise exposure to people and the environment. The most popular strategy is to adopt a precautionary approach toward nanotechnology. This stance should continue until legislation has been implemented and adopted. The “Hierarchy of control” presented in the study should be utilised through every step of the process of nanomaterials. Control measures have the advantage of being applicable to both people and the environment. The “Five criterion for responsible development of nanotechnology and their interaction scenarios” has been identified as another strategy that can aid responsible handling of CNT through its lifecycle, thereby minimising the amount of nano-waste produced. The Deming Plan-Do-Check-Act (PDCA) Cycle is a quality tool that can be used to implement the control measures and can also be used as part of the “Five criterion” implementation for risk management. The above strategies can be used individually or in combination depending on the requirements of the nano-facilities. The responsible development of nanotechnology could be more progressive by benchmarking against leading organisations in the nanotechnology field.

The information gathered from the literature review, responses from the questionnaire and the results of the experimental work have addressed all the objectives of this study.

6.3 Recommendation

Knowledge should be shared freely and readily amongst nano-facilities. This is imperative as information exists to indicate that not all nanoparticles are toxic. Sharing of knowledge will allow for best practices to be adopted in the field. Furthermore, as toxicity information becomes more voluntarily available, regulations and guidelines will be factually informed.

For this study testing was performed in duplicate. This decision was based on the cost and availability of carbon nanotubes. Furthermore replicates should be done to minimise the experimental error.

Future work requires a more detailed understanding of the fate, behaviour, uptake and distribution of CNT in the environment. In particular, as CNT does settle in the topmost layer of the soil the uptake into plants and the effects thereof should be scrutinised. Additionally, the study was based on pure CNT and nanoclay. Waste containing materials with embedded nanoparticles was not considered for this study. With the increased use of nanomaterials in commercialised products future studies should be inclusive of nanoproducts.

Guidelines, test applications and general processes applicable to bulk/macro materials are being used as a touchstone for nanotechnology. Undoubtedly a turning point is required for the handling and treatment of nano-waste. Recycling and disposal techniques must be examined for its contribution to the impact on human health and the environment. This study has shown that research and development in the nanotechnology field needs to be supported to ensure that uniform and effective frameworks are established for the recycling and disposal of this life-changing technology.

REFERENCES

Allan, J., Reed, S., Bartlett, J. and Capra, M. 2009. *Comparison of methods used to treat nanowaste from research and manufacturing*. AIOH2009 Conference. Canberra, 5-9 December 2009. University of Queensland, 1-9.

Almeida, C. 2013. *Brazil struggles to regulate emerging nanotechnology* (online). Availability: http://www.scidev.net/global/technology/feature/brazil-struggles-to-regulate-emerging-nanotechnology.html (Accessed 18 May 2015).

Alvi, M.H. 2016. *A manual for selecting sampling techniques in research*. Munich Personal Repec Archive Paper No. 70218.

Amanor, J. 2016. *A vision of nano-science: Pt 1-production* (image). Available: http://ghscientific.com/nanotech-vision-pt-1-production (Accessed 08 August 2017).

Amoabediny, G. H., Naderi, A., Malakootikhah, J., Kochi, M. K., Mortazavi, S. A., Naderi, M. and Rashedi, H. 2009. Guidelines for safe handling, use and disposal of nanoparticles. *Journal of Physics*, 170:1-13.

Anselmo, A. C. and Mitragotri, S. 2016. Nanoparticles in the clinic. *Bioengineering & Translational Medicine*, 1:10-29.

Anvari, A., Ismail, Y. and Hojjati, M. H. 2011. A study of total quality management and lean manufacturing: through lean thinking approach. *World applied sciences journal*, 12(9):1585-1596.

Arts, J.H.E, Hadi, M., Keene, A. M., Kreiling, R., Lyon, D., Maier, M., Michel, K., Petry, T., Sauer, U. G., Warheit, D., Wiench, K. and Landsiedel, R. 2014. A critical appraisal of existing concepts for the grouping of nanomaterials. *Regulatory toxicology and pharmacology,* (70):492-506.

Arora, S., Rajwade, J. M. and Paknikar, KM. 2012. *Nano-toxicology and in vitro studies: the need of the hour* (online). Available: http://www.ncbi.nlm.nih.gov/pubmed/22178382 (Accessed 16 February 2015).

Ashton, P. J., Musee, N. and Brent, A. C.2008. *A South African research agenda to investigate the potential environmental, health and safety risks of nanotechnology* (online). Available: http://www.sajs.co.za/sites/default/files/publications/html/159-1304-3-PB.html (Accessed 20 February 2015).

Austenfeld, R. B. 2001. W. Edwards Deming: The story of a truly remarkable person. *Papers for the research society of commerce and economics*, 42(1):49-102.

Avramidis, P., Nikolaou, K. and Bekiari, V. 2015. Total organic carbon and total organic nitrogen in sediments and soils: A comparison of the wet oxidation – titration method with the combustion –infrared method. *Agriculture and Agricultural Science Procedia*, 4:425-430.

Balls, M. 1994. Replacement of animal procedures: alternatives in research, education and testing. *Lab Anim*, 28(3):193-211.

Banks C.J., Johnson, D., Bednar, A., Coleman, J., Suedel, B., Boyd, R. and Steevens, J. 2010. *Leaching Silver: Soil leachability of Silver nanoparticles using a column method* (online). Available: https://www.yumpu.com (Accessed 09 May 2015)

Barbazak, W.B., Korf, I., Kadavi, C., Heyen, J., Tate, S., Wun, E., Bedell, J.A., McPherson, J.D. and Johnson, S.L. 2000. The synthetic relationship of the zebrafish and human genomes. *Genomes Research*, 10(9):1351-1358.

