MODELLING THE FATE, BEHAVIOUR AND TOXICITY OF ENGINEERED NANOMATERIALS IN AQUATIC SYSTEMS

Report to the
Water Research Commission

by

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EXECUTIVE SUMMARY

BACKGROUND

Growth in the production and use of engineered nanomaterials (ENMs) has raised concerns with regard to their potential impact on the environment, and aquatic media in particular. These materials mostly consist of metal nanoparticles and their oxides, which could be released in large quantities into aquatic environments. At present, assessment of the risk posed by these pollutants is stymied by the severe lack of information on the effects of nanoparticles on the environment, since they behave differently from their bulk material counterparts. It is therefore imperative that the potential risks that may be associated with ENMs are identified as soon as possible in what can at present still be considered to be the early stages of nanotechnology development. Therefore, this project was initiated to investigate the potential impact of ENMs on aquatic invertebrates using both experimental approaches (bioassays) and modelling methods. The chief aims of the project can be summarized as follows:

AIMS

The following were the aims of the project:

i. Development of a toxicological database of $nTiO_2$ as exemplar ENM with regard to aquatic organisms.
ii. Development of model(s) to test their functionality with respect to predicting and forecasting the risks of $nTiO_2$ in aquatic media.
iii. Identification of the most important physicochemical properties of $nTiO_2$ and its various forms that influence eventual exposure and toxicity;
iv. Investigation of the relationship between the physicochemical properties of $nTiO_2$ and their biological, chemical, and ecological effects.

METHODOLOGY

Database development

The development of the toxicological database of ENMs was accomplished through the sourcing and acquisition of scientific reports (mostly peer reviewed articles in archival journals) detailing experimental work related to the behaviour and effect of ENMs in aquatic media. Although the methodology is applicable to ENMs in general, the project scope was limited to titanium nanoparticles ($nTiO_2$), since they are among the ENMs with the most widely reported exposure and toxicity data in aquatic systems. The data described in each of these reports were collected in an Excel database. This consisted of qualitative and quantitative variables annotated by comments on the sources or quality of the data.

Neural network models

The data were used to develop multilayer perceptron neural networks to predict the *behaviour* and *toxicity* of $nTiO^2$ in aquatic media. The behaviour of the nanoparticles in water was represented by two response variables and hence two neural network models, as different sets of predictor variables were used to predict each response, namely their (i) ability to adsorb organic substances in their environment, as well as (ii) aggregation of the particulates in aqueous media.

In contrast, the effect of the particles on the environment were represented by six response variables (and hence six neural network models), namely the effect of the particles on the (i) immobilization of *Daphnia magna*, (ii) mortality of *D. magna*, (iii) growth rate of *D. magna*, (iv) growth rate of algae, (v) reproduction in *D. magna*, and (vi) biomass of algae.

(a) Adsorption

Eight predictor variables affecting the adsorptive capacity of the particles were considered in the model. There were two qualitative predictor variables, viz. aqueous composition and electrolytes and six quantitative variables, viz. organic matter, pH, ionic strength, temperature, time, and particle size.

(b) Aggregation

Fifteen predictor variables were used that broadly described three properties of $nTiO_2$, three characteristics of water chemistry, two abiotic factors, six variables for the model organisms, and one for the state of experiments conducted. Surface area concentration (SAC), structure (% anatase) and surface coating were attributes designated as the nanomaterial properties. The water chemistry was represented by the pH, ionic strength and total organic carbon (TOC), whereas abiotic factors consisted of temperature and exposure duration. The presence or absence of feeding *D. magna* during experiments, type of organism (species), developmental stage at the time of exposure, culture (axenic or mixed), age and generation of the model organisms were identified as possible contributing factors to their susceptibility during exposure. Notably, the species attribute was only applicable for modelling endpoints related to algae. Some attributes were eliminated during modelling, owing to lack of suitable variation in the data.

(c) Effect on environment

Fifteen predictor variables were used that broadly described three properties of $nTiO_2$, three characteristics of water chemistry, two abiotic factors, six variables for the model organisms, and one for the state of experiments conducted. Surface area concentration (SAC), structure (% anatase) and surface coating were attributes designated as the nanomaterial properties. The water chemistry was represented by the pH, ionic strength and total organic carbon (TOC), whereas abiotic factors consisted of temperature and exposure duration. The presence or absence of feeding *D. magna* during experiments, type of organism (species), developmental stage at the time of exposure, culture (axenic or mixed), age and generation of the model organisms were identified as possible contributing factors to their susceptibility during exposure. Notably, the species attribute was only applicable for modelling endpoints related to algae. Some attributes were eliminated during modelling, owing to lack of suitable variation in the data.

The six response variables considered were the (i) immobilization of *Daphnia magna*, (ii) mortality of *D. magna,* (iii) growth rate of *D. magna*, (iv) growth rate of algae, (v) reproduction in *D. magna*, and (vi) biomass of algae.

(d) Identification of most influential predictor variables on behaviour and toxicity of the particles

Each of the models outlined above was subjected to perturbation analysis to identify the most important or influential predictor variables, by which means aims (iii) and (iv) could be achieved.

RESULTS AND DISCUSSION

Extensive data cleaning, variable consolidation and dealing with missing variables or data were required to establish the databases from publically available scientific reports. Despite these difficulties, the data could be used to construct neural network models that could in turn be used to gain considerable insight into the variables associated with the risks posed by $nTiO_2$ to aquatic environments.

With the exception of the algae biomass (as an effect on the environment) model, the models could explain the variation in the response variables satisfactorily (with coefficients of determination or R^2 ranging from approximately 74 to 93% on test and validation data). The algae biomass model could not explain more than approximately 43% of the variation of the response variable based on test and validation data and therefore the results from this model should be treated with caution. Although the variables identified as significant predictors of the response variables could also have been derived from first principles, the models provided a quantitative assessment of the variables that could not be acquired in any other way.

CONCLUSIONS

- With the neural network models, data from different sources could be combined to generalize the behaviour and toxicity of $nTiO_2$ using reported scientific data. This facilitated the identification of complex relationships involving many variables that would otherwise not have been possible with deterministic models. This knowledge extraction and feasible inductive learning from the published data could be used to direct future research and early regulation of nanomaterials in the environment.

- In principle, the models could be applied to pilot-scale or full-scale scenarios, e.g. water bodies, to predict response patterns without requiring extensive analytical work. Output from the regression models derived from laboratory data provides generic first tier risk assessment. The natural environment comprises multiple associations of organisms and conditions, e.g. as associated with resource competition, predation, existence of other pollutants, etc., that might contribute to (i) effects to organisms that are not related to nanomaterials, and (ii) enhanced or antagonised effects that are different from those observed in pure cultures. Laboratory studies simulate aquatic systems where natural associations are simplified. The models were built on this simplification by incorporating predictor variables drawn from characteristics of nanomaterial, environmental conditions and organism factors to forecast exposure mechanisms.

- Finally, the neural network models could be integrated as management tools to predict the dynamics of $nTiO_2$ after release into aquatic systems and interrogation of the models could be used to explain the effects of the $nTiO^2$ on the environment. For example, adsorption and aggregation processes may have both negative and positive impacts on aquatic systems. An understanding of the aftermath, e.g. enhanced flocculation and removal, deposition, accumulation, etc., may influence mitigation planning. The model is flexible and can be improved by adapting it to new data to generalize risk-based outcomes in diverse aquatic systems, as and when new data are obtained.

RECOMMENDATIONS

- The quality of the neural network models is directly related to the quality of the data used in their construction. In order to improve the data, more consistent testing and reporting of descriptors should be promoted. Moreover, there is a need to measure endpoints related to behaviour and effects concurrently to reduce data ambiguity.

- Further research is required to generate experimental data at micro- and macrocosm levels where natural conditions are simulated. Data from these experiments could support scientific findings and support better management decisions on environmental risks through modelling.

- Future research studies should focus more on nanomaterials in the same state that they are likely to end up in aquatic systems. This should generate more reliable data that could support better models than those that could be derived from the use of pristine or functionalized materials, such as the $nTiO_2$ particles currently used, some of which may not find their way into aquatic systems.

- Further research should test the applicability of these models in predicting the behaviour and toxicity of other nanomaterials to establish their suitability and hence applicability in decision making for risk assessment that covers nanomaterials in general.

- Finally, environmental factors such as pH, ionic strength, and temperature and retention times are relevant environmental factors that require monitoring in the event of accidental release of ENMs to establish expected impacts and potential mitigation measures. These factors influence the kinetic transport, migration, bioavailability and effects of ENMs from the point of discharge and could be used to estimate risks of known ENMs discharged.

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ACRONYMS & ABBREVIATIONS

Δ_H	enthalpy of formation
AF	assessment factor
DLVO	Derjaguin Landau Verwey Overbeek model
DOC	dissolved organic carbon
EC_{20}	effective concentration at 20% dose
EC_{25}	effective concentration at 25% dose
EC_{30}	effective concentration at 30% dose
EC_{50}	effective concentration at 30% dose
ENMs	engineered nanomaterials
HONEC	highest observed no effect concentration
LC_{50}	lethal concentration effects at 50% dose
LOEC	lowest observed lethal concentration
$Log(EC_{50})$	logarithm of effective concentration at 50% dose
MCMC	Markov Chain Monte Carlo model
MFA	material flow analysis
NOM	natural organic matter
PEC	predicted environmental concentration
PFA	particle flow analysis
PMFA	probabilistic material flow analysis models
PNEC	predicted no effect concentration
PZC	point of zero charge
QSAR	quantitative structure activity relationship
RQ	risk quotient
TOC	total organic carbon

CHAPTER 1: BACKGROUND

1.1 INTRODUCTION

Nanotechnology entails the manipulation, precision placement, measurement, modelling or fabricating of matter at nanoscale with at least one dimension measuring 100 nm or less (Meyer and Kuusi, 2002), and on how to control the formation of two-and three-dimensional assemblies of molecular scale building blocks into well-defined nanostructures (Rosi and Mirkin, 2005) with engineered nanomaterials (ENMs) as the basic building blocks. Nanotechnology is among the core strategic technologies envisaged to provide viable solutions in addressing part of the grand challenges identified by the Department of Science and Technology (DST) (DST, 2002). Within the South African context, nanotechnology applications enjoy great support towards addressing social-related development challenges such as treating diseases (e.g. malaria, tuberculosis, HIV/AIDS, etc.), treatment and purification of potable as well as wastewater, remediation of contaminated environments, and improving energy production (DST, 2005; DST, 2010).

Moreover, the relevance of nanotechnology towards promoting sustainability and achievement of Millennium Development Goals (MDGs) recently within the South African context, and Africa in general has been acknowledged (Musee et al., 2013). To effectively exploit the benefits of nanotechnology, the DST through the National Nanotechnology Strategy (NNS) initiated a national coordinated effort to guide research in nanosciences and nanotechnology in the country (DST, 2005) – where aspects on risk are considered as part of the implementation plan (DST, 2010). It is in this context, the DST has identified the critical need for risk assessment of nanotechnology and nanosciences (Musee et al., 2010a) as priority area of focus in order to achieve the strategic view that nanotechnology should be developed in ethically and environmentally sound manner (DST, 2005).

To date, nanotechnology has matured from laboratory-based research and development phase into full commercialization of nanoproducts since the start of the 1990s. For example, presently there are numerous novel consumer products and industrial applications of nanotechnology comprising: nanoelectronics, molecular assemblies, tissue engineering, biomedicine, nanocomposites, cosmetics, paints, pesticides, and water purification modules, among others. To put the growth of nanoproducts into perspective, there were over 800 company-identified nanoproducts globally by 2008, and increased to 1700 by 2014, and expected to reach 3040 by 2020 (Woodrow Wilson International Centre for Scholars, 2014)

Consequently, the increasing production of ENMs and resultant incorporation to numerous products and applications has raised concerns on their potential associated impacts to the environment. Public inventories (Woodrow Wilson International Centre for Scholars, 2014; Nanowerk LLC, 2009) report about metal and metals oxide nanoparticulate materials that are commonly used in nanoproducts. These nanomaterials have high likelihood of being released in large quantities into the aquatic environments. In view of the rapidly increasing release and entry of ENMs into different environmental compartments (Nowack and Buchelli, 2007), especially in wastewater as conduits (Kaegi et al., 2008; Kaegi et al., 2010) and sediments as final sinks (Musee et al., 2010b); it is imperative that their potential risks get attention at the infancy phase of nanotechnology development. Such approach has the merit of promoting long-term safe, responsible, and sustainable development of nanotechnology for optimal benefit of the society especially for developing and economies in transition (Musee et al., 2013). In this context, this project was designed to investigate the potential impacts of ENMs to the invertebrates using both experimental approaches (bioassays) and modelling approaches as outlined in the funding proposal to WRC (1002316); and implemented as project K5/2107/3 titled: *Toxicity evaluation of metals and metal oxides nanoparticles to aquatic invertebrates and algae species*. The project chief objective was to be addressed through six-fold sub-objectives, namely:

1. Conduct and compile a comprehensive literature review of the published ecotoxicity data in aquatic and sediment environments;
2. Selection of nanomaterials and test organisms based on South African relevance and data needs;

3. Investigation of toxicity effects (acute and chronic) on aquatic organisms at different trophic levels;
4. Develop a risk assessment methodology based on the NMs ecotoxicity data;
5. Human capital development both for the scientists and postgraduate students working in the project; and
6. Data, information, and knowledge dissemination to different stakeholders (e.g. government departments, industry, scientists, general public, etc.) through appropriate media platforms (e.g. popular articles, journal articles, workshop presentations, note briefs, etc.)

To achieve the above objectives, four tasks were proposed where the first three objectives were primarily to be addressed experimental-based approaches. The fourth objective was to apply modelling-based approaches to predict the potential exposure and toxicity of ENMs in aquatic systems, largely using published data in the literature. Whereas the founding project's aims, objectives, and expected outcomes were novel as outlined in the original proposal, it became clear that it would be impractical to successfully implement due to budget constraints. Therefore, the project results presented herein are seeks to meet objective 4.

The choice to focus on objective 4 was informed by the fact that, in recent years, there has been a dramatic increase in the volume of data and information concerning risk assessment of ENMs published in the scientific literature (Hansen and Baun, 2012) – summarizing the number of reviews that have been done on the toxicity and exposure of ENMs over the last few years – though the data are highly fragmented. This objective was to answer several questions regarding the following; first, to what extent are these data valuable or directly applicable in informing decision-making to support adequate management of diverse array of potential risks of ENMs in the environment? Secondly, are the current modelling techniques and prediction tools suitable in establishing causal and effect mechanisms that influence exposure and toxicity of ENMs in environment media using published data? If so, how would this inform about the possibility of deriving underlying key decisions concerning their potential associated risks to different environmental compartments and organisms? Thirdly, can the inherent physicochemical properties of ENMs and the environmental factors be useful precursor factors that influence the behaviour, fate, transport, and biological effects of ENMs in the environment? If so, to what extent could these factors be used as predictive variables for the toxicity? These questions formed the basis of the findings presented in this report. Taking into account the focus of the project to address the fourth objective, the title changed to *Modelling the fate, behaviour, and toxicity of engineered nanomaterials in the aquatic systems*, and the objectives are summarized in section 1.2.

1.2 PROJECT AIMS

The following were the revised aims of the project:

1. Develop a toxicological database of ENMs to aquatic organisms from scientific reports (e.g. peer reviewed journal articles);
2. Establish the pertinent physicochemical properties of selected ENMs and their various forms that influences controlling eventual exposure and toxicity;
3. Investigate the relationship between pertinent physicochemical properties of ENMs to the biological, chemical, and ecological effects; and
4. Develop and simulate model(s) to test their functionality with respect to predicting and forecasting ENMs risks in the environmental media.

1.3 SCOPE AND LIMITATIONS

One of the key elements of this project was the limited and fragmented data reported for metals and metal oxides ENMs on aspects related to exposure and toxicity in aquatic systems. Because of the large number of static and dynamic physicochemical properties of ENMs discussed in Chapter 3, made it improbable to find in any single study that reported all the data required for modelling and comparative purposes to establish trends. This was further compounded by the large diversity of metals and metal oxides ENMs. In addition, after their release and entry into the aquatic ecosystems, the fate and behaviour mechanisms of ENMs influenced by abiotic factors (Chapter 3) were very limited, and this hindered the extent to which the reported biological effects could be linked to both ENMs static and dynamic properties. Unfortunately, such data have not been reported alongside the toxicity findings from scientific tests for many ENMs which made it difficult to correlate emission, fate, and transport of ENMs with respect to likely responses from biological organisms.

Taking into account the above challenges and limitations posed by the current state of data in this field, the scope of the project was limited to titanium nanoparticles (nTiO_2) because it is among ENMs that are widely reported in exposure and toxicity data in aquatic systems – despite the numerous gaps identified during data collection phase. Secondly, owing to wide differences of data for any given reported end-point or parameter, a set of pre-processing techniques were used to prepare data before it were used in the models for either training and/or validation purposes. Data preparation approaches adopted was semi-automatic consisting of manual and use of software where various theories, mathematical equations, and statistical modelling as described in Chapter 3. Such approach was used in acknowledgement that input model data consistency and reliability significantly influenced the robustness of the model developed, and therefore, the results derived from a given model. Thus, the study phase on data collection from the literature took an extended period of time not previously anticipated.

CHAPTER 2: LITERATURE SURVEY

2.1 INTRODUCTION

Global production of engineered nanomaterials (ENMs) and use of nanoproducts are on the rise (Musee 2011a; Gottschalk et al., 2011). Based on scientific reports, examples of some of the most commonly used ENMs in the manufacture of nano-based consumer products are presented in Table 2.1 for illustrative purposes. The production, manufacture, use and disposal points in the ENMs value chain are potential sources of their emissions into air, soil, aquatic and terrestrial ecosystems. Waste streams containing nanostructured materials, generally referred to as nanowastes, potentially pose risks to humans and the environment, and create unique challenges to waste management (Musee 2011a).

Table 2.1: Examples of most commonly used ENMs in various nanoproducts

ENM	Application	Reference
$nTiO_2$	Cosmetics, sunscreens, paints, varnishes and coatings.	Robichaud et al., 2009
nAg	Biocide agent, and composite in clinical dressing and textiles; Polymer in food industry.	Chen and Schluesener 2008 Hauri and Niece 2011
CNTs	Nanomedicine; Polymer and electronic products.	Bianco et al., 2005; Burello and Worth 2011b Baughman et al., 2002
Fullerene	Waste treatment and nanomedicine.	Hendren et al., 2011
nZnO	Cosmetics and sunscreens;	Schilling et al., 2010
	Potential biocide;	Rajendran et al., 2010
	Ceramics, rubber processing and wastewater treatment.	Wong et al., 2010
nCeO	Diesel fuel additive	Cassee et al., 2011

$nTiO_2$; nano titanium oxide, nAg; nano silver, CNTs; carbon nanotubes, nZnO; nano zinc oxide, nCeO; nano cerium oxide.

Risks associated with continued production and use of ENMs are largely unknown to manufacturers, consumers, waste regulators, scientists, government officials, and other stakeholders at various stages of the value chain. That is, informed decision making by all stakeholders on the use of nanoproducts and disposal of nanowastes is hampered by lack of substantive information and data on types, properties, volumes, concentration and forms of ENMs produced or used in manufactured products (Musee 2011a). Such information would form the basis of measuring and characterizing emissions of ENMs, establishing their fate, behaviour and effects in the ecosystems, and monitoring their associated risks and hazards to physiological and ecological systems.

Engineered nanomaterials have complex structures, properties and behaviour (Hansen et al., 2007) that determine their application in nanoproducts, fate and transport after emission, and eventual toxicity. Dissolution of ions from metal oxide ENMs determines their fate in the environment and within biological cells and organisms (Auffan et al., 2009). For example, scientific reports show that dissolved ions from metal oxide ENMs have more toxic effects to ecological bacteria (Adams et al., 2006), bacteria and crustaceans (Heinlaan et al., 2008), and biological cells (Puzyn et al., 2011) compared to their counterpart bulk materials. In aquatic sediments, research findings show that ENMs affect development of benthic organisms (Musee et al., 2010).

Nanotechnology is a rapidly advancing technology that is expected to comply with human, social, economic and environmental aspects similarly to other technologies that have previously been adopted by society. Whereas social and economic indicators of nanotechnology have largely been addressed, albeit partly in terms of benefits and profits, the human and environmental aspects have not been adequately quantified with respect to short-, medium-, and long-term risks. Presently, most attention has been directed towards evaluating the toxic effects owing to the exposure of ENMs to humans and the environment, mainly through use of experimental approaches. Toxicological studies of ENMs have mostly been conducted *in vitro* and *in vivo* to evaluate their toxicity to humans, terrestrial and aquatic environments, but the data generated are not sufficient to quantify associated risks. One of the notable aspects of the toxicological data presently accessible for ENMs is the lack of standard methods for testing and evaluating their observed effects to different receptor organisms.

Several researchers have therefore proposed the adoption of holistic and integrated approaches to quantify production volumes using extrapolated projections (Mueller and Nowack 2008; Hendren et al., 2011), to analyse life-cycle nano-risks (Gottschalk et al., 2011) and forecast risks through use of modelling techniques (Wiesner and Bottero, 2011). If the ENMs value chain is considered as a temporal and spatial system, whose source-to-sink processes are not well understood, modelling and simulation of the system are likely to offer a better understanding on the interaction of parameters underpinning their potential risks. Revisions to the models using actual data generated would further improve them, particularly with respect to providing feedback useful to decision makers in terms of monitoring and possibly mitigating various forms of risks.

Up to now, several studies have modelled the potential environmental exposure risks of ENMs, using different approaches. In this report, we highlight different models that have been applied in estimating the potential risks of ENMs in the environment. The models include (a) material flow analysis (MFA), (b) particle flow analysis (PFA), (c) stochastic modelling based on predicting risks using kinetic behaviour, abiotic and biotic interactions of the ENMs, (d) dose-response modelling, and (e) quantitative structure-activity relationship (QSAR) modelling. Each model application in the context of ENMs is described.

In addition, an analysis of risk assessment of environmental pollutants with nanoscale dimensions in terms of the models' strengths and weaknesses reviewed in this report is presented. The report concludes by highlighting salient points on modelling the ENM risks in environmental systems, and several suggestions on how this project will be pursued to meet its objectives-primarily through use of integrated modelling approaches that aids in synergistically exploiting the inherent strengths of various different modelling platform capabilities.

2.2 MODELLING ENVIRONMENTAL RISKS OF ENGINEERED NANOMATERIALS

2.2.1 Material flow analysis (MFA) models

Material flow analysis (MFA) models have been applied for the estimation of ENMs exposure to environmental compartments from production, manufacture and use of nonmaterial-based products, and recycling and disposal of waste streams with nanostructured materials. Environmental compartments, namely air, soil sediments, and water systems were considered as potential sinks of ENMs. For instance, studies have reported the application of MFA models to predict exposure concentrations of ENMs in environmental compartments in Johannesburg Metropolitan City (Musee, 2011b) and Gauteng Province in South Africa (Nota, 2011), 25 countries of the European Union (EU) (Blaser et al., 2008), United States of America (USA), EU and Switzerland (Mueller and Nowack, 2008; Gottschalk et al., 2009; 2010a; 2010b; 2011).

Owing to lack of quantitative data on the actual concentrations of ENMs released, MFA models employ predicted environment concentration (PEC) and predicted no-effect concentration (PNEC) estimates, where the risk quotient (RQ = PEC/PNEC) is used as a measure of the associated environmental risks (European Chemicals Bureau (ECB), 2003). Hypothetical postulation, extrapolations, and cognitive methods are used for the estimation of PEC values whereas the PNEC values are extrapolated using data derived from the dose-response toxicological studies reported in scientific reports as the ratio of no observed effect concentration to assessment factor (NOEC/AF)(ECB, 2003). Due to the large uncertainties and variance associated with the presently published nanoecotoxicological data of ENMs in the environment, an AF value of 1000 is mostly used in the computations.

In studies to date, model input variables to estimate PEC comprised estimated global production of ENMs scaled to regional productions, estimated quantities of ENMs used in nanoproducts, extrapolated mass flow values of ENMs from nanoproducts into environmental compartments, and comparative socio-economic and demographic data for a given region or city. Where the NOEC data were absent or insufficient, RQ was calculated using 50% lethal concentration (LC_{50}) and 50% effective concentration (EC_{50}) (Musee, 2011b); lowest observed lethal concentration (LOEC) and highest observed no-effect concentration (HONEC) (Blaser et al. 2008) in accordance to the ECB procedures (ECB, 2003). A river box model approach was used by Blaser et al. (2008) to simulate water flows where scientific information on physicochemical and dynamic properties of the river regimes were used.

The MFA model findings revealed that the $nTiO_2$ posed a great risk to aquatic ecosystems with PEC values of >0.7-16 μg L^{-1} (Mueller and Nowack, 2008, 2.7×10^{-3}-2.7×10^{-1} μg L^{-1} (Musee, 2011b) and 1.23×10^{-1}-4.292 μg L^{-1} (Nota 2011), which all yielded maximum scenario emissions of RQ_{water} >1. Whereas nAg was found to be highly toxic to aquatic system in Gauteng Province studies (PEC: 1.2×10^{-2}-4.3×10^{-2} μg L^{-1} and RQ_{water} = 11.7-42.85) (Nota 2011), its associated risks in Johannesburg Metropolitan City (Musee, 2011b) and Switzerland (Blaser et al., 2008) had RQ_{water} values ≤ 1, suggesting minimal or negligible risk to aquatic life forms. This could be partly attributed to lack of quantitative data on nAg toxicity to aquatic organisms in both scenarios, and/or underestimations, particularly in Switzerland where the PEC values were computed using the whole country's demographics.

However, the findings of Blaser et al. (2008) in Switzerland revealed that nAg colloids and not Ag ions or ENMs posed high risks to both aquatic (RQ_{water} = 20-160 μg L^{-1} as silver-zinc sulphide) and benthic micro-organisms ($RQ_{sediment}$ = 940-6800 as silver-cysteinate and 1.6-11.3 as silver-glutathionate) due to its strong sorption characteristics onto both inorganic and organic compounds. Nonetheless, data on the actual risks posed by these colloids are largely lacking, since model conversions used silver and not colloidal species. In addition, there have been scientific arguments that NOEC measurements do not account for precision and uncertainty determination (Kooijman 1996; Fox, 2008), and merit to be replaced by more certain measurements like no effect concentration (NEC).

Whereas the MFA models in Europe, USA and Switzerland estimated production volumes of ENMs using uniform country economic and demographic data to estimate PEC, models by Musee (2011b) and Nota (2011) estimated risks in specific regions in South Africa using site specific socio-economic and demographic data as means of reducing under- or over-estimation biases. Establishment of RQ for some environmental compartments (e.g. soil and sediments) in all countries was hampered by lack of toxicity data. The model by Blaser et al. (2008) accounted for 15% of nAg released from biocidal and textile nanoproducts, hence the model severely underestimated the environmental risk of nAg in aquatic systems.

The MFA models suggest that the highest risk to aquatic environment was due to $nTiO_2$, nZnO, and nAg, owing to the extensive application of these nanoscale materials in cosmetics, sunscreens, and biocides. These ENMs should, therefore, be among those studied as model bioassays for toxicity, fate, and behaviour in order to enhance our collective understanding on their potential risks to the environment. Some of the MFA models' findings are summarized in Table 2.2. Discussion of the probabilistic MFA model approaches is presented in section 2.3.1 where a combination of methods comprising probability life-cycle analysis, graph theory, Monte Carlo (MC), Markov Chain Monte Carlo (MCMC), and Bayesian inference techniques (Gottschalk et al., 2009; 2010a; 2010b; 2011) were used to improve the MFA model reported by Mueller and Nowack (2008).

For any given pollutant entering the environment, measuring downstream risks without considering the source and emission pathways is inadequate in support of sound and defensible judgments. Hence, the projection of ENMs production and distribution in different emission pathways in the development of MFA models are an important model parameter in estimating exposure risks, and the extent to which the ENMs are likely to reach different environmental compartments. The MFA models also give valuable insights to mass flow (μg L^{-1}) of ENMs into different environmental compartments, and also the prediction of their likely concentration (PEC = μg L^{-1}) in terrestrial, atmospheric, and aquatic systems after emissions. In addition, PEC provides a good measure of life-cycle mass balances of ENMs in the environment, for instance, quantities likely to be contained in nanowaste streams.

Table 2.2: Summary of MFA model findings based on ENMs risk quotient (RQ) values

	Johannesburg City, SA (Musee, 2011)		Gauteng Province, SA (Nota, 2011)		CH (Mueller and Nowack, 2008)		EU (Blaser et al., 2008)	
ENM	Prob	Max	Prob	Max	RE	HE	Min	Max
nTiO$_2$								
Soil	4.0 E-8	2.7 E-4	7.9 E-6	4.1 E-4				
Freshwater	4.0 E-2	2.7	1.23	42.92	>0.7	>16	-	-
nAg								
Soil	-	-	5.2 E-03	2.9 E-02	-	-	-	-
STP^E							<1.0 E-2 $_{\text{Ag-T}}$	1.0 E-1 $_{\text{Ag-T}}$
Freshwater	4.0 E-2	6.0 E-1	11.79	42.85	8.0 E-4	2.0 E-3	20$_{\text{Ag-ZnS}}$	160 $_{\text{Ag-ZnS}}$
Sediment	-	-	-	-	-	-	940 $_{\text{Ag-C}}$	6800 $_{\text{Ag-C}}$
							1.6 $_{\text{Ag-GSH}}$	11.3 $_{\text{Ag-GSH}}$
CNT								
Soil	-	-	9.0 E-7	5.7 E-6	-	-	-	-
Freshwater	-	-	5.1 E-3	2.0 E-2	5.0 E-3	8.0 E-3	-	-
Fullerene (C$_{60}$)								
Soil			1.6 E-9	1.3 E-8				
Freshwater			2.9 E-5	1.5 E-4				

SA: South Africa, CH: Switzerland, EU: European Union, Prob: probable scenario, Max: maximum scenario, RE: realistic scenario, HE: high emission scenario, nc: not calculated, STP^E: effluent from sewage treatment plants, $_{\text{Ag-T}}$: silver-thiosulfate, $_{\text{Ag-ZnS}}$: silver-zinc sulfide cluster, $_{\text{Ag-C}}$: silver-cysteinate organic cluster, and $_{\text{Ag-GSH:}}$ silver-glutathionate organic cluster.

The MFA models only demonstrate the expected risks to the environment and not the associated toxic effects to the ecological system. This is because the model output is expressed in dimensionless RQ values as risk indicators (where RQ >1 suggest high risk, and *vice versa*). Engineered nanomaterials have unique surface properties due to their small sizes in comparison to counterpart bulk parent material – where the latter's structural properties are used in determining their kinetics, behaviour, and potentials in the environment. However, the MFA models do not reflect the physicochemical characteristics of ENMs as model input parameters that underpin their potential risks to the receptor organisms. Physicochemical properties of nanomaterials differ remarkably from those of corresponding bulk materials, and properties of the later are not reliable predictors of the environmental risk of ENMs. In addition, MFA models are region-specific as they were developed for South Africa, Europe, USA and Switzerland based on each country's socio-economic and demographic information, and therefore cannot be replicated. To improve the capabilities of MFA models with respect to estimating ENMs exposure risks, we suggest the following:

- Physicochemical properties of ENMs, their forms of production, and use patterns of nanoproducts, expected behaviour in environmental compartments and toxic effects during exposure should be taken into account;

- Due to lack of quantitative data on production, sources of emissions, pathways and sinks of ENMs, production projections by region or country using socio-economic and demographic data may lead to unreliable estimation of the actual mass flows. This limitation can be addressed through the development, and continuous updating of inventories on the production and use of ENMs, so that these data can be used to improve the models.

- The MFA models assume steady state and homogeneous conditions for ease of computation. Nonetheless, actual states are either periodic, transient or a combination of all depending on the nature of stream/aquifer/river flows and turbulence factors. In addition, due to possible formation of inorganic or organic colloids formation and their associated sedimentation – a homogeneous system cannot be achieved; hence the mass flow kinetics would also change. Therefore, future MFA models within the context of ENMs risk exposure estimation should factor in the localized hydraulic properties of fluid media, and ENMs or colloid kinetics in different environmental compartments. This approach is likely to fundamentally alter the founding MFA models assumptions, but will considerably increase their reliability in supporting decision making.

- Adequate data on toxicological effects of ENMs in different forms to aquatic and terrestrial organisms are lacking, hence exposure assessment for some compartments cannot be quantified. There is a need to generate the nanoecotoxicological data based on standardized approaches to ensure their reliability in computing the PNEC values.

2.2.2 Particle flow analysis (PFA) model

A particle flow analysis (PFA) model is primarily hinged on the techniques used in MFA modelling as well as those outlined by Van der Voet et al., (2002) where the prediction of future chemical emissions from human society into the environment is based on delayed input or future stocks. In PFA approach, the number of particles released into the environment is evaluated as opposed to mass flow emission of the chemicals. Particle flow analysis proposes the use of particles of specific ENMs used in nanoproducts that are likely to be emitted to the environment. An explorative PFA model predicted emissions of $nTiO_2$ used in paint, sunscreen, and self-cleaning cement nanoproducts, and also for nAg used in wound dressing, textiles and electronic circuitry (Arvidsson, 2010; Arvidsson et al., 2011). Particle numbers in life-cycle phases described by Eqn. 2.1 (Arvidsson, 2010) were calculated using quantitative data.

$$\frac{\Delta N}{\Delta t} = \sum n_{in} - \sum n_{out} + n_s \qquad 2.1$$

Where, N = particle number in stock, n = particle number flows (particles/year), and n_s = source or sink of emission from the system. Model parameters used in phases were: particle size (diameter), content and properties; product density, depth and lifetime in steady state; product stock, flow and per capita stock of the country; and emission factor corrected for kinetic properties like agglomeration and aggregation. The PFA model findings are summarized in Table 2.3.

Table 2.3: The PFA model findings for $nTiO_2$ and nAg

	$nTiO_2$			nAg		
Output parameter	Paint	Sunscreen	Self-cleaning cement	Wound dressing	Textiles	Electronic circuits
Current						
Inflow, (n/year)	1.1×10^{25}	2.6×10^{25}	Negligible	4.6×10^{26}	$<8.5 \times 10^{23}$	$<6.8 \times 10^{24}$
Stock in use, (N)	1.1×10^{26}	Negligible	Negligible	Negligible	Negligible	$<6.8 \times 10^{25}$
Emission, (n/year)	1.1×10^{19}	2.6×10^{25}	Negligible	4.6×10^{21}	$<8.5 \times 10^{23}$	$<<6.8 \times 10^{24}$
Future						
Inflow, (n/year)	7.0×10^{25}	2.1×10^{26}	1.1×10^{30}	1.10^{22}-1.10^{25}	6.60^{28} - 6.1^{32}	9.1×10^{27}
Stock in use, (N)	7.0×10^{26}	Negligible	5.1×10^{31}	Negligible	Negligible	9.1×10^{28}
Emission, (n/year)	7.0×10^{19}	2.1×10^{26}	3.1×10^{21}	1.10^{21}-1.10^{24}	6.10^{28}-6.10^{32}	$<<9.1 \times 10^{27}$

From the PFA model, the highest current inflow was observed for $nTiO_2$ used in sunscreens (2.6×10^{25}) and paint (1.1×10^{25}) in comparison to its negligible use in self-cleaning cement products. This scenario was predicted not to change much in future, although there is marked increase in inflows by a factor of more than 100 and 6.4 times to self-cleaning cement and paints, respectively, where the latter increase could probably be attributed to product awareness. For nAg, inflows are high in electronic circuits ($<6.8 \times 10^{24}$), textiles ($<8.5 \times 10^{23}$), and wound dressings (4.6×10^{26}) with future estimated change in inflows to be in the order of 1.34×10^{3}, 7.76×10^{4}-$7.18 \times 10 \times 10^{8}$, and 2.39×10^{-5}-2.39×10^{-2} times, respectively. The low predicted values of nAg use in wound dressings are largely due to lack of quantitative data to aid in projecting future emission quantities.

