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Detection, Toxicology, Environmental Fate and Risk Assessment of Nanoparticles in the Aquatic Environment (DeTER)

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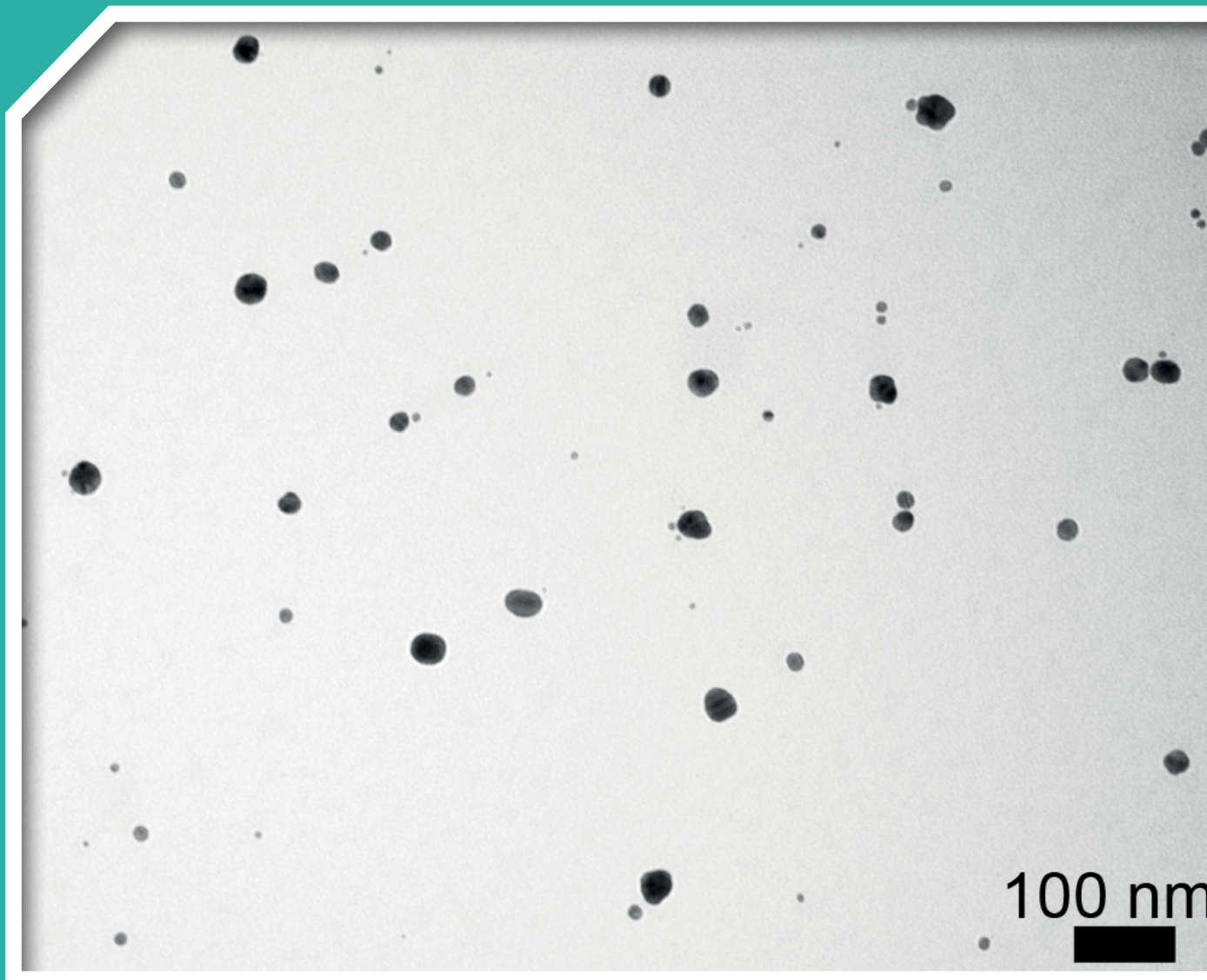

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Rialtas na hÉireann
Government of Ireland

ENVIRONMENTAL PROTECTION AGENCY

The Environmental Protection Agency (EPA) is responsible for protecting and improving the environment as a valuable asset for the people of Ireland. We are committed to protecting people and the environment from the harmful effects of radiation and pollution.

The work of the EPA can be divided into three main areas:

Regulation: *We implement effective regulation and environmental compliance systems to deliver good environmental outcomes and target those who don't comply.*

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- intensive agriculture *(e.g. pigs, poultry)*;
- the contained use and controlled release of Genetically Modified Organisms *(GMOs)*;
- sources of ionising radiation *(e.g. x-ray and radiotherapy equipment, industrial sources)*;
- large petrol storage facilities;
- waste water discharges;
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- Supervising the supply of drinking water by public water suppliers.
- Working with local authorities and other agencies to tackle environmental crime by co-ordinating a national enforcement network, targeting offenders and overseeing remediation.
- Enforcing Regulations such as Waste Electrical and Electronic Equipment (WEEE), Restriction of Hazardous Substances (RoHS) and substances that deplete the ozone layer.
- Prosecuting those who flout environmental law and damage the environment.

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- Monitoring and reporting on Bathing Water Quality.

Monitoring, Analysing and Reporting on the Environment

- Monitoring air quality and implementing the EU Clean Air for Europe (CAFÉ) Directive.
- Independent reporting to inform decision making by national and local government *(e.g. periodic reporting on the State of Ireland's Environment and Indicator Reports)*.

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- Preparing Ireland's greenhouse gas inventories and projections.
- Implementing the Emissions Trading Directive, for over 100 of the largest producers of carbon dioxide in Ireland.

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- Generating greater environmental awareness and influencing positive behavioural change by supporting businesses, communities and householders to become more resource efficient.
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- Office of Environmental Sustainability
- Office of Environmental Enforcement
- Office of Evidence and Assessment
- Office of Radiation Protection and Environmental Monitoring
- Office of Communications and Corporate Services

The EPA is assisted by an Advisory Committee of twelve members who meet regularly to discuss issues of concern and provide advice to the Board.

EPA RESEARCH PROGRAMME 2014–2020

Detection, Toxicology, Environmental Fate and Risk Assessment of Nanoparticles in the Aquatic Environment (DeTER)

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EPA Research Report

Prepared for the Environmental Protection Agency

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DISCLAIMER

The EPA Research Programme addresses the need for research in Ireland to inform policymakers and other stakeholders on a range of questions in relation to environmental protection. These reports are intended as contributions to the necessary debate on the protection of the environment.

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Executive Summary

Nanotechnology is an emerging technology that has the potential to impact on all aspects of life and the economy and is expected to form the basis of several technological innovations and advances in the 21st century. The European Commission defines a nanomaterial as “a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm–100 nm”. The production of and demand for products containing nanomaterials has increased significantly in recent years. The Nanodatabase developed by the Technical University of Denmark is a “living” inventory of commercially available products in the European consumer market that claim to contain engineered nanomaterials (ENMs) and currently lists 3037 products. Nanomaterials have a wide range of potential applications, from everyday uses (such as improvements in fabrics, paints, cosmetics and packaging) to medical applications, water and soil remediation and renewable energy production.

Nanomaterials include both nanoparticles and nano-synthesised materials. Nanomaterials can be naturally occurring, inadvertently generated or engineered. ENMs are intentionally produced and exhibit unique electrical, magnetic, optical, antimicrobial and other properties. Silver nanoparticles (AgNPs) are among the ENMs most often incorporated in nano-functionalised consumer products. AgNPs have been incorporated into a diverse range of consumer products including plastics, soaps, pastes, metals and fabrics and have applications in water and soil remediation. In the Nanodatabase, currently 539 of the 3037 products listed contain silver. Although advances in nanotechnology and the continued development of novel ENMs are expected to lead to significant societal benefits, there is increasing concern that the unique properties of ENMs may result in potential hazards for both humans and the environment. ENMs can be released into various environmental matrices during their production, use and disposal. ENMs pose a potential risk to human health through ingestion, inhalation and contact.

To date, the concentrations of AgNPs in the aquatic environment have primarily been estimated through modelling, with predicted environmental concentrations in the ng/l range. This is largely because of a dearth of appropriate detection methods. The aims of this 3-year research project were to (1) develop and implement methods for the detection of AgNPs in water; (2) determine the toxicological properties and environmental fate of AgNPs in the aquatic environment and (3) develop risk assessment protocols that can be used to evaluate the environmental fate of and likely risk from AgNPs in aquatic pathways.

The suitability of activated charcoal as a capture material for AgNPs from water was examined. Samples of 100 µg/l of AgNPs were initially generated and exposed to activated charcoal for 24 hours to examine the ability of charcoal to capture AgNPs. The decrease in silver concentration was measured using an inductively coupled plasma mass spectrometer. Following initial investigations, the surface area of the charcoal was increased, first, with a pestle and mortar and, second, by milling. The increased surface area of the milled charcoal increased the capture of the AgNPs from 11.9% to 63.6%. A hydrochloric acid leaching procedure was developed that successfully removed the captured silver, allowing the fraction captured by the charcoal to be quantified, with an average recovery rate of 94.8%. The results show that milled activated charcoal can successfully capture AgNPs from water samples. Activated charcoal therefore represents a cost-effective material for the remediation of waters impacted by AgNPs or other nano-wastes.

A multi-trophic test battery that included three trophic levels was adopted to assess the ecotoxicity of AgNPs and silver nitrate ($AgNO_3$) to the algae *Pseudokirchneriella subcapitata*, the crustacean *Daphnia* spp. and the cnidarian *Hydra attenuata.* The standard medium (Jaworski’s medium) and an ethylenediaminetetraacetic acid (EDTA)-free medium (Chu#10) were tested concurrently. An approximately 10-fold improvement in test sensitivity using EDTA-free medium was observed overall. No significant difference between the toxicity of AgNP and

the toxicity of $AgNO_3$ was observed. Both *Daphnia pulex* and *Daphnia magna* were compared using $AgNO_3$ and AgNPs. *Daphnia pulex*, with a 24-hour half-maximal inhibitory concentration (IC_{50}) of 9.3 µg/l, was less sensitive to $AgNO_3$ than *Daphnia magna*, with an IC_{50} of 1.22 µg/l. When tested with AgNPs, both species yielded similar results, with an IC_{50} of 7.85 µg/l for *Daphnia magna* and 4.2 µg/l for *Daphnia pulex*. As these IC_{50} values were substantially higher than the predicted environmental concentrations, sub-lethal end points were investigated. Fecundity was assessed in *Daphnia magna*, with the number of offspring reduced by 50% after 14 days and 75% after 28 days when cultured in 100 ng/l of AgNPs. This demonstrates that the effects of AgNPs may be seen at close to environmentally relevant concentrations on population numbers rather than single individual organisms. Assessment of the ecotoxicity of AgNPs using *Hydra attenuata* gross morphology as the end point yielded a 96-hour half-maximal effective concentration (EC_{50}) of 29 µg/l for AgNPs. The effect of silver on the regeneration of *Hydra attenuata* was the most environmentally relevant bioassay investigated as it is very sensitive and robust and *Hydra attenuata* represents benthic dwellers likely to be exposed to higher concentrations of AgNPs.

The risk assessment involved a number of interlinking stages. Stage 1 included a review of the state of the art regarding natural attenuation processes that affect ENPs in the natural aquatic environment and a review of current risk assessment strategies. In stage 2 a suite of laboratory-scale studies were conducted to better characterise the aggregation potential of AgNPs. The behavioural indications derived from stages 1 and 2 were used to develop an aquatic risk model (stage 3), which was used to characterise the likely residual levels of AgNPs in surface waters. Estimated initial values indicated a mean AgNP concentration of 4.34×10^{-2} µg/l and this was assumed as a worst-case scenario for surface water concentrations in Ireland and used as an initial input value in the risk model. Seasonal factors were incorporated in the risk model to account for potential fluctuations in organic matter and ionic strength, which have been identified as key influencers of particle stability and eventual fate in natural water systems. The predicted results from the model developed indicate that citrate-coated particles underwent greater removal than polyvinylpyrrolidone (PVP)-coated AgNPs in both stream water and lake water, with predicted removal rates after 7 days of ≈70% (stream water) and ≈67% (lake water) for citrate-coated particles and ≈45% (stream water) and ≈50% (lake water) for PVP-coated AgNPs. Predicted aquatic concentrations of AgNPs were compared with toxicity data from project partners to establish if a risk is posed by current estimated concentrations of AgNPs in natural waters. The EC_{50} values from primary producer (algae) to primary consumer (*Daphnia pulex*) to secondary consumer (*Hydra attenuata*) exposed to PVP-coated AgNPs were compared with persistent concentrations of AgNPs in freshwater systems. The concentrations of AgNPs used in the model were at levels deemed unlikely to have toxicity concerns to aquatic organisms (mean levels in water of 4.34×10^{-2} µg/l). Therefore, at current predicted water concentrations, AgNPs are unlikely to present a toxic concern to the aquatic food chain. Stages 4 and 5 used information generated to assess potential human exposure through drinking water. The model incorporated estimated removal rates for the differing treatment processes. Risk to human health was calculated based on water consumption and potential exposure to residual AgNPs using the hazard quotient (HQ). The HQ is a ratio of the possible exposure to a particular substance and the level at which it is expected that no adverse effects will occur. If the calculated HQ is less than 1 then it is expected that no adverse health effects will result from exposure. The predicted HQ indicated that there was no existing risk through the consumption of drinking water (HQ of 3.24×10^{-7} for males and 3.84×10^{-7} for females). However, the increased industrial usage of nanomaterials in many sectors, in conjunction with the persistence of AgNPs during drinking water treatment, suggests the need to constantly monitor levels and re-assess exposure through drinking water into the future.

1 Introduction

1.1 Nanoparticles and Silver Nanoparticles

In recent years there has been a proliferation in the use of engineered nanomaterials (ENMs) in numerous consumer products. Nanoparticles (NPs) are defined as “natural, incidental or manufactured materials containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm–100 nm” (EC, 2011). Amongst the ENMs most commonly incorporated in these nano-functionalised products are silver nanoparticles (AgNPs) (Zhang *et al.*, 2016). The increased interest in the use of AgNPs is evident from the increasing number of scientific publications in peer-reviewed journals including the keywords “silver nanoparticles” since 2002 (Figure 1.1).

In its bulk form Ag has been used throughout history because of its antibacterial activity (Schaller and Klauss, 2001; Alexander, 2009; Amato *et al.*, 2011; Maillard and Hartemann, 2012; Reidy *et al.*, 2013). It has also been used in its colloidal form in more recent history, with biocidal colloidal Ag registered in the USA in the 1950s (Nowack *et al.*, 2011). The known biocidal and antibacterial properties of bulk Ag are also associated with AgNPs, which has led to their incorporation in nano-functionalised products (Bone *et al.*, 2012; Cleveland *et al.*, 2012; Maillard and Hartemann 2012). AgNPs have also been incorporated into microelectronic and medical imaging products because of their electrical and thermal conductivity properties (Fabrega *et al.*, 2011; Zhang *et al.*, 2016).