Baskar, K., Sudha, V. and Tamilselvan, C. 2016. Toxicity effect of chloracetamide on earthworm (*Eisenia foetida*). *European journal of environmental ecology*, 3(1):34-37.

Beaudrie, C. 2010. *Emerging Nanotechnologies and Lifecycle Regulation: An investigation of federal regulatory oversight from nanomaterial production to end of life*. USA: Chemical Heritage Foundation.

National Nanotechnology Initiative. 2012. *Benefits and Applications* (online). 2012. Available: http://www.nano.gov (Accessed 25 March 2013).

Bertram, D. 2014. *Likert Scales* (online). Available: http://poincare.matf.bg.ac.rs/~kristina/topic-dane-likert.pdf (Accessed: 8 December 2017).

Bolarinwa, O.A. 2018. Review article: Principles and methods of validity and reliability testing of questionnaires used in social and health science researchers. *2015 Nigerian Postgraduate Medical Journal*.

Borm, P.J.A. and Berube, D. 2008. A tale of opportunities, uncertainties, and risks. *Nanotoday*, 3(1-2).

Breggin L. and Pendergrass J. 2007*. Where does the nano go? End of life regulation of nanotechnologies. Project on emerging nanotechnologies,* Woodrow Wilson International Center for Scholars, PEN 10 (online). Available: http://www.nanotechproject.org/publications/archive/pen_10_-_where_does/ (Accessed 09 February 2014).

BREC Nanotechnology Newsletter (online). 2017. Available: http://www.brec-solutions.com/docs/brec_nano_055_en.pdf (Accessed 08 September 2017).

British Standards. 2007. *Guide to safe handling and disposal of manufactured nanomaterials, PD6699-2*. United Kingdom: British Standards Institution.

British Standards Institution. 2007. *Guidance on the labelling of manufactured nanoparticles and products containing manufactured nanoparticles, PAS 130:2007*. United Kingdom: BSI Standards Limited.

British Standards Institution. 2007. *Terminology for nanomaterials, PAS 136:2007.* United Kingdom: BSI Standards Limited.

British Standards Institution. 2012. *Terminology for carbon nanostructures, PAS 134:2012.* United Kingdom: BSI Standards Limited.

Bystrzejewska-Piotrowska, G., Golimowski, J. and Urban, P.L. 2009. Nanoparticles: their potential toxicity, waste and environmental management. *Waste Management* 29(9):2587-95.

Castillo, A.M.P.D. 2013. The European and member states approaches to regulating nanomaterials: two level of governance. *Nanoethics*, (7): 189-199.

Clary, C. and Wandersee, J. 2010. Fishbone diagrams organise reading content with a “bare bones” strategy. *Science scope*. Summer (31-37).

Connett, P. 2013*. Incineration: the biggest obstacle to zero waste. In: Zero waste solution: Untrashing the Planet One Community at a Time*. United States of America: Chelsea Green Publishing.

Cozzens, S., Cortes, R., Soumonni, O. and Woodsen, T. 2012. *Nanotechnology and the Millennium Development Goals: Water, Energy, and Agri-food.* Graduate Fellowship, Universidad de Talca, Chile.

Creswell, J.W. 2014. *Research Design: qualitative, quantitative and mixed methods approaches.* 4th ed. USA. Sage Publications.

Crowley, D., Staines, A., Collins, C., Bracken, J., Bruden. M., Fry, J., Hrymak, V., Malone, D., Magette, B., Ryan, M. and Thunhurst, C. 2003. *Health and environmental effects of landfill sites and incineration of waste – a literature review*. Reports paper 3.

Curtis, H. 2013. *Revamping nanotubes* (online). Available: http://www.youris.com/nano/environment/revamping_nanotubes.kl#1x225uFRykxDm

Department of environmental affairs, 2008. *National Environmental Management Waste Act No.59 of 2008*. Capetown.

De Volder, M.F.L., Tawfick, S.H., Baughman, R.H. and Hart, A.J. 2013. Carbon Nanotubes: Present and Future Commercial Applications. *Science,* 339:535-539.

Dineshkhumar, B., Krishnakumar, K., Bhatt, A. R., Paul, D., Cherian, J., John, A. and Suresh, S. 2015. *Single-walled and multi-walled carbon nanotube based drug delivery system: cancer therapy: a review* (image). Available: http://www.indianjcancer.com/text.asp?2015/52/3/262/176720 (Accessed: 08 August 2017).

Doshi, R., Braida, W., Christodoulatos, C., Wazne, M. and O'Connor, G. 2008. Nano-aluminum: transport through sand columns and environmental effects on plants and soil communities. *Environmental Research*,106(3):296-303.

El-Temsah, Y. S., Oughton, D. H., Joner, E.J. (2013). Effects of nano-sized zero-valent iron on DDT degradation and residual toxicity in soil: a column experiment. *Plant Soil*, 368:189–200.

Environment policy committee. 2015. *Landfilling of waste containing nanomaterials andnanowaste*(online). Available:https://pdfs.semanticscholar.org/2572/dfde2d509cd38eea77e69605ea9cbd7a39ee.pdf (Accessed 16 January 2019).