The PFA forecasts provide a reasonable projection of the most used ENMs, as well as the most manufactured nanoproducts. The projections highlight potential sources of ENMs emissions to the environment, and draw attention to researchers and waste management regulators on ENMs expected in nanowaste streams. However, the model does not consider waste handling processes in order to quantify the emission pathways. In addition, the use of particle diameter poses a challenge in terms of quantifying emissions of ENMs that are non-spherical, such as carbon nanotubes (CNT), and those produced in bulk or surface coating forms.

On the basis of these limitations, further research towards improving this model is needed, for example, through the inclusion of waste emission and handling pathways, eventual disposal, and subsequent toxicity to the environment based on the particle number, rather than the mass per unit volume toxicity reported in the presently accessible literature. In addition, other ENMs need to be considered to ascertain what sort of model input parameters are critical, explore how the prediction in forecasting non-nanoparticle types of ENMs, such as fullerenes and quantum dots should be factored in the model, and validation of the model for practical applications by decision-, and policy-makers.

2.2.3 Stochastic models

Stochastic models use probabilistic approaches to predict uncertain outcomes through use of random variables whose values change as a function of time (Loucks and Van Beek, 2005). The models are applicable in domains where precise and deterministic variables are lacking. Engineered nanomaterials in buoyancy-driven flows experience stochastic movement due to rapidly changing thermal dispersion forces that result in inter-nanoparticle collisions and eventual aggregation (Routhu and Nnanna 2006). Colloidal chemistry of ENMs including agglomeration, aggregation, and sedimentation (settling) in aquatic systems constitutes an important fate mechanism that impacts on their exposure and bio-availability (Grieger et al., 2011). Given that environmental processes are non-steady state homogeneous systems but stochastic in nature (Rieckermann et al., 2011), stochastic modelling constitutes important means of predicting realistic environmental exposure and toxicity of pollutants.

2.2.3.1 Probabilistic material flow analysis (PMFA) models

An in-depth understanding of the physicochemical properties, fate, transport and toxicity of ENMs in aquatic systems is needed to develop models that improve exposure prediction of pollutants with nanoscale dimensions (Christian et al., 2008; Klaine et al., 2008; Musee et al., 2011c). Depending on the point of release, the fate and transport of ENMs in aquatic systems follow different pathways including groundwater percolation, irrigation using wastewater, surface run-offs, and sedimentation. Models for probabilistic material flow analysis (PMFA) (Gottschalk et al., 2009; 2010a; 2010b; 2011) for ENMs were developed as an improvement to the MFA model previously reported by Mueller and Nowack (2008). In the MFA model, temporal and spatial data of the river systems and emission uncertainties in environmental compartments such as sediments were not considered. Owing to the lack of empirical data or large uncertainties of the accessible data, PMFA as a stochastic model uses a combination of methods comprising probability life-cycle analysis, graph theory, Monte Carlo (MC), Markov Chain Monte Carlo (MCMC), and Bayesian inference techniques.

Variables selected in stochastic modelling are largely for determining the predictive outcome. For example, production volumes, distribution (exports), emission pathways of ENMs into environmental compartments, legislative methods and efficiency of handling wastes as well as availability of quantitative data on ENMs are among the factors that influence the PEC and subsequent RQs computation in model system boundaries (regions) (Gottschalk et al., 2009). For instance, $nTiO_2$ is the most used ENM in sunscreens and cosmetics and yielded the highest RQs in Europe, USA, and Switzerland (Table 2.4). In addition, high concentrations of ENMs in Europe and USA were found in sludge treated soil and sediment, unlike in Switzerland where soil sludge treatment is prohibited; hence more ENMs were observed in sediment and effluent from sewage treatment plants. The RQ values for CNT in aquatic systems were generally low (Gottschalk et al., 2009; 2010b) in the application phase, where CNT-based products such as electronics rarely come into contact with water, as they are firmly held in the product matrix (Hansen et al., 2007; Musee, 2011c). The RQ values from the PMFA model are summarized in Table 2.4.

Time and space variations, especially the geographical distribution of ENMs in a region or country, as well as possible dilution in river flows affect the PEC of ENMs emissions more profoundly than the transportation of ENMs in environmental compartments. Using graph theory, Gottschalk and colleagues (Gottschalk et al., 2011) developed a PMFA geo-referenced model mapping of $nTiO_2$, nZnO, and nAg PEC emissions to 543 rivers in Switzerland. Site specific uncertain emissions at point sources, geographical distribution of the ENMs, 21 river box partitioning and water flow characteristics over 20 years, and transport scenarios of the ENMs into the rivers were used as model variables.

The model PEC values (Table 2.5) show region and time dependent risk factors, and the risk profiles were found to be in the order of $nTiO_2$ >nZnO >nAg. For local and space variations, high concentrations of ENMs were found near urban areas when compared to the rural areas, except where tourists visited the rural areas. These findings concur with the arguments advanced by Musee (2011b) who suggested that the ENMs concentrations are likely to be elevated in urban centres, and illustrated this with the case of Johannesburg Metropolitan City in South Africa.

Table 2.4: Summary of PMFA model results finding based on risk quotient (RQ) values.

	USA, EU and CH (Gottschalk et al, 2009)			CH (Gottschalk et al, 2010b)	
ENM	EU	USA	CH	Mode	Median
$nTiO_2$					
Soil	4.0 E-3	2.0 E-3	1.0 E-3	1.0 E-3	3.0 E-3
SoilST	3.0 E-1	1.4 E-1	-	-	-
STP^{E}	3.5	1.8	4.3	4.28	7.58
Freshwater	1.5 E-2	2.0 E-3	2.0 E-2	2.0 E-2	4.0 E-2
nAg					
STP^{E}	61.1	30.1	55.6	55.6	88.41
Freshwater	1.1	1.7 E-2	1.03	1.03	1.73
Sediment	-	-	-	-	-
CNT					
Soil	<5.0 E-4	<5.0 E-4	<5.0 E-4	<5.0 E-4	<5.0 E-4
SoilST	<5.0 E-4	<5.0 E-4	<5.0 E-4	-	-
STP^{E}	<5.0 E-4	<5.0 E-4	<5.0 E-4	<5.0 E-4	<5.0 E-4
Freshwater	<5.0 E-4	<5.0 E-4	<5.0 E-4	<5.0 E-4	<5.0 E-4
Sediment	<5.0 E-4	<5.0 E-4	<5.0 E-4	<5.0 E-4	<5.0 E-4
Fullerenes (C_{60})					
Soil	<5.0 E-4	<5.0 E-4	<5.0 E-4	-	-
SoilST	<5.0 E-4	<5.0 E-4	-	-	-
STP^{E}	2.6 E-2	2.3 E-2	1.9 E-2	-	-
Freshwater	<5.0 E-4	<5.0 E-4	<5.0 E-4	-	-
nZnO					
STP^{E}	10.8	7.7	11	-	-
Freshwater	2.5 E-1	2.0 E-2	3.2 E-1	-	-

EU: European Union, USA: United States of America, CH: Switzerland, nc: not calculated, SoilST: soil treated with sludge, STP^{E}: effluent from sewage treatment plants.

Table 2.5: Summary of time and space PEC model findings (Gottschalk et al., 2010a).

ENM	Conservative scenario, PEC (ng L^{-1})	Optimistic scenario, PEC (ng L^{-1})
$nTiO_2$	11-1 623	2-1 618
nZnO	7.86×10^{-1}-115.93	1.43×10^{-1} – 115.57
nAg	4.6×10^{-2} – 6.76	8.3×10^{-3} – 6.74

The Gottschalk et al., (2010a) model predicted annual increase of $nTiO_2$ concentrations in Switzerland. Monte Carlo (MC), MCMC and multivariate statistical techniques were used in simulating three-tier ENMS emission scenarios, namely direct emissions, emissions from manufacture, use, as well as disposal and/or recycle. Using scientific data derived from scientific and company reports, the production, application, and transmission of ENMs into soil, surface water, air and sediment were used as model variables. Processes within an emission source and materials flow were iterated as change of rates of stock and transfer coefficients, respectively.

The densities of MCMC simulations were higher than those derived from MC simulations and suggest that the former approach yielded superior predictions (Gottschalk et al., 2010a). The model PEC values of $nTiO_2$ emissions were highest in sediments followed by soil water compartments, and air in the descending order, correspondingly. This phenomenon may be attributable to waste treatment technologies and efficiency in Switzerland.

Attempts to improve the PMFA model developed by Gottschalk et al. (2010a) have been reported (Gottschalk et al., 2010b) for $nTiO_2$, nAg, and CNT as model ENMs with inclusion of effluent compartment from sewage treatment plants, as well as evaluation on the possibilities, uncertainties, and limitations related to Gottschalk et al. (2010a) model. Bayesian inferences based on MCMC were used for iterations in instances where data were available.

The Gottschalk et al. (2010a; 2010b) models were an improvement on the work of Mueller and Nowack (2008), and offered predictions on uncertain emissions of ENMs into the environment through continuous iterations of emissions. These models used the country data for Switzerland presented by Mueller and Nowack (2008) to predict PEC for $nTiO_2$ and CNT without the likelihood of overestimating the risks. A combined model using techniques presented by Musee (2011b), Nota (2011), and Gottschalk et al. (2011) where regional emissions were considered, and Blaser et al. (2008) and Gottschalk et al. (2009) where flow partitioning considered appeared to offer improvements on the results of Muller and Nowack (2008).

Given that PMFA models are an improvement of the basic MFA models, the former exploited the stochastic prediction capabilities using modelling techniques (MC, MCMC, and Bayesian) in estimating the ENMs risks within the context of scarce and inconsistent empirical data. Similar to the MFA models, the PMFA models input variables consisted of production, manufacturing, use, recycling and disposal as the potential ENMs emission points to the environment. Other broad factors considered in PFMA individual models, different from those used in MFA, included the mass flows in temporal and spatial zoning, partitioning of emission pathways to environmental compartments as well as fate and transport. However, the abiotic and biotic environmental parameters merit more rigorous evaluation towards elucidating how inorganic and organic colloids formed and risks associated with sedimentation of these colloids will impact the aquatic organisms-with specific reference to different trophic levels.

The MFA, PFA and MFPA models suggest that there are numerous uncertainties underpinned by lack of information and quantitative data. In this context, some of the projections lead authors to conclude that certain ENMs (e.g. nAg) pose minimal risks to the aquatic organisms-though this may not be true in real environmental systems. Due to lack of adequate input data, numerous assumptions, and extrapolations have been made to compute the PEC (MFA and MFPA models), and the number of particles/particle flows (PFA model) with respect to production volumes, distribution during manufacturing, use, and disposal phases. For a rapidly evolving technology such as nanotechnology, forecasting of ENMs risks requires MC and Bayesian networks that do not follow the traditional risk assessment procedures (Wiesner and Bottero, 2011). Accordingly, modelling approaches using MC and Bayesian techniques are viewed as suitable platforms to address the potential risks of ENMs in the environmental systems in South Africa.

2.2.3.2 Probabilistic material flow analysis models

A theoretical model of Mackay and colleagues (2006) simulated aggregation of ENMs (10-1000 nm) in aqueous suspension using MCMC chemical kinetics as outlined by Gillespie (1976). The model variables consisted of particle size, particle-particle interactions and collisions, temperature and buoyancy flow properties of ENMs and fluid. The model findings were that the metastable heterogeneous aqueous suspension of ENMs depended on particle size. The model predicted that (i) the apparent solubility, and therefore, stability of ENMs in suspension was high for ENMs in the size range 10-75 nm, and approximately zero for aggregates >450 nm; (ii) settling time increased with increasing particle size as large colloids would settle out faster (iii) density had minimal effect on suspension stability up to the 75-300 nm particle size range; and (iv) time was an important variable in approximating the aggregation reactions.

This is a basic stability model in which only particle size and sedimentation retention times were considered as model variables. Other properties of ENMs that influence stability were not taken into account. Factors that were not considered in the model include physicochemical properties of ENMs in aqueous suspension that underpin reactivity and aggregation, bioavailability of ENMs and their potential toxic effects to aquatic species, and the effect that colloidal matter, such as natural organic matter may exert on the aggregate size, composition, and sedimentation properties. Given that ENMs are not inert chemical compounds, their suspension, agglomeration or aggregation potential and therefore environmental risks are largely influenced by their inherent physicochemical properties, as well as abiotic and biotic factors in aqueous media. Therefore, future MCMC-based models should consider these factors among others, though we acknowledge the complexity of the problem due to large input data sets needed, and even lack thereof of data with integrity to support substantive conclusions from the model.

2.2.3.3 The Derjaguin-Landau-Verwey-Overbeek models

The Derjaguin-Landau-Verwey-Overbeek (DLVO) theory established in the 1940s (Derjaguin and Landau 1941; Verwey and Overbeek 1948) attempts to model the colloidal stability of particles in aqueous suspension largely dependent on the total potential energy functions in the system. The DLVO theory recognizes that the total energy of colloidal stability is the summation of the potential energy of the aqueous suspension, the attractive van der Waals, and the repulsive forces. The van der Waals attractive forces are related to the Hamaker and zeta potential. Several DLVO simulations have been reported, on the agglomeration, de-agglomeration, aggregation kinetics and colloidal stability of ENMs in aqueous solutions (Guzman et al., 2006; Jiang et al., 2008; French et al., 2009; Huynh and Chen 2011; Liu et al., 2011) and biological cell cultures (Ji et al., 2010). Wong and colleagues (2010) have evaluated ENMs toxicity linked to agglomeration. A review by Petosa and co-workers (2010) has described the physicochemical interactions in deposition and aggregation of ENMs in aquatic environments, and how the interactions potentially may lead to a state of non-DLVO behaviour.

A simulation box by Liu et al. (2011), using the computational constant-number direct simulation Monte Carlo (DSMC) methods, established that the ENMs agglomeration in aqueous suspensions, and hence, the environmental stability, depended on the zeta potential, Hamaker constant, diameter of ENMs, and ionic strength of the aqueous media, based on the assumption that the agglomeration process was an irreversible process. The DSMC model is a fundamental continuous space and time process that combines steady state particle distribution in aqueous suspension and agglomerate settling.

Model ENMs studied were $nTiO_2$, $nCeO_2$, and nC_{60} whose agglomeration when suspended in 0.01-1mM ultra-pure deionized water was monitored for 24-h. The pH range of the aqueous suspension of 3 to 10 was modified using hydrogen chloride, sodium hydroxide, sodium hydrogen carbonate, and sodium chloride. The particle size distribution of the ENMs was measured using dynamic light scattering (DLS). Results showed that the agglomeration rate of $nTiO_2$ and $nCeO_2$ of 15 and 21 nm, respectively, increased over time except when sedimentation occurred with a corresponding ENMs suspension reduction by 20%-50%. Increased agglomeration was observed at the isoelectric point (IEP) where ζ = 0 mV, decreasing particle size, and increasing ionic strength, and Hamaker constant (110-550 nm at A_H = 10-90 ZJ).

Other DLVO based models have shown that the state and stability of ENMs in fluid media are governed by steric repulsive forces due to surface coatings of ENMs, zeta potential, cation valence, and particle size, hydrodynamic size (size of agglomerate or aggregate), ionic strength, Hamaker constant and pH (Jiang et al., 2008). Coating nAg with citrate and polyvinylpirrolidone (PVP) produced zero valence ENMs, both with negative charges and minimal dissolution when exposed to single (NaCl) and double ($CaCl_2$ and $MgCl_2$) valence aqueous solutions (Huynh and Chen 2011). The steric repulsion of nAg coated with PVP was higher than with the case of citrate, hence the former ENMs exhibited higher stability, although for both coatings, the colloidal stability improved in the presence of humic acid, and aggregation was enhanced at high concentrations of $CaCl_2$ (Huynh and Chen 2011).

Zeta potential is an electrostatic potential at the boundary layer of the hydrodynamic surface surrounding a particle or colloid, and is a measure of stability or dispersion of agglomerates or aggregate suspensions. An increase in ionic strength of a fluid media compresses the thickness of an electrically charged boundary layer with enhanced compression achieved from higher than lower valence ions (e.g. $Ca^{2+} > Na^+$), which reduces the zeta potential, and increases the colloid formation (Jiang et al., 2008).

A model showed that the aggregation of $nTiO_2$ increased from average particle sizes of 50-60 nm <, 500 nm, <mixed precipitates of 0.1-1.0 µm, 2-3 µm, and 60 nm at increasing ionic strengths of 4.5×10^{-3} <, 8.5×10^{-3} <, 1.65×10^{-2} M, correspondingly (French et al. 2009). Increasing agglomeration of $nTiO_2$ due to increasing ionic strength is a phenomenon observed in *in vitro* models using cell cultures (Ji et al. 2010). The sedimentation of the $nTiO_2$ colloids was observed at pH 5.8-8.2 and at ionic strengths of 8.4-9.9 $\times 10^{-3}$ M (French et al., 2009) under stationary conditions which corroborates earlier findings that high pH reduces the zeta potential and promotes aggregation or agglomeration (Jiang et al., 2008). Another model revealed that aggregate sizes of $nTiO_2$ increased as the pH approached the zero charge point (zeta potential = 0 mV, at pH 5.9 for $nTiO_2$), where sizes of 3.6 and 8.1 nm were observed at pH 4.8 and 6.2, respectively (Guzman et al., 2006). In addition, high valence of aqueous media increased colloid formation that can be accounted for by decreasing length (Debye) of electrostatic repulsion (French et al., 2009). Finally, the transportation of ENMs in environmental media was established to be influenced by the surface charges of the boundary layer, and aggregate size (Guzman et al., 2006).

Stochastic DLVO models provide insight into the behaviour of ENMs in aqueous media. The mobility of ENMs in fluid media is governed by their inherent physicochemical properties, adsorption characteristics due to the organic and inorganic strengths of the media, dispersion, agglomeration and aggregation characteristics, among other. The DLVO models have successfully been used to predict colloidal behaviour of ENMs in fluid media under different media conditions. Factors with significant influence to colloid stability or agglomeration/aggregation are the electrostatic repulsive forces, van der Waals attractive forces between particles or surfaces and fluid media potential as a function of pH and ionic strength. Given that the zeta potential is a repulsive force, it promotes dispersion. Therefore, an increase in colloidal sizes affects transportation and sedimentation properties. DLVO models also reveal that surface properties of ENMs due to coating exhibit different settling properties in comparison to the original NP resulting from differences in surface ionization potential. The stochastic Bayesian networks were shown to link ENMs sources; manufacture and product use to the associated risk pathways and uncertain parameters using MC methods.

The DLVO models are limited in that they do not demonstrate the toxicity (apart from computing the colloidal stability) and hence the risks of ENMs suspensions or colloids to the terrestrial or aquatic organisms due to settling. From a practical viewpoint, depending on the type of colloids formed, settling or sedimentation can result in toxic impacts being exacerbated to the benthic organisms in aquatic systems (Musee et al., 2010). Also, box simulations applied in the colloid studies used ultra-pure deionized water, which is not representative of actual natural environmental conditions, given that the natural environment is more complex due to organic (e.g. polysaccharides) and inorganic suspensions (presence or absence of electrolytes). The findings reported by the DLVO studies may therefore not be applicable to realistic systems.

Several authors assumed irreversibility of the agglomeration conditions and steady number of particles in applying the DLVO models. However, such conditions are “ideal” because agglomeration and fragmentation of ENMs occur simultaneously, for example, because in typical environmental conditions, plug flows and sedimentation affects the number of particles that move in and out of a boundary system. The hydrodynamic diameter of ENMs, agglomerates, and aggregates, were assumed in the computation, whereas in actual conditions, non-spherical structural properties are highly probable. Thus, to improve the DLVO findings for the ENMs, the following aspects merit consideration, including (a) the geometric and shape factors (e.g. for non-spherical surfaces), solubility as well as other sets of ENMs properties; (b) undertaking of laboratory or pilot research studies that use natural water with both organic and inorganic additives in aqueous suspensions. This may aid to simulate agglomeration, aggregation, and colloid behaviour in conditions that closely reflect the natural environment. Other variables of significance may include environmental factors, such as temperature, salinity and flows; and (c) conducting bioassays of laboratory-based toxicity studies to assess the effects of sedimentation for modified ENMs owing to the presence of organic and inorganic colloids to the aquatic organisms.

2.2.4 Dose-response models

The concept of dose-response models (DRMs) is underlined by a mathematical relationship between exposure of a substance or chemical and the effect of exposure to cells, organisms, animals or human end-points (International Programme on Chemical Safety (IPCS) 2009). The dose variables include administered, absorbed or target pollutant concentrations, dose time, and frequency. On the other hand, the end-point responses are classified as quantal, count, continuous or ordinal (IPCS, 2009). Dose-response models are generically applied in drug discovery to test the efficacy and toxicity of drugs before production at commercial scale. Standard biological reactions are evaluated using DRMs in *in vitro* and *in vivo* toxicology studies to measure the degree of chemical hazards. Various DRMs have been developed to date, and the choice of a model type to use in a given problem domain is generally at the discretion of the researcher, depending on the outcome expected, and statistical significance of the distribution.

For example, continuous and count models utilize effect outcome as a function of the dose whereas the quantal data utilize the probability of effect as a function of the dose (Ritz, 2010). Dose-response models include log-logistic, log-normal, Weibull-1 and Weibull-2 models, all having different distribution functions that can be manipulated by changing input parameters to produce desirable statistical outcomes (Ritz, 2010). Ritz (2010) suggests the need for a unified framework to consolidate DRMsand reduce inconsistencies observed in uncertainty measures due to parameterization and naming.

Most DRMs have used no observed effect concentration (NOEC), which is the largest concentration that yields no observed response. However, the NOEC values have been severely criticized due to their associated statistical problems (Kooijman, 1996; Kooijman and Bedaux, 1996; Pires et al. 2002; Fox, 2008;2010). Alternatively, Bayesian methods have been proposed for use in modelling toxicology risks where uncertainties in multiple hazards are expected (Pollino and Hart, 2005). Recently, the Bayesian modelling approaches that combine prior information and dose-response data have been tested in generating posterior data distributions, and yielded findings that supported the proposition of replacing NOEC with no effect concentrations (NEC) and hazard concentration (HC_x) estimates in ecotoxicology assessments (Fox 2010). An alternative model proposed to replace NOEC measurements is dynamic energy budget toxicology (DEBtox), which examines tissue toxicant concentration of a pollutant in a physiological process (Kooijman, 1996; Kooijman and Bedaux, 1996), based on energy derived from food uptake by micro-organisms, assimilation and use in growth, maintenance and development.

Most ENMs toxicity studies pertaining to cells, biological, and ecological organisms have used the DRMs. The data generated are used in developing other models. For example, the computation of PNEC values and the RQ in MFA models utilizes dose-response toxicology data. Also, empirical QSAR models utilize data from dose-response models. Hence, parameterization in these models is highly significant as the dose represents the exposure and frequency of application as well as the expected endpoints. The choice of parameters determines the expected quantitative and qualitative data. However, inconsistency in parameterization and statistical significance require careful analysis before selecting suitable model types. An example of dose-response model described here is the knowledge discovery from data modelling.

Knowledge discovery from data (KDD) is an interactive and iterative process of identifying a pattern and extracting useful information from complex databases (Fayyad et al. 1996). Using KDD on experimental data, Horev-Azaria et al. (2011) predicted that the dissolution of ions from cobalt ENMs aggregates mainly caused the toxic effects observed in the biological organisms. This prediction was tested *in vitro* by exposing six cell cultures representing the lung, intestines, liver, kidney and dendritic cells to Co-NPs and Co^{2+} for 48 and 72 h. Nanometer and crystal structure size, size distribution, structure, shape, and agglomeration properties of Co-NPs (10-50 nm) were characterized. J48 decision tree model was developed and accuracies of 86.6, 87.1, and 87.3% for $>EC_{20}$, $>EC_{25}$, and $>EC_{30}$ toxicity thresholds were determined, respectively. Cross-validation using Naïve Bayes conditional probability yielded an accuracy of 78.5%. Considering the model variables, concentration was the most significant predictor variable followed by interchangeable cell and material type in that order, and the least was the exposure time.

The KDD model illustrates the concept of using a theoretical framework and dose responses in predicting the cytotoxic effects of ENMs to biological cells of different environmental organisms. The model findings form the basis of generating data useful in validating other models, and also, offer insightful information on the risks of pollutants that can support the development of a regulatory framework. However, the physicochemical properties of Co-NPs and Co^{2+} that underpin the observed cytoxicity were not highlighted as well the mechanistic pathways of ENMs that may contribute to the observed eco-toxicological effects.

2.2.5 Quantitative structure-activity relationship (QSAR) models

Quantitative structure-activity relationship (QSAR) models exploit the significant correlation between the structural properties of a chemical and its biological reactivity. These relationships are often described by use of mathematical expressions (Karelson et al., 1996). Quantitative structure-activity relationship models have been effectively applied in drug discovery and chemical toxicology studies. However, their application in the domain of ENMs toxicity has been limited because of the difficulties in separating the inherent properties of NPs from their counterpart bulk forms (Alvarez et al., 2009). The characterization of molecular properties of ENMs is fundamental in supporting robust predictions of their potential toxicity in physiological and biological systems. In the QSAR paradigm, a suitable mathematical model is obtained through correlation of computed molecular descriptors for a set of chemical compounds and known experimental data in order to predict the unknown reactivity of other compounds (Puzyn et al., 2011).

Both computational and empirical strategies are useful in the development of QSAR models to predict risks. However, owing to paucity of knowledge on ENMs properties that cause biological and chemical reactions in physiological and ecological species inhibits the development of suitable models. A recent review (Burello and Worth, 2011b) highlighted the applications of QSAR models in predicting ENMs toxicity with recommendations on how to address the need of developing theoretical descriptors of ENMs by multidisciplinary teams. Identification of such descriptors is useful in guiding future ENMs design towards rendering them benign in case of incidentally interacting with biological life forms. A review on computational strategies for predicting potential nanotechnology risks (Barnard, 2009a) and perspectives on *ab initio* simulations (Barnard, 2009b) have advanced the use of algorithms suitable for assessing the likelihood of ENMs risks to the environment.

In computational approaches, theoretical descriptors can be developed by use of advanced computer software, for instance, to model the stability of ENMs and hence predict their potential ecotoxicological risks. For example, a theoretical Simplified Molecular Input Line Entry System (SMILES)-based method used by Toropov and Leszczynski (2006) illustrated the possibility of predicting Young's modulus (YM) of eight randomly selected ENMs in a training set of twenty one. SMILES method characterizes nomenclature of atoms and molecular architecture of ENMs (Toropov and Leszczynski, 2006). The YM measures the ENM stability to withstand stress or elastic stretch, and is an important structure property applicable in QSAR modelling. In addition, an *ab initio* computer simulation (Barnard 2009c) predicted the relative thermodynamic stability of single walled carbon nanotubes (SWCNTs) chemisorbed with oxygen, nitrogen, water, and humid air. The results revealed that the stability of small diameter SWCNTs improved in the presence of water due to favorable sorption characteristics. Additionally, relative humidity influenced the sorption process, an outcome useful in evaluating the fate of CNT after transport into the aquatic systems. Computational simulations of ENMs in biological environments have been presented elsewhere (Makarucha et al., 2011; Fourches et al., 2010).

Moreover, recently a predictive toxicological paradigm was proposed (Meng et al., 2009) to aid in assessing the safety of ENMs. The paradigm included the characterization of ENMs properties, *in vitro* testing of ENMs toxicity, establishment of ENMs bioactivity to molecular and cellular uptake and injury, establishment of empirical models to correlate properties to the effects observed, and model application to predict *in vivo* toxicity in physiological and ecological matrices. The significance of the model is its potential for supporting the development of protocols for testing ENMs that may lead to human diseases and environmental impacts.

The current *status quo* characterized by lack of reference standards for ENMs has led to an undesirable state of uncoordinated methods used in development of predictive QSAR models. Such models are limited by their inability to support replication of findings or to predict the bioactivity of other ENMs. Published studies show that researchers use different approaches, ENMs, and prediction descriptors in the process of developing the QSARs models. Two examples of QSAR models are highlighted to illustrate how such approaches inhibit our ability in making useful comparisons of the findings, or identifying the most significant descriptors for nano-QSARs.

A theoretical model by Burello and Worth (2011a) suggested that oxidative stress potential between a metal oxide ENM and a biological cell potentially can be predicted using the energy structure of the metal, and the adsorbed reactants ions. Six electrophilic metal oxide ENMs; TiO_2, CuO, ZnO, FeO, Fe_2O_3, and Fe_3O_4, were used as model compounds. Electronic reactivity descriptors (electronic chemical potential (ECP) and absolute chemical hardness (ACH)) (Kohn et al., 1996) were used to predict the cytoxicity effects of the metal oxide ENMs.

Assuming that the metal oxide ENMs behaved like bulk materials with sizes of 20-30 nm, model computations using conduction and valence band gap energies (Butler and Ginley, 1978; Portier, 2001; Portier et al., 2004; Lide 2005) and validation using experimental data ($R^2 = 0.84$) estimated the location of >1 eV valence band edge below redox potentials for initiating biological reactions. Electron transfer owing to redox reactions between the metal oxide ENMs and biological cells was predicted using the conduction and valence band edge positions. The model findings were that the TiO_2, CuO, FeO and Fe_2O_3 showed high probability to catalyse redox reactions within a biological cell when their conduction band lay within the band gap.

The model provides valuable insights into the computational modelling where electronic structure and reactivity behaviour of ENMs are exploited in predicting their potential risks after exposure to environmental biological systems. However, the model has several limitations. First, it does not provide predictive toxicological effects to physiological and ecological systems as a function of identifiable and relevant physicochemical properties of ENMs under question. Secondly, the use of ENMs of small particle sizes in the model development inhibits applicability for the case of larger sizes or for ENMs with surface defects.

Cytotoxicity of metal oxide ENMs decreases with increasing cationic charge. Evidently, more ionization energy is required to dissociate ions from molecules with higher cation charge. The findings were derived from an *in vitro* mathematical model by Puzyn and colleagues (2011) where enthalpy of formation (Δ_H) was used to predict $\log EC_{50}$ cytotoxicity of 17 metal oxide ENMs (sizes: 15-90 nm) against *Escherichia coli* bacteria. Enthalpy of formation was determined using semi-empirical quantum-mechanical Parametric Method 6 (PM6) and correlated with experimental data. The response variable were calculated using MatLab partial least square (PLS) and statistical Toolbox. Genetic algorithm and multiple regression (GA-MLR) modelling was performed ($R^2 = 0.85$) as well as cross validation ($Q^2_{cv} = 0.77$). The size of the ENMs was established as an insignificant determinant factor of the toxicity.

The model results showed that the toxicity of nZnO, nCuO, nNiO and nCoO to *E. coli* was higher in comparison to other ENMs. Table 2.6 indicates that nZnO, nCuO, nNiO, and nCoO have enthalpy of formation of <3000 kJ mol^{-1} with observed and predicted values of $\log 1/EC_{50} \geq 3.2$ mol^{-1}. Lower valence charges of nZnO, nCuO, nNiO, and nCoO require less energy to dissociate the electrons and form bioavailable ions. Toxicity labeling of nZnO and nCuO were found to be similar to the QSAR model findings of Liu and colleagues (Liu et al., 2011). Optical characteristics of $nTiO_2$ may have contributed to the low toxicity to *E. coli*, for instance, in the absence of light, $nTiO_2$ behaves like any other metal oxides (Puzyn et al., 2011). This model was developed based on dose-response data and the choice of using logarithmic data values as opposed to raw data may be attributed to a deliberate attempt to normalize distributions and to stabilize the variance within the data sets. The approaches of improving the model may include considering additional descriptors as well as structural and morphological properties of the ENMs.

Many QSAR models reported in the literature have used single descriptors to develop toxic effects through correlation of empirical data with the most predictive descriptor(s). Classical QSAR models usually correlate properties of a pollutant to the biological reaction leading to a mathematical relationship being established. The resultant model is then capable of predicting the toxic effects of similar pollutants or toxicants at different level(s). Owing to the unique properties of ENMs, classical QSAR models are unlikely to be appropriate in predicting toxic effects of materials with nanoscale dimensions particularly based on results obtained using biological cells. Engineered nanomaterials are categorized differently and their properties differ significantly at particle and bulk levels, and ultimately this influences on their fate and transportation in the environment due to adsorption, agglomeration, or aggregation effects. It is likely that the physicochemical properties of ENMs may form the basis of developing QSARs that are robust in predicting their toxicity effects in the environment. Therefore, future QSARs approaches of ENMs should consider the correlation of known descriptors obtained from scientific literature to accessible data as a means of identifying and qualifying physicochemical predictive descriptors.

2.3 CONCLUDING REMARKS

Modelling-based findings published in the scientific literature concerning the potential risks of ENMs in different environmental compartments have to a certain extent yielded valuable insights into improving our collective understanding on the fate, behavior, and toxicity of ENMs in environmental systems. However, one of the key literature review findings is that different model results are limited by their inability to link the physicochemical properties of ENMs to their ultimate fate, transport, and eventual toxicity under different environmental conditions.

From the reviewed reports, even where the same model was used posed the challenge of inability to translate results from one study to the next due to the variability of the factors influencing exposure and hazards of ENMs. Despite of these limitations, the reported studies offer sound in conceptualizing the potential risks of ENMs in the aquatic systems even at current infant phase of nanoecotoxicology research. From this review, we identified a gap in terms of using data-driven models in the domain of artificial intelligence to estimate the risks of ENMs to aquatic systems based on published exposure and toxicity data as described in the later chapters.

CHAPTER 3: DEVELOPMENT OF A REPOSITORY FOR FATE AND TOXICITY OF $nTIO_2$ IN AQUEOUS MEDIA

3.1 INTRODUCTION

Risk assessment of ENMs is dependent on the understanding of their physical and chemical properties, dynamics of their behaviour owing to environmental factors, the exposure mechanisms, and the potential hazards to aquatic ecologies. The availability of such information is paramount before meaningful analyses can be carried out to ascertain the potential risk of a given ENM. For example, such information is useful on the identification of the link between the input and endpoint variables as well as their associated relationships that in turn influence the risk(s) profile. In addition, the repository addressed certain information gaps as well as the identification of key controlling factors on the exposure and toxicity of ENMs. Repositories support learning, extraction, use and application of valuable information. The sourcing and organizing of data into an accessible source that could be updated is important.

Relevant data that can be characterised as available, accessible, in extractible formats, usable, and easily updated is invaluable in performing analyses especially for the purposes of prediction and modelling purposes. At the initiation phase of this study (in 2010), there were limited databases as platforms for information sharing on nanotoxicology – despite increasing large open source literature on the subject. However, over the past few years, the databases have been developed on linked to applications, commercialization, effects, articles among other aspects linked to nanotechnology as summarized in Appendix 3.1.

The repositories developed provide unstructured information on ENMs produced by companies and their applications. This information is gathered from suppliers who volunteer to provide the company information on ENMs; research articles; product information on company websites, and other documentations for example product catalogues. Other databases provide lists of nanoproducts manufactured using ENMs but some product manufacturers do not provide information on the specific nano-materials used or the quantities per product. The information contained in the databases provide useful insights on broad aspects related to the growth of nanotechnology from small scale- industry to global arena, and also avertedly the need to develop structures to govern nanotechnology industry to render it sustainable.

