

FSBI BRIEFING PAPER: NANOTECHNOLOGY IN FISHERIES AND AQUACULTURE

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EXECUTIVE SUMMARY

Nanotechnology involves the application of materials at the nanoscale to new products or processes. It is a rapidly growing industry currently worth billions of U.S. dollars, with many potential benefits to society. There are opportunities for the fisheries and aquaculture industries to use existing nanotechnologies, and also to develop new applications specific to the industry. The potential benefits of nanotechnology for fisheries and aquaculture need to be balanced against concerns for the environment and the occupational health of workers. The key points are as follows:

- Man-made nanomaterials (NMs), sometimes called engineered NMs or manufactured NMs, are novel materials that have at least one dimension <100 nm or have a primary size in the 1–100 nm range. These materials are produced in many different physical forms including nanoparticles (NPs), nanorods, nanotubes, nanospheres and nanowires. The major chemical classes include metallic NPs, carbon-based NMs [carbon nanotubes (CNTs), carbon fullerene spheres] and a wide variety of composites made of more than one chemical substance. The surfaces of NMs can also be functionalised to create many different chemistries.
- Manufactured NMs are already used in consumer products and exposure modelling predicts environmental concentrations of ng l^{-1} to low $\mu\text{g l}^{-1}$ levels of NMs in surface waters. The long-term impact of these predicted concentrations on fishes is currently unclear, and chronic effects on the environment cannot be excluded.
- Nanotechnology is already being applied in the food industry. Applications for fisheries could include nanopolymers and coatings to strengthen food packaging in order to protect delicate fish fillets. The shelf life of fish and shellfish may be improved with the use of antibacterial nanocoatings, and transparent polymer films that can help exclude oxygen from around the food product. Nanosensors on the food packaging can also be used to report the deterioration of the fish or shellfish. A public engagement programme is needed to ensure public confidence in the food uses of nanotechnology by the industry.

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- Fishes will eat food containing NMs and nanotechnology could be used to improve the delivery of micronutrients or unstable ingredients in aquafeeds. For example, the use of nanoencapsulation technology for fat-soluble vitamins, minerals and fatty acids may be advantageous. Some NMs can change the physical properties (*e.g.* buoyancy, hardness) of the feed.
- For fish health in aquaculture, nanotechnological applications include antibacterial surfaces in the aquaculture system, nanodelivery of veterinary products in fish food using porous nanostructures, and nanosensors for detecting pathogens in the water. There are many applications of nanotechnology in water purification for removing microbes, organic chemicals and metals.
- From the view point of engineering, there are a plethora of new building materials, textiles and fabrics that could be used in aquaculture engineering and on fishing vessels. These include carbon nanofibres which are over 100 times stronger than steel that could be used in cage construction, nets as well as mooring lines. The antibacterial properties of NMs may be used to prevent biofouling on marine structures.
- There is a considerable body of knowledge on the occupational health and safety of NMs. Guidance notes are available for employers from various Government agencies, including the Health and Safety Executive in the U.K. The predicted occupational health risks from NMs are probably low for fisheries workers, given that few staff would be using raw NMs or free particles. Guidance is available for safe handling by scientific staff in research laboratories.
- The potential benefits of nanotechnology to fisheries and aquaculture industries should be balanced by the risks to the environment. The colloidal behaviour of NPs is briefly outlined. Laboratory studies show that mg l^{-1} levels of free NMs are toxic to some fish and invertebrates. Sub-lethal effects are noted at $100 \mu\text{g}$ to 1mg l^{-1} of NMs and include many of the effects found with traditional chemicals such as respiratory distress, gill injury, biochemical disturbances and effects on embryonic development. The ecotoxicity data so far suggest that manufactured NMs may have relatively low toxicity compared to some traditional chemicals. However, there are many data gaps, including a lack of data on the species important to aquaculture and fisheries.
- Measurement methods for the environmental monitoring of NMs and the surveillance of NMs in products, especially food such as fish fillets, are needed.
- The report concludes, on the basis of current evidence, that the benefits of using nanotechnology in fisheries and aquaculture are considerable, and the potential hazards should not stifle innovation, and the responsible use of nanotechnology in these industries.