Environmental Protection Agency. 2008. *Carbon nanotube CNT waste disposal* (online). Available: http://www.admin.ox.ac.uk/safety/memoranda/m10-08 (Accessed 21 January 2014).

Environment Agency. 2010. *Waste acceptance at landfill sites* (online). Available: https://assets.publishing.service.gov.uk (Accessed 05 June 2016).

European Agency for Safety and Health at Work. 2012. *Good practice handling of carbon nanotubes* (online). Available:https://osha.europa.eu (Accessed 10 July 2016).

Falkner, R. and Jaspers, N. 2012. Regulating nanotechnologies: risk, uncertainty and the global governance gap. *Global environmental politics*, 12 (1). pp. 30-55.

Feitshans. I.L. 2013. *Nanotechnology: balancing benefits and risks to public health and the environment* (online). Available: http://assembly.coe.int/CommitteeDocs/2013/Asocdocinf03_2013.pdf (Accessed 08 August 2017).

Florescu, D., Lordache, A.M, Costinel, D., Horj, E, Ionete, R.E. and Culea, M. 2013. Validation procedure for assessing the total organic carbon in water samples. *Romanian Journal of Physics*, 58 (1-2):211-219.

Fuldo, C., Weber-Bruls, D. and Werth, J. 2014. Nano is nano is nano or: nanotechnology: a European legal perspective. *Nanotechnology Rev*, 3(4):401-409.

Gallessich, G. 2001. A worm is more like a human than previously thought (online). Available: https://www.news.ucsb-edu/2001/011459/worm-more-human-previously-thought (Accessed 16 January 2019).

Ganzleben, C., Pelsy, F., Hansen, S.F., Corden, C., Grebot, B. and Sobey, M. 2011. *Review of environmental legislation for the regulatory control of nanomaterials: final report*. United Kingdom. Technical University of Denmark.

Geckeler, K.E. and Premkumar, T. 2011. Carbon nanotubes: are they dispersed or dissolved in liquids. *Nanoscale Research Letters*, 6:136.

Gillham, B. 2000. *Developing a questionnaire*. London: Continuum.

Guilford, J.P. 1956. Fundamental statistics in psychology and education. *Science Education*, 41(3):565.

Gustavsson, P., Hedmer, M. and Rissler, J. 2011. *Carbon nanotubes – Exposure, toxicology and protective measures in the work environment*. Sewdish Work Environment Authority.

Haywood, G. 2012. *Safe handling and use of carbon nanotubes – safe work Australia* (online). Available: http://www.safeworkaustralia.gov (Accessed 13 January 2014).

Health and Environmental risks of nanoparticles and nanomaterials (online). Available:https://www.greenfacts.org/en/risks-nanotechnologies-nanomaterials/index.htm (Accessed 03 July 2016).

Health Council of the Netherlands. 2011. *Nanomaterials in waste*. The Hague: Health Council of the Netherlands, 2011; publication no. 2011/14E.

Health and safety executive. 2013. *The control of substances hazardous to health regulation* 2002 (online). Available: http://www.hse.gov.uk (Accessed 01 January 2014).

Heckmann, L. H., Sutherland, D. S., Besenbacher, F., Hovgaard, M. B., Autrup, H. and Scott-Fordsmand, J. J. 2011. Limit-test toxicity screening of selected inorganic nanoparticles to the earthworm *Eisenia fetida*. *Ecotoxicology,* 20:226-233.

Helgason, S. 1997. International benchmarking experiences from OECD countries. *Danish Ministry of finance conference in international benchmarking.* Copenhagen, 20-21 February 1997.

Hester, T. 2006. *RCRA regulation of wastes from the production, use and disposal of nanomaterials* (online). United States of America: American Bar Association. Available:

http://www.americanbar.org/content/dam/aba/migrated/environ/nanotech/pdf/RCRA/pdf (Accessed 31 March 2014).

Hierarchy of controls (image). 2015. Available: https://www.cdc.gov/niosh/topics/hierarchy/default.html (Accessed: 08 August 2017).

Hoang, V. and O'Malley, R. 2013. *Carbon nanotubes implementation in neural prostheses.* Conference Session A1, Paper No. 3156. University of Pittsburgh Swanson School of Engineering.

Holder, A. L., Vejerano, E. P., Zhou, X. and Marr, L.C. 2013. Nanomaterial disposal by incineration. *Environmental science processes and impacts*, 15: 1652-1664.

Ireland, I. and Morris, H. 2013. *Nanotechnology Work Health and Safety* (online). Availability: www.safeworkaustralia.gov.au/sites/SWA/whs-information/Nanotechnology /documents/nanotechnologyWorkHealthSafetyRegulatoryFramework.pdf (Accessed 09 May 2015)

International organisation for standardization. 2008. *Nanotechnologies –Terminology and definitions for nano objects – nanoparticle, nanofiber and nanoplate*, *ISO/TS 27687:2008*. United States of America: International organisation for standardization.

Invernizzi, N. 2013. Unions and NGOs positions on the risks and regulation of nanotechnology. *Vigilância Sanitária em Debate*, 1(4): 69-81.

Jackson, P., Jacobsen, N. R., Baun, A., Birkedal, R., Kuhnel, D., Jensen, K. A., Vogel, U. and Wallin, H. 2013. Bioaccumulation and ecotoxicity of carbon nanotubes. *Chemistry central journal*, 7:154

Jaisi, D.P. and Elimelech, M. 2009. Single-walled carbon nanotubes exhibit limited transport in soil columns. *Environmental Science and Technology*, 43(24): 9161-9166

Kasel, D., Bradford, S.A., Simunek, J., Putz, T., Veereecken, H. and Klumpp, E. 2013. Limited transport of functionalised multiwalled CNTs in 2 natural soils, *Environmental Pollution*, 180:152-158.