In particular, the downside of the databases is that they are limited in terms of data required to fully characterize ecological risks owing to the incompleteness of the information provided. This is because the suppliers who are the source of information do not provide full details of ENMs characteristics in the products, and hence the possible eventual hazards from exposure. This can be accounted by two-fold reasons. First, many of the repositories are business oriented hence their proprietary nature leads in providing little useful information on ENMs risks. Secondly, databases given are public but may or may not provide full access or the desired information to the end users as certain data are protected under trade legislations and agreements. Therefore, taking into account the limitations of the publicly accessible repositories, and the need for a usable repository in the context of nanoecotoxicology motivated the development of a database described in this chapter.

3.2 GOAL AND SCOPE

In this report, we describe and document the procedures applied in collating experimental data in an attempt to develop a repository. Notably, the data were keyed into the repository in a format amenable to evaluate potential risks of ENMs in the aquatic media. Among the strength of the repository is the integration and aggregation of heterogeneous data from multiple sources. It also aids in improving the consistency of the data for subsequent applications carried out in this study. In this study, the systematically formatted data were applied in predicting and modelling the fate, interaction and toxicity of $nTiO_2$, not only in aqueous media but also in actual aquatic ecosystem through simulations. The consolidation of data into a repository consisted of the following steps:

- Collection of both quantitative and qualitative data from multiple scientific sources;
- Segmentation of the heterogeneous data into related attribute-oriented repositories;
- Combination of segmented repositories into one repository;
- Identification of constraints to the data for analyses; and
- Identification and selection of relevant attributes for predictor and response variables.

The aggregate data reported in this chapter were derived from published findings from various research laboratories around the world. The results were recorded semi-continuously from replicated experimental studies under controlled conditions. Summarised results were generally reported in abstract, graphic, and image formats. The experiments were conducted either using batch or semi-batch reactors under aqueous synthetic, natural, or modified natural water conditions. The experiments were based on simplified aqueous media tests, and therefore, to a certain extent provided little information on the dynamics and complexities of ENMs found in actual aquatic systems. Constraints experienced during data collation included; lack of continuity, lack of consistency, missing relevant attributes, and inability to make postulations from the summarized information reported.

To address these limitations, multiple techniques were exploited to collate the information and address the constraints owing to the heterogeneity and forms of accessible data. This was done with the aim of applying the information gathered to analyse the behaviour and toxicity of $nTiO_2$ in the aquatic media. Knowledge and concepts from multiple fields including; biology, chemistry, physics, mathematics, aquatic ecology, computer science, social science, and engineering science were used to: (i) estimate continuous data points; (ii) construct useful predictor and target attributes relevant for this study; and (iii) transform the data for subsequent prediction and modelling analyses. Here in this report, an attempt was made to establish association among predictor variables, and the link between predictor and response variables.

3.3 METHODS AND MATERIALS

Data, information, and knowledge were collated from multiple published scientific and technical literatures such as journal articles, books, industrial brochures, institutional manuals, and company websites. The materials were either in printed or soft copy formats. Access to these sources was mainly through the internet searched engines – for example Google.

The data were collated in three steps; first, by scaling the data from reports to estimate and reproduce continuous data points from summarized formats. Secondly, transfer the data points into segmented repositories in cases where there were related attributes. The fragmented repositories stored information based on the fate of $nTiO_2$ in aqueous media and the type of target organisms exposed to the nanostructure in the media. The segmented repositories were useful during data organization and preparation processes through; construction of similar attributes across various heterogeneous data befitting analyses, and normalization of units of measurement. Thirdly, combine the fragmented repositories into one repository of usable data.

3.3.1 Type of data collated

Data on exposure and toxicity of ENMs is quantitatively and qualitatively reported in the published literature. Integrating both data types was aimed to complement the weaknesses of each type of data and to enrich an understanding associated with multidimensional attributes that influence the fate and toxicity of ENMs. Data that is difficult to measure cannot be quantified and analysed statistically but rather expressed qualitatively. For example quantitative data may not explain membrane disruption in algae or oedema in fish from exposure to $nTiO_2$, which could be explained by describing the effects observed.

3.3.1.1 Quantitative data

Quantitative data were chiefly collated from published peer-reviewed papers reported in both the texts and tables. Also, in other instances, it was done indirectly though making measurements from graphs and images by use of scaling methods. In the indirect measurement approach, a standard 30 cm plastic ruler (PENFLEX) was used to measure the scale of values represented in the 'x' and 'y' axes of graphs, and in scale bars in images. Examples of images used were those derived using transmission electron microscopy (TEM). Using the established scale, data points from graphs and images were then estimated. Appendix 3.2 illustrates scaling method used to estimate data points for pH (x-axis) variable with respect to the relative effect it exerted on the zeta potential of $nTiO_2$ (y-axis) in an aqueous media. The merit of the adopted approach was to optimize the quantity of data collated for use as input for the modelling purposes.

In certain instances, scaling of data from graphs and images were encountered as challenging. Under such scenarios, the few datum points derived from graphs were used to estimate continuous data simply by exploiting statistical models, namely: exponential, logistic, and polynomial models. An example of using the available few data and then using them to estimate a continuum of data is presented in Fig. 3.1. The data derived depict the sedimentation of $nTiO_2$ in mesocosmic water at an initial concentration of 10 mg/ℓ (Keller et al., 2010). A statistical model (blue) was developed using eight estimated datum points from the graph. The modelled data were then normalized to intercept values to moderate the concentration ratios from the polynomial models. For the exponentially modelled values, the decay rate was used to normalise value.

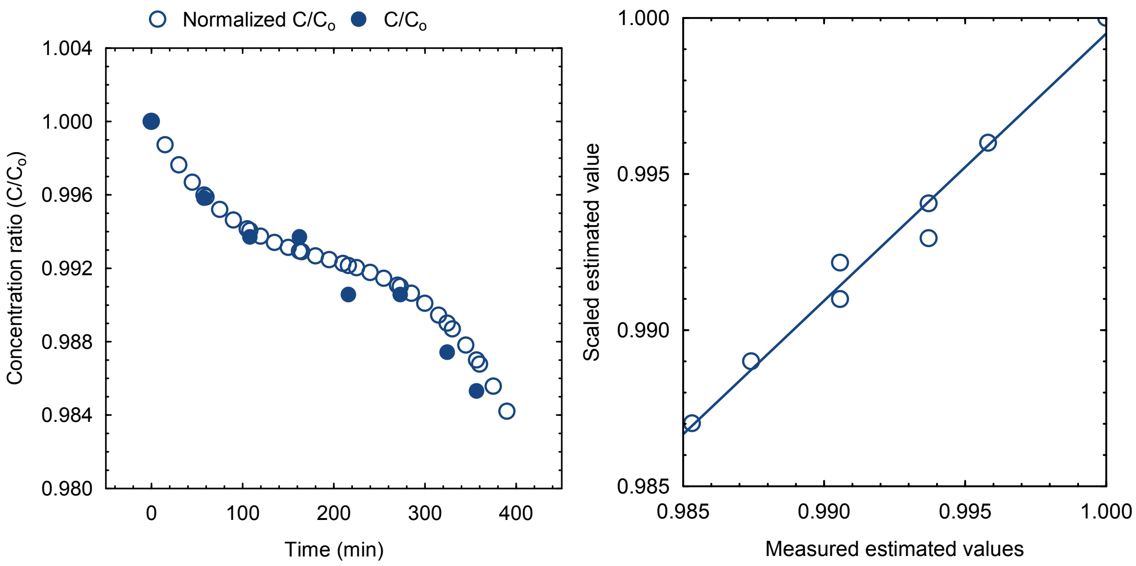

Figure 3.1: Polynomial model showing the concentration ratio of $nTiO_2$ in mesocosm water.

The model equation used was $C/C_0 = 0.9999 - 7 \times 10^{-10}(t^2) - 9 \times 10^{-5}(t)$; where t is the time in minutes. The model could fit the data well, based on the coefficient of determination of $R^2 = 0.98$ as criterion. Using the model equation, 34 data were estimated (red) over a period ranging from 0-390 minutes. The insert in Fig. 3.1 presents the linear relationship of values estimated using a scaling method and those modelled with a fit characterized by an $R^2 = 0.98$. Mathematical models or correlation equations were used to estimate missing attributes based on observed intermediate attributes. Numerical and categorical values were recorded.

Some relevant attributes were calculated using mathematical equations and modelling software. For example, for media with known concentrations of electrolytes, the concentrations of the ions in the aqueous solution were calculated and ionic strength determined using mathematical Equations developed in the PhreeQC modelling tool. Hence this form of calculation enabled the development of important predictor attributes for subsequent analyses.

3.3.1.2 Qualitative data

Qualitative data were collated from logical descriptions in published peer reviewed papers reported in the texts, figures, and tables – and indirectly depended on conceptualization, contextualization, and interpretation frameworks to account for the descriptive and interpreted empirical data. Previously, similar frameworks have been applied in social and information sciences primarily to complement quantitative research methods (Kaplan and Duchon, 1988; Babbie and Mouten, 1998). In inductive learning, conceptualization of theories is derived from prior subjective information to explain logic derived from quantitative facts (Falkenhainer and Michalski, 1986). On the other hand, contextualization is a fundamental framework in qualitative research, and is used to discover quantitative rules where information from sample occurrences may be generalized to extrapolate variables and define similar occurrence at a larger scale (Han et al., 1993). Finally, interpretation framework allows the application of all relevant information to explain a certain phenomenon. Thus, both descriptive and categorical text data were collated and recorded.

In this study, these three frameworks were buttressed by empirical paradigms to: (i) guide in distinguishing dependent and independent variables, (ii) inform on the direct and indirect relationships among the variables, and (iii) enable the construction of attribute lists that were relevant for analyses.

3.3.2 Data collection

Until now, no criterion has been developed for collating toxicology data necessary in risk assessment of ENMs. Therefore, we used the USEPA guideline developed to guide in evaluating ecological toxicity (ECOTOX) data from open literature (US EPA, 2011) specifically for pesticides. The guide provides procedures for screening, reviewing, and using both quantitative and qualitative data from literature on toxicology, and how to apply the information on risk assessment. Here, the procedures were adapted in the context of data collation from relevant scientific literature for use in analysis and risk assessment in the emerging domain of nanoecotoxicology. Using this approach as a guide, six broad categories of data were gathered, *viz*, (i) background information about experimental studies and ENMs, (ii) static physical and chemical properties of $nTiO_2$, (iii) abiotic factors in aqueous media, (iv) biotic factors in aqueous media, (v) dynamic physical and chemical properties of $nTiO_2$ in aqueous media, and (vi) toxicity of $nTiO_2$ to aquatic organisms.

All the data collated were keyed into a Microsoft Excel worksheet. Each category of data (quantitative or qualitative) had its attributes listed in columns, and attribute values were recorded in rows – where each row contained data from a specific peer-reviewed paper or report for a certain ENM. The data were broadly recorded in formats such as numerical, categorical, and text. Various attributes were listed during data collection process for three-fold reasons: first, it was based on our hypothesis that certain attributes may be useful in estimating non-reported variables through exploitation of mathematical and statistical models. Secondly, detailed subjective information was essential to construct a comprehensive repository. Lastly, all data are useful until redundancy is established during preparation and analyses stages of the study. Notably, redundancy was viewed as dependent on the intended application of the available data rather than its inherent value(s).

Historical information such as the details of authors and experimental designs were collated.
The reference attribute had details of authors, journal, year, volume, issue, page numbers, and summarized details are presented in Table 3.1.

For clarity, in this study, pristine nanoproducts refers to raw $nTiO_2$ materials, whereas formulated materials are those generally dispersed in carrier water or mineral oil as ingredients in commercial products e.g. creams and paints. Trade names such as P25 (Evonik Degussa Corporation Inc.), T-Lite SF and T-Lite S (BASF SE, Ludwigshafen, Germany), and Hombikat UV 100 (Sachtleben) were used to differentiate nanostructures. For synthesized materials or those whose trade names were not reported, attribute values were derived symbolically using their constituent crystal structure. For example, nanostructures containing rutile or anatase crystals, ‘R’ or ‘A’ symbolic names were used, respectively. If the nanostructures had mixtures of rutile and anatase crystals, the symbol ‘R-A’ was used.

Table 3.1: Information collated on the source, experimental tests, and nanostructures.

Attribute	**Examples of categories**
Type of study	*In vitro*, *In vivo*, *Ex-vivo*, ageing, stability, etc.
Reference standards	OECD 211, OECD 202, NA, ISO 7027, none, etc.
Status of experiment	Static, semi-static, continuous
Renewal rate in experiment	Recirculation, replacement, feeding of organisms, none, etc.
Treatment of media	Filtration, addition of surfactants, etc.
Nano-product	Pristine and formulated
Nanostructure	P25, T-Lite SF, T-Lite S, R, A, R-A, B, altered (Al.), etc.
Source of nanostructure	Evonik Degussa, BASF, Synthesized, etc.
Treatment of nanostructure	Filtration, dispersion, alteration, etc.

3.3.2.1 Background information about experimental studies and materials

One chief characteristic of ENMs is that no two samples from the same batch exhibit identical physical and chemical properties (e.g. size, morphology, zeta potential, etc.) when tested. The consequence is that a comparison from within and without research laboratories is unfeasible. The word ‘static’ has been rationally used to represent the unique properties of nTiO_2 nanostructures as supplied, tested and used in experimental studies. When nanostructures are released into aqueous media under varying environmental conditions (e.g. pH, NOM, ionic strength, etc.) the static properties may transform without necessarily changing their intrinsic crystal characteristics.

3.3.2.1.1 Size

Data on primary and agglomerated sizes, shape, and specific surface area of ENMs for the case of nTiO_2 were collated. The values for diverse attributes were those provided by the manufacturers of nTiO_2 or characterized during the experiments. The primary sizes of commercial nTiO_2 as supplied by the manufacturers ≤ 50 nm, and 10 nm (width) and 50 nm (length) for spherical and rod-shaped crystals, respectively. Titanium nanostructures exist as sub-micron granules that form clumps measuring ≥ 1000 nm in size. The clumps constitute ultrafine-grained nano-particles measuring ≤ 100, or fine ones measuring ≤ 400 nm due to agglomeration. Studies have revealed that grain boundaries and free surfaces of spherical ENMs cause inter-particle bonds that lead to agglomeration (Rabkin et al., 2011). This is why energy dispersive methods are used to break-up the clumps to achieve homogenous suspension of nano-crystals in aqueous media before or during bioassay tests.

Generally, size has a negative correlation with the surface area of ENMs, a relevant attribute that explains the fate and interaction kinetics of nanostructures in aqueous media. Large surface areas of ENMs increases the surface energy-which in turn causes metastability (Cao, 2004). The shape defines the surface-to-volume ratios of nanostructure crystals, and accounts for surface defects in the measurements of surface area. The shape reveals the packing density of surface atoms and electrons in crystal lattice in localized surface Plasmon resonance (Grassian, 2008; Hornyak et al., 2009; El-Badawy et al., 2010;), that play a role in the reactivity of the nanostructure in aqueous media. Moreover, the packing density signifies reactive sites of nanostructures-and accounts for the kinetic and mechanistic properties of ENMs when they interact with organisms. For example, toxicity by injury maybe caused by sharp edges of the nanoparticle puncturing cell wall membranes of organisms (Musee et al., 2011, and the references therein).

3.3.2.1.2 Structure and surface chemistry

3.3.2.1.2.1 Crystal form

Crystal structures of minerals constitute elemental compositions with mono-, poly- or amorphous crystallography that exhibit varying properties due to different arrangement of atoms in the lattice (Dakota Matrix Minerals, 2012). Naturally, titanium dioxide exhibits five forms of crystal systems and structural configurations as summarized in Table 3.2.

Table 3.2: Crystal system and structural configuration of nTiO2.

Form	Crystal system	Structure configuration	References
Anatase	Tetragonal	Dipyramidal	Macwan et al., 2011; Ralph, 2012; Reyes-Coronado et al., 2008; U.S. EPA, 2010)
Rutile	Tetragonal	Prismatic	Macwan et al., 2011; Ralph, 2012; Reyes-Coronado et al., 2008; U.S. EPA, 2010)
Brookite	Orthorhombic	Dipyramidal	Macwan et al., 2011; Ralph, 2012; Reyes-Coronado et al., 2008; U.S. EPA, 2010)
Amorphous	Mixed	Transitional	Macwan et al., 2011; Ralph, 2012; Reyes-Coronado et al., 2008; U.S. EPA, 2010)
Ilmenite	Trigonal	Rhombohedral	Ralph, 2012; U.S. EPA, 2010)

To date, brookite has no established commercial market compared to rutile and anatase forms. Notably, ilmenite is the natural form found in ores in countries such as: Australia, South Africa, Canada, and United States of America among other world leading producers. Rutile also exists as an ore, and is the most abundant and stable crystal phase (U.S. EPA, 2010). In this study, our focus was on anatase and rutile forms, and the amorphous form as they are widely reported. The amorphous form is a transitional structure and crystallizes to either anatase or rutile forms depending on the synthesis conditions. The composition of the three crystal phases vary by the type of $nTiO_2$ materials used in experiments, and generally is represented as a percentage (0-100%) of the nanostructure.

For example, Degussa's P25 is the most widely used nanostructure in experimental tests. The nanostructure consists of three forms, namely anatase (≥ 70%), rutile (≤ 20%), and amorphous (≤ 10%) crystal phases (Ohtani et al., 2010). The constituent percentages vary within batches; and hence the crystalline composition is not well established. Anatase versus rutile percentages of 80:20 (Griffit et al., 2008; Battin et al., 2009; Zhu et al., 2010; Suttinponparnit et al., 2011; Chowdhury et al., 2011; Chowdhury and Walker, 2011), 82:18 (Keller et al., 2010; Thio et al., 2011; Kulacki and Cardinale, 2012; Cardinale et al., 2012), 79:21 (Warheit et al., 2007; Planchon et al., 2013), and 86:14 (Simon-Deckers et al., 2009) have been reported. The anatase versus rutile versus amorphous phases in P25 -76.6:18.4:9 was scarcely reported (Hartmann et al., 2012). Where information on the crystal phases was not reported, the data were obtained from product catalogues of the manufacturing companies.

The size and shapes of the crystals in pure and mixed forms depends on the synthesis methods. P25 is synthesized by flame hydrolysis method, and according to the manufacturer, Evonik Degussa Inc. it is characterized as spherically-shaped with a primary size of 21 nm. Although many studies have corroborated the shape, the size of P25 is rarely replicated when re-tested in laboratories. For example, from our database, the reported average primary size of $nTiO_2$ ranged from 22 nm (Planchon et al., 2013) to 30 nm (Griffit et al., 2008; Li and Sun, 2011). Additionally, nominal sizes of 55 nm and 48 nm, and spherical and cuboid shapes of anatase and rutile crystals, respectively, have been reported in P25 mixtures (Planchon et al., 2013). Comparatively, mixtures of anatase and rutile synthesized using chlorine process acid hydrolysis reveal sizes of 9 nm and 37 nm of spherical and rod-shaped anatase and rutile crystals, respectively (Planchon et al., 2013).

3.3.2.1.2.2 Purity

The synthesis process of $nTiO_2$ materials generates impurities in the crystal structure. Impurities are defects which alter the surface chemistry of ENMs, and influence their fate and interaction in aqueous media (Badawy et al., 2011; Liu et al., 2011). In this study, purity was represented as a percentage (≤ 100.0%) of pure $nTiO_2$ in its crystal structure. For example, P25 has a purity of 99.5%, whereby the include; alumina (0.3%), silica (0.2%), and ferric oxide (0.01%) (Product information catalogue, Evonik Degussa Inc.). For charged solids in aqueous media, the complex structures and chemical composition, which includes impurities, contribute to heterogeneous surface charges (Jaroniec and Madey, 1988). Besides data on purity, the components and percentage of impurities in $nTiO_2$ nanostructures, and their effects, were found scarcely reported.

3.3.2.1.2.3 Zeta potential

Solid ENMs have charged surfaces. Unstable bonds and a high number of atoms on the surfaces of nanostructures contribute to high surface energy and reactivity of ENMs (Cao, 2004). When dispersed in aqueous media, ENMs may adhere together due to weak Van der Waals forces or disperse due to steric or electrostatic repulsion forces. Adherence forces increase flocculation and sedimentation phenomena, while repulsive forces influence the suspension of particles or associated colloids. These two forces collectively contribute to the eventual stability of the nanostructures and are strongly influenced by the aqueous media characteristics such as; ionic strength, natural organic matter, and pH described in Section 3.3.2.3.

The distribution of surface energy on charged nanostructures in aqueous solutions is described by surface potential and surface charge density. Under electrostatic condition, the surface charge density is related to the electric potential by the differential Poisson equation (Eqn. 3.1). The electric potential is related to the conductance of the electrolyte due to the presence of ions.

$$\nabla^2\psi = -\frac{\sigma}{\varepsilon} \qquad 3.1$$

where, ψ is the electric potential (V); σ is the surface charge density (C/m^2); and ε is the permittivity of the medium (C/V.m), and varies with temperature. From electrostatic fundamentals, the Poisson-Boltzmann expression (Eqn. 3.2) approximates the electric potential to the surface potential.

$$\psi = \psi_0 exp(-\kappa a) \qquad 3.2$$

where, ψ_0 is the surface potential (V); κ is the Debye-Hückel parameter, which is the thickness (Debye length) of the electrical double layer of a charged particle with reciprocal units (m^{-1}). This parameter is important in colloidal particle interaction; and a is the diameter of the particle (m).

The zeta potential is a relative surface potential index that measures the overall charge of nanostructures in aqueous media, with varying negative or positive magnitudes that are influenced by the characteristics of aqueous media. The velocity of charged particles relative to the solution is defined by the electrophoretic mobility (EPM). Laser Doppler Velocimetry technique is used to determine EPM, and the zeta potential using the Henry's Equation (Eqn. 3.3).

$$U_e = \frac{2\varepsilon \mathrm{Z} f(\kappa a)}{3\eta} \qquad 3.3$$

where, U_e is the electrophoretic mobility (10^{-8}.m^2/s.V), Z is the zeta potential (mV), $f(\kappa a)$ the Henry's function which is a correction factor. The value of $f(\kappa a)$ is 1.5 in aqueous media (Smoluchowski model) and a minimum of 1.0 in non-aqueous media (Huckel model); and η is dynamic viscosity (kg/m.s). The Smoluchowski value is applicable where >200 nm colloids are dispersed in >1 mM electrolytes, below which the Huckel value is used i.e. <200 nm particle in dilute electrolytes. The $f(ka)$ function relies on particle shape. For irregular colloidal fractals formed from $nTiO_2$ aggregates in aqueous media, relative diameter of the fractals is assumed.

In this study, where the data for the zeta potential was lacking, Eqn. 3.3 was used to make estimation. In reports where the temperature was not reported, categorical values between 20-28°C were used to estimate the permittivity. In some reports, none of the parameters, such as electrophoretic mobility and the temperature, were reported, and this rendered zeta potential estimation improbable. Notably Eqn. 3.3 is ideal for classical homogenous surface charge distribution computation. Generally, surface charges on particles exhibit high degree of heterogeneity. In aqueous media, ligands adsorb onto surfaces of charged particles and create non-uniform and heterogeneous charges (Elimelech et al., 1995). This aspect, for example, will be elucidated in the biotic properties Section 3.3.2.4.

3.3.2.1.2.4 Surface coating

Broadly we categorized $nTiO_2$ composites reported in experimental studies as bare (non-coating) or coated. Bare nanostructures refer to synthesized $nTiO_2$ which has no form of surface modification. Conversely, coated nanostructures were functionalized to suit a specific application. For example, $nTiO_2$ were coated with a biopolymer to suit their application in cosmetics and personal care products (Siddiquey et al., 2007; Jacobs et al., 2010; Lewicka et al., 2011). Surface coating is a relevant descriptor variable for predicting the fate and behaviour of ENMs in aqueous media as it influences their mobility (Lee et al., 2005; Jiang et al., 2009). The strength of the surface coating on $nTiO_2$ can also contribute to their potential persistence in the environment.

Surface coating of ENMs influences the zeta potential by enhancing electrostatic and or steric stabilization effects (Cao, 2004; Hornyak et al., 2009), and has been shown to shift the point of zero charge in aqueous suspensions (Huynh and Chen, 2011), and influences ENMs surface properties and behaviour (Chen and Mao, 2007). Whereas P25 nanostructures are bare, the surface of bare $nTiO_2$ core material in T-Lite S and T-Lite SF nanostructure from BASF are coated with inorganic materials to protect them from photo-catalytic effects and with organic materials to enhance their dispersion in formulated nanoproducts. Examples of inorganic materials used as coating include alumina (Al_2O_3) and aluminium hydroxide [$Al(OH)_3$]; and organic materials such as hydrated silica ($SiO_2.nH_2O$), polydymethylsiloxane $(C_2H_2OSi)_n$ in di-methicone/methicone silicone co-polymer, and dimethoxydiphenylsilane ($C_{14}H_{16}O_2Si$) and triethoxycaprylylsilane ($C_{14}H_{32}O_3Si$) silicone cross-polymers. The coatings undergo transformation when exposed to aqueous media, and this aspect will be addressed in detail in Section 3.3.2.5.4. Gibbsite is a mineral form of aluminium hydroxide, and the two names have been interchangeably used to refer to the aluminium-based coating.

3.3.2.2 Data on abiotic factors

Abiotic factors consists of physical and chemical attributes that defines a given ecosystem characteristics. In laboratory settings, abiotic factors are generically described as environmental conditions related to the aqueous media, photoperiod simulations, and agitation mechanisms which in turn exert influences on the fate, interaction, and subsequent toxicity of $nTiO_2$ to target aquatic organisms.

3.3.2.2.1 Physical and chemical properties of aqueous media

3.3.2.2.1.1 pH

The pH influences the zeta potential, and therefore the stability of $nTiO_2$ in an aqueous media. The ability of particle-particle, particle-colloid or colloid-colloid interactions to occur in aqueous media depends on the pH, which affects dynamic behaviour of $nTiO_2$. Titanium dioxide, like many other oxides, undergoes hydroxylation in aqueous media (Elimelech et al., 1995) or de-protonation in the presence of organic acids (Chowdhury et al., 2013). Solubility of TiO_2 is generally low, and therefore, considered inert. Thus the ionization process of oxides progresses as follows:

$$A_s - OH_2^+ \leftrightarrow A_s - OH \leftrightarrow A_s - O^- \qquad 3.4$$

where A_s is the solid surface of the oxide. The hydroxylation and de-protonation processes underpin the ability of the oxides to acquire either negative or positive surface charges as a function of the prevailing pH range. More protons are lost as the pH increases (more basic) resulting in an increasing negative charge. The reverse happens when the pH reduces (more acidic). The isoelectric point (IEP) of $nTiO_2$, which is the pH where the net surface charge of a solid molecule is neutral, is influenced by the size (Suttiponparnit et al., 2010). The point of zero charge (PZC) describes the pH at which the surface charge density is zero. The PZC is a measure of the colloidal surface charges when ligands adsorb on the surface of $nTiO_2$, or vice versa. In aqueous media, the surface charges, and therefore the PZC, of $nTiO_2$ vary depending on the crystal phase of the nanostructure, and the electrolytes and ligands present in the aqueous media. These factors affect the process in Eqn. 3.4. The pH, PZC and IEP attributes were listed were keyed into the inventory.

3.3.2.2.1.2 Turbidity

Water consists of dissolved solutes, trace micro and macro elements as well as suspended and deposited particles and colloids. When $nTiO_2$ are introduced into a solution; elements (micro and macro), particles and colloids may act as adsorption sites for the ENMs. Turbidity measurements of supernatant water are generally an indicator of the concentration of suspended particles or flocculated colloids, and therefore a measure of water quality (Lewis, 1996; Anderson, 2005; Bayram et al. 2012). Standard turbidity values for drinking water are < 1 to ≤ 5 (SANS, 2011; WHO, 2011).

In this study, turbidity was considered an intermediate attribute that could be used to estimate the concentration of $nTiO_2$ suspended, adsorbed, or deposited under different solution conditions, especially where NTU values were reported. Supernatant concentration of $nTiO_2$ nanostructures and associated crystals is a function of turbidity (Battin et al., 2009), and is affected by the electrolytes present in aqueous media (Botta et al., 2011). High NTU values account for the suspension of $nTiO_2$ particulates or colloids with wide size distribution (Von der Kammer et al., 2010), that could potentially scatter or absorb more radiant heat which in turn may reduce or increase the water temperature. Fluctuating temperatures affect oxygen solubility, and indirectly photosynthetic and aerobic aquatic organisms.

In addition, bare $nTiO_2$ in suspension may absorb energy and undergo photo-catalytic reaction that would affect organisms in the water column. Low NTU values imply that $nTiO_2$ is held in colloidal flocculants or coagulants between the supernatant and the sediment regions, or is deposited onto the sediment beds. Secondary effects on the settlement of $nTiO_2$ include sediment accumulation in shallow waters, suffocation and death of benthic organisms, accumulation and transport of toxic chemicals such as cadmium that adsorb onto $nTiO_2$ surfaces, etc.

3.3.2.2.1.3 Electrolytes, conductivity, and ionic strength

Standard media preparations have been developed to simulate natural water for use in testing the toxicity of chemical pollutants to aquatic organisms under laboratory conditions. These media include but not limited to; synthetic freshwater developed by United States Environmental Protection Agency (US EPA, 1993), standard synthetic reconstituted water (US EPA, 1990a), Elendt 4 and 7 (Elendt and Bias, 1990), algae test media (OECD 201, 1984; OECD 211, 1998), etc. Deionized water – assumed to be the ultrapure academic grade in this study-was found to be the solvent widely reported for dissolving various ionic and nutritive solutes when preparing the test media.

The concentration of cations and anions in solution is dependent on the quantity of dissolved electrolytes based on prevailing conditions including temperature and presence of other solutes, and or ions. Dissolved ions conduct electricity, the strength of which is measured as the conductivity of the aqueous solution and subsequently the ionic strength. The conductivity of ions in water is influenced by the concentration of the following cations and anions; Li^+, Na^+, NH_4^+, K^+, Mg^{2+}, Mn^{2+}, Ca^{2+}, Cu^{2+}, Fe^{2+}, Zn^{2+}, Al^{3+}, Fe^{3+}, Cl^-, SO_4^{2-}, HCO_3^-, CO_3^{2-}, F^-, NO_3^-, HSO_4^-, OH^-, $NaSO_4^-$, and $NaSO_3^-$ (Mccleskey et al., 2012). Besides specifying the media used, and some electrolytes added to obtain a specific ionic strength, most reports did not provide information on the existing ions in the media or the conductivity, and sometimes the ionic strength. Cations and anions in solution, and ionic strength are relevant attributes that influences the fate of $nTiO_2$ in solution as they control the surface charges and thereafter stability (Chowdhury et al. 2013). Some reports listed phosphate and nitrate ions in prepared synthetic media, but these are considered as nutrient ions for test organisms.

In this study, major cations and anions common in water were considered as relevant attributes comprising; Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, SO_4^{2-}, and HCO_3^- (measured as CO_3^{2-} at high pH values). The concentration of ions in ultrapure water is rarely reported. Ultrapure water has its ions stripped resulting in low conductivity (0.0555-0.1µS/cm) (Product catalogue, Millipore). The low conductivity shows the presence of very low concentration of ions. In this regard, very low concentrations of the major ions in ultrapure where no salts are added were assumed to be 10^{-9} mg/ℓ. The emphasis of common ions simplifies a relatively complex system where cationic, anionic or neutral biotic and abiotic ligands also exist in media and affects the stability and toxicity of $nTiO_2$. These include poly- and higher atomic inorganic and organic ions.

Various approaches were used to estimate the ionic strength. Mathematical equations by Lind (1970), Marion and Babcock (1976), and Russell (1976) as expressed by Eqn. 3.5-3.7, correspondingly, have been applied in calculating ionic strength using measured conductance. A review of these methods (Russell, 2011) identified Lind's Eqn. (Eqn. 3.5), as the most applicable for computing the ionic strength in surface and groundwater applications.

$$I_c = 1.4769 \times 10^{-5} K_s + 0.00015 \tag{3.5}$$

$$\log(I_c \times 1000) = 1.159 + log\left(\frac{K_s}{1000}\right) \tag{3.6}$$

$$I_c = 1.5 \times 10^{-5} K_s \tag{3.7}$$

Where, I_c is the ionic strength (mol/ℓ); and K_s is the specific conductance corrected to 25°C (µS/cm). Eqn. 3.5 was used to estimate the ionic strength of media prepared for testing the fate and toxicity of $nTiO_2$. Examples of media preparations used in experimental studies with varying ions concentrations, stated conductivities, and with or without information on ionic strength are presented in Table 3.3.

Table 3.3: Conductivity and ionic strength of types of media used to test the stability and toxicity of $nTiO_2$.

Type of media	Conductivity (µS/cm)	Ionic strength (eq/ℓ) (reported)	Ionic strength (M) (calculated)	Reference
Deionized water	0.0555-0.10	-	0.000151	Millipore website
Artificial Elendt 4	577-605	-	0.0087-0.0091	Wiench et al., 2009.
Pond water	752-879	-	0.0113-0.00131	Wiench et al., 2009.
Spring water	1108	-	0.0165	Wiench et al., 2009.
Mesocosm freshwater	372	0.0072	0.0056	Keller et al., 2010.
Stormwater	591	0.0096	0.0089	Keller et al., 2010
Seawater	37400	0.707	0.553	Keller et al., 2010

Most scientific reports do not present the concentration of ions or conductivity. In such instances, the chemical characteristics of the media used were established from refereed manuals that had standards. The concentrations of solutes in the manuals, where ultrapure water was the solvent, were used to estimate the cations and anions concentrations using the mole and mass ratios. An example of these computations are presented in Tables 3.4 and 3.5 where synthetic hard water (SHW) was used to test the *in vivo* acute toxicity of $nTiO_2$ to *Daphnia magna* (Kim et al., 2010). The SHW is a standard testing media developed by US EPA

(1993) for use in testing the toxicity of chemicals to Daphnids – and has been used widely in nano-toxicology tests as attested by data collated in this study.

Table 3.4: Computation of ions in synthetic hard water used in testing the acute toxicity of $nTiO_2$ in *Daphnia magna*.

Electrolyte (given)	Molarity (M) (reported)	Mass of solute (mg/ℓ)	Ideal ion dissociation
$NaHCO_3$	2.28E-3	192	Na^+ , HCO^-_3
$CaSO_4.2H_2O$	6.80E-4	120	Ca^{2+} , SO_4^{2-}
MgSO4	9.98E4	120	Mg^{2+} , SO_4^{2-}
KCl	1.07E-4	8	K^+, Cl^-

Table 3.5: Calculated concentration of ions in synthetic hard water.

Ions in SHW	Calculated concentration (mg/ℓ)
Na^+	52.54
K^+	4.2
Mg^{2+}	24.24
Ca^{2+}	27.94
Cl^-	3.8
SO_4^{2-}	162.72
HCO^-_3	139.46

At 25°C, the dissolved oxygen was estimated to be 8.64 mg/ℓ. Using the ion concentrations in Table 3.6, pH (7.8), dissolved oxygen (8.64 mg/ℓ), the specific conductance (554 µS/cm) and ionic strength (8.165×10^{-3} M) were estimated using PHREEQC (United States Geological Survey). There are more complex standard media including Elendt 4 and Elendt 7 that have 16-26 different dissolved solutes hence the use of the major cations and anions is an over simplification. For ideal solutions, the ionic strength is a function of the concentration of ions in solution by the relationship (Eqn. 3.8):

$$I_c = 0.5 \sum_{i=1}^{k} c_i z_i^2 \qquad 3.8$$

where c_i is the concentration of ions (mol/ℓ); and $z_i$is the charge of the ions (units). The ionic strength of solutions is not only related to the concentration of cations, but also to their activity especially for di-valence, trivalence and higher valence types of ions. Monovalence concentrations are usually equivalence to the ionic strength of aqueous media, which is not the case with divalence ions whose activity depends on the presence of other solutes in solution. The Debye-Hückel parameter defined in Eqn. 3.3 links the ionic strength from multiple dissolved salts to zeta potential by the expression:

$$\kappa = \sqrt{\left(\frac{e^2}{\epsilon kT} \times \sum_{i=1}^{k} c_i z_i^2\right)} \qquad 3.9$$

where, k is the Boltzmann constant, T is the temperature, e^2 is the electron charge, z_i is the symmetrical valence of specific ion, and c_i is the molar concentration of specific ion in aqueous solution. Besides their contribution to the ionic strength of solutions, higher valence ions influence the surface charge of $nTiO_2$ in media which is described in Section 3.3.2.2.1.3. The ionic strength influences the stability of $nTiO_2$ in aqueous solutions as it controls the zeta potential.