INTRODUCTION

AIMS AND INTENDED AUDIENCE

The audience for this briefing paper is the professionals working in the fisheries and aquaculture industries. The overall aim is to outline what nanotechnology and nanomaterials (NMs) are, and then go onto explain their potential applications in the fisheries and aquaculture industries. The document is intended as a practical guide

with some background on the chemistry of NMs and their biological effects on fishes, but the main focus will be on applications. These include considerations for new fish feeds, food packing/shelf life of fresh fish, materials for aquaculture engineering and fishing vessels, water quality technology and the delivery of fish medicines. We also provide an overview of the health and safety considerations for NMs, both to protect the health of workers and the effects on aquatic species.

WHAT IS NANOTECHNOLOGY AND WHAT IS THE INDUSTRY WORTH?

Nanotechnology involves the application of materials at the nanoscale to produce new products or processes, and with many potential benefits to society (Roco & Bainbridge, 2005). The nanotechnology industry has grown rapidly. Initial estimates (NSF, 2001) predicted a global economic value of nanotechnology at around one trillion U.S. dollars by about 2015, with a significant proportion on the theme of sustainability, including food production and agriculture. These early predictions may have been over-optimistic, but nonetheless, the global investment in nanotechnology was around 4.6 billion U.S. dollars by governments and 4.5 billion U.S. dollars by commercial companies in 2005 (Lux, 2006). The growth of the industry is exemplified by over a thousand different products containing NMs in 2009 (http://www.nanotechproject.org/inventories/consumer/analysis_draft/), and estimates of sales from nanotechnology worth around 1500 billion U.S. dollars in 2010 (<http://www.nanowerk.com/spotlight/spotid=1792.php/>). Clearly, the nanotechnology industry is an expanding market. The applications, risks and benefits have been discussed for agriculture and food production, most notably in an initial report from the Woodrow Wilson International Centre for Scholars (Kuzma & VerHage, 2006), but the current and potential applications of nanotechnology in fisheries or aquaculture have not been reviewed.

WHAT ARE NANOMATERIALS?

Man-made NMs, sometimes called engineered NMs or manufactured NMs, are novel materials with nanoscale dimensions. There are several definitions of NMs but it is generally agreed that they have at least one dimension <100 nm (Masciangioli & Zhang, 2003; Roco, 2003) or have a primary size in the 1–100 nm range (SCHENIR, 2007). These materials have been produced in many different chemical forms, the main types including nanometals [*e.g.* silver nanoparticles (NPs)], metal oxides (*e.g.* TiO₂ NPs), carbon-based materials such as carbon nanotubes (CNTs) and carbon spheres (*e.g.* C₆₀ fullerenes, often called ‘bucky balls’ by the media) as well as composites made of several substances such as nanoceramics and quantum dots (Boxall *et al.*, 2007; Stone *et al.*, 2010). The latter can include combinations of known toxic metals, such as ZnS and CdSe, used in quantum dots for new light-emitting diodes (LEDs; Bae *et al.*, 2009).

However, there is the potential for infinite numbers of chemistries for NMs. It is possible to functionalise the surface of NMs with hydroxyl groups, carboxylic acid groups, sulphate residues, *etc.* The chemicals on the surface of the NM can be covalently bound, and essentially part of the structure of the material, or be a loosely

attached surface coating (e.g. citrate-coated metal NPs). ‘Second-generation’ NMs are now also emerging with complex three-dimensional shapes, and/or containing several different chemical substances. NMs are now being produced with highly functionalized surfaces for very specific applications (e.g. functionalized CNT). It is argued that the surface chemistry of the material is partly responsible for some of the physico-chemical properties at the nanoscale (see reviews, Handy *et al.*, 2008a; Ju-Nam & Lead, 2008; Klaine *et al.*, 2008) that will inform on product development and new applications.