Silver nanoparticles can be produced using different physical and chemical methods (Fabrega *et al.*, 2011). These can be described as top-down and bottom-up methods (Tolaymat *et al.*, 2010), with the AgNPs produced in a wide variety of sizes and shapes (e.g. spheres, rods, cubes, wires and triangles) (Reidy *et al.*, 2013; Zhang *et al.*, 2016). In top-down methods bulk Ag is reduced to the nanoscale mechanically using techniques such as lithography or laser ablation (Fabrega *et al.*, 2011). In bottom-up methods Ag salts are dissolved in a solvent and a reducing agent [e.g. sodium borohydride ($NaBH_4$)] is added. The AgNPs produced can then be stabilised through the addition of a capping agent. These capping agents include

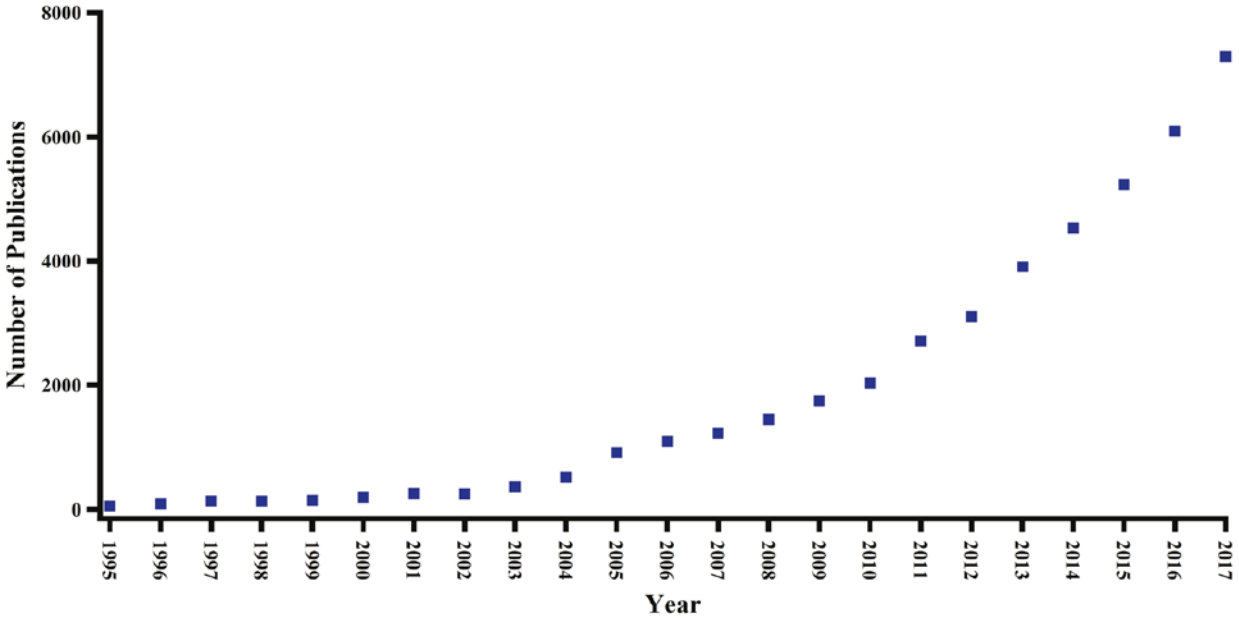


Figure 1.1. Number of publications per year on ScienceDirect.com including the keywords “silver nanoparticles”.

surfactants used in the synthesis of polymers, peptides and sugars (El Badawy *et al.*, 2010; Reidy *et al.*, 2013), with citrate, $NaBH_4$ and polyvinylpyrrolidone (PVP) being amongst the most commonly used capping agents for AgNPs (El Badawy *et al.*, 2010). The stabilising agent will influence the AgNPs' surface properties, size and shape and the aggregation of the particles (El Badawy *et al.*, 2010; Fabrega *et al.*, 2011; Reidy *et al.*, 2013; Cunningham and Joshi, 2015).

1.2 Current Applications of Silver Nanoparticles

Currently, the annual global consumption of AgNPs is estimated to range from 55 tonnes/year (Piccinno *et al.*, 2012) to 450 tonnes/year (Lazareva and Keller, 2014; Zhang, *et al.*, 2016), with levels varying based on the models applied. The annual global market for AgNPs was valued at US$1.271 billion in 2017 and is estimated to grow to US$2.597 billion by 2022 (Research and Markets, 2017).

Currently, AgNPs are incorporated into numerous nano-functionalised consumer products. These products are primarily produced because of the antibacterial properties of AgNPs. AgNPs are currently incorporated in clothing and textiles, cosmetic products, personal care products, medical devices, medicinal products, nano-functionalised plastics, food storage materials, water filters, paints and domestic appliances (Jain and Pradeep, 2005; Dubas *et al.*, 2006; Silver *et al.*, 2006; Benn and Westerhoff, 2008; Blaser *et al.*, 2008; Kumar *et al.*, 2008; Maneerung *et al.*, 2008; Abou El-Nour *et al.*, 2010; Farkas *et al.*, 2011; Cushen *et al.*, 2012; Etheridge *et al.*, 2013; Reidy *et al.*, 2013; SCENIHR, 2014; Cunningham and Joshi, 2015; Zhang *et al.*, 2016; Mackevica *et al.*, 2017). Because of the increasing use of AgNPs, it is anticipated that there will be an increase in the production of AgNPs and ENMs, the production of which is a potential source of human and environmental exposure to AgNPs (Cunningham and Joshi, 2015). Therefore, the environmental impact of AgNPs must be fully investigated.

1.3 Nanomaterial Product Inventories and Regulations

The proliferation of nano-functionalised consumer products has led to the development of a number of inventories of these products. The tracking of AgNP-containing products is a difficult task (SCENIHR 2014); this is because of the lack of regulation in this area but also because of the wide variety of products available that are sold under numerous brands. The lack of regulation is further evidenced by the fact that a number of studies have found that some products advertised by the manufacturers as containing AgNPs did not contain any detectable levels of Ag (Benn and Westerhoff, 2008; Kulthong *et al.*, 2010; Lorenz *et al.*, 2012). Therefore, the generation of inventories relying on advertised claims from the manufacturer may not be sufficient.

As part of the Project on Emerging Nanotechnologies, Vance *et al.* (2015) compiled a list of consumer product inventories and also developed the Nanotechnology Consumer Products Inventory. This inventory was last updated in December 2014, with 442 of the 1827 nano-functionalised products listed containing Ag (Project on Emerging Nanotechnologies, 2013). A more recent study examined the availability of these products in Europe (Hansen *et al.*, 2016), leading to the development of the Nanodatabase, an inventory of products claiming nano-functionalisation that are commercially available in the European consumer market [http://nanodb.dk/ (accessed 3 September 2018)]. The Nanodatabase currently lists 539 out of 3037 products as containing Ag (Nanodatabase, 2016). An inventory of AgNP-containing products was also developed by two European consumer organisations, Bureau Européen des Unions de Consommateurs (BEUC) and the European Association for the Coordination of Consumer Representation in Standardisation (ANEC); this was most recently updated in February 2013 and lists 141 AgNP-containing products (ANEC/BEUC, 2013). Other inventories are listed in the literature, for example the BUND database [http://archiv.bund.net/nc/themen_und_projekte/nanotechnologie/nanoproduktdatenbank/produktsuche/ (accessed 10 September 2018)], which is currently archived online, an inventory available at http://www.nanoproducts.de (accessed 10 September 2018) and a Japanese inventory generated by the National Institute of Advanced Industrial Science and Technology (https://www.aist-riss.jp/db/nano/index.htm), which is no longer accessible (Vance *et al.*, 2015; Hansen *et al.*, 2016).

There have been calls for the specific regulation of nanomaterials in recent years (Hansen, 2017). In

Europe the standard definition of a nanomaterial, quoted in section 1.1, is deemed insufficient by some (SCENIHR, 2010; Hansen, 2017).

In Europe the regulation of chemicals is governed by the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulations. Although nanomaterials are covered by these regulations as chemicals, there are not any nano-specific regulations (EC, 2006). Some current European regulations do have relevance to products containing nanomaterials. These include the biocidal products regulation [Regulation (EU) No. 528/2012], regulation on cosmetic products [Regulation (EC) No. 1223/2009], regulation on the provision of food information to consumers [Regulation (EU) No. 1169/2011], regulation on plastic materials and articles intended to come into contact with food [Regulation (EU) No. 10/2011], regulation related to novel foods [Regulation (EU) No. 2015/2283], Regulation (EC) No. 1333/2008 on food additives and Regulation (EU) No. 2017/745 on medical devices (SCENIHR, 2014; Rauscher *et al.*, 2017).

Within Europe, France has been the leader in terms of governmental regulation of nanomaterials. Articles L. 523–1 to L. 523–3 of the French Environment Code oblige companies to declare the quantities and uses of substances at nanoscale produced, distributed or imported to ANSES (the French agency for food safety, the environment and labour) (Ministry of Ecology, Sustainable Development, Transport and Housing, 2012). Belgium, Denmark and Norway have also initiated similar inventories of products whereas other countries such as Italy, Germany, the UK and Sweden are proposing to introduce some form of regulation (Anses, 2014; KEMI, 2015; ChemicalWatch, 2016; Hansen *et al.*, 2016). Currently, in Ireland there is a lack of knowledge across sectors on the quantities and uses of substances at nanoscale. The development of an Irish national inventory in line with other European countries is warranted.

1.4 Silver Nanoparticles in the Environment: Release, Concentrations and Fate

Because of the increase in the use of nano-functionalised products there is an increased probability of AgNPs entering the environment (Shevlin *et al.*, 2018). Exposure to AgNPs can occur at any point in the life cycle of the nano-functionalised products (Blaser *et al.*, 2008; Mueller and Nowack, 2008; Hong *et al.*, 2014; Cunningham and Joshi, 2015). Numerous studies have observed the release of AgNPs from such products. Benn and Westerhoff (2008) investigated the release of Ag from nano-functionalised socks. Geranio *et al.* (2009) investigated Ag release from fabrics in conditions mimicking washing conditions. Walser *et al.* (2011) conducted a life-cycle assessment of a nano-functionalised T-shirt in which it was determined that its production, rather than washing or disposal, was the major source of Ag emissions, because of toxic Ag emissions associated with mining. Kulthong *et al.* (2010) measured the release of Ag from nanosilver-treated fabrics into artificial sweat. Lorenz *et al.* (2012) examined the release of Ag from different textiles during a washing and rinsing cycle. Kaegi *et al.* (2010) monitored AgNP leaching from nano-functionalised exterior paints. Farkas *et al.* (2011) analysed the effluent from a commercially available nano-functionalised washing machine for Ag content. Mackevica *et al.* (2016) examined the release of AgNPs from commercially available toothbrushes. The risks associated with AgNP-functionalised plastics used for food packaging has also been investigated (Fröhlich and Roblegg, 2012; Cushen *et al.*, 2013, 2014; Hannon *et al.*, 2015). The release of Ag from Ag-containing medical devices has also previously been examined (Sussman *et al.*, 2015). For these studies it was found that different functionalised products had released different amounts of Ag; this was influenced by the method of Ag incorporation, the form of Ag and also the extraction method used (Geranio *et al.*, 2009; Sussman *et al.*, 2015). As mentioned previously, it is also of note that a number of the aforementioned studies found that some of the products did not contain detectable levels of Ag, which was not in keeping with the manufacturers' claims (Benn and Westerhoff, 2008; Kulthong *et al.*, 2010; Lorenz *et al.*, 2012).

In the environment the concentrations of AgNPs are predicted to be low (in the ng/l range). To date, there have been very few analytical studies measuring the actual concentration of AgNPs in the environment, primarily because of a lack of available capture and detection methods. (Gottschalk *et al.*, 2009; Fabrega *et al.*, 2011; Bone *et al.*, 2012; Cunningham and Joshi, 2015). The concentrations reported in the literature to date have been modelled to be in

the ng/l range (Blaser *et al.*, 2008). Studies have predicted the concentration of AgNPs in different water compartments. In US surface waters the concentrations are predicted to be between 0.09 and 0.43 ng/l and in European surface waters they are predicted to be between 0.59 and 2.16 ng/l (Blaser *et al.*, 2008; Gottschalk *et al.*, 2009; Nowack *et al.*, 2011). More recently, some initial investigations have been carried out that have measured the actual concentration of AgNPs in the environment. L. Li *et al.* (2016) measured background concentrations of AgNPs in the range 0.9–2.3 ng/l in the River Isar in Germany, with increased concentrations of 2.0–8.6 ng/l noted at waste water treatment plant discharge points. Using single particle inductively coupled plasma mass spectrometry (SP-ICPMS), Peters *et al.* (2018) detected nanosilver in all samples of surface waters, with an average concentration of 0.8 ng/l (ranging from 0.3 to 2.5 ng/l). These studies show that AgNPs are present in the environment and that convenient and cost-effective techniques need to be developed to capture and detect these AgNPs in the aquatic environment.

Once AgNPs are released into the environment numerous factors influence their ultimate fate and levels. First, it is likely that the primary route into the environment is through wastewater. Studies have predicted that AgNPs will primarily partition to the sludge in wastewater treatment plants (WWTPs) (Blaser *et al.*, 2008; Kaegi *et al.*, 2013; Doolette *et al.*, 2015), with estimates predicting that > 90% of the AgNPs entering a WWTP will partition to the sludge (Tiede *et al.*, 2010). However, the sludge may be sent to landfill or used as agricultural fertiliser, both of which could act as sources of AgNPs in the environment through leaching and surface run-off (Blaser *et al.*, 2008).

In the aquatic environment the ultimate fate of AgNPs is complicated as they can undergo numerous chemical and physical processes (Zhang *et al.*, 2016; Shevlin *et al.*, 2018). They may remain in suspension and disperse, they can aggregate or agglomerate and they can dissolve or react with different species present in the aquatic system (Luoma, 2008; SCENIHR, 2014; McGillicuddy *et al.*, 2017; Shevlin *et al.*, 2018). The form that the AgNPs take in the environment will influence their environmental impact, for example their bioavailability. The ultimate environmental fate of AgNPs is influenced by numerous intrinsic and extrinsic effects, such as nanoparticle size and shape, presence and type of coating, temperature, ionic strength, pH, dissolved oxygen levels and presence of ligands (Luoma, 2008; Liu and Hurt, 2010; Zhang *et al.*, 2011; He *et al.*, 2013; Zhang *et al.*, 2016; McGillicuddy *et al.*, 2017; Shevlin *et al.*, 2018).

Dissolution of AgNPs can be affected by the properties of the particle itself, including particle size, particle concentration and the environmental conditions, for example ambient temperature, dissolved oxygen levels, pH, presence of ligands and ionic strength (Liu and Hurt, 2010; He *et al.*, 2013; Zhang *et al.*, 2016). The solubility of smaller AgNPs is higher than that of larger AgNPs (Ma *et al.*, 2012). At higher concentrations the particles are more likely to aggregate, inhibiting dissolution as the surface area is reduced, slowing the dissolution rate (Liu and Hurt, 2010; Zhang *et al.*, 2011; Zhang *et al.*, 2016). Particle coating influences dissolution, with citrate-coated particles dissolving at accelerated rates in freshwater and seawater compared with PVP-coated AgNPs (Angel *et al.*, 2013). Dissolution is increased by higher temperatures and increasing pH (Liu and Hurt, 2010; Zhang *et al.*, 2016). Following dissolution the released ionic silver (Ag^+) can react with different species present in the aquatic environment. Ag can form a number of different species, with silver chloride (AgCl), silver sulfide (Ag_2S), silver oxide (Ag_2O) and silver(I) complexes amongst the most important Ag species formed in the aquatic environment (Zhang *et al.*, 2016). These reactions are important as the Ag species will influence its bioavailability and toxicity (Nowack *et al.*, 2011). In natural waters dissolved Ag is likely to bind to the natural particulate matter present in the water (Blaser *et al.*, 2008; Luoma, 2008; Cunningham and Joshi, 2015). Interestingly, AgNPs can be formed in the environment; this can be induced in the presence of humic acid (Dubas and Pimpan, 2008; Akaighe *et al.*, 2011; Gunsolus *et al.*, 2015). This presents a possible interference to the measurement of engineered AgNPs in the environment, particularly in regions with higher humic acid levels, for example peat-rich areas and forestry.

Particle size, particle concentration, particle coating, dissolved oxygen levels, pH, dissolved organic matter and ionic strength all influence the aggregation of AgNPs (Luoma, 2008; El Badawy *et al.*, 2010; Cunningham and Joshi, 2015; Yin *et al.*, 2015; Zhang

et al., 2016). The aggregation of AgNPs is commonly modelled using the DLVO (Derjaguine–Landaue–Verweye–Overbeek) theory, which takes account of the balance between attractive (van der Waals), repulsive and electrostatic forces (Dwivedi *et al.*, 2015; Lodeiro *et al.*, 2016; Zhang *et al.*, 2016). Different particle coatings have different impacts on particle aggregation. PVP- and branched polyethyleneimine-coated AgNPs are less likely to aggregate than dihydrogen (H_2)- and citrate-coated AgNPs (El Badawy *et al.*, 2012). Aggregation was found to increase with increasing ionic strength, whereas dissolved organic matter has been found to inhibit aggregation (Yin *et al.*, 2015). Citrate-coated AgNPs aggregated when dissolved oxygen was present in the water (Zhang *et al.*, 2011) and in an acidic environment (pH 3), whereas changing pH did not affect the aggregation of PVP-coated particles (El Badawy *et al.*, 2010). Particle aggregation and agglomeration increase the particle size and therefore increase the likelihood of the particles settling out of the water compared with individual AgNPs (Luoma, 2008). It is likely that AgNPs in environmental waters will undergo hetero-aggregation because of the high levels of naturally occurring species present in the aquatic environment (Keller and Auset, 2007; Wang *et al.*, 2015; Shevlin *et al.*, 2018).

The findings of the literature review presented in this chapter have been previously published (McGillicuddy et al., 2017; Shevlin et al., 2018).

2 Capture and Detection of Silver Nanoparticles from Water Samples

2.1 Background

To measure the levels and environmental impacts of AgNPs, suitable techniques are required to capture, detect, quantify and characterise AgNPs in natural waters (Heithmar, 2011). There are numerous challenges associated with the measurement of AgNPs in the environment (Poda *et al.*, 2011; Zook *et al.*, 2011; Sadik *et al.*, 2014). Numerous studies in recent years have investigated different methods of capturing and detecting AgNPs from aqueous samples. The different techniques examined include cloud point extraction (Hartmann *et al.*, 2013; L. Li *et al.*, 2016), ultracentrifugation (Kennedy *et al.*, 2010), ultrafiltration (Trefry *et al.*, 2010; Farmen *et al.*, 2012), hydrodynamic chromatography (Tiede *et al.*, 2009), nanofiber filtration membranes (Liang *et al.*, 2010), nanostructured nanofilters (Okello *et al.*, 2011), surface functionalised magnetic iron nanoparticles (Mwilu *et al.*, 2014) and tangential flow filtration (Maurer *et al.*, 2014).