3.3.2.2.1.4 Organic matter

Information on the quantity of organic matter (OM) and effects on the fate of $nTiO_2$ in aqueous media was collated. Organic matter comprises dissolved (≤ 0.001 µm), colloidal (≤ 450 µm), and particulate (≤ 100 µm) forms (Aiken et al., 2011) from natural and anthropogenic sources. Natural organic matter (NOM) constitutes different sizes of complex structures of hydrophilic, hydrophobic, and amphiphilic compounds with cationic, anionic and neutral charges. Characterization and quantification of NOM is challenging. Total organic carbon (TOC) is used as a representative measurement of NOM in water. In surface water and sediments, dissolved organic carbon (DOC) constitutes humic material forms (50%), low molar carboxylic and phenolic acids (≤ 25%), neutral compounds (≤ 15%), and other species (≤ 10%) (Eby, 2004). The DOC is the proportion of TOC that is neither colloidal nor particulate. Humic materials, comprising fulvic acid, humic acid and humin, are proton acceptors with a net negative charge that define the equilibrium charges and alkalinity in solution (VanLoon and Duffy, 2005).

The OM in aqueous media is characterized by the concentration of TOC. Carbon dioxide dissolved in water, and carbonic acid and carbonates formed, contribute to the measured TOC values. Characterizations of humic materials reveal that 40.7-58.7% is carbonaceous material (Eby, 2004). For sub-surface adsorption coefficients, organic matter is estimated by multiplying the carbon content measured by 1.7, a unit that is a measure of the highest percentage occurrence carbon water i.e. 58.7% (VanLoon and Duffy, 2005). Suwannee River fulvic and humic acids used in experimental studies comprise 52-55% of TOC (Product catalogue, International Humic Substances Society, Minnesota, USA). For the purposes of this study only, the organic matter was approximated to an average of 1.8 of the organic carbon expressed as (Eqn. 3.10):

$$Organic\ matter = \left[100\left(\frac{1}{60}+\frac{1}{52}+\frac{1}{55}\right) \div 3\right] * TOC = 1.8 * TOC \qquad 3.10$$

Thus, using Eqn. 3.8, the concentration of organic matter in aqueous media used in experimental studies, whose TOC values were reported, was estimated using the 1.8 conversion factor. For example, the TOC values of academic grade deionized water are 0.005-0.01 mg/ℓ (Product catalogue, Millipore) while optima water is 20 ppb[‡]. Background organic matter in deionized and optima water was calculated to be 0.009-0.018 mg/ℓ (ca. 0.0135 mg/ℓ) and 0.036 mg/ℓ respectively. The presence and complex nature of organic matter in aqueous solutions, together with other abiotic characteristics, affect the stability, biochemical reactions, and bio-availability of $nTiO_2$.

Organic matter adsorb onto $nTiO_2$ influences the stability by changing the surface charge, and therefore, the electrophoretic mobility (Chowdhury et al, 2012), that lowers aggregation (Domingos et al., 2009; Li and Sun, 2011). Interplay of combined factors, namely the pH and ions in solution, have an effect on the stability of $nTiO_2$ in the presence of humic materials. Mono-, di-, or tri-valence ions have different influence on the stability of $nTiO_2$ at various pH ranges. For example, at low pH (5), Ca^{2+} reduces the negative surface charge, while K+ increases the charge by a factor of 3, which influences the stability of $nTiO_2$ in the presence of organic matter (Chowdhury et al., 2012). At low pH (4), Fe^{3+} stabilizes $nTiO_2$ in the presence of fulvic acid, but increases aggregation at higher pH (6-8) due to bridging effects of hydroxyl compounds formed (Li and Sun, 2011). Therefore, organic matter is a useful parameter in describing the fate of $nTiO_2$ in the aquatic systems.

3.3.2.2.2 Irradiance, heat, and exposure period

3.3.2.2.2.1 Illumination and photon energy

The amount of energy produced by light, from sunlight or artificial lighting, influences the physical, physiological, and biochemical activities in the aquatic environment. Atoms and molecules absorb photon

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[‡] (http://www.fishersci.com/econm/servlet/fsproductdetail?LBCID=75327947andstorId=10652andaid=216435)

energy in specific ranges of wavelengths in the solar spectrum. The energy absorbed depends on the band gap of the quantum states (Chen and Mao, 2007), which induces electron excitation and transition in both the visible and ultraviolet regions. Titanium dioxide has a wide band gap that absorbs energy in the ultraviolet spectrum region (Faure et al., 2013). Anatase phase has a higher energy band-gap (3.2-3.3 eV) compared to the rutile phase (3.0-3.06 eV) (Li Puma et al., 2008; Reyes-Coronado et al., 2008), consequently considered to be more reactive.

Photon energy from artificial lighting in laboratory tests was identified as a relevant attribute. This attribute was rarely reported, and had to be estimated using details given about spectrum characteristics of lighting equipment used. In the database, the information of equipment used to simulate natural sunlight during experiments was collected. For example, SUNTEST CPS+ Xenon Lamp with 300-800 nm simulated sunlight (Hund-Rinke and Simon, 2006), 34-W cool white and 40-W Sylvania Gro-lux fluorescent bulbs (Cherchi et al., 2011), and 32W GE cool white fluorescent with 320-400 nm simulated sunlight (Cardinale et al., 2012). Photon energy is inversely proportional to the wavelength and can be calculated using the Planck's Equation (Eqn. 3.11):

$$E = hf = \left(\frac{hc}{\lambda}\right) \qquad 3.11$$

Where, E is photon energy (J/photon); h is the Planck's constant (6.626×10^{-34} J.s); f is the frequency (1/s); c is the speed of light (2.998×10^{8} m/s); and λ is the wavelength (m). Substituting the constants in Eqn. 3.11, and at a given spectrum wavelength, the energy per photon is;

$$E = \frac{1.986\times10^{16}}{\lambda(nm)} J.nm \qquad 3.12$$

In biological and chemical processes, the measurement of photon flux, and hence photon energy, is significant compared to irradiance. For particles, the photon energy is presented in electron volt (eV) rather than the joule (J). One electron volt is equivalence to 1.602×10^{-19} J. Accordingly, Eqn. 3.12 transforms to Eqn. 3.13, which was used to estimate photon energy.

$$E = \frac{1.2397\times10^{3}}{\lambda(nm)} eV.nm \qquad 3.13$$

Eqn. 3.13 was used to calculate the photon energy. Some articles reported the illumination as irradiance-power over the area ($W.m^2$) or photon flux-which is the number of photons per unit area per given time ($\mu moles.m^{-2}.s^{-1}$). In these instances, conversion Equations was used to obtain the equivalence photon energy. Where information on illumination was not provided, except the equipment used, the characteristics of the equipment were checked on manufacturer's websites. Other reports did not provide details of the illumination, that is neither the equipment used or the characteristics of the simulated light. An attribute, 'Illumination' was formed with binary categorical values of 'Illuminated' and 'Non-illuminated' adopted.

Generally, photon energy, >1.2 eV, has potential to induce photo-catalysis that reversibly splits water into its constituent components, including oxygen and hydrogen species. The photochemical reactivity of $nTiO_2$ lies in the 300-415 nm spectrum wavelength (Chen and Mao, 2007), which spans three spectrum regions that are UV-B (300-320 nm), UV-A (320-400 nm) and visible light (400-415 nm). The peak wavelength where energy absorption by $nTiO_2$ is high and harmful is reported to be 307-311 nm (Popov et al., 2005).

Illumination of bare $nTiO_2$ in the ultraviolet region induces photo-catalytic reaction due to absorption of photon energy. Photo-electrons from the nanostructure oxidize water to hydroxyl free radical ($^{\bullet}OH^{-}$) and hydrogen ions (H^+), whereas oxygen is reduced to form superoxide radical anions(O_2^-). The superoxide radical anion and hydrogen ions react with the metal oxide electron band to form hydrogen peroxide (H_2O_2), which is highly reactive and could penetrate cells of organisms (Banerjee et al., 2006). Photon energy absorbed by $nTiO_2$ is transferred to molecular oxygen to form singlet oxygen ($^1O_2$). The generation of ROS contributes to oxidative stress and toxicity to organisms.

Studies reveal that absorption of energy increases with decreasing size and decreases with increasing concentration, whereby backscattering has been observed at 62 nm (Popov et al., 2005). This implies that aggregation of $nTiO_2$ in aqueous media reduces energy absorption, and controls photo-catalysis and subsequent toxicity effects. Other studies reveal that anatase, rutile, and amorphous phases in P25 have independent photo-catalytic behaviour in the mixture (Ohtani et al., 2010). This behaviour is scarcely reported in fate and toxicity studies.

3.3.2.2.2.2 Temperature

Information on temperature of the media was collated. Temperature is a physical measure that affects the solubility of ions and hence ionic strength, and permittivity in aqueous media. Dissolution of ions increases with increasing temperature leading to increased conductivity. Oxygen solubility decreases with increasing temperature. The permittivity of pure water decreases with decreasing temperature, although this effect changes for aqueous solutions. This variable therefore has indirect effects on the zeta potential, and stability of $nTiO_2$ and associated colloids in aqueous media. This attribute was considered as an intermediate one.

3.3.2.2.2.3 Time

The duration of an experiment is a predictor variable of the effects that occur. Exposure time was a determining factor in aspects related to: ageing and degradation of pristine (Auffan et al., 2010; Labille et al., 2010) and formulated (Botta et al., 2011) $nTiO_2$ nanocomposites; uptake and accumulation of $nTiO_2$ in Daphnids (Hund-rinke and Simon, 2006; Zhu et al., 2010), generation of hydroxyl radicals (Xiong et al., 2011), and reactive oxygen species (Cherchi et al., 2011) of illuminated $nTiO_2$; mortality of *Daphnia magna* due to oxidative stress (Kim et al., 2010); growth inhibition in *Danio rerio* (Chen et al., 2011)(Kim et al., 2010; Chen et al., 2011); and behaviour change in Daphnia magna (Lovern et al., 2007). Acute and chronic exposure periods were reported, and varied from minutes to months.

3.3.2.2.3 Agitation mechanisms

Agitation in batch and semi-batch reactors could simulate water flow, and maintain dispersion of $nTiO_2$ composites in the media. For batch reactors, agitation was via continuous stirring using manual, magnetic, or shaking mechanisms. For semi-batch reactors, recirculation of media maintained the flow in the reactor. Information on the agitation mechanisms was collated as text values.

3.3.2.3 Data on biotic parameters

3.3.2.3.1 Aquatic organisms

Information collated on the organisms included; species, state of culture, description, habitat, age, feeding mechanisms, population and surface charge. The biological species whose information was collated comprised of: Daphnia, bacteria, fish, algae, worms, insects, snails, yeast, shrimps and insects. Information on some of the organisms is very limited. From our database, and based on the reported data collated, the frequency of organisms tested are presented in Appendix 3.3, which reveals that algae is the most tested organism whilst shrimps are least tested.

The species of organisms and their description were identified, for example, *Daphnia magna* and *Daphnia pulex* that are invertebrate crustaceans whilst *Escherichia coli MG1655* and *Bacillus subtillus CB310* were gram negative and gram positive bacteria, respectively. The state of organisms cultures were either pure (axenic) or impure. Controlled axenic cultures are preferred for simplicity as opposed to impure cultures although the latter provide a better representation of the actual aquatic habitat where species co-exist.

The habitat of aquatic organisms determines their vulnerability to interact with $nTiO_2$ in aqueous media. For example, there are organisms that dwell in the water column such as Daphnids as opposed those that dwell in the sediments e.g. worms. Information about the age of organisms described the development stage at commencement of the tests and comprised of: cells or filaments for bacteria and algae; neonates, juvenile or adults for daphnids; cell, embryo, larvae, juvenile and adult for fish, etc. The developmental stage influences the species' ability to interact with $nTiO_2$ linked to locomotion, for example, swimming capabilities of fish. The feeding mechanisms explain the ability of the organisms to ingest and accumulate the $nTiO_2$ in their gut. For instance, daphnids are water column organisms that are filter feeders that ingest particulate matter, including suspended $nTiO_2$ or associated colloids. Conversely, lugworms are sediment detritus feeders likely to feed on deposited particles or associated colloids of $nTiO_2$.

In natural habitat, population plays a role in competition for available resources. Population also informs the analytical effects to the organisms after exposure to a chemical pollutant. Examples of effects consists of; inhibition, mortality, or growth. Information on the population of the species was either the number of cells, in coliform units, or the number of organisms in a given volume of aqueous media. Organisms such as *E. coli* have a net surface charge that is negative, with charge densities and electro-kinetic potentials that are influenced by abiotic factors under various testing conditions (Chowdhury et al., 2012). As an example, the surface charge of *E. coli MG1655* was -25.3 mV in alkaline natural freshwater, and -53.4 mV in acidic synthetic water (Planchon et al., 2013). The charged surfaces influence the interaction capability of the species with toxicants, however; this attribute was scarcely reported. For example different cell structures of different strains of bacteria influence their surface charges, which in turn enhances or reduces interaction and toxicity effects when exposed to nTO_2 (Simon-Deckers et al., 2009).

Aquatic organisms are a form of organic matter. For example, microbial activity contributes to the dissolved organic carbon in solution (VanLoon and Duffy, 2005). Deionized water constitutes ≤ 1,000 colony forming units of bacteria per litre (Product catalogue, Millipore), which also contributes to TOC values measured in the ultrapure water. Organisms are colloidal biotic ligands that interact with $nTiO_2$ through ingestion and depuration (Zhu et al., 2010), biochemical reactions (Federici et al., 2007); adsorption, aggregation, and deposition (Battin et al., 2009; Chowdhury et al., 2012; Kulacki et al., 2012). These interactions have potential to contribute to accumulation and transportation of $nTiO_2$ to various water partitions, and eventual toxicity.

3.3.2.4 Data on dynamic physicochemical properties of $nTiO_2$

Concerning the synthesis of ENMs, manufacture of nanoproducts, use and disposal; information was collated on the fate of pristine and formulated $nTiO_2$ after exposure to aqueous media. Pristine $nTiO_2$ are likely to be emitted into aquatic systems from wastes generated and disposed at the extraction, functionalization, and manufacturing stages. Conversely, formulated $nTiO_2$ could be emitted during the manufacturing, use of nanoproducts such as sunscreens, and disposal stages. Most scientific studies report on the fate of bare and coated pristine $nTiO_2$, whereas very few studies have reported on the fate of formulated $nTiO_2$.

The word ‘dynamic’ in this study refers to the changes in composition and morphology of $nTiO_2$ in aqueous media. These transformations are influenced by the physical and chemical characteristics of the nanostructure as well as the abiotic and biotic factors of the media. The attributes in this section describe the endpoint variables. Importantly, the account of transformed $nTiO_2$ overlaps across the variables in specific sections because the transformations occur concurrently in aqueous solution. For example, adsorbed species result in aggregation, which influences dispersion and deposition of aggregated species depending on solution chemistry.

3.3.2.4.1 Concentration

Mass balance accounts for material flow in a bounded system. In batch or semi-batch systems, the concentration of $nTiO_2$ into the systems informs on how much is held and distributed either as adsorbed, dispersed, settled, etc. over a period of time. Background concentration of titanium in media used in laboratory tests was scarcely reported. Where the data were available, background $nTiO_2$ concentration was estimated using molar mass ratio of 59.94% of Ti in a mole of the titanium dioxide. Data on concentration was collated as reported.

Very high concentrations of $nTiO_2$ have been used in test solutions (Appendix 3.3), which does not reflect actual concentrations expected in aquatic systems. For example, Kiser and colleagues (2009) reported TiO_2

concentrations of 0.167-5.01 mg/ℓ and 0.01-0.015 mg/ℓ measured in wastewater influents and effluents, respectively. Westerhoff et al., (2011) reported concentrations of 0.302-2.06 mg/ℓ and 0.042 mg/ℓ in wastewater influents and effluents, respectively. Although this represents concentration in a state in USA, it gives a glimpse on expected concentrations of this nanostructure in aquatic systems. Therefore, the high concentration pose challenges when using laboratory results to postulate, predict, and model the aquatic behaviour, interaction and toxicity of $nTiO_2$ in real aquatic systems.

3.3.2.4.2 Adsorption

Information on adsorption species such as cadmium, biotic or abiotic ligands, in aqueous media on $nTiO_2$ was collated. The adsorption process could be described by Langmuir or Freundlich adsorption isotherms. Properties of the $nTiO_2$, the biotic and abiotic factors, influence adsorption were surface size, surface area, zeta potential, solution chemistry, and organic polymers (Pettibone et al. 2008; Li and Sun, 2011). Collision rates also influences adsorption efficiency in agitated systems but this aspect was scarcely reported. Adsorption characteristics affect aggregation, formation of fractals and deposition of flocculated or coagulated materials in solution.

3.3.2.4.3 Aggregation and formation of fractals

The hydrodynamic size and fractal dimension of $nTiO_2$ aggregates formed in aqueous media were found to be relevant attributes. Aggregates reduces surface energy, enhances deposition and sedimentation processes, and thus in turn affects bioavailability and toxicity (Hotze et al., 2010), and lowers the reactivity potential due to photo-catalytic reactions (Jassby et al., 2012). Aggregated sizes of $nTiO_2$ were reported as equivalence hydrodynamic diameters. However, information on the dimension of aggregate fractals formed was scarcely reported. Fractal dimension relates the mass and size of aggregates, and could be useful in understanding other subsequent behaviour processes of aggregated $nTiO_2$ particles or colloids in aqueous systems.

Aggregation kinetics and morphology is affected by the size of $nTiO_2$ exposed, abiotic and biotic factors in aqueous media, and the existing reaction regimes that are either reaction (slow) or diffusion (fast) limited. Conventionally, reaction limited processes produce compact high dimension fractals whilst low dimension fractals are due to diffusion limited process (Buffle and Leppard, 1995; Elimelech et al., 1995). Low fractals have been observed in reaction limited processes for small size $nTiO_2$, for instance, in the presence of organic matter (Chowdhury et al., 2013).

3.3.2.4.4 Degradation and ageing

Information on degradation and ageing of $nTiO_2$ was found to be scarcely reported. Few studies have been conducted to test these processes under different abiotic factors. Studies have reported about the degradation of surface coatings on pristine T-Lite^{TM} SF $nTiO_2$ (Auffan et al., 2010; Labille et al., 2010) un-identified $nTiO_2$ composite coated with alumina and polydimethylsiloxane (PDMS) in branded sunscreen products (Botta et al., 2011). Coincidentally, the studies were conducted in the same laboratory, which directly and indirectly raises the issue of robustness where one considers multiple variables under dynamic conditions.

In this study, the amount of silicon and aluminium released, altered structures, and new species formed were attributes identified as relevant. Favourable conditions of aqueous media promote photo- and bio-chemical processes that result in the degradation and ageing of coated $nTiO_2$ composites. The core $nTiO_2$ composite in T-Lite^{TM} SF constitutes rutile rods (90.9%) and anatase spheres (9.1%) (Labille et al., 2010) with purity of 99.5-100% (Product information catalogue, BASF). In aqueous media, the PDMS surface coating desorbs into solution and exposes the internal gibbsite coating through a surface structure change. Dissolved silicon (60%) and aluminium (1.4%), whose forms were not reported, are released into solution.

The PDMS has no measurable long term influence to environmental hazards since it degrades to form carbon dioxide, silica and water (Griessbach and Lehmann, 1999). An understanding of the dissolved species, structure alteration, and speciation of the coating material, especially gibbsite or alumina which are protective coats of the core $nTiO_2$, is significant in evaluating the fate, transport, and bioavailability of bare $nTiO_2$ in aqueous media. Gibbsite is amphoteric with both acidic and basic characteristics, and participates in hydrolysis reactions with water. The solubility and speciation properties of gibbsite are influenced by the abiotic factors which contribute to structural alteration and formation of new species (Auffan et al., 2010).

Aluminium interacts with organic (e.g. oxalic, tannic, fulvic, humic acids, etc.) and inorganic (e.g. sulphates, chlorides, carbonates, phosphates, etc.) ligands to form complex structures in solution (Driscoll et al., 1980; Sparling and Lowe, 1996). Complex compounds promote ultraviolet induced reactions due to absorption of photon energy. Solubility of aluminium is enhanced in the presence of complex ligands in the pH range of <6 and >8 (Farag et al. 1993; Parent et al. 1996). Under acidic conditions (<4), gibbsite exists in varying concentrations as aluminium hexaaqua complex ion $[(Al(H_2O)_6]^{3+}$, and its hydrolysed compounds comprising of: Al^{3+}, $Al(OH)^{+2}$, $Al(OH)_2^{+}$, and amorphous $Al(OH)_3^0$. As the pH increases, hydrolysis process decreases the Al^{3+}, and $Al(OH)^{+2}$ with $Al(OH)_2^{+}$, to diminishing values at *ca.* pH values of <6.5 and <7.5, respectively, whereas the concentration of amorphous $Al(OH)_3^0$ remains unchanged. In basic conditions >5.5, the concentration of tetrahydroxidoaluminate $[Al(OH)_4^-]$ increases with pH. Aluminium solubility is lowest at pH 6.0-7.0 (Driscoll and Schecher 1990; Witters et al. 1996). The general reaction equations for the hydrolysis and speciation of gibbsite in acid and basic conditions are summarized, thus;

Acidic conditions:

$$Al(OH)_{3_{(s)}} + H^+_{(aq)} \leftrightarrow Al^{3+}_{(aq)} + 3H_2O_{(l)} \qquad 3.14$$

$$Al^{3+}_{(aq)} + 3H_2O_{(l)} \leftrightarrow Al(OH)^{2+}_{(aq)} + H^+_{(aq)} \qquad 3.15$$

$$Al(OH)^{2+}_{(aq)} + H_2O_{(l)} \leftrightarrow Al(OH)^+_{(aq)} + H^+_{(aq)} \qquad 3.16$$

$$Al(OH)^+_{(aq)} + H_2O_{(l)} \leftrightarrow Al(OH)^0_{3_{(aq)}} + H^+_{(aq)} \qquad 3.17$$

Basic conditions:

$$Al(OH)^0_{3_{(aq)}} + OH^-_{(aq)} \leftrightarrow Al(OH)^-_{4_{(aq)}} \qquad 3.18$$

Under natural sunlight illumination, aluminium hydroxide degrades in swimming pools that have chlorine derived compounds, where structural changes increase with increasing chloride concentrations (Virkutyte et al., 2012). At pH 5, temperature rise from 0-25°C revealed a reduction in concentration of Al^{3+} by 48%, and an increase in concentration of $Al(OH)^{+2}$, $Al(OH)_2^{+}$, and $Al(OH)_3^0$ by 24%, 24%, and 1%, respectively (Lydersen, 1990). At the same pH and 25°C, Si-O-Al linkage of PDMS and aluminium hydroxide coating on T-Lite™ SF show an increased alteration from a tetrahedral to an octahedral structure by 66.67% without illumination, and by 77.78% with illumination (Auffan et al., 2010). This phenomena is attributed to desorption and dissolution of silicone and aluminium species, and the reduced linkage between the two coating materials. Octahedron is the basic structure of gibbsite with Al^{3+} bonded to six coordinated OH^- in an octahedral manner. Surface properties and dispersion stability of colloidal residues from PDMS coating depend on altered reactions (Botta et al., 2011).

In sunscreens containing coated $nTiO_2$, ageing in aqueous media releases colloids (≤ 1000 nm) whose size increases with time (Botta et al., 2011). The high organic to mineral ratio contributes to the alteration process (Huang et al., 2008; Auffan et al., 2010). Humic materials promote acidity in aqueous solutions that influence dissolution and speciation of aluminium-based coating on $nTiO_2$.

3.3.2.4.5 Deposition

Deposition of $nTiO_2$ is hinged on the stability of colloidal or particulate forms as well as their concentration in aqueous media. In this study, deposition was considered in two perspectives that occur concurrently; first, those which involve adsorption and adherence to existing surfaces in the solution including organisms.

Secondly, that which arises from sedimentation of fractal aggregates in the testing vessels. Abiotic factors, and attractive electrostatic forces influence the deposition rates (Chen and Elimelech, 2006; Fatisson et al., 2009) as well as the additive transport mechanisms (Tufenkji and Elimelech, 2004). In both batch and semi-batch reactors, either first order kinetics, logistic, or polynomial models were found applicable to account for deposition.

Various experimental approaches were used to assess the deposition or sedimentation of $nTiO_2$ in aqueous media using agitated batch reactors (Keller et al., 2010; Li and Sun, 2011), non-renewal semi-batch reactors with re-circulation (Battin et al., 2009; Kulacki et al., 2012), semi-batch porous media (Chowdhury et al., 2012; Chowdhury et al., 2011), parallel plates (Chowdhury et al., 2012; Chowdhury and Walker, 2012), and quartz crystal microbalance with dissipation (QCM-D) (Thio et al., 2011). The target attributes found applicable across the various approaches were the hydrodynamic sizes of fractal aggregates and mass of $nTiO_2$ deposited.

Statistical models, and known mathematical formulae, were used to estimate continuous target attributes and their values. First order kinetics explained the deposition of P25 (Evonik Degussa Company) and Hombikat UV 100 nanostructures(Sachtleben Company) onto microbial biofilm (Battin et al., 2009). Supernatant concentration ratios and turbidity values were used to establish the mass flux onto biofilm, and the concentration of $nTiO_2$ composites deposited. Reported data (Battin et al., 2009) was used to interpolate and extrapolate deposition and turbidity decay rates (Figure 3.2) as well as deposited mass (Figure 3.3) for both P25 and Hombikat UV 100 $nTiO_2$ in natural surface water The deposition rates for P25 and Hombikat UV 100 $nTiO_2$ onto biofilm were 0.024 min^{-1} and 0.015 min^{-1}, respectively, while the turbidity decay rates were 0.0303 min^{-1} and 0.0176 min^{-1}, respectively. Normalized deposition kinetics and turbidity values were used to estimate the concentration of nanostructures in the water column, and that deposited onto the biofilm (Figure 3.3).

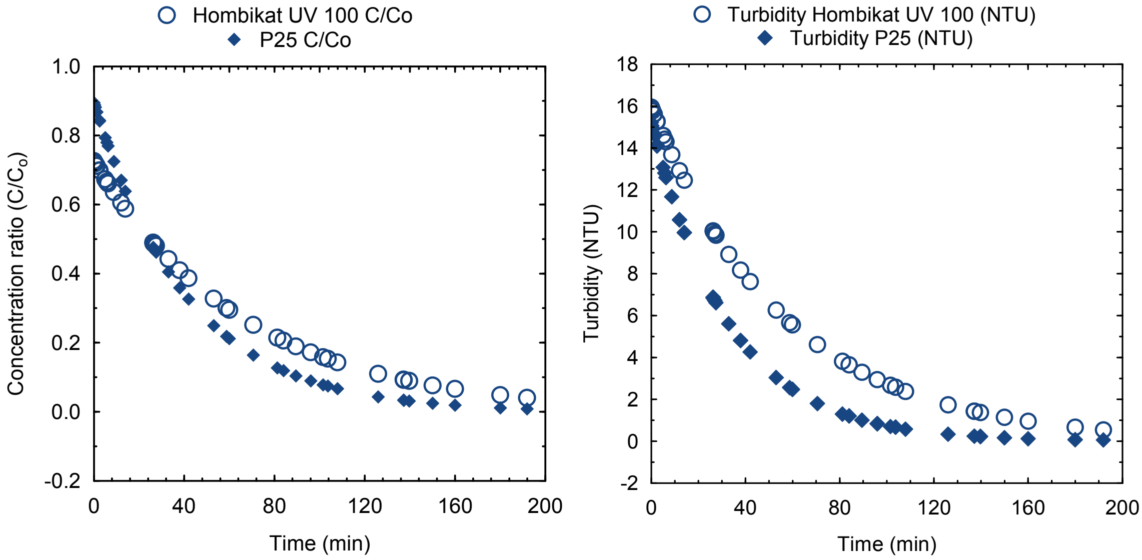

Figure 3.2: Interpolated and extrapolated values of C/Co and turbidity versus time for P25 and Hombikat UV 100 $nTiO_2$ nanostructures in natural surface water.

Data source: (Battin et al., 2009)

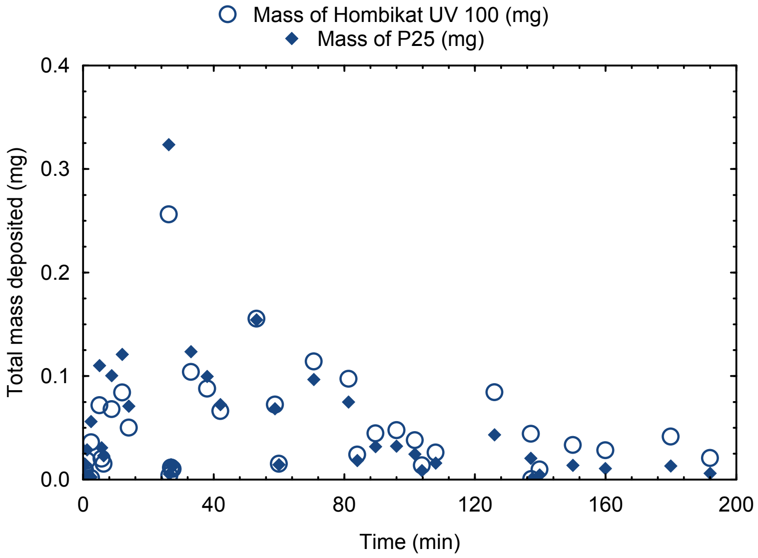

Figure 3.3: Scatterplot of time versus mass deposited onto biofilm. Data source: Bhattin et al., 2009.

Equations; $Hombikat\ UV100\ (mg/L) = 0.1447 + 0.3794\ (NTU) - 0.0036\ (NTU^2)$, R^2 = 0.9999; and $P25\ (mg/L) = 0.1291 + 0.4435\ (NTU) - 0.007\ (NTU^2)$, R^2 = 0.9994 describe the polynomial models developed for the data. The coefficients of determination, R^2, are high showing best fits for the estimated data from scaling methods.

The cumulative masses deposited were 1.873 mg and 1.82 mg for P25 and H-UV 100, respectively, after 180 minutes. In comparison with summary data reported by Battin and colleagues (Battin et al., 2009), the cumulative mass deposited were 1.64±0.04 mg and 1.88±0.04 mg, respectively, during the same period. This gives error margins of 0.372% for P25 below the reported average value but within the expected value (1.84-1.92 mg), compared with 9.82% for H-UV 100 above the reported average value and way above the expected category of values (1.6-1.68 mg). These errors could be attributed to computational methods used for estimating values from the graphs, and statistical modelling. These computational results show that estimated value have high potential (>90%) to lie within the values obtained from the studies, and hence offered good estimations.

In a similar study by Kulacki et al. (2012) a logistic model was established as suitable to model the kinetic deposition of P25 $nTiO_2$ onto algae biofilm, and hence biomass growth as function of time (0-744 minutes) under varying pH, conductivity, and organic matter in mesocosm water as exposure media. Three types of Algae (*Synedra ulna*, *Scenedesmus quadricauda* and *Stigeoclonium tenue*) were tested in pure and mixed cultures. Figure 3.5 shows the estimated mass of $nTiO_2$ deposited onto the algae biofilms. Background concentration of titanium metal in mesocosm water was incorporated in the computation.

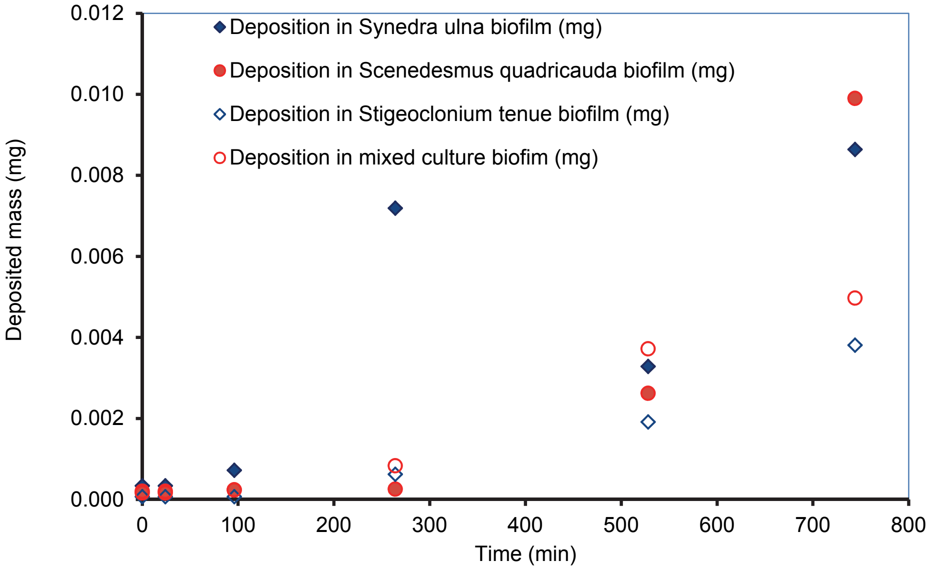

Figure 3.4: Deposition of P25 onto biofilm of three algae species and one mixed culture.

Data source: (Kulacki et al., 2012)

The computation of attribute endpoints was conducted on a case-by-case basis depending on the type of study. Noteworthy is that no single method was found as suitable for all end-point attributes estimations. First order kinetics decay was applicable to some surface water deposition and/or sedimentation processes. Deposition is also concentration depended. For example, low concentration of $nTiO_2$ in water with very high organic content experiences high dispersion due to steric repulsion. In such instances, the deposition rate is very low and does not follow a first order kinetic decay curve as shown in Figure 3.2. For example, the polynomial curve estimated the deposition of 10 mg/ℓ of $nTiO_2$ in mesocosm water with 63.45 mg/ℓ of TOC, which is equivalence to 114.21 mg/ℓ of organic matter. In addition, deposition rates in porous media such as soil, which simulates groundwater flow, have different breakthroughs. Empty bed and flow breakthrough curves were therefore used to estimate the endpoint attributes.

3.3.2.5 Toxicity endpoints

Various toxicity endpoints were reported for different species (Appendix 3.4). Based on our database, toxicity endpoints of $nTiO_2$ tested could be classified as either direct or indirect. Direct toxicity affects the organisms during exposure to the nanostructure, for example, mortality, and growth inhibition, locomotion, etc. Indirect toxicity has longer term effects compared to direct toxicity, and may impact on future generation of the organisms. For example, indirect effects comprise oxidative stress due to generation of reactive oxygen species (ROS) and DNA change.

3.4 DATA PREPARATION

Data preparation is a pre-processing step that converts original data to a new set of data. Conversion of data eliminates problems associated with the original data while preserving their important information and making them more applicable in analyses (Famili et al., 1997). The aggregate data collated in this study were secondary, and therefore, previously pre-processed at the source. However, the data had problems related to incompleteness, inconsistency and limitations arising from the following; (i) varying standards and conditions used in conducting experiments; (ii) different measurable end-points and formats; (iii) lack of relevant attributes; (iv) numerous characterization methods used to determine the properties of $nTiO_2$; and (v) missing values of relevant attributes.