NMs can be made into many different shapes including nanofilms, nanowires, nanotubes and NPs, which can be spherical or rod-shaped (Nowack & Bucheli, 2007). Second-generation materials are now introducing new shapes, including nanoneedles (high-aspect ratio crystals with sharp tips), and rosette-shaped carbon structures. The primary size and shape of the material may not be the only consideration. For example, CNTs can be polymerized into carbon fibres that may be several mm long and NPs may form aggregates or clusters that can be several hundred nm in diameter. Manufactured NMs can also be incorporated into products in many different ways, such as surface coatings, laminates or as a material dispersed within the matrix of a product (Hansen *et al.*, 2008). For example, nanosurface coatings on fabrics to make them water resistant, antibacterial nanolaminates on food packaging and the inclusion of NMs into the matrix of concrete to make it resistant to saltwater erosion. Numerous product applications have been suggested for NMs in electronics, fuel additives, building materials, textiles, paints, food, medical devices, bioremediation, waste water treatment technology and personal care products (Aitken *et al.*, 2006; Chaudhry *et al.*, 2008; Sozer & Kokini, 2009). A number of these product areas will be relevant to aquaculture and fisheries.

APPLICATIONS OF NANOMATERIALS IN FISHERIES AND AQUACULTURE

NANOMATERIALS IN FISH FOOD AND FISH PACKAGING

Nanotechnology is already being applied in the food industry (reviews, Chaudhry *et al.*, 2008; Tiede *et al.*, 2008) and interests include how NMs may influence the structure, texture and quality of food, as well as technological applications in production, processing, storage, transportation and traceability of food stuffs. There are a number of potential applications in relation to fish and shellfish production (Table I). The perishable nature of fresh fish is a long-standing concern, and any packaging that may increase the shelf life of fresh fish would be advantageous. There are several ways by which this can be achieved. First, nanopolymers and coatings are available for strengthening packaging (De Azeredo, 2009) and this could reduce the incidence of bruising or mechanical damage to packed fish fillets. Nanopackaging can be made from natural nanoscale polymers, such as cellulose and starch, or chitosan particles (De Azeredo, 2009), and are therefore likely to be biodegradable, unlike some conventional plastics (Thompson *et al.*, 2004). Strong and light nanopackaging has also been suggested for the meat industry (Lee, 2010).

Fresh fish products also perish due to microbial activity. Packaging can be made with antimicrobial and antifungal surfaces (De Azeredo, 2009; Moraru *et al.*,

TABLE I. Examples of potential applications of nanomaterials in fisheries and aquaculture

Application	Nanomaterial	Notes on usage	References
Food packaging	Clay composites, polymer films	Clay NPs introduced into biopolymer films as a barrier to prevent oxygen entry into the packaging. May be used on the clear plastic wrapping of food containers. The use of cellulose, nano silica, chitosan particles and CNTs is proposed for making lighter, stronger food packaging.	De Azeredo (2009)
Food packaging	Fluorescent dye fixed into a nano silica matrix	TiO ₂ NPs and Ag NPs as antimicrobial components of food packaging Smart labelling to detect changes in CO ₂ in the food container (e.g. CO ₂ produced by microbes). An optical sensor method to measure CO ₂ in Modified Atmosphere Packaging (MAP), based on the fluorescent pH indicator 1-hydroxypyrene-3,6,8-trisulphonate (HPTS) immobilized in a hydrophobic, organically modified silica matrix	Von Bültzingslöwen <i>et al.</i> (2002)
Water treatment	Iron oxide NPs 4–6 nm	Iron oxide NPs used in improved ultrafiltration technology. The application is proposed for cleaning drinking water, but the mode of action is improved disinfection that may be appropriate to any managed water supply	Karnik <i>et al.</i> (2005)
Water treatment	Silver NPs coated onto polyurethane foam	Proposed as a low cost and effective way of killing bacteria or providing an antibacterial filter for water treatment. Nanoparticles are stable on the foam reducing the risk of release of the material to the environment	Jain & Pradeep (2005)
Micronutrient delivery	Vitamin E NPs	A vitamin E NPs, ca. 100 nm in diameter, is proposed for use in beverages for humans by the authors. However, this is an example of a food grade nanovitamin that could also be used in fish foods	Chen & Wagner (2004)
Micronutrient delivery	Casein micelles for nanoencapsulation of nutrients	Demonstration of the utility of the nanoencapsulation technology to work for capturing vitamin D ₂ , a fat-soluble vitamin. Encapsulation technology also proposed for calcium, phosphates and proteins	Semo <i>et al.</i> (2007)
Texture and physical properties of fish food	Carbon nanotubes	Alteration of the strength and buoyancy of the food	Handy, unpubl. obs.