One aim of the DeTER (Detection, Toxicology, Environmental fate and Risk assessment of nanoparticles in the aquatic environment) project was to develop a cost-effective technique to capture AgNPs from water samples and, following this, to develop a method to quantify the concentration of the captured AgNPs. To this end, activated charcoal was chosen as the capture material to be investigated as it is a cheap material commonly used in water remediation. Following the validation of the AgNP capture technique, a quantification method was developed to quantify the AgNPs captured by the charcoal. Activated charcoal has previously been shown to successfully capture AgNPs from water samples (Gicheva and Yordanov, 2013); however, this study added electrolytes to the samples at concentrations above the critical coagulation concentration to successfully remove the AgNPs. Although the previous study showed activated charcoal to be a suitable material for AgNP capture, the addition of electrolytes is likely to limit its environmental applicability (Gicheva and Yordanov, 2013). In the DeTER project the capture of the AgNPs by the charcoal was investigated without the addition of any chemicals to aid in AgNP removal, which will be more relevant for the application of the technique in the environment.

2.2 Methodology

2.2.1 Materials and chemicals

The AgNP samples for this study were generated *in situ* immediately prior to use from commercially available AgNPs dispersed in Milli-Q water (18.3 MΩ·cm; Millipore, Bedford, MA, USA). The AgNPs used were PVP-coated, 25-nm, 5-mg/ml Econix AgNPs purchased from nanoComposix Europe (Prague, Czech Republic). A study was also undertaken using citrate-coated, 10-nm, 0.02-mg/ml AgNPs (Sigma-Aldrich, St Louis, MO, USA). Trace metal-grade hydrochloric acid (HCl) (assay 34–37%) and nitric acid (HNO_3) (assay 67–69%) [Super Pure Acid (SpA) grade; Romil™, Cambridge, UK] were used throughout the studies. The capture material used was a powdered activated carbon (Norit® CA1; Norit N.V. Amersfoort, the Netherlands), which is used in water purification and was the type used in a previous AgNP capture study (Gicheva and Yordanov, 2013).

The activated charcoal was used as purchased in initial studies; in further studies it was processed to increase its surface area by, first, grinding the charcoal using a ceramic pestle and mortar and, then, milling it in an agate ball mill (Fritsch™ Pulverisette 6 Planetary Mono Mill; Idar-Oberstein, Germany) with a rotational speed of 500 rpm for 5 minutes (repeated three times) using an 80-ml agate vial and balls (diameter 10 mm) (Healy *et al.*, 2016a; McEneff *et al.*, 2017). Granulometry studies were carried out on the activated charcoal fraction that had been ground in the pestle and mortar and the agate ball milled fraction using laser particle sizing, by introducing a slurry of the activated charcoal in isopropyl alcohol into the Hydro-G dispersion unit of a Malvern Mastersizer 2000 (Walls *et al.*, 2017). The ground fraction had a mean diameter of 341.150 µm (with a 10th percentile

diameter of 27.139 µm and a 90th percentile diameter of 1061.639 µm) and the milled fraction had a mean diameter of 22.134 µm (with a 10th percentile diameter of 6.555 µm and a 90th percentile diameter of 159.724 µm). The activated charcoal samples used in the exposure studies were prepared for a leaching procedure by freeze drying (Freezone 12; Labconco, Kansas City, MO, USA) at –50°C (Ratcliff *et al.*, 2016; Healy *et al.*, 2016b).

2.2.2 Electron microscopy of AgNPs and activated charcoal

The size of the purchased 25-nm, PVP-coated AgNPs was characterised using transmission electron microscopy (TEM) (Hitachi H7000; Hitachinaka, Japan). A 1 : 1 diluted solution of AgNPs in Milli-Q water was dropped onto a 200 mesh formvar/carbon-coated copper grid (Agar Scientific, Stansted, UK). A TEM image of the AgNPs is shown in Figure 2.1a. Two different batches of AgNPs were used during the study; the first batch had a mean diameter of 22.01 nm (with a standard deviation of 6.40 nm) and the second batch had a mean diameter of 21.92 nm (with a standard deviation of 8.11 nm). The measured size distributions of the AgNPs from batch 1 and batch 2 are shown in Figure 2.1b and c respectively.

The activated charcoal samples were examined by scanning electron microscopy (SEM). The activated charcoal was gold coated (Emitech K550; Quorum Technologies Ltd, Lewes, UK) and then subjected to SEM in secondary electron mode using a Hitachi model S-4700 (Hitachinaka, Japan). The analyses were performed at an acceleration voltage of 20 kV, an emission current of 10 µA and a working distance of 12 mm (Morrison *et al.*, 2009; R. Li *et al.*, 2016; Mahon *et al.*, 2017). Figure 2.2 shows SEM images of the activated charcoal before (a) and after (b) processing by the agate ball mill.

2.2.3 Determination of Ag

The concentrations of Ag in the samples were measured using a PerkinElmer ELAN DRC-e (Waltham, MA, USA) inductively coupled plasma mass spectrometer in standard mode and equipped with a flow injection autosampler (FIAS 93 plus) in a class 1000 clean room [International Organization for Standardization (ISO) class 6] (Staunton *et al.*, 2015). Calibration standard solutions of Ag were prepared from a customised multi-element standard (Inorganic Ventures, Christiansburg, VA, USA; 1000 µg/ml) prepared in Milli-Q water, and rhodium (^{103}Rh) and indium (^{115}In) were used as internal standards

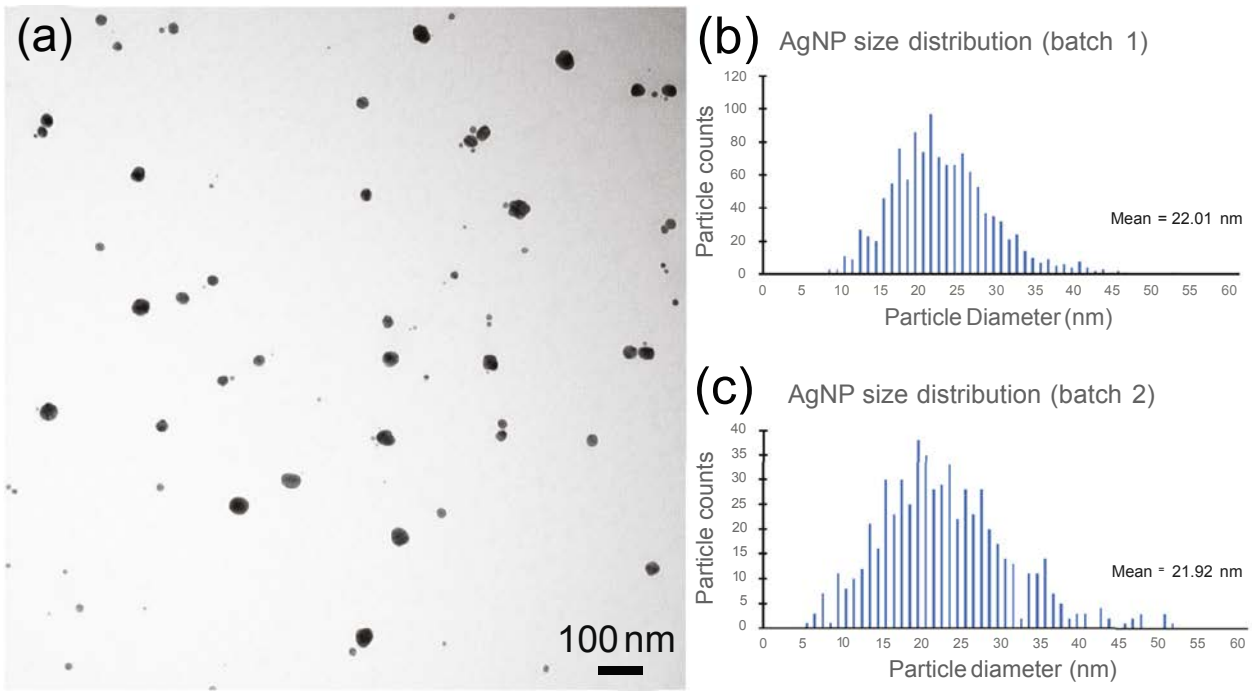


Figure 2.1. (a) TEM image of the 25-nm, PVP-coated AgNPs, (b) size distribution of the batch 1 AgNPs and (c) size distribution of the batch 2 AgNPs.

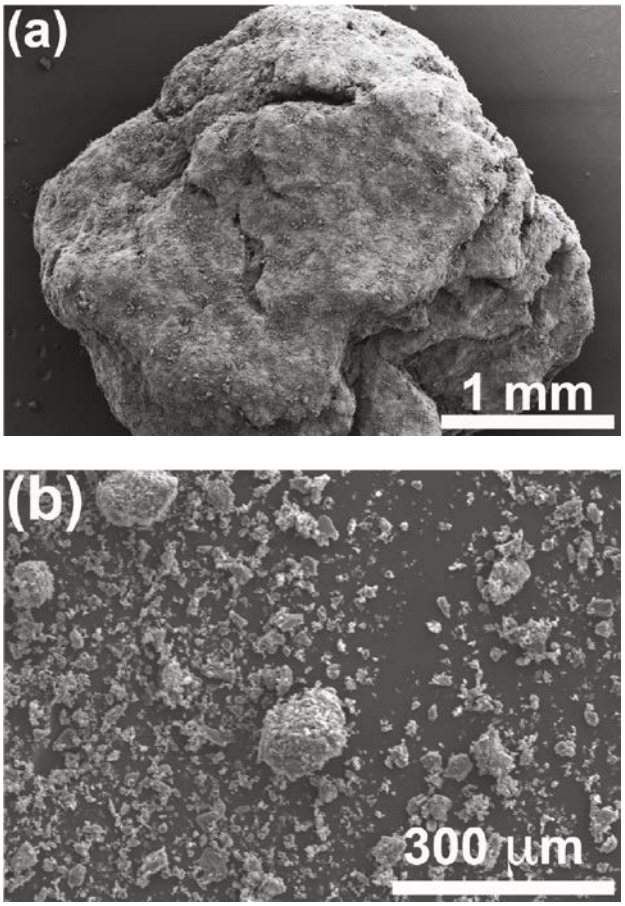


Figure 2.2. SEM images of Norit CA1 activated charcoal granules: (a) as purchased, prior to processing and (b) following processing by milling with an agate ball mill.

to account for instrumental drift and matrix effects (McGrory *et al.*, 2017; Wan *et al.*, 2017).

2.2.4 AgNP capture experiments

For the initial investigations, samples containing 100 µg/l of AgNPs were generated using Milli-Q-grade water. Next, 50 ml of the samples was exposed to 0.025 g of activated charcoal granules for 24 hours in trace metal-free centrifuge tubes (Labcon; Petaluma, CA, USA). After exposure, the charcoal was removed from the samples by syringe filtration using a 0.45-µm syringe filter. The Ag concentration in the samples, prior to and following exposure to activated charcoal, was analysed using ICPMS with the samples prepared in a 1% HNO_3 matrix. All studies were carried out in triplicate, with the samples prepared and stored in trace metal-grade Nalgene™ low-density polyethylene bottles (Thermo Scientific, USA) and the trace metal-free centrifuge tubes.

Following the initial investigations, the charcoal was physically processed, as outlined in section 2.2.1. The capture experiments were repeated using the ground and the milled activated charcoal. Further investigations of AgNP capture were conducted by varying the initial experimental parameters. The parameters varied were the initial AgNP concentration (10 µg/l, 25 µg/l, 50 µg/l, 75 µg/l and 100 µg/l), the size and coating of the AgNP (10-nm citrate-coated AgNP), sample exposure time (0, 1, 2, 4, 6, 8 and 24 hours of exposure) and the water type used in the generation of the AgNP samples [Milli-Q and environmental (canal) water].

2.2.5 Removal of captured Ag from the activated charcoal

To determine the quantity of AgNPs captured by the charcoal, a leaching procedure was developed. Initially, a HNO_3 leaching procedure was developed in which 0.1 g (dry mass) of freeze-dried activated charcoal was added to 2 ml of 70% HNO_3. This mixture was then shaken at 200 rpm for 24 hours on an orbital shaker (Orbital Shaker SSL1; Stuart Scientific, Stone, Staffordshire, UK). Following shaking the sample was syringe filtered using a 0.45-µm syringe filter and the filtrate made up to a 1% HNO_3 matrix and analysed for Ag content by ICPMS. The HNO_3 leaching procedure was not successful and therefore a second procedure was developed using HCl. This procedure again used 0.1 g of freeze-dried exposed charcoal, which was added to 2 ml of HCl (assay 34–37%); this was shaken at 150 rpm for 48 hours on the orbital shaker. The sample was then filtered with a 0.45-µm syringe filter, with the leachate prepared in a 1% HNO_3 matrix for ICPMS analysis, as previously. Blank samples of unexposed charcoal were included in the leaching procedure to act as controls, which were subtracted from the exposed charcoal results.

2.2.6 Capture of AgNPs from spiked environmental samples

Environmental water samples were collected from a pool in a fresh water canal running through a city urban environment at the final lock before the water re-enters the natural waterway at the city quays. The collected samples were divided into two groups: one was used as collected and the second was filtered prior to the experiments using the CapE system. The

CapE system is a large-volume water filtration system with a filter unit incorporating a 0.45-µm filter; the operation of the system is fully outlined elsewhere (Morris *et al.*, 2016). Spiked AgNP samples were generated using the filtered and unfiltered canal water, which were then exposed to the milled activated charcoal as outlined in section 2.2.4.

2.3 Results and Discussion

2.3.1 Capture of AgNPs by different grades of activated charcoal

Following exposure to the unaltered activated charcoal granules a reduction of 11.86% in Ag concentration was measured compared with the charcoal-free control (Figure 2.3). These results indicated that the unaltered activated charcoal granules were an unsuitable capture material for the AgNPs. To improve the AgNP capture the surface area of the charcoal was increased, as outlined in section 2.2.1; this processing resulted in three different grades of charcoal: the unaltered granules, the ground fraction (pestle and mortar) and the milled fraction (agate ball mill). Samples of AgNPs (100 µg/l) were exposed to the different grades of activated charcoal. AgNP capture increased with increasing surface area of the charcoal, with milled charcoal capturing 63.61% of the AgNPs from the sample on average compared with 37.77% captured by the ground charcoal and 11.86% captured by the charcoal granules (see Figure 2.3). These results indicate that a simple physical processing of the charcoal (i.e. milling) greatly enhances the capture of AgNPs from the samples. Previous studies of the adsorption of AgNPs by charcoal have suggested that AgNP adsorption occurs as a result of electrostatic interactions, which can be described by a mixed 1,2 kinetic adsorption model (Syafiuddin *et al.*, 2018). Another study proposed that van der Waals or London dispersion forces may be responsible for the adsorption (Gicheva and Yordanov, 2013). The total surface area and the roughness of the materials were also suggested as influencing factors in adsorption (Gicheva and Yordanov, 2013). This concurs with the findings of this study, in which increased AgNP capture was associated with increased surface area of the adsorbent. The surface of activated charcoal contains pores into which it has been suggested that AgNPs may be adsorbed (Gicheva and Yordanov, 2013; Syafiuddin *et al.*, 2018). It may be that the milling of the charcoal exposes more of these pores on the charcoal's surface, increasing the capture of the AgNPs.

2.3.2 Effect of AgNP concentration on capture by milled charcoal

The initial investigations were carried out using AgNP samples of 100 µg/l. Studies were subsequently carried out using different concentrations of AgNPs (100, 75, 50, 25 and 10 µg/l) to examine the effect that the initial AgNP concentration has on AgNP capture.

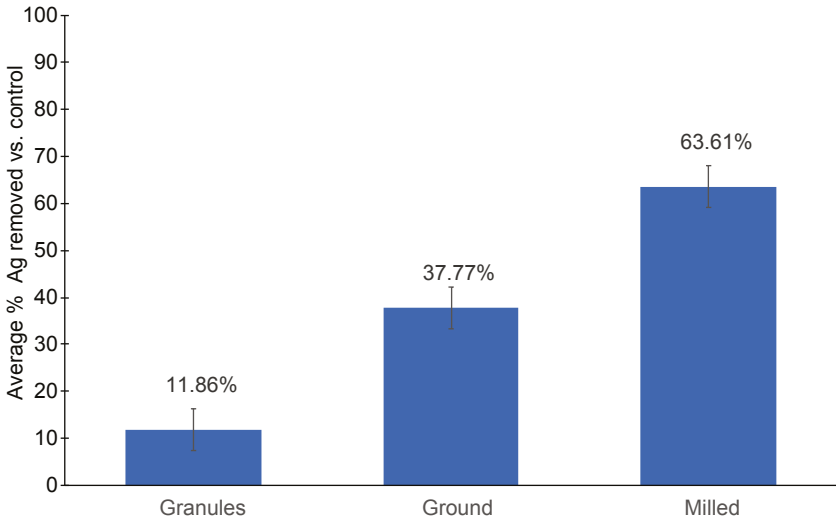


Figure 2.3. The percentage of Ag removed from 100 µg/l samples of 25-nm, PVP-coated AgNP by different grades of activated charcoal compared with carbon-free control samples following 24 hours of exposure.