Data problems prevent effective analyses using algorithms, which result in unreliable results. Quality data are needed to build repositories for subsequent analyses including knowledge discovery, prediction, modelling, and decision support and systems development. The goal of pre-processing techniques, storage and management of data in repositories is to generate quality data that is intrinsic, contextual, representative and accessible (Han and Kamber, 2009). Pre-processing techniques depend on the type of data available, and the subsequent application of the data. The data therefore needed evaluation and cleaning before further analysis. The process of data preparation was semi-automatic i.e. manually and by use of software. Theories, mathematical equations, and statistical modelling approaches were used to prepare the data. Examples of procedures and methods used to modify the data collated and construction of attributes have been described in the Section 3.3.

3.4.1 Data cleaning

Inconsistencies due to missing relevant attributes, and values some of attributes reported, made further analyses of the data collated challenging. In addition, continuity of the measurable values was lacking. For example, dynamic changes in the properties of the media arising from exposure to $nTiO_2$ and organisms were missing. The percent of missing values was quite high, and therefore, could not be ignored. To address this challenge, several methods were adopted to handle missing values. First, missing values were manually filled using information obtained from technical reports, company websites, or published scientific literature, etc. For example, if values for the physicochemical properties of P25 $nTiO_2$ were not reported, the information was obtained from the product catalogue or Evonik Degussa Company's – the manufacturer's website. The values were filled in categorical formats i.e. lower and upper bound values, which provided a broader representation of the likely missing values.

Secondly, external references, and known mathematical formulae and statistical models, were used to estimate missing attribute values. The choice of suitable Equations to be used was based on prior information of measured parameters, and standards used in the tests. For example, synthetic aqueous media used in toxicity experiments is based on standardized composition of electrolytes and nutritional compounds. The ionic strength of such media depends on the concentration and charges of ions present. Where the standard media used in the test was reported but other values were missing, the concentration of ions was calculated, and then ionic strength established. Information of the behaviour of $nTiO_2$, and its interaction with organisms, in aqueous media is irrelevant without knowledge of the solution chemistry that influences it. Lastly, missing values that could not be filled by two methods described were left blank for further analyses and estimation using data mining techniques.

3.4.2 Data consolidation

Information obtained from various sources was consolidated by merging the data into groups based on similar properties. For example: (i) attributes for dynamic properties of the $nTiO_2$ e.g. deposition, aggregation, degradation, etc., and (ii) the organism exposed to the nanostructure e.g. bacteria, daphnia, fish, etc. The data were matched by comparable attributes in the same group. It was noted that the names and units of measurement for many attributes were inconsistent. Thus conversion tables were used to convert the units into SI format to improve consistency. Some units had no direct conversion formulae, and generalization was adopted.

Redundant attributes were identified by testing the association between and among attributes. Some attributes could be used to derive others. In this study, such attributes were referred to as intermediates and were used to construct target attributes. Correlation analyses were performed to measure the strength of the association. For example, given attributes x_i and y_i, for $i = 1.....k$, the Spearman's rank correlation (Eqn. 3.19) – a non-parametric measure of dependence – was used to detect the correlation.

$$\rho = \frac{\sum_i (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_i (x_i - \bar{x})^2 \sum_i (y_i - \bar{y})^2}} \quad 3.19$$

where $\bar{x}$ and $\bar{y}$ are the means of x_i and y_i. For $\rho > 0$; x_i and y_i have a positive correlation where both attributes values increases or decreases simultaneously. For $\rho = 0$, x_i and $y_i$are independent of one another; and for $\rho < 0$, x_i and y_i, have a negative correlation where values of one attribute increases as those of the other decreases.

Highly positive or negative correlation indicates a strong relationship between the attributes but does not establish causation between the variables. For example, the concentration of monovalence electrolytes, such as sodium chloride (NaCl) is equivalence to the ionic strength of the aqueous solution. In correlation analyses, the ionic strength, and the concentration of Na^+ and Cl^- ions would have strong positive correlation; hence two of the three attributes would therefore be considered as redundant. Similarly, the primary size of $nTiO_2$ has high negative correlation with the specific surface area, and one of these two attributes would be redundant. Redundancy, however, is not a measure of causal effect in analyses. Correlation informs about attributes to include in subsequent analyses.

3.4.3 Data conversion

Relevant attributes were constructed using data collated for other attributes. For example, the concentration of $nTiO_2$ could be derived from turbidity measures (Figure 3.4), and deposited mass (Figures 3.5 and 3.6). Depending on the prevailing conditions, especially the solution chemistry, the surface charge of $nTiO_2$ in aqueous media could have a positive charge at low pH but a negative charge at high pH. This attribute is influenced by the point of zero charge. Hence, an attribute pH-PZC was constructed. The surface area per unit mass of ENMs increases and imparts on the interface process when the nanostructures are emitted in aquatic systems. In this case, the surface area concentration becomes a relevant attribute to describe these processes – and represented as number concentration (Fissan, 2012), or mass concentration (Suttiponparnit et al., 2010). Because number concentration was scarcely reported, therefore the mass surface area concentration (SAC) attribute was developed using surface area and concentration of $nTiO_2$ in solution. In this study, the SAC attribute represents different sizes, different morphologies (i.e. spherical or rod shaped dimensions), and concentrations. Other attributes were constructed from the empirical values of endpoints. For example, mortality was derived from survival (%) by basic subtraction from 100%.

From the collated data, values of multiple target attributes were combined to reduce the endpoints. For example, ROS in liver, kidney, brain, gills in fish were combined using additive methods to one attribute, namely 'Oxidation'. This was done for other organisms. Generalization methods were also applied to the data. This was done to expand categorical attributes. For example, the survival rates between 0-40% were categorized as 'Low'', 40-70% as 'Medium', and >70% as 'high'.

3.4.4 Predictive analysis of data

In tandem with correlation analyses amongst variables described in Section 3.4.2, preliminary regression model analyses were performed on data four different data mining techniques, namely: (i) Classification and Regression Trees (CandRT), (ii) Random Forest, (iii) Boosted Trees, and (iv) Neural Networks. The four methods were used to first, test their capability to predict response variables; secondly, test their performance in predicting unknown values with known predictor values; and lastly, to establish the strength of descriptor variables to response variables. For example, the prediction of zeta potential as a response variable using eight variables, *viz*: pH, ionic strength, surface area concentration, % of anatase and rutile crystal phases, organic matter, duration and temperature. The data mining analyses were performed separately (Figure 3.5), and as an ensemble (Figure 3.6). In both cases, 373 data values were used to train models that were used to predict 29 data values of zeta potential. The ensemble averages the performance of the four models to yield better prediction. Table 3.6 summarizes the models' performance that shows lower error rates and high coefficients of determination for Boosted trees and Neural Networks.

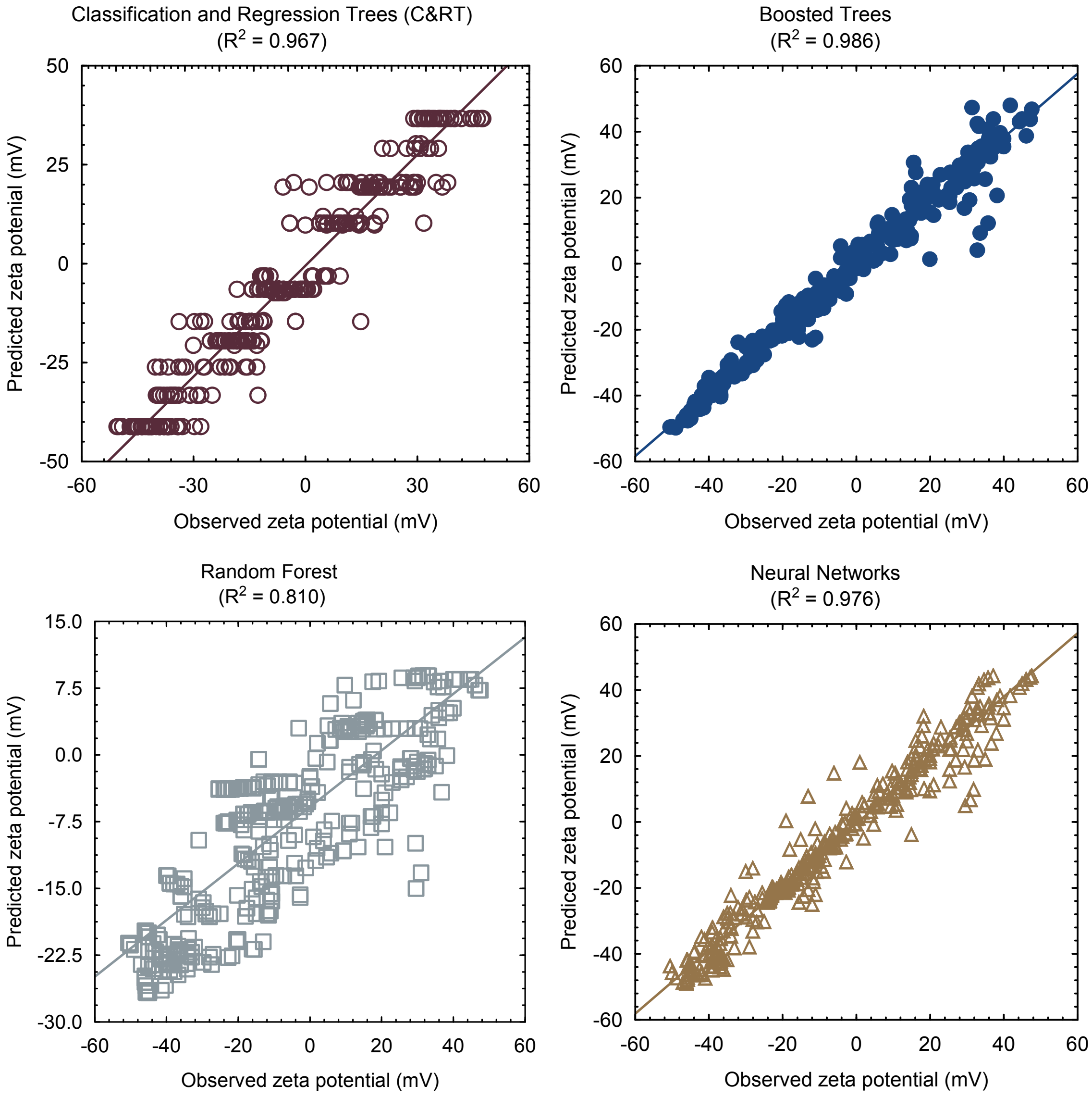

Figure 3.5: Prediction of zeta potential using Classification and Regression Trees (*Top Left*); Boosted Trees (*Top Right*); Random Forest (*Bottom Left*) and Neural Networks (*Bottom Right*). Data source: (Suttinponparnit et al., 2011; Planchon et al., 2013)

Table 3.6: Outcome of regression models of data mining algorithms

Data mining algorithm	Prediction error rate	Coefficient of determination (R^2)
Boosted trees	17.95	0.986
Classification and regression trees (C & RT)	39.03	0.967
Neural Networks	27.75	0.976
Random forest	309.26	0.810

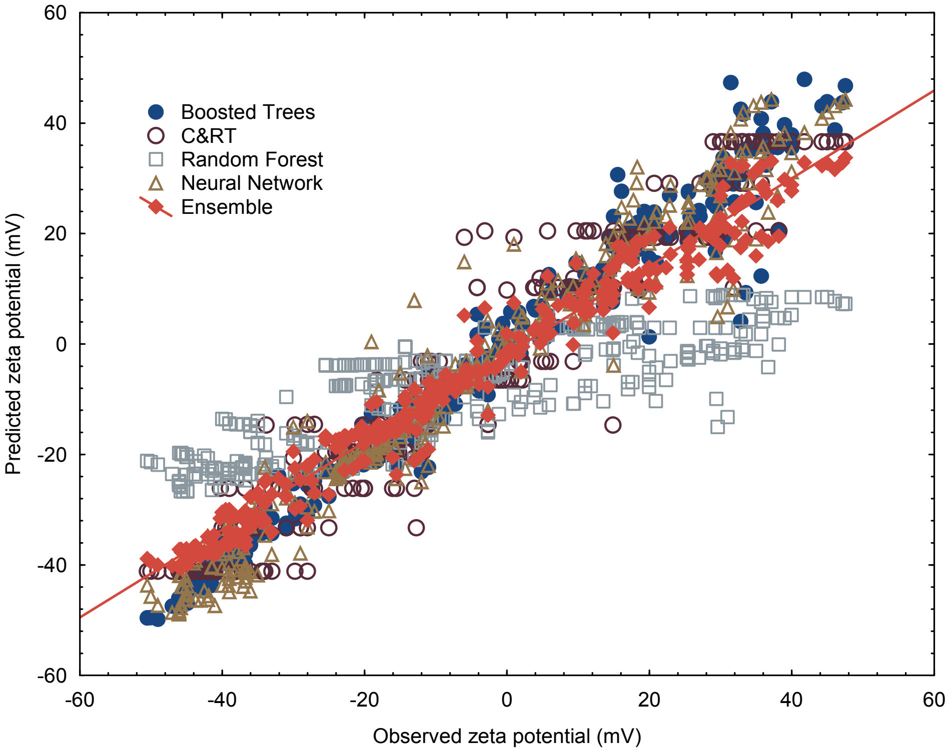

Figure 3.6: Ensemble model that averages individual model prediction of zeta potential.

Boosted trees and Neural Network algorithms had high prediction performance with coefficients of determinations at 0.986 and 0.976 respectively. These techniques will be pursued in future studies. The prediction capability of each model and combined (ensemble) is outlined in Table 3.7. The performance of data mining algorithms is unique based on various factors including data quantity and quality. Consequently, it is difficult to rank them unless further analytical and error tests are performed on more data scientific data.

From Table 3.8, it is observed that surface coating has zero influencing effect possibly owing to lack of variations since only 9 values (2.24% of the reported data used) represented coated $nTiO_2$ while the rest had uncapped surfaces. In ideal aqueous solutions – for example simulated water – zeta potential is highly influenced by pH and ionic strength. In addition to these variables, it is observed that ENMs characteristics i.e. surface area concentrations and crystal phases are important predictor variables based on their prediction performance in Boosted trees, CandRT, and Random forest models. All the three models ranked anatase and rutile equally which could be attributed to their negative correlation i.e. one variable opposes the other. Either of the two variables could be eliminated in subsequent analyses due to redundancy. Using Neural Networks, the prediction of zeta potential was highly influenced by duration, temperature, surface area concentration, organic matter, anatase crystal and pH (≥ 17-100%). Rutile and ionic strength variables had ≤ 5% influence. This model relates surface energy to time and temperature variations and how these environmental parameters can influence interactions.

Table 3.7: Performance of models in predicting zeta potential with known input variables

Data No.	Boosted Trees	Classification and Regression Trees (CandRT)	Neural Networks	Random Forest	Ensemble
1	45.8423	19.3149	20.24352	-8.8594	19.1353
2	51.8825	19.3149	20.98761	-7.1575	21.2569
3	20.9502	19.3149	22.77716	-0.5214	15.6302
4	31.4957	36.6301	28.95044	-0.1856	24.2226
5	34.0399	36.6301	40.57964	0.5353	27.9462
6	44.6148	19.3149	18.91964	-4.1213	19.6820
7	47.9474	19.3149	19.67099	-2.4194	21.1285
8	17.0210	19.3149	21.48332	3.2385	15.2644
9	26.6545	36.6301	27.80014	3.4961	23.6452
10	28.6278	36.6301	40.09237	4.2171	27.3918
11	47.0557	19.3149	9.80615	-3.5840	18.1482
12	45.9268	19.3149	10.42274	-1.8821	18.4456
13	19.3970	19.3149	11.94350	3.5720	13.5569
14	29.0643	36.6301	17.70445	3.8296	21.8071
15	30.5110	36.6301	33.03568	4.5505	26.1818
16	-15.8138	-6.5392	41.92771	-9.2712	2.5759
17	-15.8138	-6.5392	28.39292	-9.2712	-0.8078
18	-16.0127	-6.5392	-6.00314	-10.5697	-9.7812
19	-16.0127	-6.5392	-6.00314	-10.5697	-9.7812
20	-16.7959	-6.5392	-6.00314	-10.5697	-9.9770
21	-18.9364	-33.2600	-6.00314	-6.0496	-16.0623
22	-18.9364	-33.2600	-6.00314	-6.0496	-16.0623
23	-18.7375	-33.2600	43.30689	-5.6390	-3.5824
24	-18.9364	-33.2600	-6.00314	-6.0496	-16.0623
25	-18.9364	-33.2600	-6.00314	-6.0496	-16.0623
26	-18.7375	-33.2600	43.30689	-5.6390	-3.5824
27	-18.9364	-33.2600	-6.00314	-6.0496	-16.0623
28	-18.9364	-33.2600	-6.00314	-6.0496	-16.0623
29	-18.7375	-33.2600	42.92597	-5.6390	-3.6776

Data source: (Wiench et al., 2009; Suttinponparnit et al., 2011; Planchon et al., 2013)

Table 3.8: The strength of variables in predicting the zeta potential.

	Variable Importance (%)			
Variable	**Boosted Trees**	**Classification and Regression Trees**	**Neural Networks**	**Random Forest**
pH	100	100	17.87	100
Ionic strength (mM)	57	55	2.82	43
Surface area concentration (m^2/ℓ)	67	54	93.41	35
Anatase (%)	39	29	20.73	12
Rutile (%)	39	29	4.8	15
Temperature (^oC)	17	15	95.35	6
Organic matter (mg/ℓ)	22	17	47.55	8
Duration (hours)	17	15	100	8
Surface coating	0	0	0	0

3.5 CONCLUDING REMARKS

In this chapter, an approach and its conceptual framework were adopted to develop a repository characterized with attributes identified as useful for the prediction, modelling and simulation of effects and behaviour of $nTiO_2$ in the aquatic systems. Because of lack of data and differences observed data from one study to the next, various techniques were adopted to improve of the quality collated from the published literature. The collated data were rendered amenable for the purposes of predicting and simulating the fate and effects of $nTiO_2$ through four major processes of data cleaning, consolidation, conversion to similar units, and data's ability to predict the response variables, missing values, and establish the strength of the link between descriptor variables to the response variables. Data derived from the activities described in this chapter was applied in modelling the behaviour and effects of $nTiO_2$ described in Chapters 4 and 5, respectively.

CHAPTER 4: MODELLING OF ENGINEERED NANOMATERIALS' BEHAVIOUR USING ARTIFICIAL NEURAL NETWORKS: A CASE OF NANO-TIO_2 IN AQUEOUS ENVIRONMENT

4.1 INTRODUCTION

Demand for present and future use of ENMs in products is increasing with an estimated market growth index of 25% per year between 2000 and 2020 (Roco et al., 2010). During their life cycle, ENMs are directly or indirectly released into environmental matrices. In aquatic ecosystems, for example, the interaction of ENMs with biota is influenced by their behaviour and fate mechanisms as related to their physicochemical properties in manufactured products (Nowack et al., 2012), and water chemistry (Batley et al., 2013; French et al., 2009; Lin et al., 2010; Petosa et al., 2010), as discussed in Chapter 3.

Environmental risk assessment of potential hazardous substances evaluates the well-being of exposed flora, fauna and ecosystems that support human life (Mihelcic and Zimmerman, 2010). Risk assessment of ENMs as raw materials in consumer products and industrial applications is necessary, but difficult to conduct, as their occurrence, exposure and impacts remain inadequately understood or measured in the environment. Moreover, lack of full characterization of individual ingredients of ENMs during production and manufacturing processes limits our ability to undertake risk assessment. This is further exacerbated by inconsistencies in the production statistics of ENMs (Schmid et al., 2008; Nightingale et al., 2008; Robichaud et al., 2009; EPA, 2010; Aschberger et al., 2011; Hendren et al., 2011; Piccinno et al., 2012; Keller et al., 2013) and lack of mandatory legislation on labelling of nanoproducts (Gruère, 2011), which in turn compounds the uncertainty of their emissions into the environment.

Information on the number and types of formulation matrices of nanoproducts may reveal the potential release and eventual entry of ENMs into the environment during different phases of their life cycles. Report of Project on Emerging Nanotechnologies (2014) listed 1 880 nanoproducts, where nano-silver (nAg) and nano-titanium dioxide ($nTiO_2$) were the first and second most popular ENMs in products, respectively. Studies have reported the content of $nTiO_2$ in nanoproducts (Contado and Pagnoni, 2010; Lomer et al., 2000; Samontha et al., 2011; Weir et al., 2012), revealing a 2-in-3 probability of labelling (Lomer et al., 2000), but also with preferential application without adherence to standards (Weir et al., 2012). Studies report bulk ENMs (>100 nm) in tested products, but reveal 36% ultrafine particles (<100 nm) (Weir et al., 2012). These findings indicate the uncertainty associated with the downstream release and impact of ENMs.

In the environment, specific monodisperse engineered and composite polydisperse natural nanomaterials, as well as transformed polydisperse ENMs co-exist or are likely to exist (Casals et al., 2008; Nowack et al., 2012). The aquatic ecosystem is complex, owing to chemical, physical, and biological colloids (Buffle and Leppard, 1995b) underpinned by micromolecules, and partly explains why the interactions of ENMs with the complex environment are poorly understood. This challenge is likely to remain unresolved for the foreseeable future, owing to a lack of specialised analytical techniques for *in-situ* characterization and monitoring of ENMs in real time (Abbott and Maynard, 2010; Von der Kammer et al., 2012). Techniques previously developed for testing organic and inorganic compounds are presently being used to estimate the presence of ENMs in composite environments and biota (Von der Kammer et al., 2012) – despite their limitations in dealing with pollutants with nanoscale dimensions in aquatic systems.

For example, at present, few studies have (i) characterized and quantified elemental titanium (Ti) in wastewater effluent (Kiser et al., 2009; Westerhoff et al., 2011) and surface water run-off (Kaegi et al., 2008; Kaegi et al., 2010), and (ii) determined the concentration of Ti in wastewater effluent (Khosravi et al., 2012), sludge (Johnson et al., 2011; Khosravi et al., 2012) and freshwater in rivers (Neal et al., 2011) using quantitative techniques, such as acid (Johnson et al., 2011; Kaegi et al., 2008; Kaegi et al., 2010; Kiser et al., 2009; Neal et al., 2011; Westerhoff et al., 2011) or oxidant digestion (Khosravi et al., 2012), element detection, and stoichiometric calculation for $nTiO_2$. However, these tools have not been able to distinguish and estimate the impact of nano- and bulk forms of ENMs from their natural counterparts.

As discussed in Chapter 1, modelling studies have forecasted likely ENM concentrations discharged into different environmental compartments. Many assumptions were used to approximate data uncertainties in the models reported. However, to the authors' knowledge no previously reported results have been published on the fate and behaviour of ENMs in aquatic systems, particularly based on the use of data-driven models, and this forms the thrust of this work.

In this chapter, we present a modelling approach that can forecast the potential risks of ENMs, specifically "as produced" materials as a starting point. At nanoscale, production methods influence the properties of ENMs where batches bear unique physicochemical identities that are enhanced during the manufacturing phase of nanoproducts. To illustrate the functionality of the proposed model, published data are used to model the behaviour of $nTiO_2$, whose fate is influenced by the environmental chemistry (e.g. NOM, pH, ionic strength, etc.), as discussed in Chapter 3. Previously the substance flow analysis models considered continuous life-cycle emission of ENMs, where no distinction was made between pristine (as produced) and functionalised (as formulated) properties of the materials. Our model considers each life cycle stage (e.g. production, storage, use, etc.) as a discrete stage that builds into a continuum along the value chain as data become more available.

With this background, the specific objectives of this Chapter are (i) to develop a framework that identifies suitable input and output variables for data-based model(s) designed to represent the exposure mechanisms of nano-TiO_2 in aquatic systems, (ii) to assess the influence of the input variables on the output variable(s), and (iii) to develop models using the most important input variables to predict the behaviour of $nTiO_2$ in aqueous environments. Multilayer perceptron (MLP) neural networks are used for this purpose. Two case studies are presented to illustrate the functionality of the proposed models.

4.2 METHODOLOGY

4.2.1 Conceptual framework of the modelling approach

A generalised conceptual framework of the modelling approach is illustrated in Fig 4.1, using directed graphs to link cause to the effect of ENMs. The approach focuses on ENMs after their release into aquatic environments at any stage during their life cycles. The aquatic environment is defined by physical, biological, and chemical characteristics which in turn influence the behaviour, fate, and impact of ENMs in the aquatic ecosystems as discussed in Chapter 3.

In the scientific literature, modelling studies have exploited both deterministic and probabilistic approaches to estimate the partitioning of ENMs in the environment. However, these approaches are yet to be applied to forecast the potential behaviour, fate, and impact of ENMs after release. This is particularly so regarding the complex interrelationships between the properties of ENMs and characteristics of the environment using published experimental data. The framework proposed herein underlines the important role of identifying and selecting input variables based on the properties of ENMs and aqueous environmental characteristics as basis of developing a robust predictive model.

To collate the data used to develop the model, scientific reports on the behaviour and fate of ENMs in aqueous environment were critically reviewed, and data analysed as discussed in Section 3.4. Here we focused on $nTiO_2$ for reasons articulated in Section 1. This was conducted to elucidate the interacting variables from the laboratory experimental data that significantly impact on the exposure mechanisms of $nTiO_2$ in aquatic media. Laboratory-based data were derived using natural water and aqueous solutions aimed to establish the plausible behaviour and fate of $nTiO_2$ as a model for the natural aquatic environment. The data collected indicated that batch reactors used in the simulated studies adequately accounted for discrete time-controlled processes that are representative of lentic aquatic ecosystems.

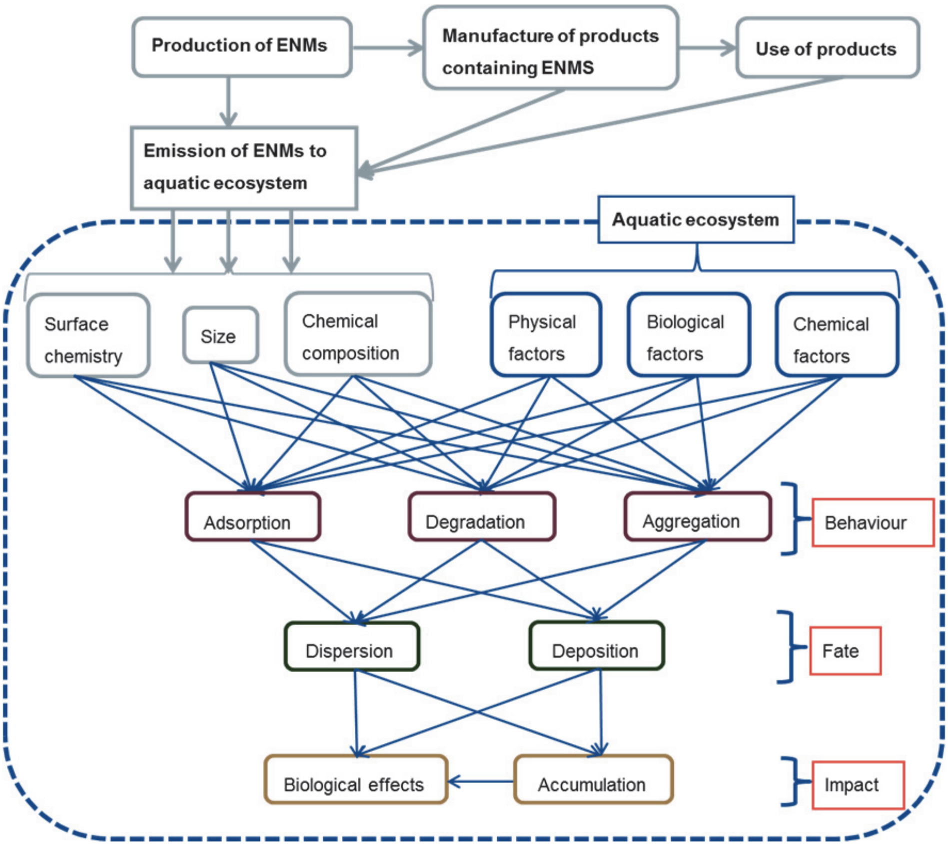

Figure 4.1: Conceptual framework illustrating the behaviour, fate, and impact of ENMs in aquatic ecosystems influenced by ENMs properties and aquatic system characteristics.

Assumptions made in this study were motivated by data and information availability in the scientific reports for the $nTiO_2$, as explained in Chapter 3. First, the intrinsic properties of $nTiO_2$ exploited by industry in nanoproducts are fixed at the point of exposure. Most laboratory experiments have reported data on uncoated pristine $nTiO_2$ – a form representative of ENMs released at the production stage of the value chain. Based on this assumption, various types and forms of $nTiO_2$ used in commercial products could be differentiated. This included functionalized (Faure et al., 2013), doped (Abazovic et al., 2009), and/or coated (Botta et al., 2011) $nTiO_2$. Second, the physical, chemical and biological composition of water and duration of exposure influence the behaviour and fate mechanisms of ENMs. Third, the surface area concentration of the particles expressed in square metres per litre was used in our model as an integrated variable comprising size, morphology, and the concentration parameters as discussed in Chapter 3. The use of an integrated variable helped to reduce the dimensionality of the input variable space, bridged the data gaps on structural properties of ENMs, and incorporated the concentration of ENMs into our proposed model.

Fourth, temperature, pH, and ionic strength are significant water chemistry variables that account for the mechanistic processes of the fate and behaviour of ENMs in aqueous environments. Both pH and temperature are easy to measure and can be detected in grab samples, while the ionic strength represents electrolytes in the aqueous environment. These three variables adequately represent the characteristics of the aqueous environment for the purpose of simple risk assessment, especially where specialized equipment for further analysis is lacking.

In summary, the proposed modelling approach is based on the use of fundamental scientific principles and experimental data in published scientific reports to develop predictive models. These models can be used to identify future research needs in understanding the potential risks of ENMs in the environmental systems.

4.2.2 Colloid kinetics and selection of target endpoints

The general principles and theoretical approaches governing the electrokinetic mobility of charged particles in aqueous systems were reviewed. Generally, ENMs can be described as colloids, as the size of aquatic colloids range from 1 to 1000 nm (Buffle and Leppard, 1995a). Moreover, the properties and behaviour of colloidal systems are strongly dependent on electrical charges of the particles. In addition to hydrodynamic factors, these attractive and repulsive forces play a significant role in influencing transport mechanisms and colloidal interaction in ENMs. In this study, two behaviour mechanisms, namely adsorption and aggregation, were selected as target endpoints, as they are described by widely reported experimental data in the scientific literature. In contrast, other endpoints, such as degradation or surface coatings on pristine $nTiO_2$ as reported in cosmetics (Auffan et al., 2010; Labille et al., 2010), was not considered in this study, owing to the limited availability of data.

The pH is an ion concentration scale that defines the surface charge of colloids (Hunter 1981). Surface charges develop at the interface of interacting liquid-solid or solid-solid phases through hydroxylation of metal oxides in aqueous environment (Elimelech et al., 1995; Hunter 1981) and (de)protonation of adsorbed organic substrates (Chowdhury et al., 2013; Pettibone et al., 2008; Hunter 1981). Ionization of hydrogen and hydroxyl ions (Eqns. 4.1 and 4.2) (Zhao et al., 1993) and adsorption of cationic, anionic and neutral molecules exert control on the net surface charge (Eqn. 4.3)(Hunter, 1981).

$$(OH^-) \leftrightarrow^{H^+}_{OH^-} (H_2O) \leftrightarrow^{H^+}_{OH^-} (H_3O^+) \quad \text{… adsorption of water} \tag{4.1}$$

$$(MO^-) \leftrightarrow^{H^+}_{OH^-} (MOH) \leftrightarrow^{H^+}_{OH^-} \left(MOH_2{}^+\right) \text{… metal surface hydroxyl} \tag{4.2}$$

$$\sigma_0 = F(\Gamma_{H+} - \Gamma_{OH-}) \tag{4.3}$$

where MO is the metal oxide, σ_0 is the net surface charge, and Γ_{H+} and Γ_{H-} are adsorption densities. Titanium dioxide is an intermediate oxide with a net charge of zero at pH range of 5.6-6.2 (Elimelech et al., 1995) – which is the point of zero charge (PZC). For rutile titanium dioxide, Nernst's model (Eqn. 4.4) demonstrates the pH's influence on surface potential around the PZC (Larson and Attard, 2000).

$$\psi_0 = \frac{kT}{ze} In \frac{[pH]}{[pH]_{PZC}} \tag{4.4}$$

where, ψ_0 is the surface potential; k is the Boltzmann constant; T is the temperature; and ze is the proton charge. Hence the pH difference between a given pH and pH_{PZC} is a useful input variable in our model.

Zeta potential is a measurable quantity that represents the surface potential of colloids, and controls their interaction(s), and subsequently stability. In this work, zeta potential was considered as an intermediate variable that affected the coagulation, flocculation, and sedimentation kinetics of ENMs. On the other hand, ionic strength is a function of electrolyte ion concentration and valency (Eqn. 3.8). In ideal low ionic strength environments, the surface charges of ENMs are positive below the pH_{PZC}, but negative above it. Increasing the ionic strength reduces the surface charge towards the PZC value, due to increased thickness of the double layer, a Debye-Hückel parameter (Eqn. 3.9), that associated with high attractive forces between interacting colloids and enhanced aggregation. The Debye-Hückel parameter links the ionic strength to the zeta potential.

Complex adsorbed organic ligands of acidic, dissolved, surfactants among other forms are surface functional groups that donate protons to metal oxides (Eqn. 4.5). Physical sorption reverses the sign of the zeta potential around the PZC and chemisorption shifts the PZC depending on ion concentration (Hunter 1981). Depending on the pH, the dissociation constant of organic adsorbents influence the surface potential at the interaction site, as expressed by the Boltzmann Eqn. (Eqn. 4.6).

$$-COOH \rightarrow -COO^{-} + H^{+} \tag{4.5}$$

$$K_a = \frac{v_-}{v_0}[H^+]exp\left(\frac{-e\psi_0}{kT}\right) \tag{4.6}$$

where K_a is the dissociation constant of functional groups adsorbed on the surface of charged particles, and v_- and v_0 are the number of negative and neutral functional groups at the interaction sites having different proton concentration, respectively. A combination of physical (Van der Waals), chemical (chemisorption), and ionic (electrostatic) factors influences the adsorption processes (Nowack et al., 2012). Conventional Langmuir and Freundlich isotherms model surface coverage of adsorbates onto colloid adsorbents, where the former accounts for the chemisorption (Eqn. 4.7), and the latter adsorption in low concentrated aqueous environment (Eqn. 4.8).

$$\theta_s = \theta_{max}\frac{K_{ads}C_s}{1 + K_{ads}C_s} \tag{4.7}$$

$$\theta_s = K_{ads}C_s^{1/n} \tag{4.8}$$

where, θ_s is the quantity of adsorbed species, θ_{max} is the saturated adsorbate quantity, K_{ads} is the adsorption constant that varies with adsorbate, C_s is the equilibrium concentration of the adsorbate in solution, and $1/n$ is the Freundlich isotherm constant intensity parameter.

Transport mechanisms in aqueous environment influence the mobility and interaction of colloids through collision and attachment processes, and similar aspects have been proposed for the ENMs. Aggregation is a second-order rate whereby collision frequency between two particles that coalesce together, and is expressed by the Smoluchowski model (Eqn. 4.9).

$$J_{ij} = K_{ij}n_in_j \tag{4.9}$$

where J_{ij} is the collision frequency between the *i*-th and *j*-th particles, K_{ij} is the second-order collision rate constant, and n_i and n_j is the concentration of *i*-th and *j*-th particles, respectively. The Smoluchowski model estimates the continuous collisions between particles within a spatial regime but excludes hydrodynamic factors as well as organic and inorganic colloid interactions with particles.