CNT, carbon nanotubes; NP, nanoparticle.

2003), and nano silver is particularly noted for its antibacterial properties (Mühling *et al.*, 2009). The oxidation of food, either through microbial activity or simply due to the presence of oxygen, is a well-known issue for the shelf life of fish (Richards *et al.*, 1998). Oxygen-scavenging films incorporating nano titanium have been suggested (De Azeredo, 2009), or selective gas-permeable films that exclude oxygen from the container (transparent nanoclay polymers; Priolo *et al.*, 2010). Similarly, nanotechnology could be exploited to detect the gases released from deteriorating fish fillets, such as carbon dioxide (Von Bültzingslöwen *et al.*, 2002). Fish oils are also used widely in the food industry and nanoencapsulation with maltodextrin combined with a surface-active biopolymer of starch or whey protein concentrate is suggested as a method to preserve the flavour and quality of fish oil used in food processing (Jafari *et al.*, 2008).

There are numerous potential applications of NMs in aquafeeds. The addition of antimicrobial or antifungal agents (above) equally applies to preserving sacks of fish food. However, nanotechnology may offer some significant advantages in the delivery of micronutrients or other less stable ingredients to the fish. NMs may be used to enclose or coat (nanoencapsulation technology) nutrients that would normally degrade, such as fatty acids, or have limited assimilation efficiency across the gut of fishes, because they are poorly soluble (*e.g.* fat-soluble vitamins). Nanoencapsulation technology has been suggested for carotenoids, trace minerals, vitamins and fatty acids, with increasing bioavailability being the main goal (Acosta, 2009; see Bouwmeester *et al.*, 2009). For example, nano-micelles made from casein are proposed as a vehicle for hydrophobic ingredients like vitamin D2 (Semo *et al.*, 2007). Nanoscale mineral supplements might provide a source of trace metals, without the extensive faecal losses normally associated with mineral salts (*e.g.* Fe salts; Carriquiriborde *et al.*, 2004). Nanoforms of sodium selenite are already proposed to improve selenium absorption in ruminants (Romero-Perez *et al.*, 2010). NMs may also offer an alternative to organic forms of food supplements, where antinutritional factors (incidental pesticides, toxic metals, *etc.*) in the ingredient can sometimes be a problem (Berntssen *et al.*, 2010).

In addition to improving the bioavailability and stability of the food ingredients, NMs may be used to alter the physical properties of fish food. Food wastage and pollution in aquaculture due to poor food stability, texture or inappropriate buoyancy of the pellet is a long-standing problem (Handy & Poxton, 1993). Small additions of NMs can dramatically change the physical properties of food pellets. For example, the additions of single-walled carbon nanotubes (SWCNTs) to trout food can result in a hard pellet that does not fragment easily in water (Handy, unpubl. obs.). Rainbow trout readily eat food containing NMs (up to 100 mg kg⁻¹ TiO₂ NPs, Ramsden *et al.*, 2009; 500 mg kg⁻¹ C₆₀ and 500 mg kg⁻¹ SWCNT, Fraser *et al.*, 2010) without loss of appetite or growth rate. Adding a few mg of an NM to fish feed to modify the physical properties of fish pellets would therefore seem a practical proposition for the aquafeed industry. Clearly, fish nutrition trials would be needed for each new feed formulation containing NMs, but this would be a normal procedure in developing commercial fish food (see below for detection of NMs and their effects on fish health).