Table 2.1. The percentage of Ag removed from different concentrations of 25-nm, PVP-coated AgNP samples by milled activated charcoal following 24 hours of exposure

Initial AgNP concentration	Average % Ag removal vs. charcoal-free control
100 µg/l	65.89
75 µg/l	68.07
50 µg/l	73.04
25 µg/l	81.61
10 µg/l	89.92

The percentage of Ag captured by the milled charcoal increased with decreasing initial concentration (Table 2.1). Therefore, at lower concentrations the system captures a higher proportion of the AgNPs. At a 100 µg/l initial AgNP concentration, 65.89% of the AgNPs were captured; this increased to 89.92% at an initial AgNP concentration of 10 µg/l. These findings are encouraging as AgNP concentrations in the aquatic environment are in the low ng/l range (Blaser *et al.*, 2008; Gottschalk *et al.*, 2009; R. Li *et al.*, 2016; Peters *et al.*, 2018), suggesting that at environmentally relevant concentrations milled charcoal could capture a significant portion of the AgNPs. Lower capture percentages at higher concentrations could be due to the saturation of the charcoal by the blocking of the charcoal's pores by the captured AgNPs; this could then inhibit further AgNP capture by the charcoal. It is clear from this study that milled activated charcoal is a suitable material for the capture of AgNPs from water samples; however, should it be applied to environmental waters, care would have to be taken to ensure that the charcoal does not cease capturing the AgNPs through saturation.

2.3.3 Effect of different AgNP size and coating on capture by milled charcoal

All previous capture experiments were conducted using 25-nm PVP-coated AgNPs. However, nano-functionalised products will contain AgNPs with different coatings and of different sizes. It is therefore imperative that a technique to capture AgNPs from environmental waters must capture different AgNP types. This was investigated for the milled activated charcoal technique by comparing the capture of samples generated using 10-nm citrate-coated AgNPs with the capture of the 25-nm PVP-coated AgNPs. PVP- and citrate-coated AgNPs were selected for this study as they are two of the most commonly used capping agents for AgNPs (El Badawy *et al.*, 2010). Samples of the AgNPs (100 µg/l and 10 µg/l) were exposed to the milled activated charcoal as previously. In total, 76% of the 10-nm citrate-coated AgNPs were captured from the 100-µg/l sample whereas 94% of the 10-nm citrate-coated AgNPs were captured from the 10-µg/l sample. This compares favourably with the 25-nm, PVP-coated particles, with 66% and 90%, on average, of the AgNPs captured in the 100-µg/l and 10-µg/l samples respectively (Table 2.2). The milled activated charcoal is therefore capable of capturing AgNPs of differing sizes and coatings, which is important as the AgNPs entering the environment will have different sizes and coating.

2.3.4 Effect of exposure time on capture of AgNPs

The effect of the time that the AgNPs were exposed to the activated charcoal was examined. When the samples were filtered immediately following exposure to the charcoal, 46.9% of the AgNPs were captured; this compared with 63.6% of the AgNPs captured from samples exposed for 24 hours (Figure. 2.4). This demonstrated that increasing the exposure time increases the amount of Ag captured by the charcoal. However, even short exposure times resulted in a significant capture of the AgNPs, showing that even short-term exposure to the activated charcoal offers some benefit in terms of AgNP capture from water samples. Although this study found that, overall, the increased exposure time resulted in increased

Table 2.2. The percentage of Ag removed from samples of two different concentrations generated using two different commercially available AgNPs following 24 hours of exposure to milled activated charcoal

Initial AgNP concentration	% Ag captured	
	10-nm, citrate-coated AgNPs	25-nm, PVP-coated AgNPs
100 µg/l	76.28	65.89
10 µg/l	94.12	89.92

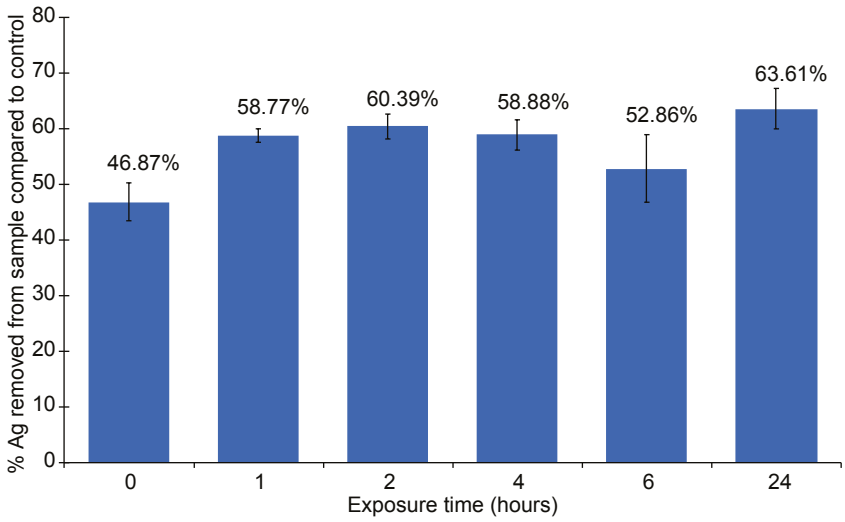


Figure 2.4. The percentage of Ag removed from 100 µg/l samples of 25-nm, PVP-coated AgNPs by milled activated charcoal following different exposure times.

AgNP capture, there was some difficulty associated with some of the measurements, with a large error associated in particular with the 6-hour measurement, which has a much lower than expected average.

2.3.5 Leaching of captured Ag from activated charcoal following exposure

The quantification of the Ag captured by the milled charcoal is important as it will provide information on the levels of AgNPs in the environment in which it is placed. Therefore, a suitable technique is required to remove the captured Ag from the charcoal. The initial HNO_3 leaching procedure proved unsuccessful, recovering only an average of 3.6% of the Ag captured by the charcoal. The second leaching procedure developed using HCl recovered an average of 94.83% (standard deviation 5.51%), of the Ag captured by the charcoal, with recoveries ranging from 86.67% to 101.93% (Figure 2.5). The HCl extraction procedure is therefore a suitable method to quantify the Ag captured by the milled charcoal and could be developed as a technique for the possible quantification of AgNP levels in the environment, should milled activated charcoal be used as the capture method.

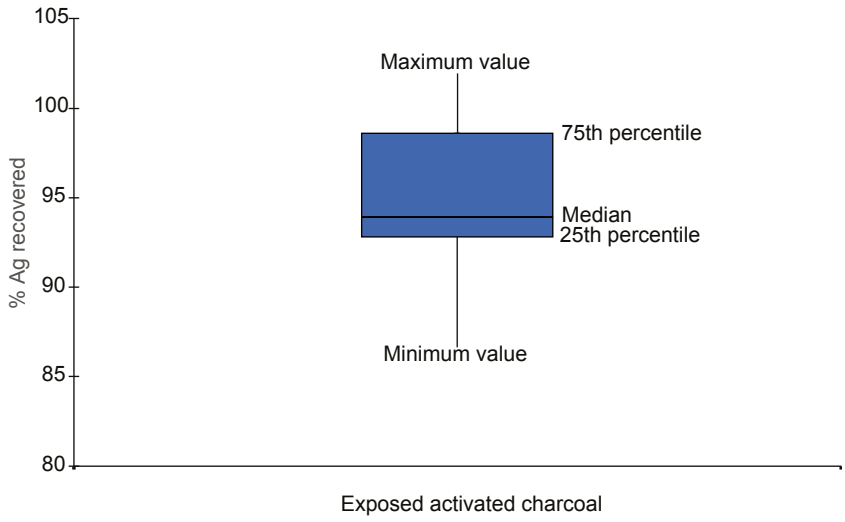


Figure 2.5. Box and whisker plot showing the recovery of Ag from activated charcoal that had been exposed to samples containing AgNPs using a 30% HCl leaching procedure.

2.3.6 Capture of AgNPs from spiked environmental samples

Milled activated charcoal has been shown to capture AgNPs from water samples generated using Milli-Q water. It must also be capable of the capture of AgNPs from environmental waters in which the ambient parameters will be more complicated than in Milli-Q samples. Following exposure of the spiked canal water samples to the milled charcoal an average of 88.7% and 87.3% of the Ag in the sample was captured from the filtered and unfiltered canal samples respectively. These findings demonstrate that the milled charcoal is a suitable material for AgNP capture from the aquatic environment. The AgNPs in the canal water samples were found to behave differently from the AgNPs in Milli-Q water. The canal samples were spiked to have an initial concentration of AgNPs of 100 µg/l; however, the actual concentration prior to exposure to the charcoal was found to be much lower. This may be the result of the AgNPs undergoing some chemical reactions immediately on exposure to the canal water, for example attaching to particulate matter or reacting with chemical species present in the canal waters and settling from the suspension. This occurred in both the filtered and the unfiltered canal water, with average concentrations of AgNPs measured prior to exposure of 25.31 and 25.96 µg/l respectively. These findings show the uncertainties surrounding the fate of AgNPs in environmental waters. In the study undertaken with the canal water it was not possible to determine the actual concentration of AgNPs in the water because the sample volumes were too low in the laboratory studies. Therefore, further studies should be undertaken using larger volumes of water, possibly adapting the CapE system, or focusing sampling on areas suspected to have high levels of contamination to determine the burden of AgNPs in environmental waters.

The findings presented in this chapter have been previously published (McGillicuddy *et al*., 2018).

3 Ecotoxicological Assessment of the Effect of Silver Nanoparticles and Silver Nitrate on Freshwater Aquatic Organisms

3.1 Background

The possible fates of AgNPs following introduction to the wastewater system have been described by several authors (Fabrega *et al.*, 2011; Bleaker *et al.*, 2015); however, the behaviour of these AgNPs once they reach surface waters remains elusive and subject to local environmental parameters and potentially even seasonal influences (Ellis *et al.*, 2018). Additionally, the lack of information on the specific types of AgNPs in use in Ireland and their concentrations in the aquatic ecosystem means that making an informed risk assessment is challenging. This underpins the need for a more targeted environmentally relevant ecotoxicological assessment of AgNPs. The modern approach to ecotoxicological assessment advocates a multi-trophic test battery that should include three trophic levels. This approach was adopted in this study, which investigated the ecotoxicity of AgNPs and silver nitrate ($AgNO_3$) to the algae *Pseudokirchneriella subcapitata*, the crustacean *Daphnia* spp. and the cnidarian *Hydra attenuata.*

3.1.1 Pseudokirchneriella subcapitata

As primary producers, algae form the basis of the aquatic food chain. Algal toxicity tests form part of the basic information required for the toxicity assessment of potential environmental hazards recommended by the Organisation for Economic Co-operation and Development (OECD) and prescribed by REACH regulations in the European Union (EU) (ECHA, 2017). The validated procedures pertaining to this toxicity test are outlined in ISO 8692: 2012 and OECD 201 (International Organization for Standardization, 2012a). The prescribed end point for this toxicity test is algal growth inhibition. The test duration is 72 hours, which although relatively short does allows for multi-generational effects because of the rapid growth rate of algae. The end point is the E_rC_{50} value, which is the median effective growth rate-inhibiting concentration.

Several studies have already looked at the effects of certain AgNPs on the freshwater algae *Pseudokirchneriella subcapitata* using the traditional ISO 8692: 2012 methodology. A 72-hour E_rC_{50} for AgNPs of 20–30 nm was reported to be 190 µg/l by Griffitt *et al*. (2008). Ivask *et al*. (2014) reported size-dependent toxicity ranging from 180 to 1140 µg/l using citrate-coated AgNPs ranging from 10 to 80 nm in diameter. Kennedy *et al*. (2010) reported a 48-hour E_rC_{50} of 18.4 µg/l using PVP-coated 40-nm AgNPs, expressed as total Ag.

The validity criteria for the *Pseudokirchneriella subcapitata* bioassay include media characterisation and the growth rate in the control, which should be in excess of a 67-fold increase after a 72-hour incubation period. The medium prescribed by ISO 8692: 2012 is Jaworski's medium (JM). This medium is the standard used for most freshwater algal culture and testing. However, it contains the chelating agent, ethylenediaminetetraacetic acid (EDTA). The binding of EDTA to metals in solution appears to have an effect on test sensitivity because of reduced bioavailability. In the sections relating to toxicity assessment using *Daphnia magna*, the REACH legislation, Commission Council Regulation (EC) No. 440/2008, suggests limiting the use of EDTA and chelating agents in test media for testing ionising chemicals or metallic toxicants; however, this interference is not mentioned in relation to the prescribed media for algae (EC, 2008). An EDTA-free medium, Chu#10 (Anderson, 2005), was selected for this study and tested concurrently with JM for comparison. The medium was further modified and optimised to improve growth rates and make them comparable with those of JM.

3.1.2 Daphnia magna *and* Daphnia pulex

A number of studies report the acute toxicity of several AgNP variants to *Daphnia* spp. Asghari *et al*. (2012) compared the toxicity of two Ag colloids, a powdered

suspension and Ag^+ (from $AgNO_3$). The toxicity of the 1- to 13-nm water-based colloidal suspension was very similar to that of $AgNO_3$, with a half-maximal effective concentration (EC_{50}) for $AgNO_3$ of approximately 2 µg/l. In contrast, a powdered suspension of AgNPs ranging in size up to 161 nm (86% 1–45 nm) yielded a far less toxic response, with an EC_{50} of 187 µg/l. The study concludes that the toxic responses are dependent on the chemical characteristics and aggregation properties of the AgNP (Asghari *et al.*, 2012).

Blinova *et al*. (2013) assessed the toxicity of PVP-coated AgNPs in artificial freshwater and natural waters from a number of lakes and rivers. A significant difference in toxicity between the different test matrices was reported, with natural water attenuating toxicity substantially. It was also noted that the toxicity of AgNPs was 10-fold lower in this study than that of $AgNO_3$ in all matrices tested.

More recent studies have investigated the effects of test matrices on Ag toxicity. Hu *et al*. (2018) reported a significant difference between the toxicity of AgNP and that of $AgNO_3$ in the media specified in ISO 6341: 2012 and surface water, with the latter reducing the toxicity of both Ag species.

An assessment of Ag bioaccumulation in reproducing daphnids over 7 days demonstrated that the first two broods of *Daphnia magna* reproduced in AgNP were affected whereas only the first brood was affected by $AgNO_3$ (Pakrashi *et al.*, 2017). The authors concluded that AgNPs are likely to have adverse effects on the daphnids for longer than $AgNO_3$.

In this study, the toxic effects of Ag^+ (from $AgNO_3$) and 25-nm PVP-coated nanospheres on *Daphnia pulex* and *Daphnia magna* were investigated using the acute immobilisation end point. The effects of the two Ag types on *Daphnia magna* fecundity were also investigated.

3.1.3 Hydra attenuata

Giese *et al*. (2018) reported that it is almost impossible to predict the environmental concentrations of AgNPs because of the dynamic nature of the environment and changing influences; however, using a combination of models and measurements on a number of ENMs they suggest a predicted environmental concentration (PEC) of 0.03–2.79 ng/l in surface water.

Although it is clear from existing ecotoxicological data that the toxicity of AgNP (and $AgNO_3$) to *Daphnia* is unlikely to be in the range of the PECs, more recent ecotoxicological models of water column distribution suggest that benthic organisms such as *Hydra attenuata* are likely to be exposed to substantially higher concentrations of AgNPs. The model by Giese *et al*. (2018) went further than previous models such as that by Blaser *et al*. (2008) by separating predictions into different parts of the water column based on different chemical fates. They suggested that 0.02–33.67 µg/l could accumulate in the sediment in a 100% AgNP degradation scenario and 0.19–470.65 µg/l in a 100% persistent AgNP scenario. This could be of particular importance to benthic organisms such as crustaceans and other invertebrates as well as organisms in higher trophic levels that consume them such as *Hydra attenuata*, as they are likely to come into contact with higher concentrations than other animals that dwell in the general water column.

A number of different end points are available for *Hydra attenuata* bioassays. Morphological effects, effects on regenerative ability and deoxyribonucleic acid (DNA) damage using the Comet assay were investigated in this study.

3.2 Materials and Methods

Analytes used were PVP-coated 25-nm Econix AgNPs from nanoComposix, as described in Chapter 2, and $AgNO_3$ from Sigma-Aldrich.