Combining Brownian motion, drag forces, and sedimentation model in the Smoluchowski model – the collision rates expressed in Eqn. 11 account for different aqueous fluid regimes, namely: (i) perikinetic aggregation rate , K_{ij}^{p} , that incorporates Brownian-diffusion rate from Stokes-Einstein model (Eqn. 4.10), (ii) orthokinetic aggregation rate, K_{ij}^{o} , from Camp and Stein model that considers laminar with fixed particle concentration (Eqn. 4.11), and (iii) differential sedimentation rate, K_{ij}^{ds}, where particles and aggregated forms settle out of a suspension in accordance to Stokes' Law (Eqn. 4.12) (Elimelech et al., 1995).

$$K_{ij}^{p} = \frac{2kT}{3\mu}\frac{\left(a_i + a_j\right)^2}{a_ia_j} \tag{4.10}$$

$$K_{ij}^{o} = \frac{4}{3}G\left(a_i + a_j\right)^3 \tag{4.11}$$

$$K_{ij}^{ds} = \left(\frac{2\pi g}{9\mu}\right)(p_s - \rho)\left(a_i + a_j\right)^3\left(a_i - a_j\right) \tag{4.12}$$

where, μ is the viscosity of the solution, a_i and a_j are the radii of *i*-th and *j*-th particles respectively, G is the shear rate, g is the acceleration due to gravity, and p_s and ρ is the density of the particle and solution, respectively. Aggregation process increases the size of interacting surfaces, which form fractals of irregular shapes. The size of fractals influence Brownian motion in orthokinetic process where the collision rate in Eqn. 4.11 is modified to Eqn. 4.13 to include the fractal dimensions of aggregated colloids.

$$K_{ij}^{o} = \frac{4}{3}\, G a_0^3 \left(i^{1/df} + j^{1/df}\right)^3 \qquad 4.13$$

where, a_0 is the radius of the primary particles, i and j are the *i*-fold and *j*-fold aggregate sizes respectively, and d_f and is the fractal dimension. Aggregated size reduces the surface area of interaction, which influences the overall stability of the colloids. At pH above PZC, aggregate fractals of humic acid adsorbed on $nTiO_2$ increase with increasing size of the ENM in monovalence potassium ion environment, whereas ionic strength has reverse effects on the size of the fractals (Chowdhury et al., 2013). The fractal packing density is used to determine the hydrodynamic size of colloidal aggregates formed (Eqn. 4.14)

$$R_i = a_o \left(\frac{i}{\varphi_o}\right)^{1/df} \qquad 4.14$$

where, R_i is the hydrodynamic size of colloid aggregates, φ_o is the packing density. Sedimentation of symmetrically spherical colloids in concentrated suspensions has been described using boundary conditions of the slipping plane and the hydrodynamic size, based on improved Ohshima's theory (Carrique et al., 2001). Stokes' law is used to estimate the sedimentation velocity of neutral and impervious colloids in static settlement, whereas Eqn. 4.15 (Carrique et al., 2001) explains the sedimentation velocity of charged particles in the direction of the gravitational force field.

$$\mu_{sed} = \frac{2h(R_{ih})}{(R_{ih})} g \qquad 4.15$$

where, μ_{sed} is the sedimentation velocity of charged spherical colloids, R_{ih} is the hydrodynamic radius of the *i*-th colloid, and h is the radial function of the colloid at the slipping plane. From Eqn. 4.17, the Stern layer influences colloid settlement, whereby standard sedimentation, a dimensionless value which is the ratio of sedimentation rate of charged particles and the uncharged ones, is ascribed to this (Carrique et al., 2001). Low sedimentation rates associated with repulsive forces imply stable regimes (Pettibone et al., 2008). The widely used model of Richardson and Zaki (1954) describes the hindered settling of particles (Eqn. 4.16).

$$v_s = \mu_{s,r}(1 - \mathfrak{R}\emptyset_s)^n \qquad 4.16$$

where v_s is the effective settling velocity; $\mathfrak{R}$ is approximately equal to 1; $\emptyset_s$ is the volumetric concentration of primary particles; and n is the Reynolds number of particles, where $2.5 < n < 5.5$. Eqn. 4.16 implies that the effective velocity of the particles is strongly influenced by the concentration of the particles in the suspension.

However, this settling velocity by itself does not explain the deposition rate of colloids in aquatic environments, where deposition kinetics is determined by the build-up, containment and elimination of colloids in the aqueous environment (Elimelech et al., 1995). Colloidal build-up is similar to aggregation and involves collision and attachment. In attractive double layer energy systems, the combined diffusion-convection-Derjaguin-Landau-Verwey-Overbeek (DLVO) expression in Eqn. 4.17 (Elimelech, 1994) explains the deposition rate influenced by colloidal and hydrodynamic mechanisms. With short double layer separation distance, the DLVO attractive energy explains colloid interaction Eqn. 4.18.

$$F = -\nabla(w_a + w_r) + \frac{4}{3}\pi a_i^3(\rho_s - \rho)g \qquad 4.17$$

$$w_a = \frac{-A}{(12\pi D^2)} \qquad 4.18$$

where, F is the external force vector, w_a is the attractive energy, A is the Hamaker constant, and D is the particle separation distance.

The fundamental principles described in this section have been applied to bulk charged colloidal materials found in the aquatic environment. Engineered nanomaterials do not behave like their bulk counterparts, owing to their morphology and unique properties in different environments. For example, the PZC of $nTiO_2$ shifts with changes with particle size, e.g. from 6.5 to 8.1 for sizes increasing from 3.6 to 8.1 nm (Mudunkotuwa and Grassian, 2010). To begin to understand whether these principles are applicable to ENMs, models that predict the target endpoints using multiple input variables comprising characteristics of both the ENMs and the aqueous media are necessary. To develop such models, we used data obtained from experimental laboratory studies to predict the attributes of selected endpoints, i.e. (i) the adsorbed quantity of organic species onto $nTiO_2$ (mg of species/g of $nTiO_2$), and (ii) the hydrodynamic size of the aggregated colloids (nm). Other than these laboratory data, there are limited experimental data to model the settlement and deposition of particles and aggregated forms of $nTiO_2$ in aqueous environments.

4.2.3 Neural network modelling: Case studies

A multilayer perceptron neural network (MLP-ANN) in Statistica® was used to predict target endpoints. All input variables were normalized by feature scaling over [0, 1] (Eqn. 4.19).

$$x' = a + (b - a)\frac{(x - x_{min})}{(x_{max} - x_{min})} \qquad 4.19$$

where x' is the normalized value, x is the original value from a given study, x_{max} and x_{min} are the maximum and minimum values of any specific variable, respectively, and a and b are arbitrary values between [0, 1]. In this study, the assigned values of a and b were 0.1 and 0.9, respectively. 70%, 20% and 10% of the data records were randomly selected for training, testing and validating purposes, respectively.

The number of hidden layers between the input and output layers were randomly assigned based on the number of input variables, and ranged between 3 and 14. Weight decay parameters of 0.001 to 0.01 were used to prevent overfitting of data (Bishop, 2006), while the Broyden-Fletcher-Goldfarb-Shanno (BFGS) learning algorithm was used to train the network for up to 10 000 iterations. Model selection was based on sum of squares errors between the actual and predicted outputs of each endpoint attribute. Variable importance was assessed using global sensitivity analysis of the trained models based on perturbation of the individual input variables and the effect this had on the response of the model (adsorption or aggregation).

4.2.3.1 Modelling of adsorption

The adsorption of organic substances comprised oxalic, adipic, citric, fulvic and humic acids, as well as natural organic matter onto $nTiO_2$ was predicted. These substances were grouped under '*type of organic material'* variable. The choice to use these organic materials was based on accessible representative data in scientific reports. Organic substances have different structures, dissociation constants and unique proton binding characteristics, which drive their adsorption behaviour in aqueous environment. For example, adipic and oxalic acids are dibasic, whereas citric is tribasic. Hence both forms of acids produce different conjugated functional groups on the surface of colloids and their subsequent charge effects. Conversely, fulvic and humic acids, and natural organic matter are complex acids comprising both elemental compounds and acid functional groups (http://www.humic substances.org).

Two qualitative variables, type of organic material and cations present in natural or simulated water and seven quantitative variables; concentration of organic matter (mg/ℓ), pH (unit less), ionic strength (IS)(mM), temperature (^{o}C), duration of exposure (hours), anatase crystal phase (%), and surface area concentration (SAC) were selected as model input variables. Experimental buffers used in experiments were combined and expressed as part of type of organic material. The presence of monovalent and divalent ions that contribute to ionic strength as well magnitude of surface charge of ENMs constituted the 'cations' variable. The qualitative variables were assigned arbitrary numerical values during model training, testing and validation. These values were internally recoded by Statistica® as appropriate for nominal qualitative variables.

Adsorbed mass in the reviewed literature was reported either on the basis of mass or surface area of the adsorbent. Molecular mass of organic substances and specific surface areas of $nTiO_2$ were used to convert either mass to surface area units or vice versa. Data were obtained from scientific reports (Table 4.5), whereby 265 records were available to develop the model.

4.2.3.2 Modelling of aggregation

The objective of this section was to predict the hydrodynamic size (nm) of $nTiO_2$ due to aggregation in natural and simulated water. One hundred and ninety eight records of available pre-processed and analyzed data from scientific reports (Table 5.2) were used in modelling. Predictor variables were similar to those applied in modelling the adsorption of natural organic matter (NOM) on nTiO2 in Chapter 5 namely; two qualitative variables – type of organic matter and cations, and seven quantitative variables – concentration of NOM (mg/ℓ), pH (unit less), ionic strength (mM), temperature (oC), duration of exposure (hours), anatase crystal phase (%), and surface area concentration (SAC)(m2/ℓ). However, due to lack of continuity observed in scientific reports, the cases for qualitative variables used in adsorption and aggregation modelling were dissimilar and not comparable.

The organic substances comprised NOM distinguished by natural or simulated water systems reported in the study, oxalic, fulvic and humic acids as well as inorganic carbon based on carbonic acid from dissolved salts. The quantity of estimated inorganic carbon excluded potential dissolved carbon dioxide owing to lack of information in scientific literature. In aquatic environments, cationic and anionic species coexist as a consortium, and therefore reduce the likelihood of individual ionic species influencing aggregation. The possibility of combining all cations as a predictor of the hydrodynamic size of $nTiO_2$ was explored. Data was randomly divided accordingly for training, testing and validation and used in the model.

4.3 RESULTS AND DISCUSSION

The best model for adsorption could explain approximately 99.1% of the variance in the response ($R^2 = 0.991$) (Fig. 4.2). The choice of input variables was established to have had a signifcant influence on the performance levels of the networks. For example, when the size of the $nTiO_2$ was omitted on the basis of its known inverse relationship with surface area, the network performance declined to $R^2 = 0.938$, and had several negative outlier values (results not inluded).

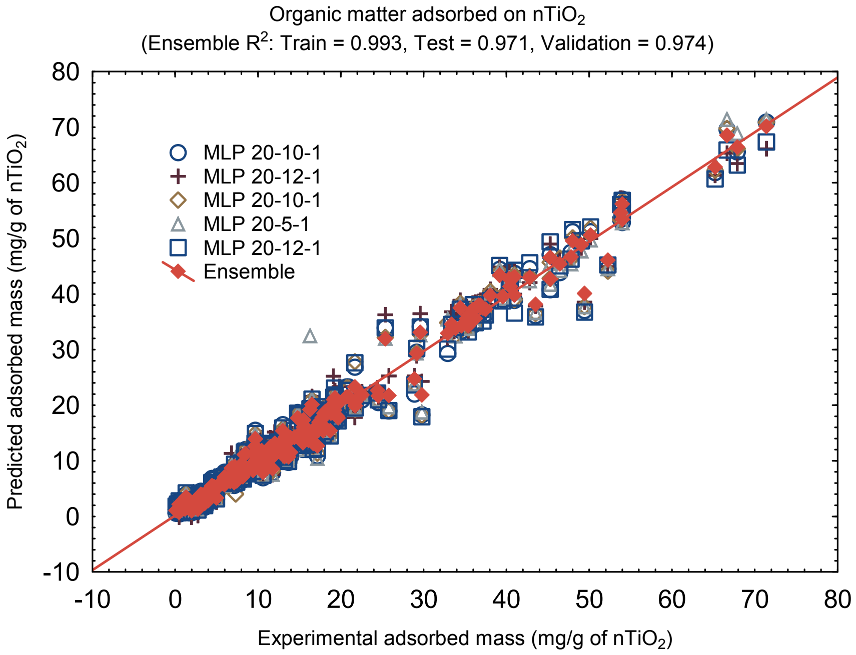

Figure 4.2: Comparison of experimental and predicted values of organic matter adsorbed onto $nTiO_2$ surface.

Similarly the best model for hydrodynamic size could explain 81.3% of the variance of the response variable (Fig. 4.3). The low prediction of aggregation compared to adsorption behaviour where similar input variables were used could be attributed to the data distribution of the target endpoint where concentration was in the 200-400 nm range (18.7%), and 1000-1200 nm range (13.64%) compared to the 2000-2400 nm range (2%). It can also be explained by heterogeneity of input variables that might have resulted in less homogeneous training, testing and validatiion. Even though similar variables were used to model both behaviours, the broad range of properties – for example cations – influenced the model performances.

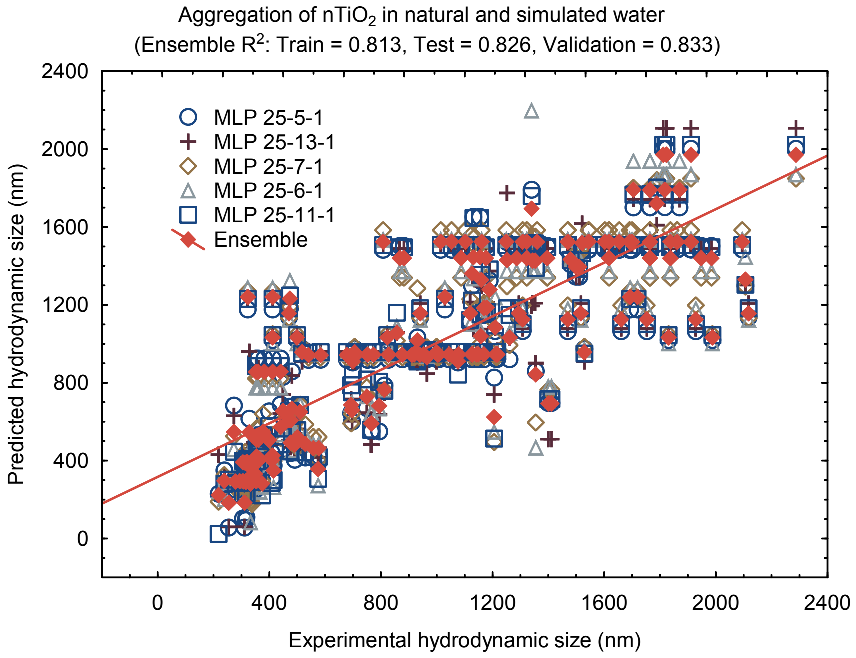

Figure 4.3: Comparison of experimental and predicted values of hydrodynamic size of $nTiO_2$ owing to aggregation.

4.3.1 Adsorption and aggregation results

The type of organic material and cations signifcantly influence both adsorption and aggregation behaviour of $nTiO_2$ (Table 4.1) when compared to the concentration of the organic compounds. The number in brackets explain the variable importance for aggregation different with adsorption (Table 4.1). This shows that the characteristics of different organic, inorganic or biological matter that exists in the environment would influence the kinetic mechanisms of ENMs after discharge. Size and concentration of $nTiO_2$, temperature and duration of exposure enhance adsorption of organic species onto the surface of $nTiO_2$ (Fig. 4.3). This corroborates earlier findings (Khan et al., 2005; Uddin et al., 2007), where adsorption energy and temperature were found to influence the adsorption capacity of organic compounds. Moreover, the fraction of adsorbed species on the adsorbent is dependent on interaction time which reduces with increasing concentration (Elimelech et al., 1995).

Results showed size as the third most influential variable on adsorption (Fig. 4.4). This corroborates the findings of Pettibone et al. (2008), where adsorption rate was not dependent on particle size, but the adsorption capacity of oxalic and adipic acids on 5 nm $nTiO_2$ particles was greater than that on 32 nm particles. Divalent cations (e.g. Mg^{2+}, Ca^{2+}, etc.) enhance adsorption of organic matter onto $nTiO_2$ at a pH above the PZC, compared to monovalent cations (e.g. Na^+, K^+, etc.) (Erhayem and Sohn, 2014). At a pH above the PZC, the surface charge of $nTiO_2$ is negative, and therefore, attracts positive divalence ions. The divalent ions bind with negatively charged functional acid groups via chemisorption (Chowdhury et al., 2012).

The pH, ionic strength as well as size and concentration of $nTiO_2$ were other influencing factors on aggregation. High concentration of organic carbon increases steric stabilization of $nTiO_2$ at low ionic strength and increases aggregation at high ionic strength (Keller et al., 2010). Complex surface adsorption can lead to irreversible processes (Mudunkotuwa and Grassian, 2010), which modifies the surface structure of $nTiO_2$. Adsorption contributes to aggregation (Simon-Deckers et al., 2009) and the size of $nTiO_2$ affect the aggregate size and the fractal sizes formed (Chowdhury et al., 2013). The particle concentration influences aggregation (Cardinale et al., 2012; Keller et al., 2010; Li and Sun, 2011; Suttiponparnit et al., 2010; Xiong et al., 2011), and large aggregate sizes reduce the surface area (Planchon et al., 2013). Natural organic matter has an inverse effect on aggregation (Keller et al., 2010; Ottofuelling et al., 2011), while biosorption of organisms promote dispersion (Horst et al., 2010) and surface charges influence aggregation (Godinez and Darnault, 2011).

Table 4.1: Summary of variable importance in predicting adsorption and aggregation behaviour of $nTiO_2$.

	Variable importance (Rank)		
Variable	**Adsorption**	**Aggregation**	**Additive ranking**
Type of organic material	154	10.05 (1)	164.05
Cations	110.04	9.17 (2)	119.21
Surface area concentration (SAC) (m^2/L)	55.21	4.31 (5)	59.52
Temperature (^oC)	52.8	2.51 (7)	55.31
Duration (hours)	33.55	1.36 (8)	34.91
pH	24.67	8.85 (3)	33.52
Anatase (%)	13.05	3.5 (6)	16.55
Concentration of organic matter (mg/ℓ)	12.43	1.05 (9)	13.48
Ionic strength (mM)	3.52	8.33 (4)	11.85

Adsorption and aggregation are important behavioural characteristics that influence the partitioning and potential bioavailability of $nTiO_2$ in the aquatic environment. Subsequent differential settlement, deposition, and sediment burial of particulate or aggregated $nTiO_2$ forms in aquatic environments influence how nanomaterials interacts with aquatic fauna and flora at different trophic levels, and the impact on the aquatic ecosystem health. For example, adsorption modifies the surface charge of $nTiO_2$ through neutralization or bridging, and in turn the electrostatic or steric stability (Li and Sun, 2011). Adsorbed organic acids also lower the attractive forces at the PZC and promote dispersion (Mudunkotuwa et al., 2008). Therefore, the stability of ENMs points to high accumulation potential in the water column regime, enhanced transport, and bioavailability to water column organisms. The suspended material can also enhance turbidity which constrains natural oxygen exchange between the surface and the aerobic aquatic organism in the subsurface. Conversely, under conditions where the ENMs stability is poor, aggregation leads to their sedimentation where risk to benthic organisms increases (Musee et al., 2010).

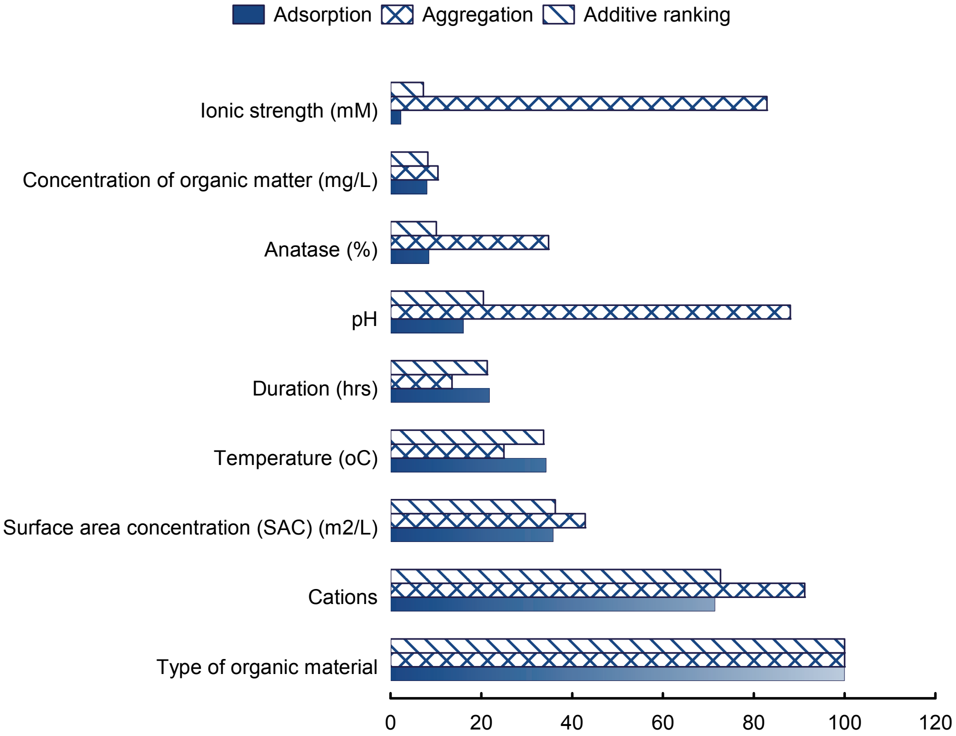

Figure 4.4: Variable importance of predictor variables influencing adsorption and aggregation behaviour of $nTiO_2$.

Table 4.2: Summary of best performing Neural Networks.

Target / Topology	Training error	Test error	Validation error	Epoch	Hidden activation	Output activation
Adsorption						
20-10-1	1.68	4.69	4.41	117	Tanh	Tanh
20-12-1	1.91	4.86	2.15	139	Tanh	Tanh
20-10-1	1.48	3.88	4.36	156	Tanh	Tanh
20-5-1	1.83	4.57	4.55	182	Tanh	Tanh
20-12-1	1.66	5.10	4.26	115	Tanh	Tanh
Aggregation						
25-5-1	51573.24	44338.55	45602.21	58	Tanh	Tanh
25-13-1	55581.72	40375.29	55337.04	40	Tanh	Tanh
25-7-1	48476.42	47349.94	56968.65	60	Tanh	Tanh
25-6-1	56483.54	29789.04	57036.62	37	Tanh	Tanh
25-11-1	49406.68	44831.81	57386.05	59	Tanh	Tanh

Hence, the estimated concentration of $nTiO_2$ in the water column or sediments indicates different risk levels for different aquatic ecologies. For example, research studies suggest that long term effects may occur when $nTiO_2$ interacts with biological organisms such as heterogeneous bacterial biofilms (Battin et al., 2009), algae (Cardinale et al., 2012), and *Escheridia coli* (Chowdhury et al., 2013; Planchon et al., 2013).

4.4 CONCLUSIONS

The characteristics of ENMs, specifically $nTiO_2$ in this study, are dissimilar even from the same batch. Standardizing these properties to estimate their kinetic behaviour using classical deterministic models would be challenging. Neural Networks, a data mining algorithm, reveals the possibility of extracting useful information from multiple and fragmented data to predict adsorption and aggregation behaviour of $nTiO_2$. The computational model generalizes the relationships of predictor and response variables. The models explain relationships based on scientific principles associated with the behaviour of colloids in aquatic environments. The findings show that irrespective of data variations depending on the source, data mining algorithms exploit the differences to predict the potential phenomena of ENMs in aquatic environment.

Table 4.3: Summary of the characteristics and codes of aqueous composition variable.

Aqueous composition	Reference
Optima water, [1]HEPES,Oxalic acid	Pettibone et al., 2008
Optima water, [2]MES, Adipic acid	Pettibone et al., 2008
Optima water, Oxalic acid	Pettibone et al., 2008
Optima water, Citric acid	Mudunkotuwa and Grassian, 2010
Optima water, [2]MES,Citric acid	Mudunkotuwa and Grassian, 2010
Optima water, [1]HEPES,Citric acid	Mudunkotuwa and Grassian, 2010
Deionized water, [3]SRHA	Erhayem and Sohn, 2014, Chowdhury et al., 2012
Deionized water, [4]SRNOM	Erhayem and Sohn, 2014
Deionized water, [5]SRFA	Erhayem and Sohn, 2014,Li and Sun, 2011
Optima water, [6]Aqueous NOM	Pettibone et al., 2008
Santa Barbara sea water	Keller et al., 2010
Mesocosm freshwater	Keller et al., 2010
Deionized water, [6]Aqueous NOM	Li and Sun, 2011, Ottofuelling, et al., 2011
Peat bog water	Ottofuelling et al., 2011
[7]EPA very soft water	Ottofuelling et al., 2011
[8]EPA moderately hard water	Ottofuelling et al., 2011
Groundwater	Ottofuelling et al., 2011
Lakewater	Ottofuelling et al., 2011
Seawater	Ottofuelling et al., 2011
Tapwater	Ottofuelling et al., 2011
Wastewater inflow	Ottofuelling et al., 2011
Wastewater outflow	Ottofuelling et al., 2011
[9]EPA very hard water	Ottofuelling et al., 2011

[1]HEPES, (4-2(-hydroxyethyl)-1-piperazineethanesulfonic acid buffer; [2]MES, 2-(N-morpholino) ethanesulfonic acid buffer; [3]SRHA,Suwannee River humic acid; [4]SRNOM, Suwannee river natural organic matter; [5]SRFA, Suwannee river fulvic acid; [6]Aqueous NOM, background natural organic matter in deionized or optima water that is 1.8 multiplied by measured organic carbon; [7,8,9] synthetic water prepared using standard preparations by Environment Protection Agency, USA.

Table 4.4: Summary of data used in predictive modelling of adsorption behaviour of $nTiO_2$.

Properties of $nTiO_2$	Properties of aqueous solution	Organic matter	Study
5 nm, 219 ± 3 m^2/g, 32 nm, 41 ± 2 m^2/g, Anatase, 500-6000 mg/ℓ, Structured and Amorphous Material Inc.	Optima water, pH 2, 10 mM HCl, 20 mM NaCl, pH 5.5, 20 mM NaCl, MES, pH 6.5, HEPES, 20 mML NaCl salt, $25°C$, 2 hours contact time.	Oxalic and adipic acids, 0.5-2.2 mM	Pettibone et al., 2008
4 nm, 219 ± 3 m^2/g, Anatase, 2000 mg/ℓ, Structured and Amorphous Material Inc.	Optima water: pH 2, HCl, pH 4, HCl, pH 6, MES, pH 7.5 HEPES, 30 mM NaCl salt, $20°C$, 24 hours contact time.	Citric acid, 0.1-5 mM	Mudunkotuwa and Grassian, 2010
21 nm[1], 500 mg/ℓ, Anatase-rutile mixture[1].	Deionized water, pH 4.8 and 7.8, 10-100 mM, NaCl, KCl, $CaCl_2$ and $MgCl_2$ salts,$22°C$, 48 hours contact time.	Humic and fulvic acids, natural organic matter, 10 mg/ℓ.	Erhayem and Sohn, 2014
18.4 ± 6.0 nm^2, 10 mg/ℓ, Anatase-rutile mixture[2], P 25 Evonik Degussa, New Jersey.	Deionized water, pH 5 and 7, 10 mM, KCl, $CaCl_2$, $23°C$, 24 hours contact time.	Humic acid, 1 mg/ℓ	Chowdhury et al., 2012.

[1] http://www.sigmaaldrich.com/catalog/product/Aldrich/718467?lang=enandregion=ZA; 2 Chowdhury et al., 2011.

Table 4.5: Summary of data used in predictive modelling of aggregation behaviour of $nTiO_2$.

Properties of $nTiO_2$	Properties of aqueous solution	Organic matter	Study
5 nm: 219 ± 3 m^2/g, 50 mg/ℓ, 32 nm: 41 ± 2 m^2/g, 100 mg/ℓ, Anatase, Structured and Amorphous Material Inc.	Optima water, pH 2, 10 mM HCl, 20 mM NaCl,$25°C$, 24 hours contact time.	Oxalic acid, 5 nm: 0.05 mM, 32 nm: 0.1 mM	Pettibone et al., 2008
27 ± 4 nm, 51.5 ± 3 m^2/g, anatase (82%) and rutile (18%), 10, 50, and 100 mg/ℓ, Evonik Degussa 4168063098, USA.	Seawater: pH 8.05, K+ (377.80 mg/ℓ), Ca2+ (398 mg/ℓ), Mg2+ (1361.4 mg/ℓ), ionic strength (0.707 eq./ℓ) Mesocosm freshwater: pH 8.38, K^+ (22.28 mg/ℓ), Ca^{2+} (17.66 mg/ℓ), Mg^{2+} (11.37 mg/ℓ), ionic strength (0.00718 eq./ℓ) $22 \pm 2°C$, 24 hours contact time.	Natural organic matter: Seawater, 54 µM C, Mesocosm freshwater, 5,283 µM C.	Keller et al., 2010
5 nm, 1 mg/ℓ, [1]219 m^2/g Anatase, Structured and Amorphous Material Inc.	Water characteristics, refer to Table Deionized water, pH 8 10 mM,$23°C$, ≤ 48 hours contact time.	Fulvic acid, 1 mg/ℓ.	(Domingos et al., 2009)
19.8 nm, 57 m^2/g, anatase (88%) and rutile (12%), 25 mg/ℓ, P 25, Evonik Degussa, Germany.	Refer to Table 1 for water characteristics (Codes 114-123), pH 3-10, 0.30-146.5 mM, K^+ (1.0-340 mg/ℓ), Ca^{2+} (2.0-430 mg/ℓ), Mg^{2+} (2.0-1300 mg/ℓ), Fe^{3+} (0.2-3.5 mg/ℓ), $25°C$, 15 hours contact time.	Natural organic matter, 0.9-121.5 mg/ℓ.	Ottofuelling et al., 2011
30 nm, 40 m^2/g, 50 mg/ℓ, P 25, Evonik Degussa, Germany.	Deionized water, pH 4, 6, 8, 5 mM, Fe^{3+}(≤ 0.2 mM), $22.5°C$, 24 hours contact time	Fulvic acid, 0.5, 2.5 mg/ℓ.	Li and Sun, 2011

[1]Pettibone et al., 2008

CHAPTER 5: MODELLING OF THE ENVIRONMENTAL IMPACTS OF NANO-TIO2 ON ALGAE AND *DAPHNIA MAGNA* USING NEURAL NETWORKS

5.1 INTRODUCTION

Risk assessment comprise four distinctive aspects, namely hazard, dose-response, exposure assessments, and risk characterization (Mihelcic and Zimmerman, 2010). As emerging pollutants, environmental hazard assessment of ENMs is yet to be established due to lack of analytical techniques for in situ studies as described in Chapter 4.Therefore, the linkage of effects from *in vitro* and *in vivo* studies to ecological impacts is difficult. Dose-response assessments have gained momentum since they were first reported in 2004 (Oberdörster 2004), but are characterized by uncertainties accruing from the generation of multiple sources of data, for example, arising from using different sample preparations and analytical techniques under different laboratories conditions. In addition, due to high costs, higher doses of ENMs – for the ease of detection – than what would be practically emitted into the environment have been used to test acute toxicity in small sample populations of aquatic organisms (Heinlaan et al. 2008). The extrapolation of data from these findings may not reveal chronic toxicity impacts common at low levels (Mihelcic and Zimmerman, 2010).

On the other hand, exposure assessment entails evaluation of the occurrence and magnitude of chemical toxicants to receptor organisms. Similarly, lack of knowledge on the quantities of ENMs emitted into the environment, limits what can be achieved with this methodology. Besides models that have been exploited to estimate likely environmental concentrations of ENMs, only a few scientific reports have documented their isolated in situ occurrence in the environment (refer to Chapter 4). The exposures of ENMs in different environments from sources such as industrial plants, commercial businesses, freshwaters, etc. remain to be fully reported.

The uncertainties on risk assessment of ENMs are propagated to the risk characterization phase – which entails integration of assessment processes. Until now, there are no known acceptable levels of risks associated with ENMs apart from those due to their bulk forms. The effective concentration (EC) (Aruoja et al., 2008;Hartmann et al., 2010) and lethal concentration (LC) (Zhu et al. 2010; Aruoja et al., 2008) are some of the standard toxicity endpoints that have been adopted to estimate acute and chronic toxicity of ENMs to target organisms. The LC is based on solubility of a chemical pollutant. Given $nTiO_2$ has negligible solubility in water (Planchon et al., 2013), the use of this endpoint as a measure of toxicity plausibly introduces high uncertainties.

Multiple factors influence the toxicity of a chemical pollutant in the environment, hence renders risk assessment highly complex. The aquatic ecosystem consists of multiple biotic fauna and flora that interact with the abiotic factors (e.g. temperature, sunlight, particulates, etc.). Ecosystems have carrying capacities beyond which the influx of materials that either influences diminishing or blooming population would need to be regulated. As such, an understanding on the functions of food web, bioaccumulation of pollutants, and toxicity impacts are relevant aspects in risk assessment (Mihelcic and Zimmerman, 2010). Research findings indicate that the toxicity of ENMs is dependent on the species of exposed organisms (Clément et al., 2013), developmental stage and generation of the species (Jacobasch et al., 2014), absence or presence of food (Campos et al., 2013), and structure of ENMs, for example, anatase shows more toxic effects than rutile form of $nTiO_2$ (Clément et al., 2013). Rutile and anatase $nTiO_2$ have distinct properties even in mixtures (Ohtani et al., 2010) attributed to differences in their structure and photon energy. In addition, duration of exposure, concentration, and aggregation influence toxicity (Clément et al., 2013).

To contribute in addressing the uncertainties posed by lack of a systematic method to assess and characterize the risks of ENMs in aquatic ecosystems, in this work, we proposed and developed a modelling method that seeks to identify the most relevant/significant predictor variables for aquatic toxicity based on available published scientific data. Our model forms basis of identifying parameters that need to be tested and reported in experimental studies (for effects and behaviour) to generate data useful to extrapolate findings to aquatic ecosystems. On the other hand, the model seeks to serve as a guideline helpful in assessing risks of ENMs where few measurable variables are available. Long term effects of ENMs can be elucidated using population growth rates of exposed organisms as opposed to focusing on toxic concentrations of ENMs. For a given period of exposure, growth, survival and fecundity of organisms are intermediate endpoints to the abundance or reduction of population responses whereas mortality as an endpoint signifies the surviving species.

5.2 METHODOLOGY

The conceptual framework for modelling the impacts of $nTiO_2$ to model organisms consisting of eleven algae and one *Daphnia* species in aquatic ecosystems is depicted in Figure 4.1 as described in Section 4. The model organisms used in this study play an important role in the aquatic food chain; namely the algae and *Daphnia* as members of primary producers and primary consumers, respectively. The motivation for the choice of these organisms was based on an understanding that potential long-term biological effects of ENMs may have impacts on the food chain (Figure 5.1). To date limited mixed aquatic organisms have been tested either *in situ* or in laboratory mesocosms to understand the interplay of food availability and predation in the presence of ENMs; and therefore, such a scenario will not be considered in this study due to lack of data.