Kersante, A., Martin-Laurent, F., Soulas, G. and Binet, F. 2006. Interactions of earthworms with atrazine-degrading bacteria in an agricultural soil. *FEMS Microbiol Escol*, 57:192-2005.

Khalid, P., Hussain, M.A., Suman, V.B. and Arun, A.B. 2016. Toxicology of Carbon Nanotubes - A Review. *International Journal of Applied Engineering Research*. 11(1):148-157.

Khan, I. A., Aich, N., Afrooz, A. R. M. N., Flora, J. R. V., Ferguson, P. L., Sabo-Attwood, T., Saleh, N. B. 2013. Fractal Structures of Single-Walled Carbon Nanotubes in Biologically Relevant Conditions: Role of Chirality vs. Media Conditions. *Chemosphere*, 93:1997-2003.

Kim, H. 2016. A study on the utilization of the earthworms *Eisenia fetida* and *Eisenia Andrei* for the disposal of ploymers. *International Journal of Environmental Science and Development,* 7(5).

Kolodziejczyk, B. 2016. *Nanotechnology, nanowaste and their effects on ecosystems: a need for efficient monitoring, disposal and recycling* (online). Available: https://sustainabledevelopment.un.org (Accessed 09 September 2017).

Kolodziejczyk, B. 2016. *What is nanowaste and why should we worry about it?* (online). Available: https://www.weforum.org/agenda/2016/02/what-is-nanowaste-and-how-will-it-affect-us (Accessed 17 January 2019).

Kreyling, W. G., Semmler-Behnke, M and Chaudhry, Q. 2010. A complementary definition of nanomaterial. *Nano Today*, 5:165-168.

Kruger, M., Ndebele, P., and Horn, L. 2014. *Research in ethics in Africa: A resource for research ethics committees.* Sun Media: Stellenbosch.

Kumar, R. 2011. *Research methodology a step-by-step guide for beginners*. 3rd edition. London: Sage Publications.

Lam, C., James, J.T., McCluskey, R., Arepalli, S. and Hunter, R.L. 2006. A Review of Carbon Nanotube Toxicity and Assessment of Potential Occupational and

Environmental Health Risks. *JSC Toxicology Group Space Life Sciences.* 36(3): 189-217.

Lava, N. 2016. *The basics of mad cow disease (online)*. Available: https//www.webmd.com/brain/mad-cow-disease-basics (Accessed 04 July 2018).

Lee, C., Kim, J.Y., Lee, W.I., Nelson, K.L., Yoon, J. and Sedlak, D.L. 2008. Bactericidal effect of zero-valent iron nanoparticles on escherichia coli. *Environmental Science and Technology*, 42(13):4927-4933.

Lee, C.W., Mahendra, S., Zodrow, K., Li, D., Tsai, Y.C., Braam, J. and Alvarez, P.J. 2010. Developmental phytotoxicity of metal oxide nanoparticles to Arabidopsis thaliana. *Environmental Toxicology and Chemistry*, 29(3):669-675.

Leung, S.W., Williams, B., Jesus, K.D. And Lai, J.C.K. 2017. Critical review of removal of nanomaterials in waste streams. In: *IOP Conference Series: Earth and environmental science*, 68 (1)

Liebert, M.A.1991. Final report on the safety assessment of chloracetamide. *Journal of American colledge of toxicology*, 10(1). Inc Publishers.

Li, Z., Greden, K., Alvarez, P., Gregory, K. and Lowry, GV. 2010. Adsorbed polymer and NOM limits adhesion and toxicity of nano scale sero-valent iron (NZVi) to e.coli. *Environmental and Science Technology*, 44(9):3462.

Liu, W.T. 2006. Nanoparticles and Their Biological and Environmental Applications. *J Biosci Bioeng*. 102(1):1-7

Lionetto, M.G., Calisi, A. and Schettino, T. 2012. Earthworm biomarkers as tools for soil pollution assessment (online). Available: https://www.intechopen.com/books/soil-health-and-land-use-management/earthworm-biomarkers-as-tools-for-soil-pollution-assessment (Accessed 30 January 2018).

Lowry, G. V., Hotze, E. M., Bernhardt, D. D. D., Pedersen, J. A. and Wiesner, B.X. 2010. Environmental occurrences, behaviour, fate and ecological effects of nanomaterials: an introduction to the special series. *Journal of environmental quality*, 39:1867-1874.

Lozano, P. and Berge, N. D. 2012. Single-walled carbon nanotube behaviour in representative mature leachate (online). Available: http://www.ncbi.nlm.nih.gov/pubmed/22608471 (Accessed 09 May 2015).

Lu, C.W., Yang, J.C. and Lin, C.J. 2014. Treatment of helicobacter pylori infection: current status and future concepts. *World Journal of Gastroenterol*, 20(18):5283-5293.

Luoma, S. 2013. Nanosilver: weighing the risks and benefits. *Environmental health perspectives*, 121:7.

Luus, K. 2007. Asbestos: Mining exposure, health effects and policy implications. *Mcgill Journal of Medicine*, 10(2):121-126.

Lux, Lumens and Watts: Our Guide (online). 2008. Available: https://greenbusinesslight.com/resources/ligthing-lux-lumens-watts/ (Accessed 08 August 2018).