3.2.1 Pseudokirchneriella subcapitata *bioassay*

Growth inhibition of *Pseudokirchneriella subcapitata* [Culture Collection of Algae and Protozoa (CCAP) strain 278/4] was assessed following the procedures and conditions outlined in the ISO 8692: 2012 algal growth inhibition bioassay, with some adaptations regarding the medium. The medium was substituted for Chu#10 EDTA-free medium as described in Anderson (2005). This medium was reduced to half-strength and supplemented with the vitamin trace solution and metals trace solutions as described in ISO 6341: 2012 and Anderson (2005) for JM.

Algal cells were counted by haemocytometer; the number of cells/ml was calculated using equation 3.1

and the average specific growth rate was calculated using equation 3.2.

$$\text{Algal cells / ml} = \frac{n \times 10^3}{0.02} \quad (3.1)$$

$$\mu = \frac{\ln X_n - \ln X_0}{T_n} \quad (3.2)$$

Validation was completed using reference chemical $K_2Cr_2O_7$, optimisation was completed using $AgNO_3$ and testing was carried out with both $AgNO_3$ and AgNPs. A minimum of three independent tests were carried out in triplicate per treatment using 25-ml volumes and a seeding density of 1×10^4 cells/ml.

3.2.2 Acute Daphnia *bioassay*

The acute immobilisation tests were performed in accordance with ISO 6341: 2012, with some modifications. US Environmental Protection Agency (EPA) moderately hard water was used as the test water and diluent as per the recommendations of REACH legislation 440/2008 for the testing of nanomaterials (EC, 2008).

Tests were carried out in batches of five daphnids per test vessel and four vessels (i.e. 20 daphnids per treatment). Validation was completed using reference chemical $K_2Cr_2O_7$, optimisation was completed using $AgNO_3$ and testing was carried out with both $AgNO_3$ and AgNPs. Gravid females were isolated and, once hatched, age-synchronised neonates less than 24 hours old were used for testing. This was repeated for both *Daphnia pulex* (from Blades Biological Ltd, Edenbridge, UK) and *Daphnia magna* Straus.

3.2.3 Daphnia magna *fecundity assessment*

This test was carried out in 20 replicates (five daphnids per replicate) under semi-static conditions at a fixed concentration of 0.1 µg/l of AgNPs (and control). Gravid female *Daphnia magna* were isolated and age-synchronised neonates less than 24 hours old were removed to individual 25-ml cultures in 50-ml beakers. This is the F1 (first filial) generation. These beakers were incubated under the same lighting and temperature conditions as for the acute toxicity test as outlined in the previous section. Every 48 hours, new neonates were counted and removed from the culture. The neonates were counted in this F2 (second filial) generation and retained for feeding to *Hydra attenuata* for a 28-day assessment of bioconcentration.

3.2.4 Hydra attenuata *morphology assessment*

Hydra were released from the sides and bottom of their culture bowl with fingertips. Budding *Hydra* were identified under the dissecting microscope and nine hydranths were isolated into rinsing wells of a 24-well multi-well plate (sterile culture wells) containing approximately 2-ml aliquots of prepared AgNPs in *Hydra* medium (this step was repeated for each AgNP treatment and control).

Three budding hydranths were taken from the rinsing wells and placed into each well of a prefilled 24-well multi-plate. This step was repeated for each treatment concentration three times (n = 9). *Hydra attenuata* were obtained as a gift from Dr Brian Quinn (University of the West Scotland).

3.2.5 Hydra attenuata *regeneration assessment*

Hydra were gently detached from the sides and bottom of their culture bowl with gloved fingertips. Adult *Hydra* were identified under the dissecting microscope and isolated with long-form glass Pasteur pipettes into glass petri dishes. Using a sterile scalpel blade, the *Hydra* were dissected by careful incisions to separate the gastric region from the basal disc and the hypostome. The dissected *Hydra* gastric regions, which included the budding region, were transferred with a short-form glass Pasteur pipette into rinsing wells of a 24-well multi-well plate containing 2-ml aliquots of prepared *Hydra* medium (this step was repeated for each treatment concentration of AgNPs).

Each adult *Hydra* was taken from the rinsing well and placed into another well of the 24-well multi-well plate. This step was repeated for each treatment concentration five times, that is, five adult *Hydra* per treatment. Concentrations of the test chemical were prepared. The concentrations used in the study were 0, 4, 8, 12, 16 and 20 µg/l. Each well had a test volume of 2 ml of test chemical or control *Hydra* medium.

3.3 Results

3.3.1 Pseudokirchneriella subcapitata

It was noted that the growth rates in the EDTA-free medium were lower than the prescribed limits set out in ISO 8692: 2012, which required a minimum 67-fold increase in cell number in 72 hours. This growth rate was met by JM. A comparative analysis of growth numbers was carried out. This analysis assessed the growth rates in JM and EDTA-free medium, as well as for algae tested in EDTA-free medium having been cultured and passaged a number of times in JM prior to the test. It was also noted that the use of EDTA-free medium significantly increased the sensitivity of the test to both $AgNO_3$ and AgNPs ($p < 0.05$). For this reason, both the E_rC_{50} and the control growth rates were compared using $AgNO_3$. The results from this analysis are shown in Table 3.1 and Figure 3.1.

As shown in this figure, the sensitivity of *Pseudokirchneriella subcapitata* increases (as shown by reducing E_rC_{50} values) when the algae is cultured in EDTA-free medium, that is, the algae require less Ag to yield the same toxic effect. The 72-hour E_rC_{50} for AgNPs tested in JM was 6.76 µg/l, reducing to 1.89 µg/l when cultured in JM, passaged once in EDTA-free medium and then tested in EDTA-free medium and further reducing to 0.68 µg/l when cultured and tested purely in EDTA-free medium. An approximate 10-fold improvement in test sensitivity was observed overall. The growth rate also decreased from a 75-fold increase in 72 hours in JM to a 45-fold increase in 72 hours in EDTA-free medium.

The effect of AgNPs on the *Pseudokirchneriella subcapitata* growth rate over 72 hours under varied conditions is shown in Figure 3.2. The different test conditions included the testing of algae in JM having been cultured continuously in JM, testing in EDTA-free medium having been cultured in JM and finally testing in EDTA-free medium having been cultured for only one passage in EDTA-free medium following continuous culture in JM. It is evident from the results that the toxicity of AgNPs was reduced by the presence of JM in the test matrix. This suggests that the Ag is being rendered less bioavailable in JM than in EDTA-free medium, thus improving the sensitivity of the test. This finding has not previously been quantified and reported for *Pseudokirchneriella subcapitata*.

No significant difference between the toxicity of AgNP and that of $AgNO_3$ was observed over 72 hours ($p > 0.05$), as shown in Figure 3.3. As previously

Table 3.1. Summary of the effects of AgNPs and $AgNO_3$ on a multi-trophic test battery including the algae *Pseudokirchneriella subcapitata* and the freshwater invertebrates *Daphnia pulex*, *Daphnia magna* and *Hydra attenuata*

Test		AgNP (25 nm, PVP coated)		Ag^+ from $AgNO_3$	
Species name	Parameter	Median effective concentration[a] (µg/l)	95% CI (µg/l)	Median effective concentration[a] (µg/l)	95% CI (µg/l)
Pseudokirchneriella subcapitata	JM	6.76	5.28–8.66	6.74	5.72–7.94
	EDTA free[b]	0.70	0.59–0.85	0.68	0.58–0.79
	Combination[c]	1.89	1.40–2.55	1.86	1.79–1.94
Daphnia magna	US EPA – acute	7.85	5.8–10.7	1.22	0.97–1.55
Daphnia pulex	US EPA – acute	4.2	3.4–5.0	9.3	5.8–13.0
	US EPA – fecundity	Reduced by 33% on day 8 and 80% on day 12 cultured in 0.1 µg/l of AgNPs			
Hydra attenuata	Morphology	29	18–50	35	25–52
	Regeneration	7.24	5.2–10.0	6.98	4.9–9.7
	Comet assay	Same as Morphology			

[a]***Pseudokirchneriella subcapitata*** **data are E_rC_{50};** ***Daphnia*** **spp. data are IC_{50};** ***Hydra attenuata*** **data are EC_{50}.**

[b]**EDTA-free medium adapted from Chu#10 and used for all culturing and testing.**

[c]**Combination medium is algae cultured in JM, passaged once in EDTA-free medium and then tested in EDTA-free medium.**

CI, confidence interval.

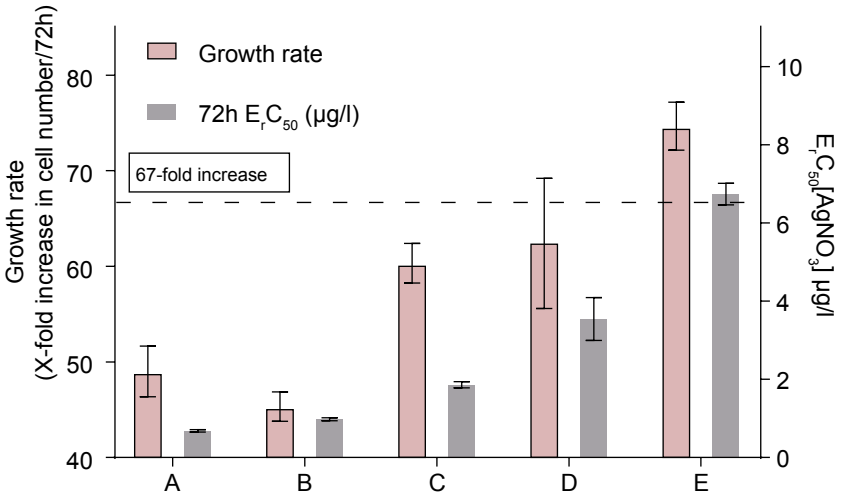


Figure 3.1. Comparison of test sensitivity to $AgNO_3$ and algal growth rates under different media conditions (n = 3, standard error of the mean indicated). A, cultured and tested in EDTA-free medium; B, cultured in JM, three passages in EDTA-free medium and tested in EDTA-free medium; C, cultured in JM, two passages in EDTA-free medium and tested in EDTA-free medium; D, cultured in JM, one passage in EDTA-free medium and tested in EDTA-free medium; E: cultured and tested in JM.

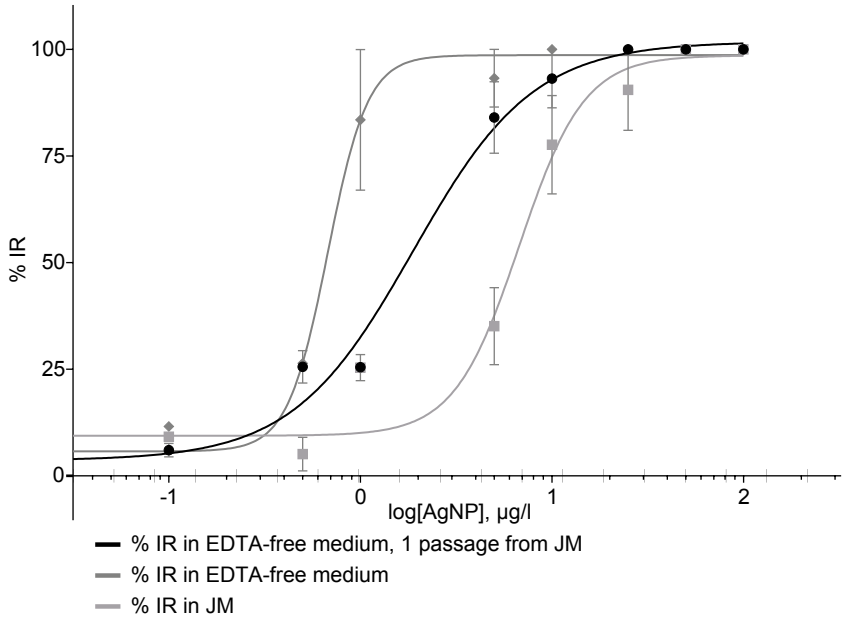


Figure 3.2. Comparison of the toxicity of AgNPs for *Pseudokirchneriella subcapitata* over 72 hours in three different types of media: JM, EDTA-free medium and one passage in EDTA-free medium and testing in EDTA-free medium following continuous culture in JM (n = 9, standard error of the mean indicated). IR, rate of inhibition.

described, when tested and cultured in EDTA-free medium, AgNPs yielded a 72-hour E_rC_{50} of 0.68 µg/l and $AgNO_3$ yielded a 72-hour E_rC_{50} of 0.702 µg/l. This did not demonstrate a significant difference (p > 0.05) in toxicity between the two species of Ag over 72 hours.

3.3.2 Daphnia

When *Daphnia pulex* were tested with $AgNO_3$, the observed 24-hour half-maximal inhibitory concentration (IC_{50}) was 9.3 µg/l [95% confidence interval (CI) 5.8–13.0 µg/l]. The assessment of the effect of $AgNO_3$ on *Daphnia magna* over 24 hours yielded different results

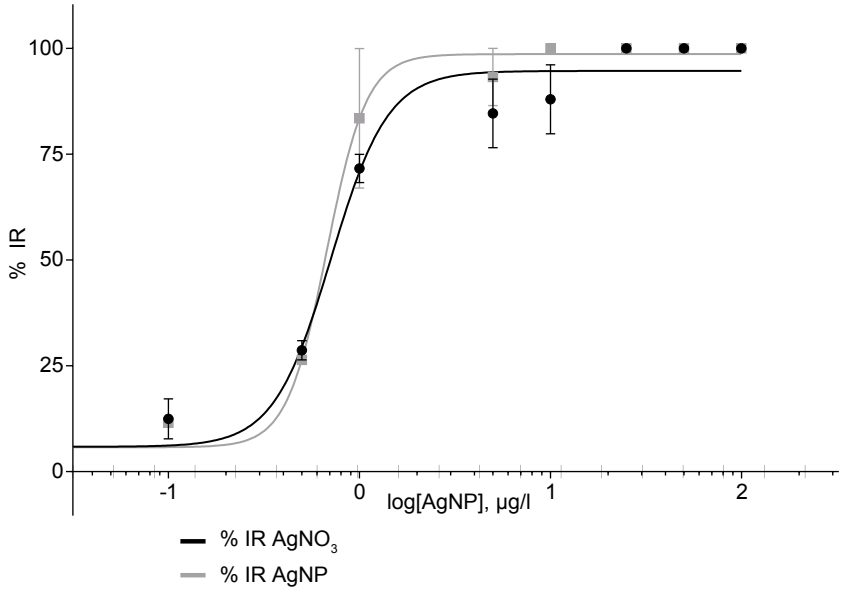


Figure 3.3. Comparison of toxicity of $AgNO_3$ and AgNPs to *Pseudokirchneriella subcapitata* over 72 hours in EDTA-free medium (*n* = 6, standard error of the mean indicated).

($p < 0.05$), with an IC_{50} observed of 1.22 µg/l (95% CI 0.97–1.55 µg/l). When tested with AgNPs, *Daphnia pulex* yielded similar results to those for $AgNO_3$, with a 24-hour IC_{50} value of 42 µg/l (95% CI 3.4–5.0 µg/l). In comparison, the IC_{50} observed was 7.85 µg/l (95% CI 5.8–10.7 µg/l) for *Daphnia magna* when treated with the same 25-nm PVP-coated nanospheres.

The effects of AgNPs on the fecundity of *Daphnia magna* were assessed by culturing continuously in a semi-static treated medium containing 0.1 µg/l of AgNPs. The results are shown in Figure 3.4. The number of offspring produced had reduced by 33% after 8 days and by 80% after 12 days, a level that was maintained for the remainder of the 28-day study. The reduction in the number of offspring was significant ($p < 0.05$) from day 8 onwards when assessed using the Sidak two-way ANOVA procedure on GraphPad Prism 7.

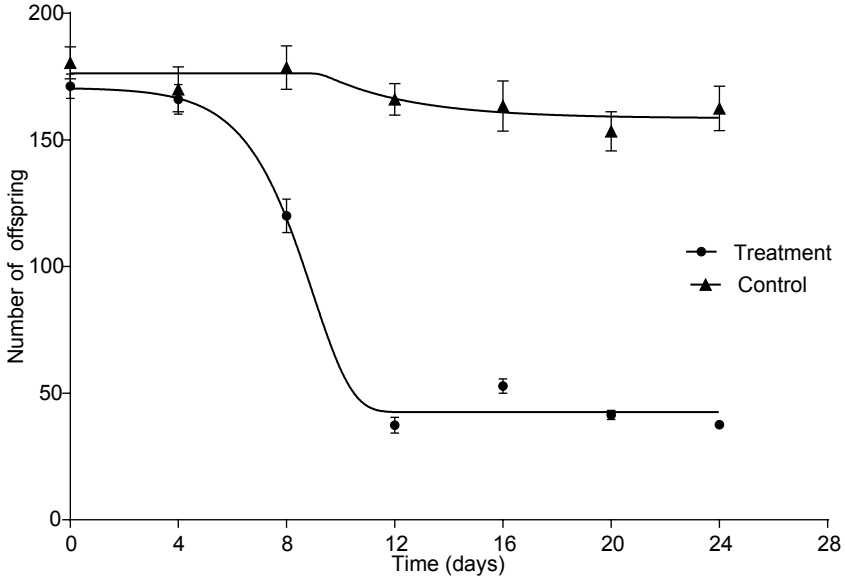
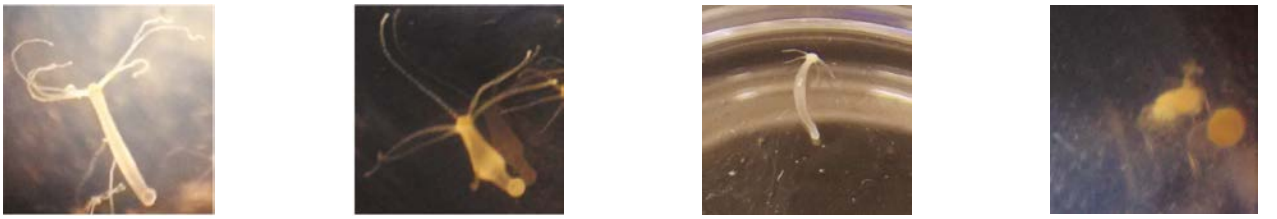


Figure 3.4. Effect of AgNPs at a concentration of 0.1 µg/l on *Daphnia magna* fecundity over 28 days in semi-static culture (*n* = 3, standard error of the mean indicated).