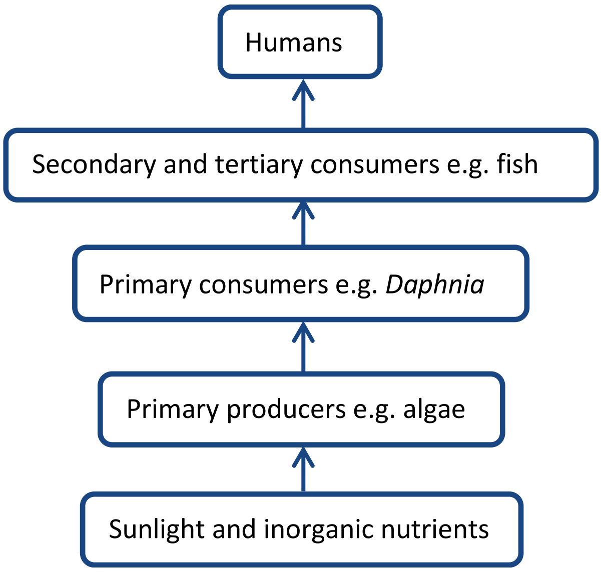

Figure 5.1: A simplified food chain in an aquatic system
Modified from: Mihelcic and Zimmerman (2010)

As before, the data for modelling were obtained from scientific reports, and the results for *Daphnia magna* and algae are summarized in Table 5.1 and 5.2, respectively. Six target endpoints were chosen, i.e. (i) three organism-level responses, namely immobilization and mortality of *D. magna*, and growth rates of *D. magna* and algae, and (ii) two population responses that were reproduction in *D. magna* and biomass abundance of algae. All endpoint attribute tuples were designated outcomes equivalence to normalized percentages of negative control data, which tests the health of organisms (United States Environmental Protection Agency 2002).

Table 5.1 Summary of data used in predictive modelling of the effects of $nTiO_2$ to *D. magna*

Properties, source and test concentration of $nTiO_2$	Test media properties and standards	Study
140±44 nm (agglomerate size): 38.5 m^2/g, anatase (21%) and rutile (79%), coated with alumina (7%) and silica (1%); and 380 nm (agglomerates): 5.8 m^2/g, rutile (100%), coated with alumina (1%), concentration (0.1-100 mg/ℓ). Both from DuPont Inc.	Reconstituted water: pH 7.5 [1], Ionic strength (IS) (0.35 mM) [2], TOC (0.38 mg/ℓ) [2], temperature 20.1 and 20.2 °C, exposure time (48-hours). Test standard: OECD 202, 2004	(Warheit et al. 2007)
32 nm (primary size), 45 m^2/g, anatase (100%), 0.01-100 mg/ℓ; and, 15 nm (primary size), 240 m^2/g, anatase (100%), 0.01-100 mg/ℓ. Uncoated powders from Alfa Aesar	Reconstituted Daphnia culture media: pH 8 [3], IS (8.43 mM) [2], TOC (9.27 mg/ℓ) [2], 22 °C, 48 and 72-hours. OECD 202, 2004	(Clément et al. 2013)
<40 nm (primary size), <200-800 nm (agglomerated size in test media) 50 m^2/g [4], anatase (70%) and rutile (30%), 0.5-10 mg/ℓ, uncoated nanoparticles from Sigma Chemicals, St. Louis. MO, USA.	Synthetic hard water: pH 7.8, IS (8.16 mM) [2], TOC (27.46 mg/ℓ) [2], 25 °C, 48 to 504-hours. US EPA 1993	(Kim et al. 2010)
<40 nm, 50 m^2/g [4], anatase (70%) and rutile (30%), 0.5-10 mg/ℓ, uncoated nanoparticles from Sigma Chemicals, St. Louis. MO, USA.	Synthetic moderately hard water: pH 7.6, IS (4.24 mM) [2], TOC (13.73 mg/ℓ) [2], 25°C [5], 48 to 504-hours. US EPA 1993	(Kim et al. 2014)
P25: 50 m^2/g, anatase (80%) and rutile (20%), uncoated powder from Degussa, Essen, Germany. 0.1-5.0 mg/ℓ	Reconstituted Daphnia culture media: pH 8 [3], IS (8.43 mM) [2], TOC (9.27 mg/ℓ) [2], 22 °C, 24, 48 and 72-hours. Modified OECD 202, 2004	(Zhu et al. 2010)
P25: 21 nm (primary size), 50 m^2/g [6], anatase (80%) [6] and rutile (20%) [6], 1.19-6.0 mg/ℓ, uncoated powder from Degussa, Essen, Germany.	Elendt 4 media: pH 8.2 [1], IS (8.51 mM) [2], TOC (9.27 mg/ℓ) [2], 20°C 504-hours. OECD 211, 2008	(Jacobasch et al. 2014)
NM-103: 60 m^2/g, rutile (100%), coated with alumina and siloxane; **NM-104**:60 m^2/g, rutile (100%), coated with alumina, siloxane, and glycerol, both from Joint Research Centre (JRC) in European Union (EU) repository; and **P25**: 21 nm (primary size), 50±15 m^2/g, anatase (78%), rutile (14%) and amorphous (8%), uncoated from Evonik Degussa, Germany. Concentration (1.0 and 10 mg/ℓ).	Synthetic hard water: pH 8 [3], IS (8.23 mM) [2], TOC (27.46 mg/ℓ) [2], 22 °C, 504-hours. ASTM, 1980	(Campos et al. 2013)

[1] Estimated from minimum and maximum values in test standards applied; [2] Computed using concentration of salts specified in test standards; [3] (Zhu et al. 2010); [4] Sigma Aldrich website; [5] (Kim et al. 2010); [6] Evonik Degussa website.

Control data served as baseline conditions for organisms to thrive without chemical pollution – in this case ENMs – where normalization defined the effects arising from exposure. Negative control in experiments tests the health of the organims. Abbott's formula (Abbott 1925) was used to normalize immobilization and mortality data whose units were reported as percentages in scientific reports. Conversely, the formula described in OECD guidelines (OECD, 2011) was used to normalize data from specific concentration exposures to the control. The normalization of data were aimed to reflect $nTiO_2$ effects through standardizing different units published in diverse sources for comparable endpoints, for example, fecundity and number of off-springs for *D. magna* in the reproduction endpoint.

Table 5.2 Summary of data used in predictive modelling of the effects of $nTiO_2$ to algae.

Properties, source and test concentration of $nTiO_2$	Test media properties and standards, and species	Study
140±44 nm (agglomerate size): 38.5 m^2/g, anatase (21%) and rutile (79%), coated with alumina (7%) and silica (1%); and 380 nm (agglomerates): 5.8 m^2/g, rutile (100%), coated with alumina (1%); both from DuPont Inc. Concentration (0.1-100 mg/ℓ).	Synthetic algal assay procedure nutrient media: pH 7.5 [1], Ionic strength (IS) (1.001 mM) [2], TOC (2.15 mg/ℓ) [2], temperature (23.8 °C), exposure time (72-hours), sunlight (8938 lux). Test standard: OECD 201, 1984. Species: *Pseudokirchneriella subcapitata.*	(Warheit et al. 2007)
Hombikat UV100: <10 nm (primary size), 288 m^2/g, anatase (67.2%) and amorphous (32.8%), zeta potential (-23 mV), uncoated from Sachtleben Chemie GmbH; **P25**: ca. 30 nm (primary size), 47 m^2/g, anatase (72.6%), rutile (18.4%) and amorphous (9%), zeta potential (-21 mV), uncoated from Frederiksen, Ølgod, Denmark; **Hombitan LW-S**: ca. 300 nm, 11.5 m^2/g, anatase (100%), zeta potential (-25 mV), uncoated from Sachtleben Chemie GmbH. 0.6-250 mg/ℓ.	Algal test Media: pH 7.5, 7.6 and 7.6 for Hombikat-UV100, P25, and Hombitan-LW-S $nTiO_2$ respectively, IS (1.5 mM), TOC (7.16 mg/ℓ) [2], 20°C, 72-hours, sunlight (growth light intensity of 80-105 µE/m^2/s). Modified ISO 8692, 2002. *Pseudokirchneriella subcapitata.*	(Hartmann et al. 2010)
25-70 nm (primary size), 18.6 m^2/g [3], anatase (100%) [3], uncoated nanoparticles from Sigma Aldrich. 6-48 mg Ti/L.	Algal medium water: pH 5.5, IS (1.41 mM) [2], TOC (7.16 mg/ℓ) [2], 24 °C, 24, 48 and 72-hours, sunlight (Irradiance at 400-500 nm). Test standard: OECD 201, 1984. *Pseudokirchneriella subcapitata.*	(Aruoja et al. 2008)
Ca. 300 nm (agglomerate size), 51.5 m^2/g [4], anatase (82%) and rutile (18%), uncoated nanoparticles from Evonik Degussa Corporation 4168063098. 50-300 mg/ℓ	Sterilized soil water media: pH 8.38, IS (7.18 mM), TOC (63.45 mg/ℓ), 18 °C, 72-504-hours, sunlight (Irradiance at 360-400 nm, 0.002-0.004 W/m^2). Test method [5]. *Clamydomonas moewusii, Chlorella vulgaris, and Scenedesmus quadricauda*.	(Cardinale et al. 2012)
27±4 nm (primary size), 50±15 m^2/g, anatase (82%) and rutile (18%), uncoated nanoparticles from Evonik Degussa Corporation CAS No. 13463-67-7. 50-300 mg/ℓ.	Sterilized soil water media: pH 8.38, IS (7.18 mM), TOC (63.45 mg/ℓ), 18 °C, 24-600-hours, sunlight (Irradiance at 1.13 W/m^2). Test method [5]. *Anabaena spp.*, *Navicula subminiscula*, *Nitzschia pusilla*, *Oscillatoria spp.*, *Planothidium lanceolatum*, *Scenedesmus quadricauda, Selenastrum minutum, Spyrigyra communis, Stigeoclonium tenue, and Tabularia fasciculate.*	(Kulacki and Cardinale 2012)

[1]Estimated from minimum and maximum values in test standards applied; [2] Computed using concentration of salts specified in test standards; [3](Takeda et al., 2009); [4](Keller et al., 2010); [5](Watanabe, 2005).

Predictor variables consisted of fifteen attributes that broadly describes three properties of $nTiO_2$, three characteristics of water chemistry, two abiotic factors, six variables for the model organisms, and one for the state of experiments conducted. Surface area concentration (SAC), structure (% anatase) and surface coating were attributes designated as the nanomaterial properties. The water chemistry was represented by the pH, ionic strength and total organic carbon (TOC), whereas abiotic factors consisted of temperature and exposure duration. Unlike in the fate and transport models described in Chapter 4, where natural organic matter (NOM) was estimated using TOC values, similar estimates were not performed in this model, because the only source of carbon was from inorganic compounds added to purified water as per OECD guidelines (OECD, 2011). The presence or absence of feeding *D. magna* during experiments, type of organism (species), developmental stage at the time of exposure, culture (axenic or mixed), age and generation of the model organisms were identified as possible contributing factors to their susceptibility during exposure. Notably, the species attribute was only applicable for modelling endpoints related to algae. Some attributes were eliminated during modelling, due to lack of suitable variation in the data. For example, the development stage (i.e. cells, filaments or coliform units) and age of organisms were similar and unique to each algae species, and therefore; these attributes were eliminated in biomass abundance modeling.

Exposure conditions impacts on the likely interaction(s) between organisms and the toxicants. The last attribute, state of experiment, was explored based on observations of exposure conditions reported in literature that included: static (non-agitated) or semi-static (agitated) systems, renewal or non-renewal of either water or concentration of $nTiO_2$, and frequency of the renewal – all of which may affect exposure mechanisms. Generally organisms are not exposed to uniform concentrations of nTiO2 due to these materials tendency to adsorb, aggregate and settle in static and semi-static systems, therefore, the use of SAC attribute in this model to some extent is an over-estimation. Agitated vessels show better toxicity outcomes of $nTiO_2$ (Kim et al. 2014), whereas adherence to test vessels (Hartmann et al., 2010), and settlement (Jacobasch et al., 2014; Kim et al., 2014; Federici et al., 2007; Zhu et al., 2010) have been observed as limiting exposure factors.

Missing data of $nTiO_2$ properties and water chemistry were estimated as described in Chapter 3. Standard procedures followed during toxicity tests were critically evaluated – and aqueous media used identified to aid in estimating cations, anions, and ionic strength. Data on the properties of ENMs were either estimated from company websites or other scientific reports where similar materials had been used to conduct toxicity tests. Except in immobilization and mortality data, negative values of normalized effects signified an increase in organism's growth rate, reproduction in *D. magna* or biomass abundance in algae, and vice versa where positive values were computed. From the reviewed studies, zeta potential and aggregate size of the nanomaterials during exposure and under similar conditions were found scarcely reported (Campos et al., 2013), and hence the feasibility of modelling the behaviour and fate of nanomaterial as a plausible contributing factor to reported toxicity effects was found difficult. The pH of aqueous environment changes during exposure (Hartmann et al. 2010) but this was also not widely reported phenomenon. As a result, variables in used in the models were considered to be discrete static and acute or chronic exposure was expressed in hours.

The data handling used for modelling was prepared as described in Chapter 4. Neural networks' multilayer perceptron modelling tool was used to predict the endpoints using suitable predictor variables that were preselected based on their statistical relevance to each attribute. The number of tuples used in modelling were 42 (immobilization of *D. magna*), 190 (mortality of *D. magna*), 66 (growth rate in *D. magna*), 92 (growth rate in algae), 144 (reproduction in *D. magna*), and 482 (biomass abundance of algae). Data was randomly partitioned into 70%, 15% and 15% for the respective training, testing, and validation procedures. Other modelling procedures were identical to those described in Chapter 4.

5.3 RESULTS AND DISCUSSION

Generally, algae and daphnids are representative organisms used to evaluate chemical toxicity in the environment. Shifting focus from minimum concentration-based risks, the study modelled target endpoints as percentages of observed effects to the organisms using laboratory derived data to explain the potential ecological impacts in the natural aquatic environment. Based on available data, similar predictor variables uniuqely influence targeted response attributes as observed in Table 5.1 and 5.2 as well as Figures 5.2, 5.7 and 5.10. Table 5.3 summarises model networks.

5.3.1 Immobilization and mortality of *Daphnia magna* exposed to $nTiO_2$

The model explained 97.9% mobility and 83.5% mortality of *D. magna* exposed to $nTiO_2$ that generalised well on unseen data based on testing and validation responses (Figure 5.2 and 5.3). A broad range of effects (0-100%) on the Cladocera mobility (Figure 5.2) could be attributed to water conditions, mechanistic properties of $nTiO_2$ and organism-$nTiO_2$ interactions during exposure. Surface coating and the age of the organism had the highest influence on immobilization, followed by duration of exposure and surface area concentration (Table 5.1). Immobilization could be explained by physical, chemical and biological mechanisms of interaction.

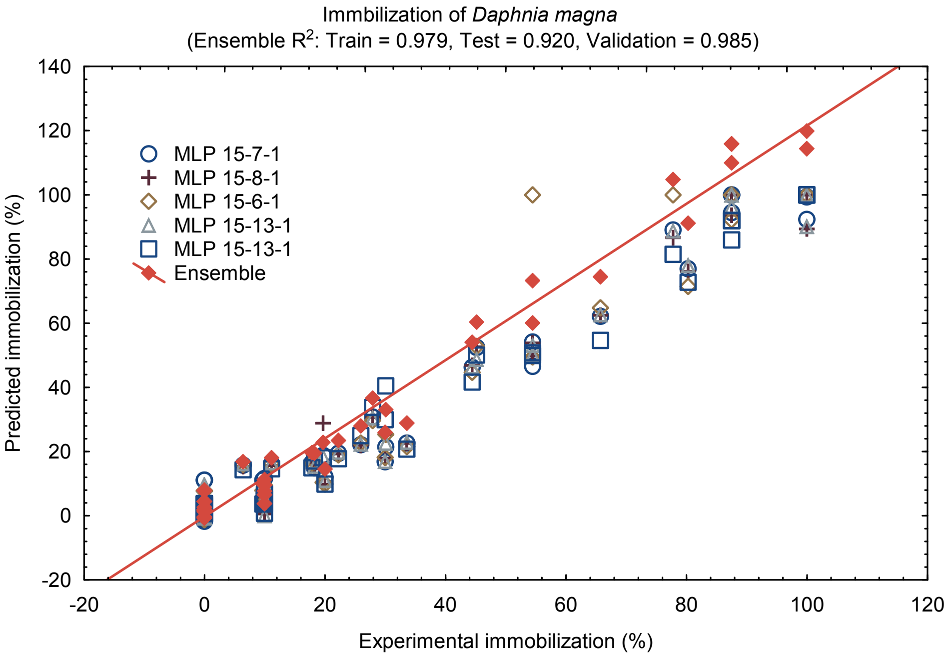

Figure 5.2: Comparison of experimental and predicted immobilization of *D. magna.*

Physically, the data used in modelling comprised 81% of uncapped nanomaterial, which implies likely enhanced adsorption and aggregation of exposed charged $nTiO_2$ surfaces. Organisms have charged surfaces (Chowdhury et al., 2012; Planchon et al., 2013). Depending on the magnitude of the surface energy, electrostatic attraction of organisms-nanoparticles and nanoparticles-nanoparticles would lead to attachment and accumulation of $nTiO_2$ on the Cladocera surface. Daphnids have small sizes whose magnitude has been attributed to their sensitivity to inorganic toxicants (Vesela and Vijverberg, 2007). The small sizes – that also vary with age of organisms – might explain immobility of *D. magna* owing to accumulated mass of $nTiO_2$ on their body surfaces.

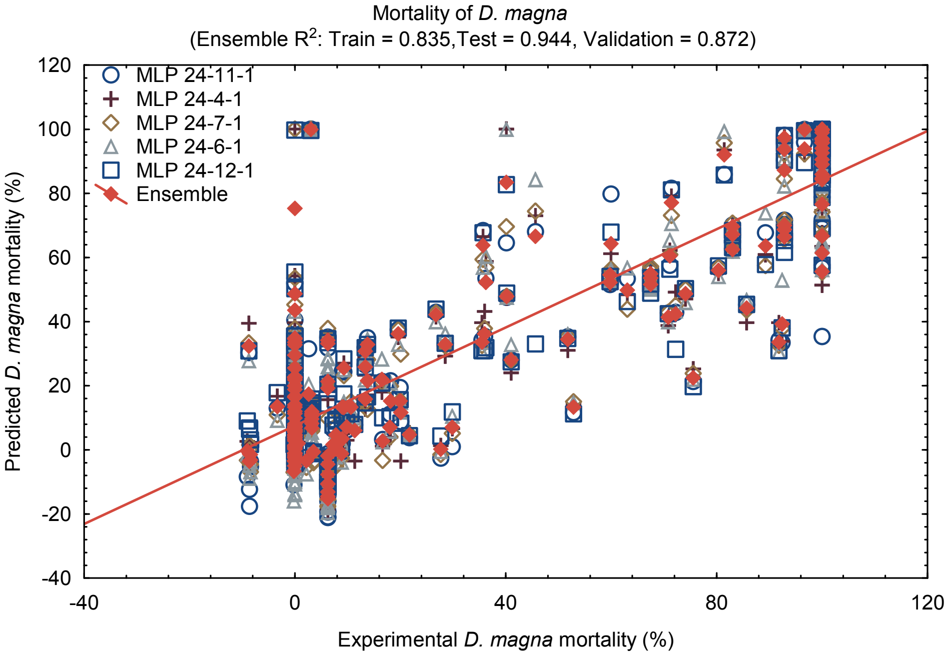

Figure 5.3: Comparison of experimental and predicted mortality of *D. magna.*

Biochemically, defence mechanisms of *D. magna* – for example – immunity factors could counter the effects of the pollutant and slow down the organism's behaviour during the process depending on the exposure period. The ENMs have different sizes and shapes, which in the colloidal form would have potential mechanistic effects on the membrane of exposed organisms. Such occurrence could cause injuries leading to immobilization as a process of healing or impairment. Behaviour changes in *D. magna* have been reported – for example increased hopping rates, heart and appendage beats and reduced post-abdominal curling that are attributed to exposure time to $nTiO_2$ (Lovern et al., 2007).

Table 5.1: Importance of variables influencing immobilization, mortality, and inhibited growth and reproduction of *D. magna* exposed to $nTiO_2$.

	Variable importance ranking			
Variable	**Immobilization**	**Mortality**	**Inhibited growth rate**	**Inhibited reproduction**
Surface coating	139.5	-	1.31 (*4*)	-
Organism age	113.75	8.03 (*3*)	-	8.51 (*3*)
Duration (hours)	75.19	5.61 (*4*)	-	2.44 (*7*)
SAC (m^2/l)	32.96	4.85 (*5*)	1.33 (*3*)	9.64 (*2*)
pH	19.73	2.15 (*8*)	1.01 (*8*)	1.8 (*8*)
Total organic carbon (mg/ℓ)	19.72	1.08 (*10*)	1.04 (*5*)	1.8 (*8*)
Ionic strength (mM)	19.65	3.67 (*7*)	1.02 (*7*)	6.03 (*4*)
Temperature (^{o}C)	19.25	1.19 (*9*)	-	3.86 (*5*)
Anatase (%)	17.51	3.68 (*6*)	1.04 (*5*)	3.1 (*6*)
Organism feeding	1	12.51 (*2*)	2 (2)	13.7 (*1*)
Organism generation	-	12.87 (*1*)	3.52 (1)	-
Development stage	-	1 (*11*)	1(*9*)	1 (*10*)

Note: The italicized numbers in brackets indicate the significant value of the variable in estimating target endpoint.

Although the data used in the modelling study is not comprehensive, it can be deduced from Table 5.1 that the species of receptor organisms, the aquatic food web, exposure duration as well as the characteristics of chemical toxicants play a significant role in explaining the mortality of biological organisms during exposure. Mortality of *D. magna* was influenced by the generation, absence or presence of food, age, duration of exposure in addition to size, concentration, morphology and crystal phase of $nTiO_2$ (Table 5.2). The low influence of environmental conditions and developmental stage to *D. magna* mortality could imply secondary effects of enhancing interactions and equal effects that lead to death during their lifecycle, respectively.

Low concentrations of $nTiO_2$ have been modelled or environmentally analysed in aquatic ecosystems and are unlikely to cause adverse acute effects to biological organisms. However, their migration, partitioning in different environmental compartments as well as persistence could lead to accumulation in organisms that is transferred in the food chain or generationally in off-springs. The *D. magna* are filter feeders that could ingest, accumulate and/or depurate $nTiO_2$ over a period of time depending on the presence or absence of food. The concentration of toxicant, duration of exposure presence and absence of food influences accumulation and depuration of $nTiO_2$ in *D. magna,* whereby high accumulation and depuration mechanisms at longer exposure and depuration times are observed in the presence of food (Zhu et al., 2009). Feeding was reported in 48% of the data used in modelling, that illustrates accumulation and hence mortality. Accumulation of nanomaterials in the gut of biological organisms may impair their metabolism leading to death.

The absence of data could not allow the use of surface coating as an input variable to compare with immobilization characteristics. Nevertheless, immobilization of *D. magna* would likely enhance their vulnerability to predators leading to death in a natural environment. Susceptibility to predation, as well as impacts to accumulated toxicants, depends on the age of the organisms that might have a bearing on development of valuable appendages and efficiency of depuration at the time of exposure.

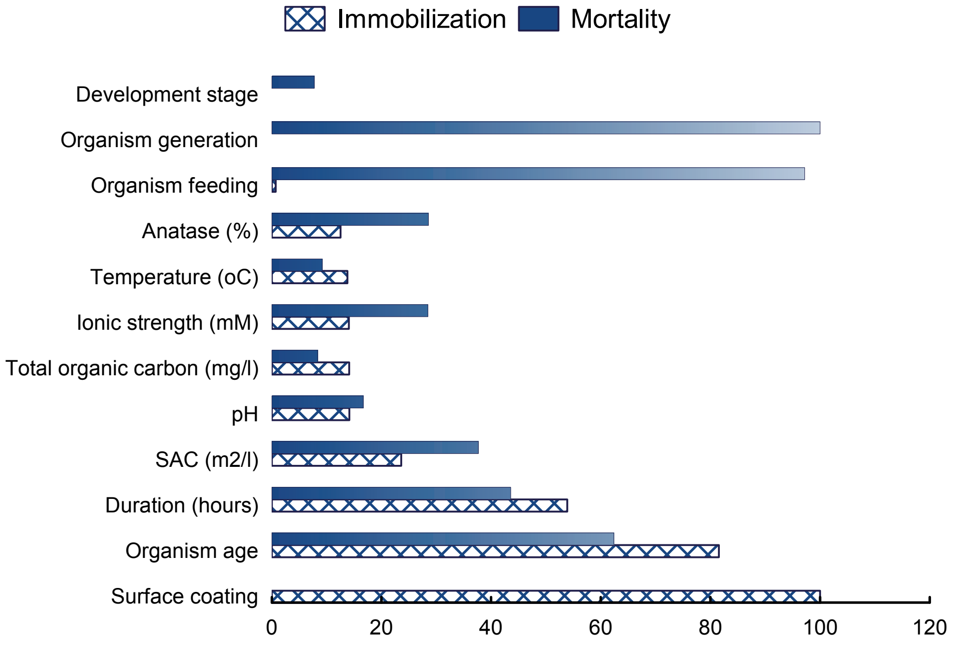

Figure 5.4: Important variables influencing immobilization and mortality of *D. magna* exposed to $nTiO_2$.

5.3.2 Inhibition to growth rate and reproduction of Daphnia magna exposed to $nTiO_2$

The model predicts the inhibited growth rate response by a 0.795 coefficient of determination (Figure 5.5). High distribution (42%) of inhibited growth is projected in the 10-40% range followed by 13.8% in the 40-70%, 10.1% in the 70-90% and 34.1% in the <10% ranges. On the other hand, the model estimates 67.62% of >50% inhibited reproduction response with high projections (51.3%) lying in the 70-100% inhibition (Figure 5.6). Compounded effects of $nTiO_2$ that might accumulate in offsprings of *D. magna* at a given age, availability of food, bioavailability of toxicants and their mechanistic properties as well as an enabling environment (Table 5.1) to the growth and reproduction capability of the Cladocera.

Potential genetic and mortality impacts are relfected in the population due to inhibited growth rate and reproduction. Long term-effcets have been reported in *D. magna* exposed to $nTiO_2$ including high bioconcentrataion factors, retardation and reproductive defects (Zhu et al., 2009). When fecundity is impared due to reproductive defects, the population is reduced and vice versa. Population dynamics of aquatic organisms have an impact on the maintenance of ecologial balance including the food chain. The model findings are in agreement with the Lotka equation that attributes population growth in ecological habitats to the age of female organisms, the survival rate and fecundity (Campos et al., 2013).

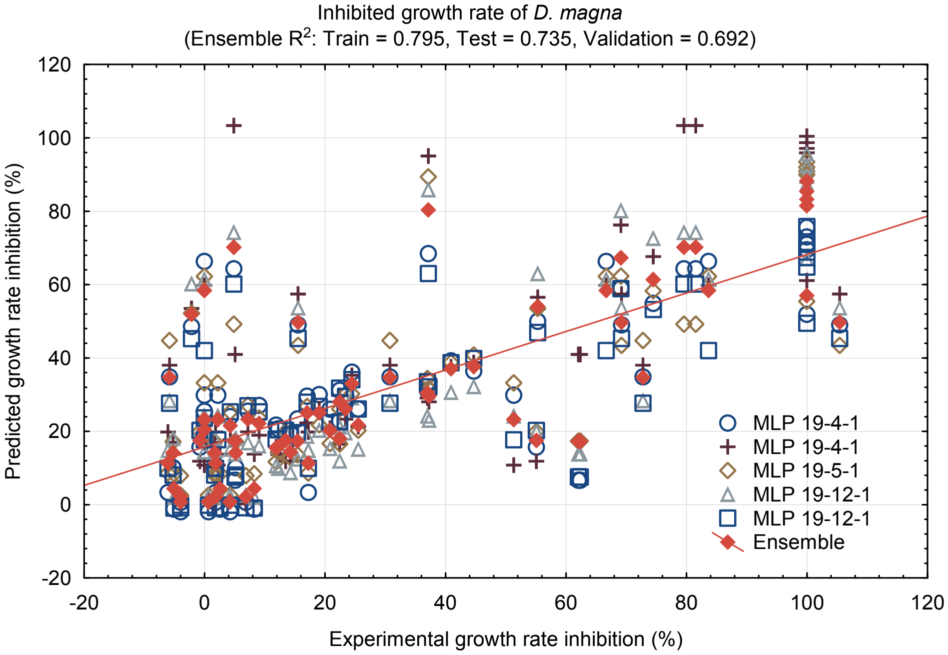

Figure 5.5: Comparison of experimental and predicted growth rate inhibition of *D. magna.*

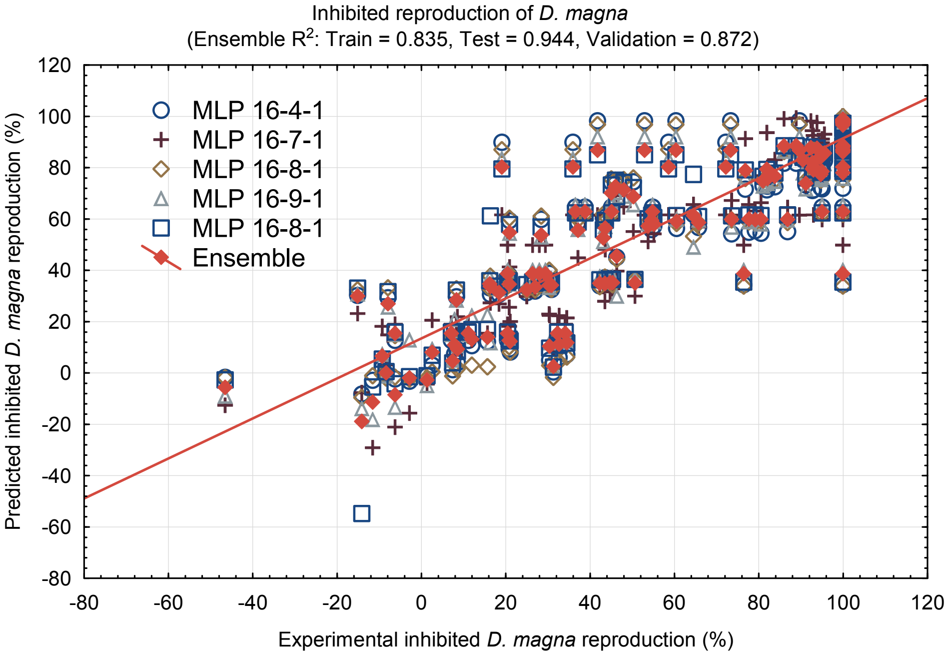

Figure 5.6: Comparison of experimental and predicted inhibition of reproduction of *D. magna.*

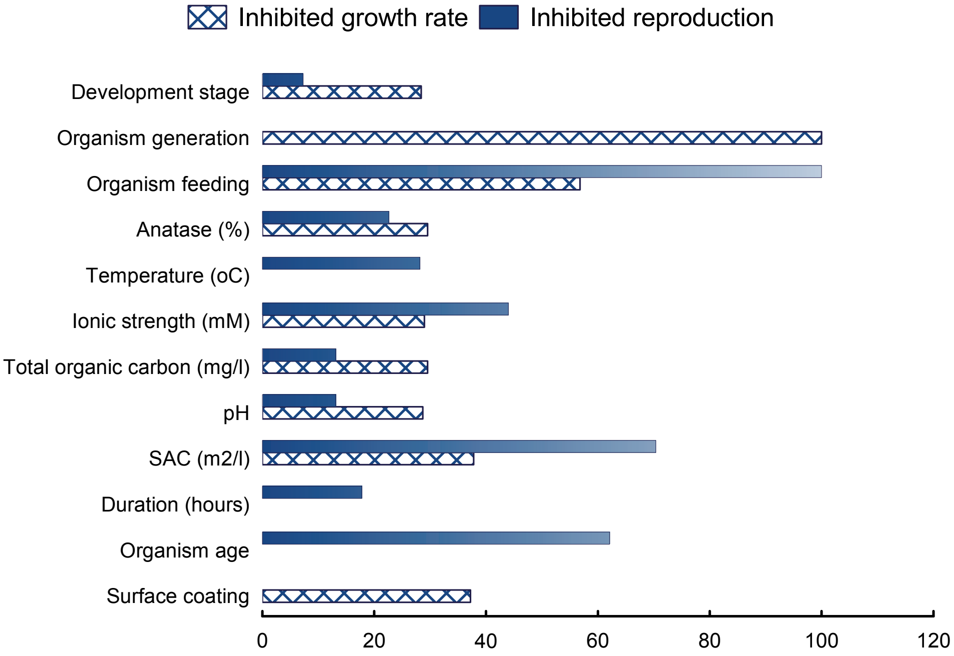

Figure 5.7: Important variables influencing inhibition of growth rate and reproduction of *D. magna*.

5.3.3 Inhibition to growth rate and abundance of algae exposed to $nTiO_2$

The prediction of inhibition to algae growth rate (97.8%) and biomass bloom (91.7) were high and the models generalized well with unseen data based on testing and validation outcomes (Figure 5.8 and 5.9). In the presence of $nTiO_2$, duration of exposure, pH, temperature and organism species are significant factors in explaining inhibited growth of algae.

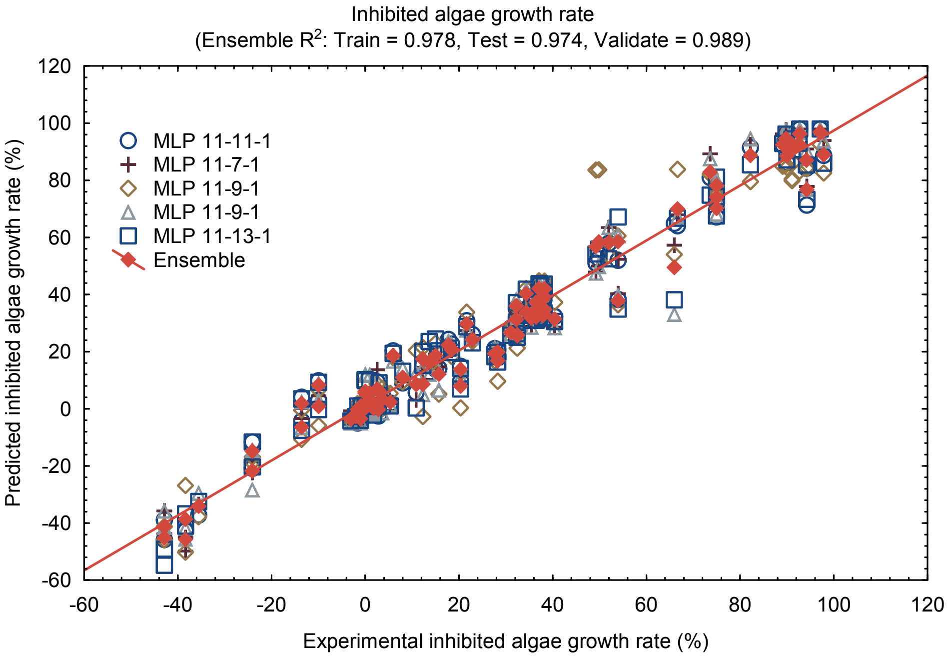

Figure 5.8: Comparison of experimental and predicted inhibition of growth rate of algae.