Of course, a question remains over public opinion, and whether the consumer would be happy to eat a fish that may contain manufactured NMs. The situation so far for nanotechnology is very different from the situation that occurred with

genetically modified foods (GM foods). The evidence suggests that public perception is generally supportive of nanotechnology, and this is in part due to efforts to ensure that information about NMs were in the public domain at an early stage (Anderson *et al.*, 2005; Handy & Shaw, 2007). However, some risk analysis of the potential long-term effects of manufactured NMs in human food is required. For the aquaculture industry, the exposure risk would relate to the edible parts of the fish, such as the muscle. Data so far indicate that metal NP levels in fish tissues from diets containing mg kg^{-1} amounts of metal NPs are likely to be of the order of ng g^{-1} or low μg amounts (see TiO_2 in Ramsden *et al.*, 2009). There is currently no evidence that such levels would be toxic to humans via the diet, but we should also be mindful that mammalian studies can show very slow clearance of NMs from the body (*e.g.* carbon black; Lam *et al.*, 2004). The human health risks, if at all, are therefore likely to be associated with a life time of eating fishes with ng or lower amounts of NMs. However, this type of concern is not a new problem. An acceptable risk from persistent chemical in edible fish (*e.g.* pesticides, mercury) is identified in legislation on allowable levels of feed contaminants in aquaculture (Berntssen *et al.*, 2010). The same approach can be applied to NMs. For some NMs, there are bulk powder forms (not nanoscale) that have been used in the food industry for decades. For example, the anatase form of TiO_2 (E171) is widely used as a whitening agent in the food industry (Powell *et al.*, 2000). In these cases, safety testing to confirm any difference between the existing bulk chemical and the nanoform could be done. Overall, the potential benefits of manufactured NMs in food are considerable, and the safety issues are very manageable problems, which should not be a barrier to the innovative use of nanotechnology in aquafeeds or the use of NMs in the packaging of fish fillets. However, while the public are generally not adverse to nanotechnology (Anderson *et al.*, 2005), it would be prudent to do further social science research on the specific issue of public acceptability of NMs in edible fish products. There also needs to be a programme of public engagement to explain the uses of nanotechnology by the industry as well as the benefits and risks to the consumer. For example, public confidence in the food safety of edible fish will be essential to the sustained use of nanoingredients in fish feeds.

FISH HEALTH IN AQUACULTURE

The potential benefits of nanotechnology in intensive aquaculture are considerable. Defra (2009) estimates that annual fish/shellfish production in aquaculture is worth £43 million to the U.K. alone, with a further £22 million from the ornamental fish trade (*e.g.* goldfish, *Cyprinus auratus*, Linnaeus, 1758) and £400 million in dry goods sales. For long-term food security, Defra (2009) identifies the strategic importance of developing land-based aquaculture to replace fishing (alongside attempts to conserve wild fish stocks). Land-based aquaculture has some advantages, such as ease of access to the fish, and some ability to avoid pollution events that occur in the natural environment. However, a number of technological challenges have remained, for example, dealing with the considerable nitrogen waste from the aquaculture system (Handy & Poxton, 1993). Here, nanotechnology may provide a step change in our technological capability, *e.g.* a U.S. patent is already filed for 'smart membranes' to remove nitrates from water (patent number: 7632406, issued 15 December 2009). Nanotechnology has an immense role to play in

providing new construction materials for water filtration and purification (see Handy & Shaw, 2007), water quality monitoring and new approaches to fish health diagnostics.