Control
(Wilby score: 10)

12.5 µg/l
(Wilby score: 10)

25.0 µg/l
(Wilby score: 7)

50.0 µg/l
(Wilby score: 4)

75.0 µg/l
(Wilby score: 0)

100.0 µg/l
(Wilby score: 10)

150.0 µg/l
(Wilby score: 0)

200.0 µg/l
(Wilby score: 0)

Figure 3.5. Photographic results for AgNP vs. *Hydra attenuata* following 96-hour exposure, showing a representative hydranth for each concentration.

3.3.3 Hydra attenuata

The results of the assessment of the morphological effects of $AgNO_3$ on *Hydra attenuata* are presented in Figure 3.5. The EC_{50} for $AgNO_3$ was observed to be 35 µg/l (95% CI 25–52 µg/l). An anomalous result was noted at the concentration of 100 µg/l. This result was repeated in three independent tests and was determined to be a "real" result. The improved viability of *Hydra* at this concentration was also observed when tested with AgNP. This concentration (100 µg/l) was treated as an outlier and omitted from the calculation of the EC_{50} as it erroneously inflates the EC_{50}, suggesting that the Ag is less toxic than it actually is. With this concentration included in the analysis, the EC_{50} was shown to be 66 µg/l, whereas with the 100 µg/l concentration excluded as an outlier the EC_{50} reduces to 35 µg/l, suggesting a higher degree of toxicity.

Figure 3.5 shows representative photographic results for hydranths following 96-hour exposure to AgNPs at different concentrations. As can be seen from the photographs, exposure to the control and 12.5 µg/l concentration have little effect on the organisms; they have healthy, long flowing tentacles that are spread out, demonstrating a Wilby score of 9–10. At a concentration of 25 µg/l, the body of the hydranths became blanched and the tentacles shortened and began to "club", suggesting a Wilby score of approximately 6–7. At a concentration of 50 µg/l there is further morphological degradation and the Wilby score reduced further to 3–4. The *Hydra* exposed to 75 µg/l of AgNPs for 96 hours is completely destroyed. Similar observations were found for *Hydra* exposed to concentrations of 150 and 200 µg/l. *Hydra* exposed to a concentration of AGNPs of 100 µg/l, however, remained almost completely unaffected. The EC_{50} for AgNPs with the 100 µg/l value omitted was 29 µg/l (95% CI 18–50 µg/l), as previously described. The effects of AgNP and Ag^+ were not significantly different ($p < 0.05$).

The 96-hour EC_{50} values determined for the effects of $AgNO_3$ and AgNP on *Hydra* regeneration were 6.98 µg/l (95% CI 4.9–9.7 µg/l) and 7.24 µg/l (95% 5.2–10 µg/l) respectively. There was no evidence of a significant difference in the mean Wilby scores at any of the concentrations tested ($p > 0.05$).

As indicated by the Comet assay, DNA damage did not increase the sensitivity of the test substantially beyond that of the morphology end point, with similar IC_{50} values reported. The anomalous survival at a treatment concentration of 100 µg/l was also observed using DNA damage as the endpoint, as assessed using the Comet assay.

3.4 Discussion

3.4.1 Addressing the analytical problem

The initial objective of this research was to assess the ecotoxicological effects of AgNPs in the freshwater environment. It quickly became apparent that the tools available to ecotoxicologists were not suitable for this application. As a result, the project evolved into the adaptation of existing bioassays to make them more suitable for the testing of AgNPs, thus optimising the sensitivity of the tests for the specified analyte. An improvement in test sensitivity was of particular importance because of the low AgNP PECs in the ng/l range. Essentially, the research endeavoured to contribute to an enhanced ecotoxicological toolbox for the assessment of the effects of AgNPs on select freshwater organisms.

This study became an endeavour to find the most sensitive bioassays by adaptation of test conditions and the utilisation of lethal, chronic or sub-lethal end points to make the assessment as environmentally relevant as possible. This approach was applied to all three test organisms, that is, *Pseudokirchneriella subcapitata, Daphnia* spp. and *Hydra attenuata.*

It was noted from the literature that there was limited information available on which AgNPs are in widespread use and are most likely to end up in the freshwater receiving environment (McGillicuddy *et al.*, 2017). It was also noted that there was no statutory registration system or database of ENMs within the EU, with the exception of in France. On 11 June 2018, the establishment of two EU databases of ENMs was announced (European Observatory for Nanomaterials, 2018), NanoData and eNanoMapper. NanoData is a database of products on the market including patents, projects and publications whereas eNanoMapper provides a repository of toxicological data. Unfortunately, these toxicological data are primarily mammalian in nature, relating more to primary toxicity to end users than to the environment. As such, the cradle-to-grave assessment of ENMs and AgNPs remains incomplete.

Although moves to harmonise testing have been ongoing through REACH, ISO and OECD legislation, there seems to be a somewhat disjointed approach to bioavailability and related interference for ecotoxicological assessment. For example, the interference of EDTA with bioavailability in *Daphnia magna* is noted in Council Regulation (EC) No. 440/2008 (EC, 2008), in which the use of EDTA-free medium prescribed by the US Environmental Protection Agency (2002) is recommended. The same recognition is not given to the interference of EDTA with AgNPs in algal testing, with Council Regulation (EC) No. 440/2008 (EC, 2008) maintaining its support for JM as set out in ISO 6341: 2012 guidelines (International Organization for Standardization, 2012b) for the algal growth inhibition bioassay. This issue was addressed in the assessment of AgNP toxicity using *Pseudokirchneriella subcapitata* in this study.

3.4.2 Pseudokirchneriella subcapitata

The presence of EDTA in algal growth media, a known chelator of Ag ions, was identified as a potential interference. Comparisons and correlations between the ISO medium, which contains EDTA, and an adapted Chu#10 EDTA-free medium were produced using the ISO-prescribed reference chemical $K_2Cr_2O_7$, Ag^+ and AgNPs. The most sensitive and robust method was selected and used as the basis for analysis, that is, EDTA-free medium. This approach improved the sensitivity of the test 10-fold. This improvement in sensitivity was evidenced by a reduction in the E_rC_{50} value from 6.76 µg/l in ISO medium to 0.68 µg/l in EDTA-free medium. This line of investigation and subsequent findings clearly demonstrate the impact that test matrices and systems have on test sensitivity and, by default, perceived toxicity. It also underpins the need to understand the chemistry of the analyte and the culture media and its components. No significant differences in the toxicities of AgNP and Ag^+ were observed, with the 72-hour E_rC_{50} for the latter observed to be 0.7 µg/l. This adds weight to the hypothesis that it is Ag^+ that exerts its toxicity and not the Ag in its nanoparticle form.

3.4.3 Daphnia magna *and* Daphnia pulex

The effects that media components have on the bioavailability of AgNPs are accounted for in the *Daphnia* bioassay. The US EPA has produced guidelines for the testing of nanomaterials including the use of EDTA-free medium in contrast to the ISO medium (JM), which contains the chelator. The US EPA EDTA-free medium was used for all testing. The same reference chemical $K_2Cr_2O_7$ as well as Ag^+ and AgNPs were assessed. Both *Daphnia* species,

Daphnia pulex and *Daphnia magna*, were compared using $AgNO_3$ and AgNPs.

It was found that *Daphnia pulex*, with a 24-hour IC_{50} of 9.3 µg/l, was less sensitive to $AgNO_3$ than *Daphnia magna*, with an IC_{50} of 1.22 µg/l. The increased sensitivity of *Daphnia magna* is possibly as a result of their larger size and hence greater filter feeding volume or throughput.

When tested with AgNPs, both species yielded results similar, with an IC_{50} of 7.85 µg/l for *Daphnia magna* and 4.2 µg/l for *Daphnia pulex*.

As these IC_{50} values are still substantially higher than the PECs, sub-lethal end points were investigated. Fecundity was assessed in *Daphnia magna* and the number of offspring reduced by 50% after 14 days and 75% after 28 days when cultured in 100 ng/l of AgNPs. This demonstrates that the effects of AgNPs may be seen at close to environmentally relevant concentrations on population numbers rather than single individual organisms. This test brings the toxicity assessment into the ng/l range and closer to the PECs, further demonstrating the importance of chronic testing.

3.4.4 Hydra attenuata

Assessment of the ecotoxicity of AgNPs using *Hydra attenuata* gross morphology as the end point yielded a 96-hour EC_{50} of 29 µg/l. This was shown not to be significantly different from the 96-hour $AgNO_3$ EC_{50} of 35 µg/l, thus further substantiating the hypothesis that $AgNO_3$ can be used as a proxy for AgNPs.

As with the other test systems, sub-lethal and discrete end points were also employed to improve test sensitivity. These included the effect of Ag^+ and AgNPs on the ability of the hydranths to regenerate and on DNA damage, as assessed by the Comet assay. Surprisingly, the Comet assay did not prove to be substantially more sensitive than the gross morphology end point.

Regeneration on the other hand was a more sensitive test, with an EC_{50} of 7.24 µg/l, which is a substantial improvement and environmentally relevant because of the potentially higher concentrations of Ag in the sediment. Future work should be carried out to elucidate the underlying mechanism behind the 100 µg/l survival anomaly. It is hypothesised that a defence mechanism is being induced and then subsequently overwhelmed at higher concentrations. A clear understanding of this mechanism and the enzymes involved may prove to be a sensitive measurement tool in the risk assessment of Ag^+ or AgNPs in future studies. For example, if the inducible defence mechanism involves heat shock proteins (HSPs), quantitative analysis of the specific HSPs by real-time polymerase chain reaction (PCR) analysis may provide a particularly sensitive end point following long-term exposure at ultra-low ng/l concentrations similar to the PECs.

4 Risk Assessment of the Environmental Fate of Silver Nanoparticles through the Aquatic Environment

4.1 Background

This risk assessment study involved a number of interlinking stages, which are depicted in the schematic presented in Figure 4.1. The first stage involved a review of the state of the art regarding natural attenuation processes that affect ENMs in the natural aquatic environment and a review of current risk assessment strategies. From there, a suite of laboratory-scale studies was conducted to better characterise the aggregation potential of AgNPs. The behavioural indications derived from stages 1 and 2 were used to develop an aquatic risk model (stage 3), which was used to characterise likely residual levels of AgNPs in surface waters. In stages 4 and 5 this information was used to assess potential human exposure through drinking water.

4.1.1 State of the art

A review of current state of the art in relation to the fate and behaviour of AgNPs through the aquatic environment was conducted to identify the key driving processes likely to influence these activities. The fate and behaviour of these unnatural forms of materials remain uncertain and therefore warrant increased research to understand and assess the potential risks that these materials may pose to the environment and human health (Meesters *et al.*, 2014; Quik *et al.*, 2014). Because of the complexities of natural freshwater systems, much of the available data relating to fate and behaviour is based on predictive models or bench-scale experiments conducted under controlled/fixed environments. This makes them difficult to compare with data from dynamic natural waters without considering inherent uncertainties and assumptions. Extrapolation of behavioural data to accurately mimic natural systems needs to consider a degree of uncertainty. The review identified natural attenuation processes from the literature pertaining to ENMs and AgNPs that are likely to determine the fate and behaviour of these materials in natural waters. It remains unclear how aged and partially transformed

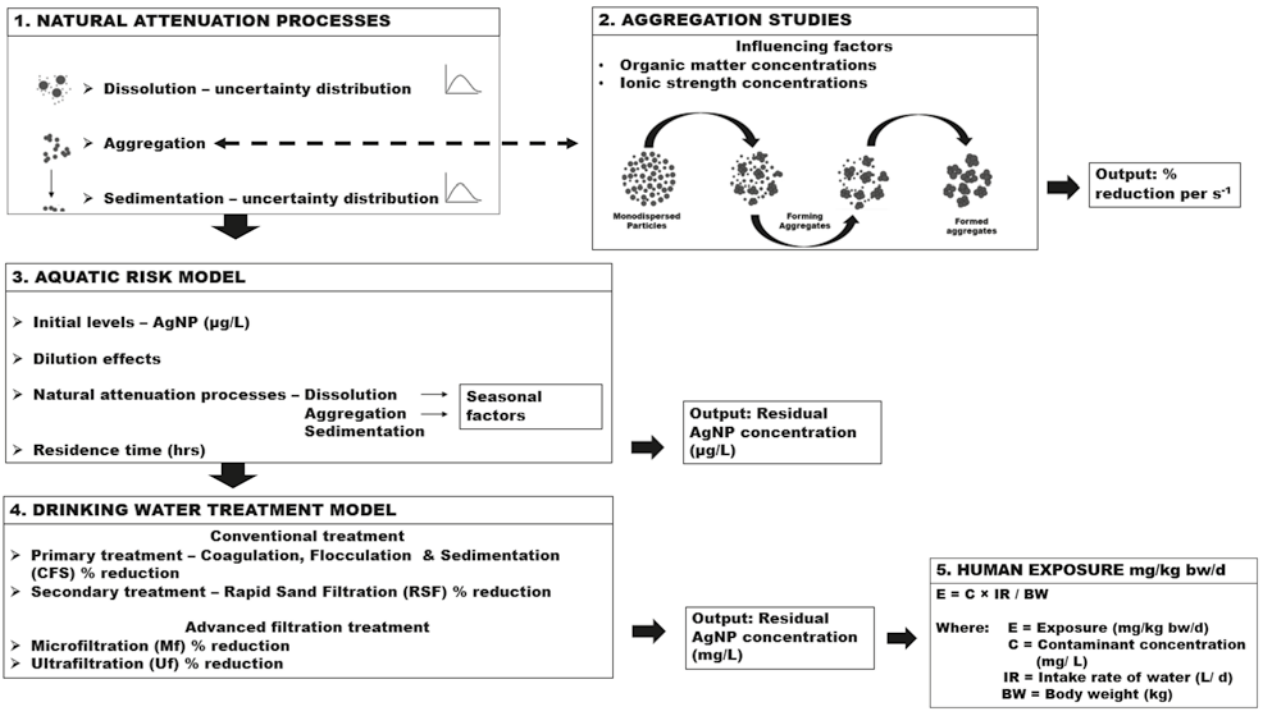


Figure 4.1. Stages involved in the risk assessment study.

particles will perform over time. Natural attenuation processes include dispersion, dissolution, adsorption, aggregation and sedimentation (Hartmann *et al.*, 2014). The process of dissolution and the release of surface Ag^+ is somewhat mitigated by the presence of polymeric surface coatings, therefore lowering the toxicity concerns of Ag^+ release (Shevlin *et al.*, 2018). Hetero-aggregation is likely to dominate in natural waters because of increased levels of naturally occurring geo- and bio-colloids such as viruses, bacteria, proteins, DNA, spores, algae, protozoa and other micro-organisms (Keller and Auset, 2007; Wang, *et al.*, 2015). The growth in the use of nanotechnology has not been matched by an increase in risk assessments of this technology so its implications for the environment and human health are still largely unknown. This study highlights the need for specific risk assessment approaches for metallic ENMs and puts this into context with regard to informing environmental policy and potential nanoparticle influences on environmental/human health. As part of this work a scientific paper was published (Shevlin *et al.*, 2018) focusing on ENM characteristics and the influence of physical, chemical and biological processes occurring in aquatic systems that are likely to impact the fate of metallic ENMs.

4.2 Aggregation Studies

Having identified the natural attenuation processes associated with nanoparticles, this study further investigated the aggregation potential of PVP-coated AgNPs under varying environmentally relevant conditions. Ubiquitous colloid material such as natural organic matter, ionic strength and the surface chemistry of AgNPs will likely govern particle–colloid interactions driving the formation of aggregates. In this study 25-nm, PVP-coated AgNPs were exposed to varying concentrations of organic matter and ionic strength solutions in a range of experimental scenarios. A surrogate colloid (silica beads) was used as an attachment medium to aid in the formation of hetero-aggregates in the attachment process and removal of AgNPs from the system. Samples were abstracted from the medium over time and centrifuged to separate the silica beads from solution prior to ultraviolet (UV) analysis of the supernatant to estimate removal of PVP-coated AgNPs from solution. The influence of organic matter and ionic strength varied across all experimental scenarios.