The data used in modelling comprised 96.3% in the 8.0-8.5 pH range, 2.1% in the 7.0-7.5 range and 1.6% in the 5.0-5.5 pH range. Prevalent low pH has been found to inhibit algae growth rate and subsequent reduced bloom of some algae species, which indicates that the growth pH in this model generally promoted growth. The Arrhenius equation describes the growth rate of algae as a function of temperature and available nutrients – in this model the total organic carbon – where Van't Hoff rule that doubles biochemical reaction with every 10°C temperature increase apply (Goldman and Carpenter, 1974). Growth and decay kinetics are a function of time predicted well by the model. The distribution of temperature values in data used were 19.6-20°C (52.2%), 23.6-24°C (34.8%) and 18-18.4°C (13%), which gives a significant 6°C temperature difference that can affect growth rates. The interaction of algae with toxicants depends on their physiology. Diverse algae species would react differently to adverse effects during exposure to $nTiO_2$ (Cardinale et al., 2012).

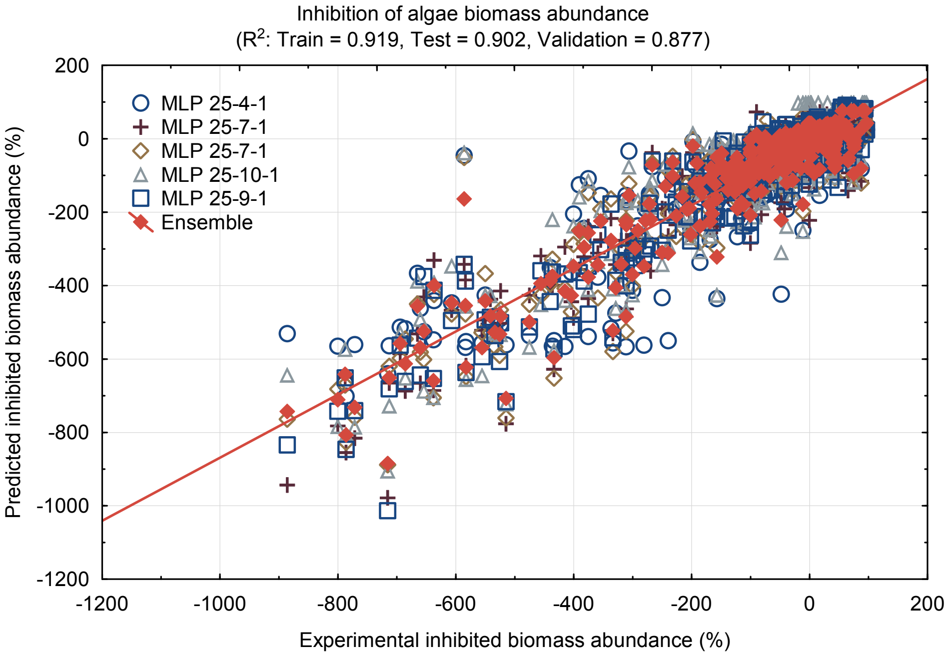

Figure 5.9: Comparison of experimental and predicted inhibition of growth rate of algae.

From the data used in modelling, the model predicted 34.65% impediment to biomass bloom in the range of 0-100% and biomass development of 46.1% in the region of 0-100% due to exposure to $nTiO_2$ as illustrated in Figure 5.9. From Table 5.2 and Figure 5.10, it can be deduced that biomass development is a function of the characteristics of the nanomaterial, the species of biological organism, duration of exposure and heterogeneity of ecological system. The data had high distribution for uncapped $nTiO_2$ (98%) and mixed cultures (96.3%). The relative importance of surface coating in this model could be attributed to aggregation behaviour of the nanomaterials and settlement, a phenomenon that by-passes bio-interaction with algae species and enhances biomass growth or attachment on the surface of the algae that promotes inhibition.

The energy balance of algae is maintained by photosynthetic reactions whereas photo catalysis of $nTiO_2$ is enhanced by adsorbed photon energy. The interplay of algae and $nTiO_2$ during exposure might trigger a chain of either positive or negative photo-induced biochemical reactions that negatively or positively influence biomass development.

For simplicity of controlled environments, scientific ecotoxicity studies focus on using axenic cultures even though actual aquatic systems have heterogeneous cultures of multiple species. Mixed cultures have interactive toxicity through antagonistic or enhancement effects (Mihelcic and Zimmerman, 2010). Scientific reports reveal that mesocosms (mixed water cultures simulations) with a high carbon content (63.454 mg/ℓ) contribute to biomass abundance due to bioavailable nutrients resulting from suppressed organisms such as bacteria (Kulacki and Cardinale 2012). At high concentrations, anatase $nTiO_2$ normally promotes root growth due to its antibacterial properties (Clement et al., 2013).

Table 5.2: Importance of variables influencing inhibited growth and biomass abundance of algae species exposed to $nTiO_2$.

	Variable importance ranking	
Variable	**Algae growth rate inhibition**	**Algae biomass abundance inhibition**
Duration (hours)	322.40	4.77 (*3*)
pH	311.50	1.01 (*7*)
Temperature (^{o}C)	217.90	1.02 (*6*)
Species	179.23	9.10 (*2*)
Ionic strength (mM)	164.98	1.01 (*7*)
Total organic carbon (TOC)(mg/ℓ)	164.71	1.01 (*7*)
Anatase (%)	160.44	1.00 (*8*)
Surface area concentration (SAC)(m^2/L)	32.18	3.82 (*5*)
Surface coating		9.24 (*1*)
Culture		3.94 (*4*)

Note: The italicized numbers in brackets indicate the significant value of the variable in estimating target endpoint.

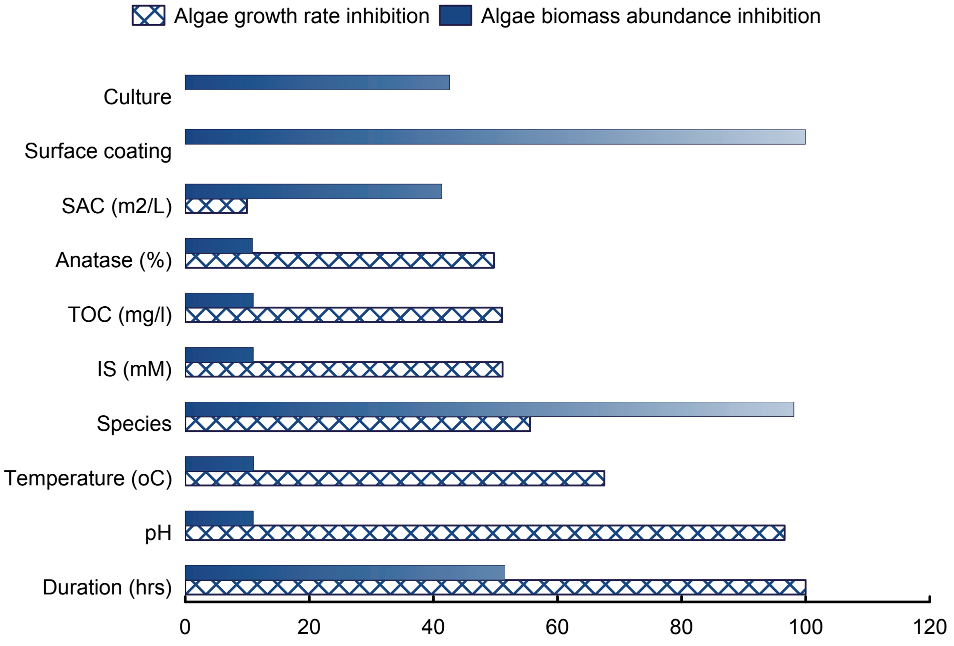

Figure 5.10: Important variables influencing inhibition of growth rate and biomass abundance of algae species.

Inhibition of algae biomass abundance increased with increasing $nTiO_2$ concentration, irrespective of species (Kulacki and Cardinale, 2012). This is a phenomenon represented by the surface area concentration variable in this model. Algae species contain carbon in different proportions, for example, 1 x 10^8 cells of *Chlorella vulgaris* species comprises 0.3 mg of carbon (Campos et al. 2013). Growth rate and biomass abundance of photosynthetic algae provide energy that sustains the food web and balances ecosystems. Inhibition and promotion of these functions could have ecological impacts upstream. Algal bloom could increase carbon storage in the aquatic systems but also lead to eutrophication that has adverse ecological impacts. For example, decaying algae emits geosmin and methyl isoborneol, which contribute to taste and odour problems in freshwater (Mihelcic and Zimmerman, 2010).

Table 5.3: Summary characteristics of the best performing neural network models

Target response	Topology	Training error	Test error	Validation error	Epoch	Hidden activation	Output activation
D. magna immobilization	MLP 15-7-1	21.45	15.81	21.26	69	Tanh	Tanh
D. magna immobilization	MLP 15-6-1	58.28	21.2	23.87	55	Tanh	Tanh
D. magna mortality	MLP 24-6-1	215.13	108	131.48	70	Tanh	Tanh
D. magna mortality	MLP 24-12-1	252.19	80.19	133.27	40	Tanh	Tanh
Inhibited *D. magna* growth rate	MLP 19-4-1	224.14	351.30	453.16	14	Tanh	Tanh
Inhibited *D. magna* growth rate	MLP 19-4-1	189.66	309.30	480.21	17	Tanh	Tanh
Inhibited *D. magna* reproduction	MLP 16-7-1	132.66	52.96	54.43	61	Tanh	Tanh
Inhibited *D. magna* reproduction	MLP 16-6-1	125.24	63.19	63.77	53	Tanh	Tanh
Inhibited algae growth rate	MLP 11-11-1	18.60	27.40	15.85	219	Tanh	Tanh
Inhibited algae growth rate	MLP 11-7-1	32.54	32.60	16.62	133	Tanh	Tanh
Inhibited algae biomass abundance	MLP 25-4-1	3602.96	3385.65	863.96	63	Tanh	Tanh
Inhibited algae biomass abundance	MLP 25-7-1	2603.52	2531.80	885.64	39	Tanh	Tanh

5.4 CONCLUSIONS

The model findings illustrate the functionality of using MLP-NN to predict the effects of ENMs to aquatic organisms. Even though only two organisms were used in this model, the findings attest the strength of predictor variables in forecasting the generic impacts of $nTiO_2$ to the exposure mechanisms and organism species. Consistency and reliability of the data used significantly influences the robustness of the model developed. Generally, low quality data were used in this study because most of it was derived in axenic static and semi-static cultures to test acute and chronic toxicities of $nTiO_2$. These tests may, however, overlook the contribution of other factors not related to ENMs, such as predation and resource competition, if the organisms were exposed in micro-or mesocosm cultures. In such regimes, the response of organisms to exposed ENMs may be different.

Data from published reports point to the use of synthesized materials as model ENMs where few were functionalised for product application such as NM-103 and NM-104 (Rasmussen et al. 2014). Thus the possibility of these materials reaching the aquatic system is unknown and likely to be confined to un-intentional discharges during production. After their release and entry into the aquatic ecosystems, the behaviour and fate mechanisms of the ENMs are likely to influence how they interact with as well as affect biota. Such data have not been reported alongside the toxicity findings from scientific tests which make it difficult to correlate emission, fate, and transport of ENMs with respect to likely responses from biological organisms. Therefore, in future research studies focus should be on using functionalised ENMs that find applications in nano-products in testing their toxicity to aquatic organisms, as well as in designing experiments that include mechanistic transport of ENMs. Data from such proposed form of studies would generate consistent data that is useful for developing modelling tools.

CHAPTER 6: OVERAL CONCLUSIONS AND RECOMMENDATIONS

6.1 ENVIRONMENTAL MODELLING OF ENMS

6.1.1 Opportunities

Modelling provides an attractive strategy for estimating potential risks of ENMs and the mechanisms that elucidate linkages of their physicochemical properties and abiotic factors to observed biological effects. Generally, reports suggest that data derived from laboratory tests have low quality compared to that generated in microcosm and mesocosm tests, which leads to high uncertainties when assessing ecological effects (Miyamoto et al. 2008). Notwithstanding the uncertainties involved, data derived from acute and chronic exposure tests are less costly and easier to manage, and could generate data that is useful in modelling for first tier risk assessment. Data discordancy due to multiple testing standards used can be gainfully turned into learning significant patterns that explain complex phenomena.

Non-linear models provide feasible inductive learning of complex environments. Functional relationships amongst interacting variables that influence complex behaviour and toxicity mechanisms of $nTiO_2$ are explained by exploratory discovery of unforeseen structures from laboratory derived datasets using multilayer perceptron data mining algorithm. Neural networks is a powerful modelling tool that can learn from diverse data and predict non-linear relationships (Swingler 1996) hence its application in modelling data from different sources in this study. Model findings provide a platform for interpreting likely effects in the environment for early regulation of chemical pollutants or facilitating feedback for further research.

A broad range of predictor variables were used in the models to estimate the behaviour and toxicity impacts responses. These variables were selected on the backdrop of their potential to influence the movement and interaction of ENMs in the environment in the event of inadvertent or accidental discharge – for example treated effluents released into rivers, terrestrial spills washed into water-bodies through surface run-off, release from painted walls, etc. An understanding of the physical and chemical properties of the ENMs, and their behaviour – adsorption and aggregation – informs about the potential kinetic transport mechanisms that inhibit or enhance their ability to migrate from the point of discharge. The temperature, pH, ionic strength and duration are significant environmental variables that would enhance reactivity and toxicity effects in the event of a discharge. These variables are not only easier to measure when assessing the risks for control but also in estimating the extent of damage to the affected ecosystem.

Even though comprehensive analysis of compounded effects of $nTiO_2$ to target organisms in mixed cultures has not been adequately tested, the model identifies organsism heterogeneity of multiple species as important contributors to algae bloom or inhibition. Multiple species in natural environments interact uniquely – for example, compete for resources, predate, progress or expire in the presence of other chemical pollutants, etc. that might contribute to effects not related to nanomaterials observed in pure cultures.

6.1.2 Challenges

The data from published reports point to the use of synthesized materials as model ENMs, few of which are functionalised for product application such as NM-103 and NM-104 (Rasmussen et al. 2014). The possibility of these materials reaching the aquatic system is unknown and might likely be confined to un-intentional discharge during production. After their release into aquatic ecosystems, the behaviour and fate mechanisms of the ENMs are likely to influence how the nanomaterials interact with and affect biota. Such data have not been reported alongside toxicity findings which hinder the connection of behaviour and fate of ENMs and effect responses from receptor biological organisms.

General inconsistency in the reporting of data for certain variables considered by the authors as useful in modelling were observed. These included environmental conditions under which the tests were conducted, and the properties of $nTiO_2$ used. For instance, the size distribution of $nTiO_2$ in particulate or aggregated forms is an important variable, as it reveals the potential partitioning of ENMs in aqueous environments. However, in scientific reports, the distribution of different sizes of $nTiO_2$ has inconsistently been reported in terms of number, volume, and intensity. As a result, it made it difficult to convert one unit to the other in an attempt to create a single attribute, and ultimately to reduction the dimensionality of the input variable space.

Harmonizing data from diverse sources poses a challenge especially in selecting model predictor variables. For example, multiple sizes of the different structural forms of $nTiO_2$ exist in test media (Planchon et al. 2013) where mono- and hetero-aggregation occurs but average sizes of these aggregated forms with limited percent distribution (Aruoja et al. 2008) have been reported. Measured sizes vary based on the instruments used (Simon-Deckers et al. 2009). The choice of using surface area concentration (SAC) in our modelling was to moderate potential problems of using numerous sizes of ENMs as a predictor variable. Despite this, adherence of the nanomaterial to test vessels (Hartmann et al. 2010) and settlement (Jacobasch et al. 2014; Kim et al. 2014; Federici et al. 2007; Zhu et al. 2010) might limit the SAC as a predictor variable.

Zeta potential is a measure of the surface charge of ENMs. This attribute was found poorly reported or reported as electrophoretic mobility (EPM). While it is possible to compute zeta potentials from the values of EPM, it becomes challenging when the environmental conditions in a given study are only partially reported or not reported at all. On the other hand, addition of $nTiO_2$ has been shown to influence the pH of the aqueous environment (Pettibone et al., 2008). However, in most studies aqueous conditions are reported at the beginning of the experiments, but rarely during or at the end of these experiments, where variations have been observed (Mudunkotuwa and Grassian, 2010).

Crystalline $nTiO_2$ comprises anatase, rutile and amorphous phases. Research findings show that even from the same batch, mixed structures of $nTiO_2$ have different proportions of the mentioned forms (Ohtani et al. 2010). Characteristics of $nTiO_2$ are measured either in purified or synthetic aquatic media at the beginning of toxicity tests (Jacobasch et al. 2014) using selected doses and various analytical methods. Continual characterisation in different dose-response treatments was generally not conducted or scarcely reported making it problematic to generalize and apply these data in modelling. During exposure, research findings show that $nTiO_2$ concentration affects the pH of aqueous environment (Pettibone et al. 2008; Hartmann et al. 2010) as well as the aggregated size (Domingos et al. 2009; Jacobasch et al. 2014; Cardinale et al. 2012). The pH is one of the parameters that influence the behaviour of nanomaterials in aqueous environments. Hetero-aggregation due to adsorption of $nTiO_2$ on target organisms influences interaction mechanisms that lead to eventual toxicity effects (Campos et al. 2013; Hartmann et al. 2010). Generalization of $nTiO_2$ characteristics at different treatment doses might affect the robustness of the model due to consistency and reliability issues of the data used.

Based on the data challenges experienced, the study conservatively imputed useful missing data and used fewer data records based on available and complete data were to develop the models. This procedure might have contributed to some statistical uncertainty and the errors observed in the model predictions. However, the models form a successful basis of explaining the complex interrelationships of multiple variables that would influence the risks of ENMs in the aquatic environment and can be refined or improved when quality and substantive data becomes available.

6.2 CONCLUSIONS

Modelling using multilayer perceptron neural network (MLP-NN) technique accomplished the following objectives; firstly, multiple sources of data were combined to formulate descriptor variables that are potential ecological stressors. The model effectively generalized the behaviour and toxicity of $nTiO_2$ using reported scientific data and addressed the dilemma of understanding complex relationships amongst multiple influencing parameters that have been simplified previously in deterministic models. Inference from this knowledge and feasible inductive learning that is drawn would provide feedback for further research and early regulation of the nanomaterial.

Secodly, non-linear relationship patterns modelled using experimental data could be applied to pilot or field scale scenarios, e.g. water bodies, to predict response patterns without extensive analytical work. The output from the regression models provide generic first tier risk assessment demonstrated by using laboratory findings. The natural environment comprises multiple associations of organisms and conditions e.g. resource competition, predation, existence of other pollutants, etc. that might contribute to; (i) effects to organisms that are not related to nanomaterials, and (ii) enhanced or antagonised effects that are different from those observed in pure cultures. Laboratory studies simulate aquatic systems where natural associations are simplified. The models were built on this simplification by incorporating predictor variables drawn from characteristics of nanomaterial, environmental conditions and organism factors to forecast exposure mechanisms.

Thirdly, MLP-NN model could be integrated as a management tool to predict the dynamics of $nTiO_2$ after release into aquatic systems and explain their subsequent effects. For example, adsorption and aggregation processes may have both negative and positive impacts to aquatic systems. An understanding of the aftermath e.g. enhanced flocculation and removal, deposition, accumulation, etc. may influence decisions on risks that support mitigation planning. The model is flexible and can be improved by adapting it to new data to generalize risk-based outcomes in diverse aquatic systems, as and when new data are obtained.

6.3 RECOMMENDATIONS

The MLP-NN modelling technique generalizes well the relationship of multiple variables even where data uncertainty is experienced. However, inconsistent data may contribute interpretation errors that affect subsequent application. Our recommendation is based on the improvement of the accuracy of the model and its robust application in decision making in risk assessment. Firstly, we suggest that experimental designs should incorporate consistent testing and reporting of descriptors that are relevant for modelling. More importantly, there is a need to measure endpoints related to behaviour and effects concurrently to reduce data ambiguity. Secondly, we propose further research to generate experimental data at micro- and macrocosm levels where near natural conditions are simulated. Data from these experiments could buttress scientific findings and support better management decisions on environmental risks through modelling. Thirdly, we propose that that future research studies should consider using functionalised nanomaterials formulated in nano-products and have potential to be released into the aquatic system. Findings from these studies would generate reliable data that supports robust prediction models compared to model pristine or functionalized $nTiO_2$ currently used, some of which may not find their way into the aquatics. We propose that further research should test the applicability of these model in predicting the behaviour and toxicity of other nanomaterials to establish its suitability and hence applicability in decision making for risk assessment that covers nanomaterials in their generality. Finally, environmental factors such as pH, ionic strength, and temperature and retention times are relevant environmental factors that require monitoring in the event of accidental release of ENMs to establish expected impacts and potential mitigation measures. These factors influence the kinetic transport, migration, bioavailability and effects of ENMs from the point of discharge and could be used to estimate risks of known ENMs discharged.

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APPENDIX: SUPPORTING INFORMATION FOR CHAPTER 3

APPENDIX 3.1: ILLUSTRATIVE EXAMPLES OF DATABASES AND DATA TYPE

Database developers	Type of data	Access status	References
Nanowerk LLC	Information on companies, laboratories, nanomaterial and characteristics, nano-products and application, training, articles.	Public	http://www.nanowerk.com/nanotechnology_databases.php
Nano Science and Technology Institute (NSTI)	Business, internet protocol, research, and contacts on nanotechnology and industries.	Internal	http://www.nsti.org/about/database.html
US EPA's Clean-up information (CLU-IN)	Profiles on nanotechnology-based projects in remediation of chemical pollutants	Public	http://www.clu-in.org/databases/
Project on Emerging Nanotechnologies	Inventory on consumer products containing nano materials.	Public	http://www.nanotechproject.org/cpi/
American National Standards Institute's Nanotechnology Standards Panel (ANSI-NSP)	Standards and related documents on products and processes developed from various research centres and regulatory institutions on Nano science and Nano-toxicology.	Public	http://nanostandards.ansi.org/tiki-index.php?page=Published+Documents
The Silver Institute	Commercial information and use of silver metal and its products	Public	https://www.silverinstitute.org/site/publications/
Organization for Economic Corporation and Development (OECD)	Research projects on the safety of manufactured nano-materials, and impact of nanoparticles to health and environment from scientific articles.	Public	http://webnet.oecd.org/NanoMaterials
The European Consumers Organization (BEUC) and The European consumer voice in Standardization (ANEC)	Inventory on consumer products that contain nanomaterials.	Target group	www.beuc.org/Content/Default.asp?PageID=2142, MS Excel Files
Danish Consumer Council, Danish Ecological Council, and Danish Technical University	Inventory on consumer products that contain nanomaterials.	Target group	http://nanodb.dk/
US EPA-Nanoscale Materials Stewardship Program (NMSP)	Information on production, characterization, and manufacture of nano-products from volunteering companies.	Public	http://www.epa.gov/oppt/nano/stewardship.htm
Nano Health-Environment Commented Database (NHECD)	Impact of nanoparticles to health and environment from scientific articles.	Public	http://nhecd.jrc.ec.europa.eu/content/discover-our-intelligent-search

APPENDIX 3.2: SCALING AND ESTIMATION OF DATA POINTS FROM GRAPHS IN PUBLISHED SCIENTIFIC REPORTS

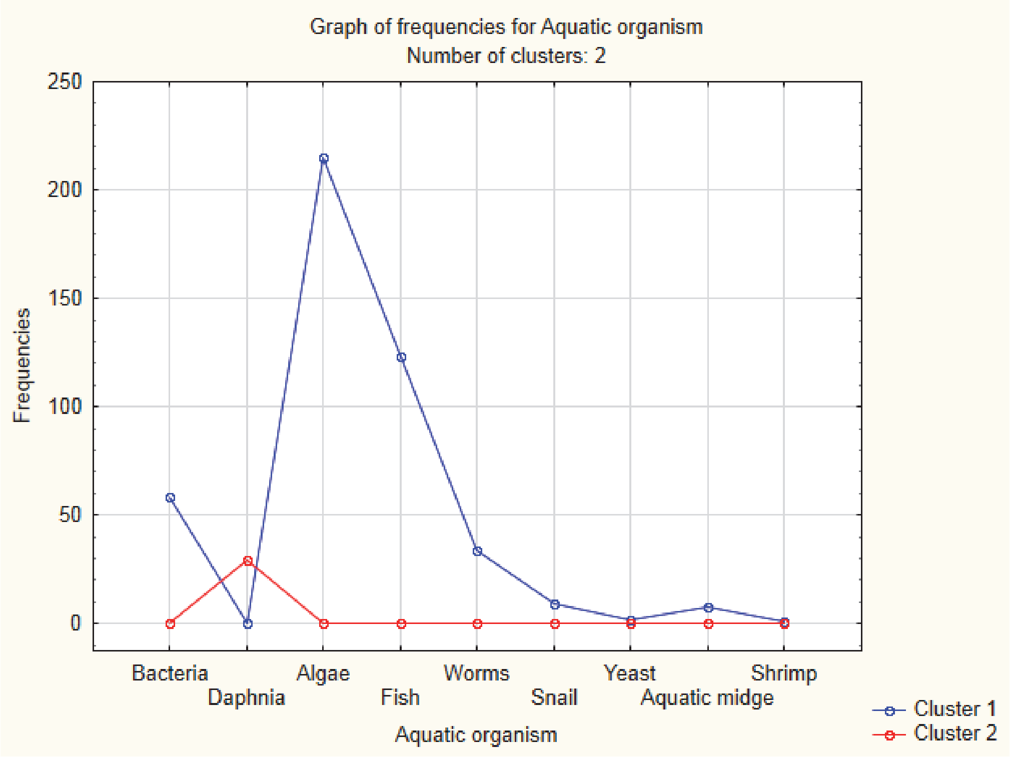
	X-1 Scale of pH (units)	**X-2** pH value (units)	**X-3** Scale value of pH (units)	**X-4** pH (Unit)	**Y-1** Scale of ζ (units)	**Y-2** Value of ζ (mV)	**Y-31** Scale value of ζ, R (units)	**Y-41** ζ, R (mV)	**Y-32** Scale value of ζ, R-A (units)	**Y-42** ζ,R-A (mV)	**Y-33** Scale value of ζ, P25 (units)	**Y-43** ζ, P25 (mV)
1	14.35	2.00	11.00	1.53	7.00	10.00	25.00	35.71	26.00	37.14	33.30	47.57
2	14.35	2.00	14.35	2.00	7.00	10.00	23.50	33.57	25.00	35.71	33.00	47.14
3	14.35	2.00	17.00	2.37	7.00	10.00	23.00	32.86	24.25	34.64	32.25	46.07
4	14.35	2.00	20.00	2.79	7.00	10.00	21.50	30.71	23.25	33.21	31.50	45.00
5	14.35	2.00	22.00	3.07	7.00	10.00	20.50	29.29	23.00	32.86	31.00	44.29
6	14.35	2.00	25.00	3.48	7.00	10.00	19.00	27.14	22.00	31.43	29.25	41.79
7	14.35	2.00	28.00	3.90	7.00	10.00	16.00	22.86	21.25	30.36	28.00	40.00
8	14.35	2.00	30.00	4.18	7.00	10.00	14.50	20.71	21.00	30.00	25.50	36.43
9	14.35	2.00	32.00	4.46	7.00	10.00	13.00	18.57	20.25	28.93	20.50	29.29
10	14.35	2.00	35.00	4.88	7.00	10.00	10.10	14.43	18.00	25.71	13.50	19.29
11	14.35	2.00	38.00	5.30	7.00	10.00	7.00	10.00	12.30	17.57	6.80	9.71
12	14.35	2.00	40.00	5.57	7.00	10.00	4.00	5.71	8.50	12.14	4.00	5.71
13	14.35	2.00	43.00	5.99	7.00	10.00	0.00	0.00	3.80	5.43	1.50	2.14
14	14.35	2.00	45.00	6.27	7.00	10.00	-2.00	-2.86	0.00	0.00	0.00	0.00
15	14.35	2.00	48.00	6.69	7.00	10.00	-5.00	-7.14	-4.00	-5.71	-3.00	-4.29
16	14.35	2.00	50.00	6.97	7.00	10.00	-7.00	-10.00	-6.00	-8.57	-4.00	-5.71
17	14.35	2.00	53.50	7.46	7.00	10.00	-10.00	-14.29	-7.50	-10.71	-6.00	-8.57
18	14.35	2.00	57.40	8.00	7.00	10.00	-12.80	-18.29	-9.00	-12.86	-7.50	-10.71
19	14.35	2.00	60.00	8.36	7.00	10.00	-14.25	-20.36	-10.00	-14.29	-8.25	-11.79
20	14.35	2.00	63.00	8.78	7.00	10.00	-15.80	-22.57	-11.25	-16.07	-11.00	-15.71
21	14.35	2.00	65.00	9.06	7.00	10.00	-16.00	-22.86	-12.00	-17.14	-12.80	-18.29
22	14.35	2.00	68.00	9.48	7.00	10.00	-16.00	-22.86	-13.00	-18.57	-14.25	-20.36
23	14.35	2.00	70.00	9.76	7.00	10.00	-16.00	-22.86	-13.50	-19.29	-15.25	-21.79
24	14.35	2.00	73.00	10.17	7.00	10.00	-16.25	-23.21	-14.40	-20.57	-16.00	-22.86
25	14.35	2.00	75.00	10.45	7.00	10.00	-16.50	-23.57	-15.00	-21.43	-16.50	-23.57
26	14.35	2.00	78.00	10.87	7.00	10.00	-16.40	-23.43	-15.90	-22.71	-16.50	-23.57
27	14.35	2.00	80.00	11.15	7.00	10.00	-16.50	-23.57	-16.00	-22.86	-16.80	-24.00
28	14.35	2.00	83.00	11.57	7.00	10.00	-16.80	-24.00	-17.80	-25.43	-17.80	-25.43

Data source: (Planchon et al., 2013).

For clarity, the columns in Appendix 3.2 have been numbered as X-1 to X-4 to represent the computation values for the X-axis variable, which is the pH. The Y-1 to Y-43 columns represent the Y-axis values used in computation of zeta potential (ζ) of three types of nTiO_2 composites tested in aqueous media. The Y-41 column represents the zeta potential for rutile (R); Y-42 column represents the zeta potential for rutile and anatase mixture (R-A) that was synthesized in the laboratory; and Y-43 represents the zeta potential column for P25 (rutile and anatase phase mixture that is a product of Evonik Degussa Company). The generic equation used to estimate the data points using the scaling methods was;

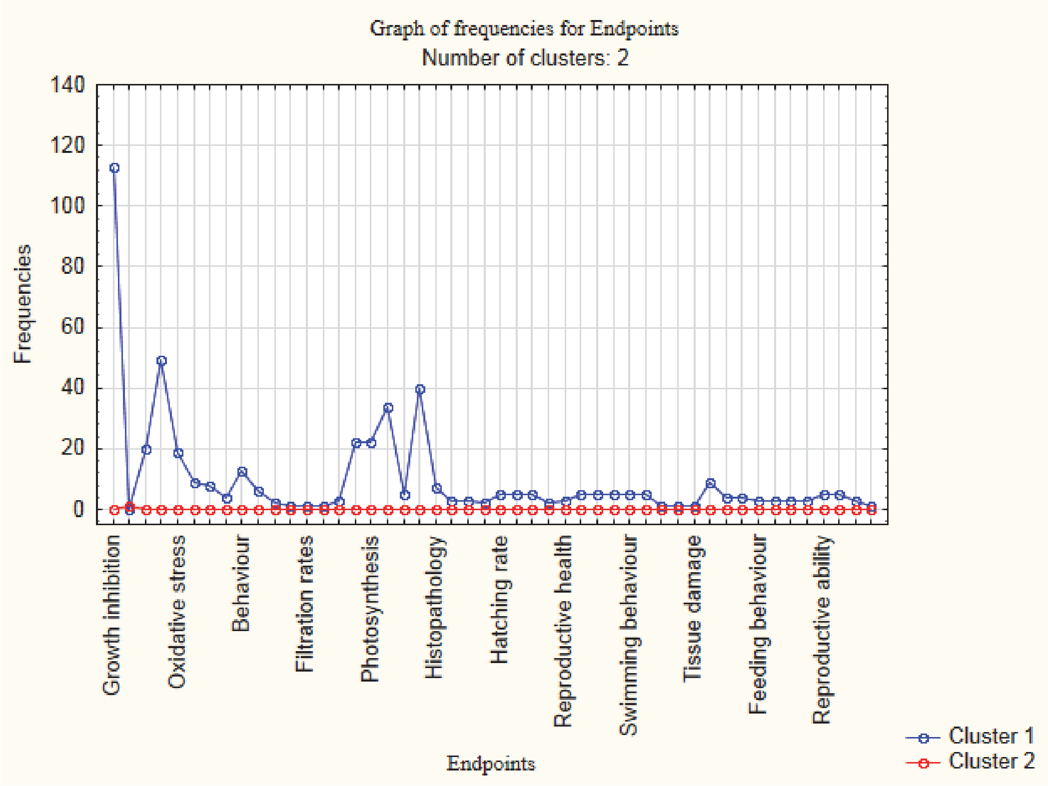
$$\text{Data value (units)} = \frac{\text{the length of the data point on the graph/image (mm)} \times \text{the actual value represented by the scale (units)}}{\text{The actual length on the graph/images (mm)}}$$

APPENDIX 3.3: DISTRIBUTION OF TESTED ORGANISMS AT DIFFERENT CONCENTRATIONS OF NTIO$_2$ AND ENDPOINTS



Organism	Cluster 1	Cluster 2	Total	Concentration of nTiO$_2$ (mg/ℓ
Bacteria	58	0	58	1.00-5000.00
Daphnia	0	29	0	0.01-20000.00
Algae	215	0	215	0.01-1067.72
Fish	123	0	123	0.10-1000.00
Worms	34	0	34	24.00-3000.00
Snail	9	0	9	5.0-500.0
Yeast	2	0	2	625.00-20000.00
Aquatic midge	8	0	8	7.00-20.00
Shrimp	1	0	1	20000.00
Total	450	29	479	

APPENDIX 3.4: DISTRIBUTION OF ENDPOINTS REPORTED IN TESTING THE TOXICITY OF DIFFERENT ORGANISMS AT DIFFERENT CONCENTRATIONS OF NTIO$_2$



Endpoint	Cluster 1	Cluster 2	Total
Growth inhibition	113	0	113
Inhibition of luminescence	0	1	1
Adsorption of nTiO2	20	0	20
Mortality	49	0	49
Oxidative stress	19	0	19
Cell transformation	9	0	9
Immobilization	8	0	8
Reproduction ability	4	0	4
Behaviour	13	0	13
Reproduction	6	0	6
Growth rate	2	0	2
Accumulation of nTiO2	1	0	1
Filtration rates	1	0	1
Ingestion rate	1	0	1
DNA damage	3	0	3
Biomass growth	22	0	22
Photosynthesis	22	0	22
Metabolism	34	0	34
Growth rate inhibition	5	0	5
Biomass production	40	0	40
Histopathology	7	0	7

Endpoint	Cluster 1	Cluster 2	Total
Biochemical changes	3	0	3
Haematology	3	0	3
Tissue ion variations	2	0	2
Hatching rate	5	0	5
Embryo survival	5	0	5
Malformations	5	0	5
Physiology	2	0	2
Reproductive health	3	0	3
Hatchability	5	0	5
Survival	5	0	5
Malformation	5	0	5
Swimming behaviour	5	0	5
Accumulation	5	0	5
Locomotive behaviour	1	0	1
Physiological mechanisms	1	0	1
Tissue damage	1	0	1
Cytoxicity	9	0	9
Genotoxicity	4	0	4
DNA oxidation	4	0	4
Feeding behaviour	3	0	3
Burrowing behaviour	3	0	3
Uptake	3	0	3
Cytotoxicity	3	0	3
Reproductive ability	5	0	5
Offspring survival	5	0	5
Anti-oxidation enzyme activity	3	0	3
Cell viability	1	0	1
Total	**478**	**1**	**479**