Lyn, T.E. 2009. Deaths, lung damage linked to nanoparticles in China. *Science News*. 09 August. Available: https://www.reuters.com/article/us-china-nanoparticles-idUSTRE57I1Y720090819 (Accessed 22 June 2019).

Malsch, I. 2014. Nano-education from a European perspective: nano-training for non-R&D jobs. *Nanotechnol Rev*, 3 (2): 211-221.

Manoharan, D.P. 2011. *Nanotechnology may revolutionise the world* (online). Available: http://14.139.186.108/jspui/handle/123456789/1564 (Accessed 26 March 2013).

Manzetti, S. and Andersen, O. 2013. Carbon nanotubes in electronics: background and discussion for waste handling strategies. *Challenges*. 4(1):75-85.

Marchant, G.E., Sylvester, D. J. and Abbott, K. W. 2009. What does the history of technology regulation teach us about nano oversight. *Journal of Law, Medicine and Ethics,* Winter: 724-731.

Marquardt, K. D. 2013. *A lifecycle risk assessment framework of CNT in the pharmaceutical industry.* Master of Science in Science, Technology, and Environmental Policy, University of Minnesota.

Matsoso, M.L. and Benedict, O.H. 2015. Critical success factors towards the implementation of total quality management in small medium enterprises a comparative study of franchise and manufacturing business in Capetown. *Investment management and financial innovations*, 12 (3)

McAlpine, K. 2010. Slack nanosafety (online). Available: http://www.rsc.org/chemistryworld/News/2010/January/31011001.asp (Accessed 09 May 2015).

McIntyre, R.A. 2012. *Nanotechnology (Economic Aspects) Economic growth Management* (online), 95 (1): 1-2. Available: http://www.freepatentsonline.com (Accessed 26 March 2013).

McKenna, P. 2008. *Smelly sock treatment leak silver nanoparticles* (online). Available:https://www.newscientist.com/article/dn13602-smelly-sock-treatment-leaks-silver-nanoparticles/amp (Accessed 17 January 2019).

Mckim, C.A. 2017. The value of mixed methods research: a mixed methods study. *Journal of mixed methods research*, 3(2):202-222.

Mcleod, S.A. 2018. Questionnaire (online). Available: https://www.simplypsychology.org/questionnaires.html (Accessed 03 June 2018)

Miralles, P., Church, T.L. and Harris, A.T. 2012.*Toxicity, Uptake, and Translocation of Engineered Nanomaterials in Vascular plants* (online). Available: http://pubs.acs.org/doi/abs/10.1021/es202995d (Accessed 02 May 2015).

Mohajan, H.K. 2017. *Two criteria for good measurements in research: validity and reliability*. Annals of Spiru Haret University, 17(3): 58-82

Mugo, F W. 2002. *Sampling in research* (online). Available: http://trochim.human.cornell.edu/tutorial/mugo/tutorial.htm (Accessed 12 December 2017).

Murashov, V., Schulte, P., Geraci, C. and Howard, J. 2011. Regulatory Approaches to Worker Protection in Nanotechnology Industry in the USA and European Union. *Industrial Health*, 49: 280-296.

Murthy, B. P., Kishore, S. A. and Surekha, P. 2011. Acute Toxicological Effects of Multi-Walled Carbon Nanotubes (MWCNT). In: Naraghi, M. Carbon Nanotubes - Growth and Applications (online). (Ed). India. In-Tech. Available: http://www.intechopen.com (Accessed 17 March 2014).

Musee, N. 2011. Nanowastes and the environment: Potential new waste management paradigm. *Environment International*, 37:112–128.

National Industrial Chemicals Notification and Assessment Scheme, 2012. *Human Health Hazard Assessment and Classification of Carbon Nanotubes*. Safe works Australia.

International POPs Elimination Network (IPEN) Nanotechnology Working Group. 2012. *Nanotechnology and the environment: A mismatch between claims and reality* (online). Available: www.eeb.org/documents/090713-OECD-Environmental-Brief.pdf (Accessed 31 March 2014).

Nanotechnology regulation and the OECD. 2015 (online). Available: http://ecostandard.org/wp-content/uploads/nano-reg-oecd.pdf (Accessed 02 December 2017).

Navjot, S., Ramash, E. and Kumar, K. 2014. An artificial soil ingredient substitute for the earthworm toxicity testing (coco peat). *International journal of environmental science and toxicology*, 2(6):81-87.

Nickel, C., Gabsch, S., Hellack, B., Nogowski, A., Babick, F., Stintz, M. and Kuhlbusch, TA. 2015. Mobility of coated and uncoated TiO2 nanomaterials in soil columns-applicability of the tests methods of OECD TG 312 and 106 for nanomaterials. *Journal of Environmental Management*, 157:230-237.

Niemeyer, K. 2011. *What is Nanotechnology? Environmental Impact of carbon nanotubes* (online). Available: https://www.google.com/amp/s/what isnano.wordpress.com/2011/01/27/environmental-impact-of-nanotubes/amp/ (Accessed 17 March 2014).

Nowack, B., David, R.M., Fissan, H., Morris, H., Shatkin, J.A., Stintz, M., Zepper, R. and Brouwer, D. 2013. Potential release scenarios for carbon nanotubes used in composites. *Environmental international* (59):1-11.