4.2.1 Materials and methods

For this study 25-nm, PVP-coated Econix AgNPs were purchased from nanoComposix Inc. (San Diego, CA, USA); these were provided in a 25-ml stock suspension. The mass concentration (5.2 mg/ml) was determined using a Thermo Fisher X Series inductively coupled plasma mass spectrometer (Warrington, UK). Humic acid powder (10 g) was purchased from Sigma-Aldrich (St. Louis, MO, USA). A stock solution of 50 parts per million (ppm) was made with Milli-Q water and mixed on a vortex mixer (MMR International) for 1 minute initially and prior to use in each scenario. Potassium nitrate (99% KNO_3) was purchased from Alfa Aesar (Heysham, UK). A stock solution of 1 M was made with Milli-Q water and mixed on a vortex mixer (MMR International) for 1 minute. Silica beads (acid washed) with a size class of < 106 µm were purchased from Sigma-Aldrich (Wicklow, Ireland). A 20 g/l stock solution was made with Milli-Q water and mixed on a vortex mixer (MMR International) for 1 minute initially and prior to use.

4.2.2 Experimental scenarios

Water conditions were established over nine different experimental scenarios incorporating combinations of increasing organic matter and ionic strength over a range of conditions likely to represent Irish river water physiochemical conditions (Table 4.1). Typical ranges of organic matter and ionic strength recorded in Ireland were sourced from water monitoring station readings for the period 2007–2009 (EPA, 2011) and used to develop realistic fresh water scenarios for the model (Table 4.1).

Table 4.1. Experimental water scenarios

Scenario	Organic matter (mg/l)	Ionic strength (mM)
1	1.0	1.0
2	10.0	1.0
3	20.0	1.0
4	1.0	10.0
5	10.0	10.0
6	20.0	10.0
7[a]	1.0	100.0
8[a]	10.0	100.0
9[a]	20.0	100.0

[a]Concentrations of ionic strength in scenarios 7, 8 and 9 are likely to reflect proximity to coastal/transitional waters.

4.2.3 UV spectroscopy analysis

The optical properties of AgNPs can be used to determine the stability of particles in solution. Particle destabilisation and formation of aggregates alter the optical properties as a result of the delocalisation of electrons through the electron sharing between neighbouring particles. The delocalisation causes a shift in the surface plasma resonance to lower energies resulting in scattering of peaks to longer wavelengths in the infrared spectrum (nanoComposix, 2012).

Absorbance readings were taken at 405 nm and 650 nm to cover a range in the infrared spectrum that could account for the presence of background material as particles are removed. Absorbance readings minus the blank (Milli-Q water) were plotted for removal/attachment of PVP-coated AgNPs as a function of time. All experiments were performed in triplicate. Absorbance readings were collated in a Microsoft Excel spreadsheet; calculated mean values were used to determine the slope value for each scenario using the following method.

Mean absorbance readings were calculated for each time period using equation 4.1.

$$M_{abs} = \frac{R_1 + R_2 + R_3}{3}, \quad (4.1)$$

where M_{abs} is the mean absorbance and R_x is the reading for the replicate x.

The blank reading was then deducted from the M_{abs} reading using equation 4.2.

$$C_0 = M_{abs} - B_r, \quad (4.2)$$

where M_{abs} is the mean absorbance, B_r is the blank reading and C_0 is the concentration absorbance at 405 nm.

The C_0 values from equation 4.2 for each scenario and time series were then evaluated using equation 4.3 to determine the potential attachment and removal of the AgNPs under the varying environmental scenarios:

$$R\alpha = \ln\left(\frac{C_0}{C}\right) \quad (4.3)$$

where R(α) is the estimated percentage removal, C_0 is the original concentration at t = 0 and C is the concentration after time (t) has elapsed. Slope values represents the (α) attachment rate. Estimated removal per second was calculated to determine potential removal from a water column under the various experimental scenarios. The exponential decay of the AgNPs was calculated using equation 4.4.

$$E = e^{-rt}, \quad (4.4)$$

where E is the exponential decay, r is the calculated slope value and t is time (seconds).

Percentage removal estimations over time were calculated using the Goal Seek function in the Microsoft Excel software package. The Goal Seek function allows a selected target value to be used to calculate an unknown value. Setting the E value to the desired percentage, Goal Seek can back-calculate to estimate a t value necessary to decrease the initial concentration to the percentage value. Target values of 50%, 90% and 95% removal were used with the Goal Seek function and equation 4.4 to estimate the time necessary to achieve the target values.

4.2.4 Results

Nanoparticle removal in this study is defined as attachment to surrogate colloid material through particle and colloid interactions leading to hetero-aggregate formation and subsequent sedimentation. Removal rates of PVP-coated AgNPs over the range of scenarios indicated particle removal varied considerably depending on the aquatic conditions (Table 4.2). The experimental scenarios used in this study indicate that AgNPs are likely to interact with suspended colloids in water systems and, over time, will be removed by aggregation and subsequent settling or will be carried downstream in a waterbody.

Based on an initial concentration of 1 ppm, PVP-coated AgNPs could be removed by as much as 95% over ≈2 to ≈42 hours, depending on the media conditions. The literature suggests that organic matter can inhibit particle aggregation whereas increasing ionic strength can promote particle destabilisation; however, this was not consistent in this study. This may be because of the complex nature of organic matter and its influence on surface stability and particle surface modification. The results of this study contribute to data pertaining to the fate and behaviour of AgNPs under environmentally relevant conditions.

Table 4.2. Estimated percentage removal of 25 nm PVP-coated AgNPs (1.0 ppm) over time

Scenario	Conditions		50% removal (hours)	90% removal (hours)	95% removal (hours)
	Organic matter (mg/l)	Ionic strength (mM)			
1	1.0	1.0	3.21	10.66	13.87
2	10.0	1.0	9.63	31.98	41.61
3	20.0	1.0	2.41	8.00	10.40
4	1.0	10.0	0.48	1.60	2.08
5	10.0	10.0	1.93	6.40	8.32
6	20.0	10.0	6.42	21.32	27.74
7	1.0	100.0	0.48	1.60	2.08
8	10.0	100.0	0.64	2.13	2.77
9	20.0	100.0	1.93	6.40	8.32

4.3 Aquatic Risk Assessment Model

Having characterised some of the aquatic behavioural aspects of nanoparticles, the next part of this study focused on modelling the fate and behaviour of AgNPs in natural aqueous systems considering the influence of selected natural attenuation processes. Complex media such as freshwater systems are dynamic and vary considerably both spatially and temporally. In this study, an aquatic risk assessment model was developed to assess the likely fate and behaviour of AgNPs in this complex and dynamic environment. The model focuses on three behavioural processes (dissolution, aggregation and sedimentation) identified in the literature to be likely to influence both AgNP fate and behaviour. The model focuses on PVP- and citrate-stabilised AgNPs and how effective the behavioural processes are at influencing their removal from a natural water system. A mass balance model was developed using an initial AgNP estimated water input concentration value (prior to *in situ* processes), based on a combination of reported and predicted literature values. Probability distributions were developed for each process using the Microsoft Excel software add-in @RISK 7 (Palisade Software, Ithaca, NY, USA) and run for 10,000 iterations.

Estimated initial values indicated a mean AgNP concentration of 4.34×10^{-2} µg/l and this was assumed as a worst-case scenario for surface water concentrations in Ireland and was used as an initial input value in the risk model. Seasonal factors were incorporated in the risk model to account for potential fluctuations in organic matter and ionic strength, which have been identified as key influencers of particle stability and eventual fate in natural water systems.

Other factors considered included dilution effects and effects of the key processes of dissolution, aggregation and subsequent sedimentation on the likely removal of AgNPs over time. Residues of AgNPs were assumed to persist in the water column or exist as free Ag^{+}, unsettled aggregates or sedimented aggregates. The predicted results from the model indicate that citrate-coated particles are removed to a greater extent than PVP-coated AgNPs in both stream water and lake water (Tables 4.3 and 4.4). Water flow is likely to transport the residues to transitional waters where the increase in ionic strength will likely increase aggregation and rapid sedimentation.

The seasonal physiochemical water conditions appeared to have limited impacts on removal potential for both citrate- and PVP-coated AgNPs in this model. There were low levels of dissolution of both particle types over the time period of the model run. Increased

Table 4.3. Predicted residual concentration and percentage reduction from the initial concentration of 4.34×10^{-2} µg/l of PVP-coated AgNPs in the water column after 7 days using the aquatic risk model

Watershed	Season	Residual concentration (µg/l)	% reduction
Stream water	Spring	2.32×10^{-3}	46.54
	Summer	2.36×10^{-3}	45.62
	Autumn	2.41×10^{-3}	44.47
	Winter	2.36×10^{-3}	45.62
Lake water	Spring	2.16×10^{-3}	50.23
	Summer	2.17×10^{-3}	50.00
	Autumn	2.20×10^{-3}	49.31
	Winter	2.16×10^{-3}	50.23

Table 4.4. Predicted residual concentration and percentage reduction from the initial concentration of 4.34×10^{-2} µg/l of citrate-coated AgNPs after 7 days using the aquatic risk model

Watershed	Season	Residual concentration (µg/l)	% reduction
Stream water	Spring	1.31×10^{-3}	69.81
	Summer	1.32×10^{-3}	69.58
	Autumn	1.32×10^{-3}	69.58
	Winter	1.30×10^{-3}	70.05
Lake water	Spring	1.46×10^{-3}	66.36
	Summer	1.43×10^{-3}	67.05
	Autumn	1.45×10^{-3}	66.59
	Winter	1.46×10^{-3}	66.36

organic matter and ionic strength had minimal effects on dissolution while stability may have been controlled solely by the presence of the polymeric coating present on the particle surface. The process of aggregation incorporated in the model dominated the potential removal while driving the progressive process of sedimentation. Water systems are invariably turbulent and vary considerably as a result of elevation, depth, geology, etc. and this can impact on the rates of sedimentation and potential mobility of aggregates over a range of distances.

Predicted aquatic concentrations of AgNPs were compared with toxicity data from project partners (Athlone Institute of Technology) to establish if a risk is posed by the current estimated concentrations of AgNPs in natural waters. The EC_{50} values for the primary producer (algae), primary consumer (*Daphnia pulex*) and secondary consumer (*Hydra attenuata*) exposed to PVP-coated AgNPs were compared with persistent concentrations of AgNPs in freshwater systems. The concentrations of AgNPs used in the model were at levels deemed unlikely to have toxicity concerns to aquatic organisms. The results indicated that algae had an EC_{50} value of 0.67 µg/l over a 72-hour period. Therefore, at current predicted water concentrations, AgNPs are unlikely to present a toxic concern to the aquatic food chain.

4.4 Drinking Water Exposure

Human exposure to AgNPs was estimated using a mass balance model based on surface water concentrations of AgNPs likely persisting and readily available during surface water abstraction for human consumption. Abstracted surface water for drinking water purposes is treated at municipal drinking water treatment plants to remove undesirable contaminants (EPA, 2002). Current Irish Drinking Water Regulations (S.I. No. 122 of 2014) do not include a defined acceptable limit for Ag or nanomaterials in the legislation (Oireachtas, 2014). The World Health Organization (WHO) and European Food Safety Authority (EFSA) have set guideline limits for the ingestion of Ag. Both organisations have set levels in mg/l/day for drinking water (SCENIHR, 2013). The WHO limit is based on a no observable adverse effect level (NOAEL) for humans. This limit is based on a lifetime exposure (70 years or 25,550 days), giving a NOAEL of 0.39 mg/person/day or 0.005 mg/kg of body weight/day (body weight = 70 kg). The WHO suggests that a tolerable level of 0.1 mg/l is acceptable when Ag compounds are used for water purification purposes. EFSA has a recommended limit of 0.05 mg/l and 0.05 mg/kg in water and food, respectively, based on the same information (SCENIHR, 2013). The US EPA suggests a reference dose (RfD) of 5.0 µg/kg of body weight/day for chronic oral exposure to Ag (SCENIHR, 2013).

The model incorporated estimated removal rates for the differing treatment processes. Three commonly used coagulants, aluminium sulfate [alum, $Al_2(SO_4)_3$], ferric chloride ($FeCl_3$) and ferric sulfate ($FeSO_4$), were incorporated in the primary and secondary treatment process. Advance treatments (microfiltration and ultrafiltration) were represented through several scenarios. The use of sequential treatments for the effective removal of emerging contaminants has been suggested (Rodriguez-Narvaez *et al.*, 2017). The scenarios modelled are summarised as follows: scenario 1: rapid sand filtration (standard process employed in Irish drinking water treatment plants); scenario 2: rapid sand filtration + microfiltration; scenario 3: rapid sand filtration + microfiltration + ultrafiltration.

Probability distributions were developed for each treatment process to account for uncertainty in the data and capture likely removal rates. Residuals in post-treated drinking water were compared with daily drinking water consumption patterns for various demographic groups in Ireland. The potential human exposure to AgNPs from consumption of drinking

water was calculated using equation 4.5 (ATSDR, 2017):

$$E = C \times IR/BW \quad (4.5)$$

where E is exposure (mg/kg of body weight/day), C is contaminant concentration (mg/l) (from the aquatic risk assessment study), IR is the intake rate of water (l/day) and BW is body weight (kg).

The exposure dose from consumption of AgNP residues in post-treated water was compared with the RfD value (as the most pessimistic heath-based guidance value currently available) to determine the risk to each demographic group in the model. It is calculated using the hazard quotient (HQ) according to the formula in equation 4.6:

$$HQ = D/RfD \quad (4.6)$$

where D is the exposure dose (mg/kg of body weight/day) and the RfD is 0.005 mg/kg of body weight/day.

The resulting value was compared with threshold limits presented in Table 4.5 to determine the likely risk to human health.

The risk to human health based on water consumption and potential exposure to residual AgNPs using the HQ equation is shown in Figures 4.2 and 4.3. The HQ score for conventional treatment (scenario 1) for each type of coagulant is presented in Table 4.6.

The predicted HQs indicated that there was no existing risk (Table 4.6) to either demographic group through consumption of drinking water with residual concentrations of AgNPs based on the data used in this model. The performance of the different coagulants used in this model indicated a greater removal potential using alum, followed by $FeCl_3$ and $FeSO_4$ (Figure 4.2). Scenario 1 showed a mean residual concentration of 2.35×10^{-4}, 2.13×10^{-3} and 1.90×10^{-3} µg/l, respectively, persisting prior to disinfection. The HQ indicated that there was no existing risk from AgNP residues following scenario 1 for all coagulants used (Table 4.6). Advanced treatment processes can significantly reduce the

Table 4.5. Threshold for HQ risk limits

HQ value	Risk
< 0.01	No existing risk
0.1–1.0	Risk is low
1.1–10	Risk is moderate
> 10	Risk is high

Source: Lemly (1996) and Clarke *et al.* (2016).

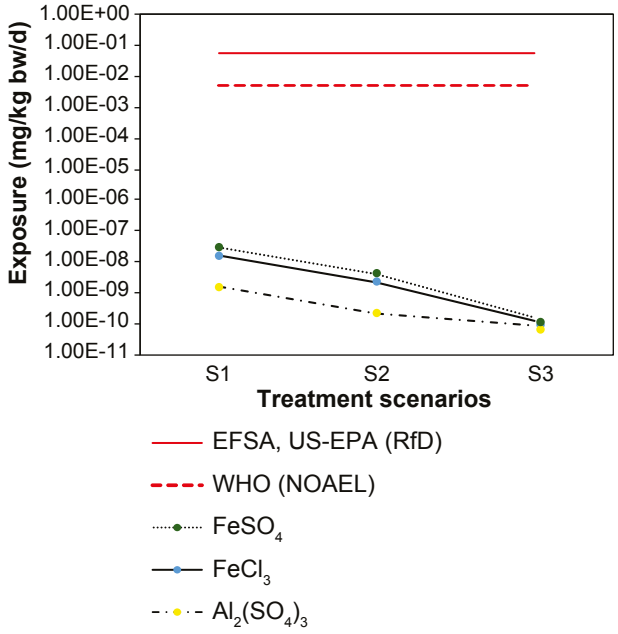


Figure 4.2. Predicted adult male (18–64 years) exposure to AgNPs following consumption of post-treated drinking water compared with regulatory guideline limits for ingestion (mg/kg of body weight/day) (based on macro-scale Ag).