Water quality is, of course, a critical factor in fish health. The standard concerns include ensuring water quality for the immediate needs of the species (*e.g.* dissolved oxygen levels, temperature, salinity), removal of nitrogen wastes as well as the interactions of these parameters (see review, Handy & Poxton, 1993). There is also a concern about pathogens. Indeed, fish disease is arguably one of the main threats to intensive aquaculture systems (Toranzo *et al.*, 2005). The antimicrobial properties of NMs such as nano titania and nano silver (Mühling *et al.*, 2009) can be exploited to reduce the build-up of bacteria in the aquaculture system. In addition to adding antibacterial NMs to food, this could include antibacterial coatings on the sides of fish tanks and pipe work to prevent biofouling. Similar to polymers and films used for food packaging, these NMs can be fixed to the surfaces so that the threat to the sensitive biofiltration systems used in re-circulating aquaria is negligible.

Nanomedicine is a rapidly growing aspect of nanotechnology (review, Freitas, 2005) and there is an opportunity to use these technological advances to monitor and improve fish health. The poor stability of pharmaceuticals in natural water has inevitably led to many fish medicines being delivered via the food, or accepting that much of any aqueous treatment may be simply washed away. NMs have been exploited to make new drug delivery systems for humans, and these may also be used for veterinary medicines including those for fishes. The approaches include solid core drug delivery systems (SCDDS), which involve coating a solid NP with a fatty acid shell to contain the drug of interest. This methodology works at relatively low temperature and pressure, making it especially useful for heat-sensitive or labile pharmaceuticals (Mitchell & Trivedi, 2010). Porous NMs can also be used as a drug delivery matrix. For example, mesoporous silica particles can be used for the controlled release of substances (Stromme *et al.*, 2009). This latter technology could, for example, be used for delivering fish vaccines. Nanosensors are also becoming available to detect pathogens. It is now possible to detect single virus particles using electrical nanosensors (Patolsky *et al.*, 2004), and clearly nanosensors will have an important role to play in disease diagnosis in the immediate future.

Nanotechnology also has applications in water purification. These include the use of antibacterial NMs to remove pathogens directly or to enhance photodegradation of pathogens with ultraviolet (UV) treatment (Li *et al.*, 2008). The use of gold and silver NPs in water purification to remove low concentrations of halogenated compounds like pesticides and heavy metals is reported, with several products being commercially available (review, Pradeep & Anshup, 2009). Nanofiltration methods are also suggested over traditional micron scale filtration to greatly improved removal of foreign substances from water (Zodrow *et al.*, 2009). The application of ZnO nanofibres on thin films has also been used for photocatalytic degradation of organic chemicals such as trichlorophenol (Aal *et al.*, 2009). Clearly, nano-based water purification technology is rapidly emerging, and with some products commercially available, it may only be a relatively small step to adapt these approaches for use in aquaculture systems.

ENGINEERING OF AQUACULTURE SYSTEMS, BOATS AND FISHING GEAR

Nanotechnology offers a plethora of new building materials, textiles, fabrics and electronic devices (see review on nanoproducts, Aitken *et al.*, 2006). For engineering in aquaculture, any material that can offer an increase in strength for fish cage construction without adding extra weight would be advantageous. Here, CNTs are particularly noteworthy. CNT fibres are light weight and very strong. New generation CNT fibres have a strength to weight ratio that is *ca.* 30 times higher than Kevlar and 117 times that of steel (Chang *et al.*, 2010). This makes CNT fibres the strongest material known to mankind. Traditional mooring lines can be strengthened by weaving CNT fibres into the strands of the rope, and tensile strengths exceeding 10–20 GPa are expected, exceeding much of the current rope technology (Chae & Kumar, 2006). The antibacterial properties outlined earlier for other NMs can also be used to prevent the biofouling of marine structures. For the latter, there may be some concerns about introducing dissolved metals into the environment where the antibacterial mechanism involves free metal ion toxicity (*e.g.* probably the case with Ag NPs). However, other NMs show poor solubility and use reactive oxygen chemistry as the antibacterial mechanism (*e.g.* TiO₂), and could be incorporated into a stable antibacterial coating.