OECD Guideline for Testing of Chemicals. 1984. Test 207: *Earthworm, Acute Toxicity Tests.*

OECD Guideline for Testing of Chemicals. 2004. Test 312: *Leaching in Soil Columns.*

OECD. 2011 Nanosafety and the OECD: the first five years 2006-2010. 2011 (online) Available: www.oecd.org/chemicalsafety/nanosafety/47104296.pdf (Accessed 02 December 2017).

Ong, F. and Sheriff, R. E. 2009. *Distance learning of engineering based subjects: A case study* (image). Available: https://www.ou.nl/Docs/Campagnes/ICDE2009/Papers/Final_Paper_113Ong.pdf (Accessed 08 August 2017).

Organisation for Economic Corporation and Development. 2016. *Physical-chemical parameters: measurements and methods relevant for the regulation ofnanomaterial*(online).Available: http://www.oecd.org/officialdocuments/publicdisplaydocument.pdf (Accessed 31 March 2014).

Organisation for Economic Co-operation and Development Guidance document for neurotoxicity testing.2004. Available: http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2004)25&doclanguage=en (Accessed 03 July 2017).

Part, F., Grebler, S., Huber-Humer, M. and Gazso, A. 2015. Environmentally relevant aspects of nanomaterials at the end of life phase – part 2: Waste recycling and disposal. *Journal of Nanotrust dossiers*, 044:1-6.

Petersen, E.J. and Henry, T.B. 2012. Nanomaterials in the Environment: Methodological considerations for testing the ecotoxicity of carbon nanotubes and fullerenes: a review. *Environmental toxicology and chemistry*, 31(1):60-72.

Petersen, E.J., Flores-Cervantes, D.X., Bucheli, T.D., Elliott, L.C.C., Fagan, J.A., Gogos, A., Hanna, S., Kagi, R., Mansfield, E., Bustos, A.R.M., Plata, D.L., Reipa, V., Westerhoff, P. and Winchester, MR. 2016. Quantification of carbon nanotubes in environmental matrices: current capabilities, case studies, and future prospects. *Environmental Science and Technology*, 50:4587-4605.

Preston, C.J., Sheinin, M. Y., Sproat, D. J. and Swarup, V. P. 2010. The novelty of nano and the regulatory challenge of newness. *Nanoethics*, (4): 13-26.

Petratos, P. 2015. Complexity, regulation and nanotechnology. *Regulation*, Spring: 7-9.

Perry, D. and Perry, G. 2008. Improving interactions between animal right groups and conservation biologists. *Conservation biology*, 22(1):27-35.

Purdue University. 2010. *Nanoparticle Safety and Health Guidelines* (online). Available: http://ubes www.purdue.edu (Accessed 12/02/2013).

Raja, P., Huynh, M. and Khabashesku, V.N. 2015. *Safe handling and disposal of nanostructured materials.* In: Offshore Technology Conference. Houston Texas, 04-07 May 2015.

Ray, P.C., Yu, H., and Fu, P.P. (2009). Toxicity and Environmental Risks of Nanomaterials: Challenges and Future Needs. *Journal of Environmental Science and Health.* Part C, Environmental Carcinogenesis & Ecotoxicology Reviews, 27(1): 1–35.

Rajasekar, S., Philominathan, P. and Chinnathambi, V. 2013. *Research Methodology* (online). Available: https://arxiv.org (Accessed 13 December 2017).

Recytube. 2012. *Increasing the use of recycled carbon nanotube (CNT) compounds for technical applications (online)*. Available: http://www.faperin.com/docs/RECYTUBE%20press%20release_%20English.pdf (Accessed 13 January 2014).

Remedios, C., Roasrio, F. and Bastos, V. 2012. Environmental Nanoparticles Interactions with Plants: Morphological, Physiological and Genotoxic Aspects. J*ournal of Botany*, 2012:8

Richard, O. 2005. *Nanotechnology and the environment: Risks and rewards* (online). Available: www.elsevier.com/locate/marpolbul (Accessed 09 May 2015.)

Rickards, G., Magee, C. and Artino, A.R. 2012. You can't fix by analysis what you've spoiled by design: developing survey instruments and collecting validity evidence. *Journal of Graduate Medical Education*, 4(4):407-410.

Russel, W.M.S., Burch, R.L. and Hume, C.W.1992. *Principals of humane experimental technique*. Universities federation for animal welfare, Edition 2.

Safe Work Australia.2012. *Safe handling and use of carbon nanotubes*. Australia. Safe Work Australia.

Saugeron, B. 2012. European NGO's publish position paper on nanomolecule regulation (online). Available: http://www.ecomundo.eu/en Accessed 01 February 2014).

Sauser, B. 2009. *A new recipe for rocket fuel* (online). Available: https://thewere42.wordpress.com (Accessed 11 November 2016).

Schauerman, C.M., Ganter, M.J., Gaustad, G., Babbitt, C.W., Raffaelle, R.P. and Landi, B.J. 2012. Recycling single-walled carbon nanotube anodes from lithium ion batteries. *Journal of Materials Chemistry*, 22:12008.

Schopke, C. 2017. *Uranium and toxic metal uptake by the earthworm Eisenia hortensis in contaminated soil.* Masters, Norwegian University of Life Sciences.

Schulte, P.A., Geraci, C.L., Murashov, V., Kuempel, E.D., Zumwalde, R.D., Castranova, V., Hoover, M.D., Hodson, L. and Martinez, K.F. 2014. Occupational safety and health criteria for responsible development of nanotechnology. *Journal of Nanoparticle Research*, 16:2153.