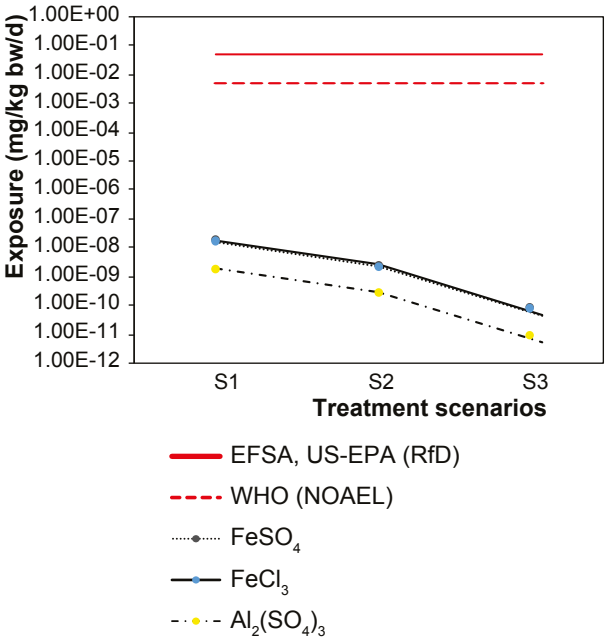


Figure 4.3. Predicted adult female (18–64 years) exposure to AgNPs following consumption of post-treated drinking water compared with regulatory guideline limits for ingestion (mg/kg of body weight/day) (based on macro-scale Ag).

presence of AgNPs to several orders of magnitude below regulatory limits. The final stage of treatment in drinking water is the process of disinfection; because of a lack of data relating to the influence of chlorination on residual AgNPs it was assumed that chlorination had no impact on the final level of residual AgNPs. The increased industrial usage of nanomaterials in many sectors, in conjunction with the persistence of AgNPs through drinking water treatment, suggests the need to constantly monitor levels and re-assess exposure through drinking water into the future.

Table 4.6. HQ scores for AgNP exposure after conventional drinking water treatment (scenario 1) using three coagulants

Sex	$Al_2(SO_4)_3$		$FeCl_3$		$FeSO_4$	
	Mean	HQ	Mean	HQ	Mean	HQ
Male	1.62×10^{-9}	3.24×10^{-7}	1.47×10^{-8}	2.94×10^{-6}	1.33×10^{-8}	2.66×10^{-6}
Female	1.92×10^{-9}	3.84×10^{-7}	1.76×10^{-8}	3.52×10^{-6}	1.57×10^{-8}	3.14×10^{-6}

5 Conclusions

- Owing to their numerous applications, the release of AgNPs into the environment is highly likely. Their impact on the environment is difficult to determine because of the low concentrations and the complicated reactions that they undergo.
- Milled activated charcoal was found to be a suitable material to capture AgNPs from water samples and shows potential as a cost-effective material for the capture and remediation of AgNPs, and possibly other nano-wastes, from the aquatic environment.
- A HCl leaching technique was developed to successfully quantify the Ag captured by the milled activated charcoal, recovering an average of 94.8% of the captured AgNPs. This leaching procedure, combined with the application of the milled activated charcoal to environmental water, could provide a method to quantify AgNPs in the environment.
- An EDTA-free medium should be utilised to improve test sensitivity by reducing interference with chemicals that influence the bioavailability of the AgNPs.
- Ag^+ from $AgNO_3$ is a suitable proxy for AgNPs as no significant difference in toxicity between them was observed in any test investigated in this study
- The effect of Ag on the regeneration of *Hydra attenuata* is the most environmentally relevant bioassay investigated as it is very sensitive and robust and *Hydra attenuata* represents benthic dwellers likely to be exposed to higher concentrations of AgNPs.
- Hetero-aggregation of ENMs with naturally occurring geo- and bio-colloids is likely to be the dominant natural attenuation process in natural waters. ENMs are likely to be transported to transitional waters where the increase in ionic strength will likely increase aggregation and rapid sedimentation.
- The concentrations of AgNPs used in the model were at levels deemed unlikely to have toxicity concerns to aquatic organisms.
- For the scenarios considered, there is no existing risk from AgNP residues following the consumption of drinking water.

6 Recommendations

- An Irish national inventory of all products containing nanoparticles should be established and updated on a regular basis. A similar legislative framework to that in operation in France could be considered, which would require industry reporting to the inventory and ensuring the quality and accuracy of the information gathered.
- Further investigations should be carried out to determine the levels of AgNPs in the Irish environment. These studies should allow for examination of their impacts on the environment and associated risks.
- This study substantiates the need for international bodies such as the EU, ISO and OECD to develop and harmonise a suite of ecotoxicology bioassays for the risk assessment of Ag nanomaterials, taking cognisance of bioavailability issues, test matrix interference and the very low PECs.
- Given the rapid uptake of nanotechnology in various sectors, and the consequential likely increased environmental release of ENMs, environmental and human exposure will change and should be constantly monitored and re-evaluated.

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Abbreviations

Ag	Silver
Ag^+	Ionic silver
$AgNO_3$	Silver nitrate
AgNP	Silver nanoparticle
$Al_2(SO_4)_3$	Aluminium sulfate
ANSES	Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (French agency for food safety, the environment and labour)
BUND	Bund für Umwelt und Naturschutz Deutschland (Friends of the Earth Germany)
CI	Confidence interval
DeTER	Detection, Toxicology, Environmental fate and Risk assessment of nanoparticles in the aquatic environment
DNA	Deoxyribonucleic acid
EC	European Commission
EC_{50}	Half-maximal effective concentration
EDTA	Ethylenediaminetetraacetic acid
EFSA	European Food Safety Authority
ENM	Engineered nanomaterial
EPA	Environmental Protection Agency
E_rC_{50}	Median effective growth rate-inhibiting concentration
EU	European Union
$FeCl_3$	Ferric chloride
$FeSO_4$	Ferric sulfate
HCl	Hydrochloric acid
HNO_3	Nitric acid
HQ	Hazard quotient
HSP	Heat shock protein
IC_{50}	Half-maximal inhibitory concentration
ICPMS	Inductively coupled plasma mass spectrometry
ISO	International Organization for Standardization
JM	Jaworski's medium
$NaBH_4$	Sodium borohydride
NOAEL	No observable adverse effect level
OECD	Organisation for Economic Co-operation and Development
PEC	Predicted environmental concentration
ppm	Parts per million
PVP	Polyvinylpyrrolidone
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RfD	Reference dose
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
UV	Ultraviolet
WHO	World Health Organization
WWTP	Wastewater treatment plant

AN GHNÍOMHAIREACHT UM CHAOMHNÚ COMHSHAOIL
Tá an Ghníomhaireacht um Chaomhnú Comhshaoil (GCC) freagrach as an gcomhshaol a chaomhnú agus a fheabhsú mar shócmhainn luachmhar do mhuintir na hÉireann. Táimid tiomanta do dhaoine agus don chomhshaol a chosaint ó éifeachtaí díobhálacha na radaíochta agus an truaillithe.

Is féidir obair na Gníomhaireachta a roinnt ina trí phríomhréimse:

Rialú: *Déanaimid córais éifeachtacha rialaithe agus comhlíonta comhshaoil a chur i bhfeidhm chun torthaí maithe comhshaoil a sholáthar agus chun díriú orthu siúd nach gcloíonn leis na córais sin.*

Eolas: *Soláthraímid sonraí, faisnéis agus measúnú comhshaoil atá ar ardchaighdeán, spriocdhírithe agus tráthúil chun bonn eolais a chur faoin gcinnteoireacht ar gach leibhéal.*

Tacaíocht: *Bímid ag saothrú i gcomhar le grúpaí eile chun tacú le comhshaol atá glan, táirgiúil agus cosanta go maith, agus le hiompar a chuirfidh le comhshaol inbhuanaithe.*

Ár bhFreagrachtaí

Ceadúnú
Déanaimid na gníomhaíochtaí seo a leanas a rialú ionas nach ndéanann siad dochar do shláinte an phobail ná don chomhshaol:
- saoráidí dramhaíola *(m.sh. láithreáin líonta talún, loisceoirí, stáisiúin aistrithe dramhaíola)*;
- gníomhaíochtaí tionsclaíocha ar scála mór *(m.sh. déantúsaíocht cógaisíochta, déantúsaíocht stroighne, stáisiúin chumhachta)*;
- an diantalmhaíocht *(m.sh. muca, éanlaith)*;
- úsáid shrianta agus scaoileadh rialaithe Orgánach Géinmhodhnaithe *(OGM)*;
- foinsí radaíochta ianúcháin *(m.sh. trealamh x-gha agus radaiteiripe, foinsí tionsclaíocha)*;
- áiseanna móra stórála peitril;
- scardadh dramhuisce;
- gníomhaíochtaí dumpála ar farraige.

Forfheidhmiú Náisiúnta i leith Cúrsaí Comhshaoil
- Clár náisiúnta iniúchtaí agus cigireachtaí a dhéanamh gach bliain ar shaoráidí a bhfuil ceadúnas ón nGníomhaireacht acu.
- Maoirseacht a dhéanamh ar fhreagrachtaí cosanta comhshaoil na n-údarás áitiúil.
- Caighdeán an uisce óil, arna sholáthar ag soláthraithe uisce phoiblí, a mhaoirsiú.
- Obair le húdaráis áitiúla agus le gníomhaireachtaí eile chun dul i ngleic le coireanna comhshaoil trí chomhordú a dhéanamh ar líonra forfheidhmiúcháin náisiúnta, trí dhíriú ar chiontóirí, agus trí mhaoirsiú a dhéanamh ar leasúchán.
- Cur i bhfeidhm rialachán ar nós na Rialachán um Dhramhthrealamh Leictreach agus Leictreonach (DTLL), um Shrian ar Shubstaintí Guaiseacha agus na Rialachán um rialú ar shubstaintí a ídíonn an ciseal ózóin.
- An dlí a chur orthu siúd a bhriseann dlí an chomhshaoil agus a dhéanann dochar don chomhshaol.

Bainistíocht Uisce
- Monatóireacht agus tuairisciú a dhéanamh ar cháilíocht aibhneacha, lochanna, uiscí idirchriosacha agus cósta na hÉireann, agus screamhuiscí; leibhéil uisce agus sruthanna aibhneacha a thomhas.
- Comhordú náisiúnta agus maoirsiú a dhéanamh ar an gCreat-Treoir Uisce.
- Monatóireacht agus tuairisciú a dhéanamh ar Cháilíocht an Uisce Snámha.

Monatóireacht, Anailís agus Tuairisciú ar an gComhshaol
- Monatóireacht a dhéanamh ar cháilíocht an aeir agus Treoir an AE maidir le hAer Glan don Eoraip (CAFÉ) a chur chun feidhme.
- Tuairisciú neamhspleách le cabhrú le cinnteoireacht an rialtais náisiúnta agus na n-údarás áitiúil *(m.sh. tuairisciú tréimhsiúil ar staid Chomhshaol na hÉireann agus Tuarascálacha ar Tháscairí)*.

Rialú Astaíochtaí na nGás Ceaptha Teasa in Éirinn
- Fardail agus réamh-mheastacháin na hÉireann maidir le gáis cheaptha teasa a ullmhú.
- An Treoir maidir le Trádáil Astaíochtaí a chur chun feidhme i gcomhair breis agus 100 de na táirgeoirí dé-ocsaíde carbóin is mó in Éirinn.

Taighde agus Forbairt Comhshaoil
- Taighde comhshaoil a chistiú chun brúnna a shainaithint, bonn eolais a chur faoi bheartais, agus réitigh a sholáthar i réimsí na haeráide, an uisce agus na hinbhuanaitheachta.

Measúnacht Straitéiseach Timpeallachta
- Measúnacht a dhéanamh ar thionchar pleananna agus clár beartaithe ar an gcomhshaol in Éirinn *(m.sh. mórphleananna forbartha)*.

Cosaint Raideolaíoch
- Monatóireacht a dhéanamh ar leibhéil radaíochta, measúnacht a dhéanamh ar nochtadh mhuintir na hÉireann don radaíocht ianúcháin.
- Cabhrú le pleananna náisiúnta a fhorbairt le haghaidh éigeandálaí ag eascairt as taismí núicléacha.
- Monatóireacht a dhéanamh ar fhorbairtí thar lear a bhaineann le saoráidí núicléacha agus leis an tsábháilteacht raideolaíochta.
- Sainseirbhísí cosanta ar an radaíocht a sholáthar, nó maoirsiú a dhéanamh ar sholáthar na seirbhísí sin.

Treoir, Faisnéis Inrochtana agus Oideachas
- Comhairle agus treoir a chur ar fáil d'earnáil na tionsclaíochta agus don phobal maidir le hábhair a bhaineann le caomhnú an chomhshaoil agus leis an gcosaint raideolaíoch.
- Faisnéis thráthúil ar an gcomhshaol ar a bhfuil fáil éasca a chur ar fáil chun rannpháirtíocht an phobail a spreagadh sa chinnteoireacht i ndáil leis an gcomhshaol *(m.sh. Timpeall an Tí, léarscáileanna radóin)*.
- Comhairle a chur ar fáil don Rialtas maidir le hábhair a bhaineann leis an tsábháilteacht raideolaíoch agus le cúrsaí práinnfhreagartha.
- Plean Náisiúnta Bainistíochta Dramhaíola Guaisí a fhorbairt chun dramhaíl ghuaiseach a chosc agus a bhainistiú.

Múscailt Feasachta agus Athrú Iompraíochta
- Feasacht chomhshaoil níos fearr a ghiniúint agus dul i bhfeidhm ar athrú iompraíochta dearfach trí thacú le gnóthais, le pobail agus le teaghlaigh a bheith níos éifeachtúla ar acmhainní.
- Tástáil le haghaidh radóin a chur chun cinn i dtithe agus in ionaid oibre, agus gníomhartha leasúcháin a spreagadh nuair is gá.

Bainistíocht agus struchtúr na Gníomhaireachta um Chaomhnú Comhshaoil
Tá an ghníomhaíocht á bainistiú ag Bord lánaimseartha, ar a bhfuil Ard-Stiúrthóir agus cúigear Stiúrthóirí. Déantar an obair ar fud cúig cinn d'Oifigí:
- An Oifig um Inmharthanacht Comhshaoil
- An Oifig Forfheidhmithe i leith cúrsaí Comhshaoil
- An Oifig um Fianaise is Measúnú
- Oifig um Chosaint Radaíochta agus Monatóireachta Comhshaoil
- An Oifig Cumarsáide agus Seirbhísí Corparáideacha

Tá Coiste Comhairleach ag an nGníomhaireacht le cabhrú léi. Tá dáréag comhaltaí air agus tagann siad le chéile go rialta le plé a dhéanamh ar ábhair imní agus le comhairle a chur ar an mBord.

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Detection, Toxicology, Environmental Fate and Risk Assessment of Nanoparticles in the Aquatic Environment (DeTER)

Authors: Eoin McGillicuddy, Iain Murray, David Shevlin, Liam Morrison, Martin Cormican, Andrew Fogarty, Enda Cummins, Peter Dockery, Patrick Dunlop, Neil Rowan and Dearbháile Morris

Nanotechnology is an emerging technology that has the potential to impact on all aspects of life and the economy and is expected to form the basis of several technological innovations and advances in the 21st century. In this report, a 3-year study was undertaken to (1) develop and implement methods for the detection, characterisation and quantification of silver nanoparticles in water; (2) determine the toxicological properties and environmental fate of silver nanoparticles in the aquatic environment; and (3) develop risk assessment protocols that can be used to evaluate the environmental fate and likely risk from silver nanoparticles in aquatic pathways.

Identifying Pressures

This report demonstrates that, to date, the concentrations of silver nanoparticles in the aquatic environment have primarily been estimated through modelling, largely because of a dearth of appropriate detection methods, with predicted environmental concentrations in the ng/l range. This report also identifies that research is urgently required to determine the full extent of nanomaterial use and production in Ireland across all sectors in order to develop appropriate risk assessment and risk management policies and guidelines.

Informing Policy

This research informs the development of policies on the regulation and standardisation of nanomaterials by building capacity in the area of the environment and health. It informs the following national regulations and EU directives with regard to engineered nanomaterials: the Water Framework Directive (2000), the Groundwater Directive (2006), Good Agricultural Practice for Protection of Waters (2010), the Environmental Quality Standards Directive (2008), the Floods Directive (2007), the Marine Strategy Framework Directive (2008), the Urban Waste Water Directive (1991), the Nitrates Directive (1991), the Bathing Water Directive (2006), the Drinking Water Directive (1998), the Biocidal Products Directive (1998), the Biocidal Products Regulation (2012), the framework regulation on food contact materials and articles (FCM Regulation) (2004), the regulation on active and intelligent food contact materials (AIM Regulation) (2009) and the regulation on plastic materials and articles intended to come into contact with food (PIM Regulation) (2011).

Developing Solutions

This research demonstrates that activated charcoal represents a cost-effective material for the remediation of waters impacted by silver nanoparticles or other nano-waste. It provides valuable evidence that EDTA-free media should be used to improve toxicity test sensitivities. This research reveals that the concentrations of silver nanoparticles used in the model developed were at levels deemed unlikely to have toxicity concerns to aquatic organisms and, for the scenarios considered, there is no existing risk from silver nanoparticle residues following the consumption of drinking water.

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