OCCUPATIONAL HEALTH AND SAFETY OF NANOMATERIALS

There is an emerging literature on the health and safety of manufactured NMs, with reports from regulatory bodies such as the Health and Safety Executive in the U.K. (Aitken *et al.*, 2004) and from similar agencies in other countries, notably the U.S.A. (NIOSH, 2009). There are also useful health and safety networks that conveniently bring together a wide variety of health and safety information (*e.g.* Safenano, <http://www.safenano.org/Home.aspx/>; NanoimpactNet, <http://www.nanoimpactnet.eu/>). Understandably, considerable attention has been given to the workers involved in the manufacture of NMs, and situations where workers could be exposed to ultra-fine dusts containing the raw materials (Donaldson *et al.*, 2002). However, this type of exposure will be less relevant to staff in the fisheries and aquaculture industry, where most of the likely contact will be with commercial products containing NMs. Any commercial product such as new building materials, or water filtration material, would be subject to the normal safety evaluation for consumer goods prior to public sale, although the details of exactly how consumers might be exposed over the entire life of product are still being debated (Thomas *et al.*, 2006). This also raises the issue of product labelling so that consumers (including fish farmers) can make informed choices about the use (or not) of nano-containing products. This labelling should not only be informative of content or ingredients but also have hazard labelling. In occupational settings, many of the material safety data sheets (MSDS) used for 'in-house' risk assessments currently do not contain nano-specific hazard information. This is partly because the international hazard data information system is based on chemical formulae, not physical structure. Thus, the safety data sheets for graphite and pristine carbon fullerenes (both pure carbon from a chemical classification perspective) might contain the same toxicity information, being derived from data on the traditional form of the chemical (see Handy & Shaw, 2007 for discussion). This anomaly

in the safety information is now recognized, and various international efforts (the International Standards Organisation, Organisation for Economic Co-operation and Development) are trying to resolve this issue for NMs.

However, there may be some concerns for research and development activities in the aquaculture industry. For example, in situations where workers are preparing new feed formulations containing NMs in the laboratory. Occupational exposure to NMs in the research laboratory has also been investigated (Demou *et al.*, 2009; Tsai *et al.*, 2009). The evidence suggests that routine procedures with a few grams of manufactured NMs, such as sonication and stirring, do not result in any appreciable worker exposure when normal precautions are used in the laboratory (*e.g.* gloves, a suitable dusk mask or use of a ventilated fume cupboard). It is therefore not envisaged that elaborate personal protective clothing will be needed for routine research activities, but of course, researchers are required to carry out chemical risk assessments for NMs before they start work, as with any other laboratory procedure.

ENVIRONMENTAL PROTECTION

FATE AND BEHAVIOUR OF NANOMATERIALS IN THE ENVIRONMENT

The presence of nanoscale material in the environment is not new, and natural NMs have likely existed since the earth was formed (Handy *et al.*, 2008b). In water, this natural material is often referred to as ‘natural colloids’, defined as particulate matter in the 1 nm to 1 μm size range, which may comprise of macromolecular organic material (*e.g.* humic and fulvic acids, peptides, protein) as well as colloidal inorganic species (hydrrous iron and manganese oxides; see Buffle, 2006). In soil and sediments, natural NPs include clays, organic matter and iron oxides (Klaine *et al.*, 2008 and references therein). However, the presence of natural NMs in the environment does not mean that organisms are already adapted to manufactured NMs. This is not the case, with materials being engineered for specific chemistries and surface properties that are not readily found in nature.