Senjen, R. 2009. Nanotechnologies in the 21st century: Nanomaterials – health and environmental concerns (online). Available: http://www.eeb.org (Accessed 03 May 2015).

Shah, S.N., Shah, Z., Hussain, M. and Khan, M. 2017. Hazardous effects of titanium dioxide nanoparticles in ecosystems. *Bioinorganic chemistry and applications*. Article number 4101735.

Singh, N.B. and Agrawal, S. 2013. Science of advanced materials, *JSM Chem J* (1):1003.

Soler, B.M.D., Aires, M.D.M. and Morales, M.M. 2015. *Occupational safety and hygiene 111*. Taylor and Francis group, London Ed5.

South Africa. 1993. *Occupational Health and Safety Act No. 85 of 1993* (online). Available: http://www.labour.gov.za (Accessed: 27 January 2016).

Soutter, W. 2012. *Dealing with Nanowaste: Can nanoparticles be recycled?* (online). Available: www.azonano.com (Accessed 03/03/13).

Stemler, S. 2001. An overview of content analysis practical assessment, research and evaluation (online). Available: http://pareonline.net/getvn.asp?v=7&n=17 (Accessed 12 December 2017).

Suarez, J.G. 1992. *Three experts on quality management*. Department of the TQLO Navy Office: 92(2).

Suppan, S. 2013. *Nanomaterials in Soil, our Future Food Chain?.* Institute for Agriculture and Trade Policy (online). Available: https://www.researchgate.net/publication/290447996 (Accessed 29 June 2016).

Tavakol, M. and Dennick, R. 2011. Making sense of Cronbach's alpha. *International Journal of Medical Education,* 2:53-55.

Taylor, R. and Allen, A. 2006. Waste Disposal and Landfill sites: Potential Hazards and Information Needs. In: WHO, World Health Organization. Protecting Groundwater for Health: Managing the Quality of Drinking Water Resources (online). Available: http://www.who.int/water_sanitation_health/publications/PGWsection1.pdf (Accessed 10 April 2014).

Terekhova, V. A. and Gladkova, M. M. 2013. Engineered Nanomaterials in Soil: Problems in Assessing their Effect on Living Organisms. *Eurasian Soil Service*, 46(12):1203-1210.

Theodore, L. and Kunz, R.G. 2005. *Nanotechnology: Environmental Implication and Solutions*(online).Available: http://samples.sainsburysebooks.co.uk/9780471711698_sample_384770.pdf (Accessed 09 May 2015)

Thomas, D.R. 2003. *A general inductive approach for qualitative data analysis*. School of population health. University of Auckland, August.

Todea, A. M., Stahlmecke, B., Asbach, C. and Kuhlbusch, T. A. J. 2003. Combustion of CNT-containing composite materials in a laboratory scale incinerator (online). Available:http://eac2013.cz/EAC%20abstracta/aerosol-based%20Nanotechnology/kuhlbusch-Thomas-A-J_aerosol-based_Nanotechnology_20130228-101904_8328157923.pdf (Accessed 10 February 2014).

Tortzen, A. 1999. *Waste in Denmark*. Danish Environmental Protection Agency (online). Available: www.seas.columbia.edu (Accessed 10 November 2017).

Ukpabi, C.F., Akubugwo, E.I., Ibiam, U.A., Agbafor, K.N., Ezeigbo, R.O. and Wogu, C. 2013. Fertiliser application effect on heavy metal composition and biochemical responses of earthworm Eisenia fetida (savigny) in a sandy farm soil. American Journal of Biochemistry, 3(3):74-79.

Van Dooren, J. 2011. *Carbon nanotubes have unexpected negative impact on environment* (online). Available:http://www.bitsofscience.org (Accessed 04 May 2015).

Viswanath, B. and Kim, S. 2016. Influence of nanotoxicity on human health and the environment: The alternative strategies. *Reviews of Environmental Contamination and Toxicology*, 242:61-104.

Vejerano, E. P., Leon, E. C., Holder, A. L. and Marr, L. C. 2014. Characterization of particle emissions and fate of nanomaterials during incineration (online). *Royal Society of Chemistry.* Available: http://pubs.rsc.org (Accessed 10 February 2014).

Watson, G. 2004. *The legacy of Ishikawa* (online). Available: http://www.asq.org (Accessed 11 September 2017).

Wijnhoven, S. W. P., Peijnenburg, W. J. G. M., Herberts, C. A., Hagnes, W. I., Oomen, A. G., Heugens, E. H. W., Roszek, B., Bisschops, J., Van Zijverden, M., Sips A. J. A. M. and Geertsma, R. E. 2009. Nanosilver – A review of available data and knowledge gaps in human and environmental risk assessment. *Nanotoxicology*, 3(2):109-138.

Williams, A. 2003. How to write and analyse a questionnaire. *Journal of Orthodontics*, 30: 245-252.

Willits, F. K., Theodori, G. L. and Luloff, A. E. 2016. Another look at Likert scales. *Journal of rural social sciences*, 31(3): 126-139.

Workgroup for safe markets. 2017. *Toxic chemicals in products* (online). Available:http://safemarkets.org/toxic-chemicals-in-products/overview (Accessed 08 April 2018).

Yang, S., Wang, X., Jia, G., Gu, Y., Wang, T., Nie, H., Ge, C., Wang, H. and Liu, Y. 2008. Long-term accumulation and low toxicity of single-walled carbon nanotubes in intravenously exposed mice. *Toxicology Letters*, 181 (3):182-189.

Yildirimer, L., Thanh, N.T.K., Loizidou, M. and Seifalian, A.M. 2011. Toxicological considerations of clinically applicable nanoparticles. *Nano Today*, (6): 585-607.

Zhang, J., Bello, D., Isaacs, J.A., Jozokos, T. and Mead, J. 2017. The effects of recycling on the structure and properties of carbon nanotube-filled polycarbonate. *Materials for energy, efficiency and sustainability: TechConnect Briefs 2017*:308-311.

Zhang, P., Ma, Y., Zhang, Z., He, X., Zhang, J., Guo, Z., Tai, R., Zhao, Y. and Chai, Z. 2012. Biotransformation of Ceria nanoparticles in cucumber plants. *American Chemical Society*, 6(11):9943-9950.

ADDENDUM 1: ETHICS CLEARANCE LETTER

DUT
DURBAN
UNIVERSITY OF
TECHNOLOGY

Institutional Research Ethics Committee
Research and Postgraduate Support Directorate
2nd Floor, Berwyn Court
Gate 1, Steve Biko Campus
Durban University of Technology

P O Box 1334, Durban, South Africa, 4001

Tel: 031 373 2375
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7 September 2018

Ms D R Naidoo
138 Camper Drive
Havenside
Chatsworth

Dear Ms Naidoo

ACKNOWLEDGEMENT OF RECEIPT OF APPLICATION FOR ETHICAL APPROVAL

Title: Recycling and Disposal of Carbon Nanotubes

The Institutional Research Ethics Committee acknowledges receipt of your research proposal received on 4 September 2018 which is to be reviewed via the expedited process.

PLEASE NOTE THAT THIS IS NOT AN ETHICS APPROVAL LETTER

Yours Sincerely

Professor J K Adam
Chairperson: IREC

DUT DURBAN UNIVERSITY OF TECHNOLOGY
2018 -09- 07
INSTITUTIONAL R... COMMITTEE
P O BOX 1334 ... SOUTH AFRICA

ADDENDUM 2: PILOT AND MAIN STUDY QUESTIONNAIRE

DURBAN UNIVERSITY OF TECHNOLOGY

The following questionnaire has been designed to collect information on the Recycling and Disposal practices of Engineered Nanomaterials (ENMs) in research laboratories. The results from this questionnaire will be used to develop a strategy for laboratories using ENMs.

Please answer each of the following questions by placing a cross (X) in the appropriate box.

The Following Scale is applicable:
5 = Strongly Agree, 4 = Agree, 3 = Neutral, 2 = Disagree, 1= Strongly Disagree

	Strongly Agree	Agree	Neutral	Disagree	Strongly Disagree
1. It necessary for a Certificate of Assurance (COA) and/or Material Safety Data Sheet (MSDS) to be available for ENMs?					
2. The COA and/or MSDS contain adequate information for the Recycling and Disposal of ENMs?					
3. The information on the COA / MSDS directly related to ENMs is lacking?					
4. The information on the COA / MSDS directly related to ENMs is more suited to your bulk/macro materials / counterparts?					
5. There are procedures in place for the Recycling and Disposal of ENMs in my organisation?					
6. The procedures in my organisation takes into account the accepted/validated guidelines/standard practices for the Recycling and Disposal of nanomaterials?					

	Strongly Agree	Agree	Neutral	Disagree	Strongly Disagree
7. I am knowledgeable about how nanowaste is currently removed in my organisation?					
8. The nanowaste generated by my organisation is removed using a reputable waste removal company?					
9. The waste disposal certificate does not list the ENMs waste as nanowaste?					
10. I am familiar with the waste manifest for the disposal of nanowaste generated through my research?					
11. I have access to the waste manifest for the disposal of my nanowaste?					
12. My organisation has considered the effects of nano engineered materials waste on the environment?					
13. My organisation allows for the recycling / reusing options of nano engineered materials.					
14. I use conventional Personal Protective Equipment (PPE) when handling nanomaterials?					

ADDENDUM 3: LETTER OF CONSENT AND CONFIDENTIALITY

DUT DURBAN UNIVERSITY OF TECHNOLOGY

Title of the Research Study:
Recycling and Disposal of Carbon Nanotubes manufactured at a selected organisation.

Principal Investigator/researcher:
Denise Naidoo, Btech Quality Management and ND Analytical Chemistry.

Brief Introduction and Purpose of the Study/ Nature of the Study:
The Strategies for Recycling and Disposal of carbon nanotubes need to be considered because inefficient Recycling and Disposal of nano-waste has been identified as a potential risk to humans, animals and the environment. In order to address the safety concerns associated with inefficient recycling and disposal of nano-waste via landfill sites, the potential toxicity of nanoparticles in soil must be understood. Suitable strategies will guide the safe management for the recycling and disposal of carbon nanotubes so that the risks can be minimised.

Risks or Discomforts to the Participant:
No risks or discomforts have been identified for this study.

Benefits:
A better understanding of the potential effects of engineered nanomaterials (ENMs) on the environment and the life it supports. This will further inform manufacturers about the development of the ENMs for safer and better controls.

Reason/s why the Participant May Be Withdrawn from the Study:
Participants may be withdrawn from the study at any time at their request. There will be no adverse consequences for participants that withdraw.

Remuneration:
There will be no remuneration of any kind.

Costs of the Study:
The participants will not be responsible for any costs.

Confidentiality:
The anonymity and confidentiality of the correspondents will be respected throughout the study.

Thank you in advance for taking the time to participate.