Currently, there are no routine methods for measuring manufactured NMs in the environment, and consequently, estimates of environmental levels are mainly derived from conceptual models of NP release. This modelling gives estimates of likely manufactured NM concentrations in surface waters (rivers, lakes) in the ng l^{-1} to low $\mu\text{g l}^{-1}$ range (Boxall *et al.*, 2007; Gottschalk *et al.*, 2009; Nowack & Bucheli, 2007). These are only predictions and the fate and behaviour of manufactured NMs in important systems, such as the oceans, are poorly understood. Traditionally, the manufacturing base for the chemical industry has been located on estuaries where waste water can be discharged. Although the levels of NMs have not been quantified yet, it seems likely that marine effluents are being produced that contain manufactured NMs. Klaine *et al.* (2008) summarised the potential fate and behaviour of manufactured NMs in the marine environment. Briefly, the alkaline pH and high ionic strength (NaCl concentrations) in sea water will tend to promote the agglomeration or aggregation of NMs, and deposition of NMs is expected in coastal sediments, as well as in the different zones of the oceans (Fig. 1). So, the exposure of benthic species in the coastal zones seems likely, and eventually pelagic species in the open

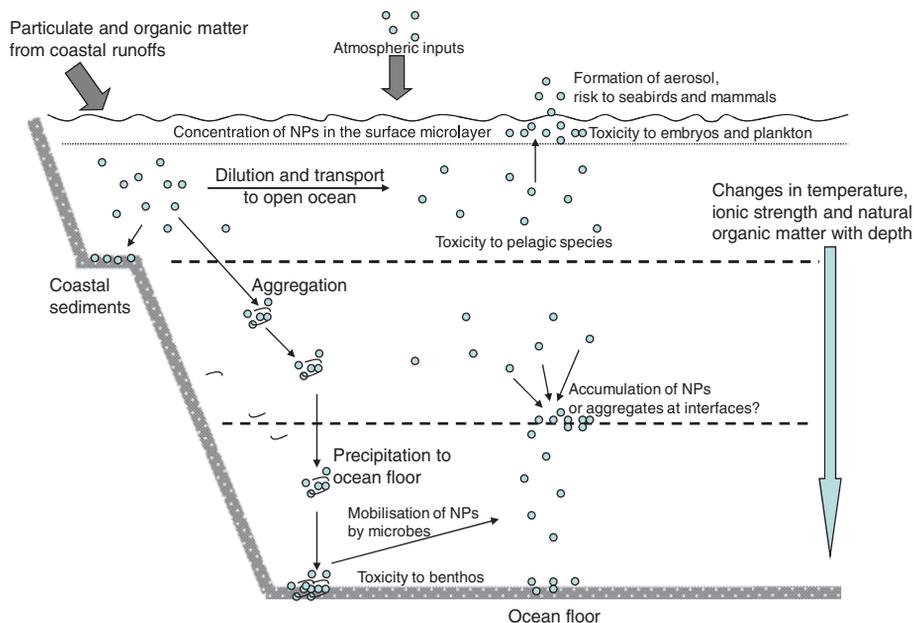


FIG. 1. Schematic diagram outlining the possible fate of nanoparticles (NPs) in the marine environment and the organisms at risk of exposure. Surface inputs could be coastal pollution and atmospheric deposition. The pH of sea water (typically pH 8) and high ionic strength will promote aggregation processes, and NPs may therefore precipitate onto coastal or deep ocean sediments. However, chemistry will change with depth. Microbes and diatoms may be able to remobilise NPs that have accumulated on/in sediments. It remains unclear whether NPs will accumulate in current mixing zones in the ocean, and present a risk to organisms that feed at these interfaces. Some manufactured NPs may have surface properties that allow them to remain dispersed in saline conditions, and these NPs could accumulate due to viscous properties and surface tension effects at the surface microlayer of the ocean with consequent risks for larvae and planktonic organisms in the surface microlayer. Redrawn from Klaine *et al.* (2008).

ocean as well. There may also be a risk of NMs accumulating in the surface microlayers of the oceans, where viscous and surface tension properties could trap NMs in the microlayer at the ocean surface (Simpkiss, 1990; Wurl & Obbard, 2004).

However, much of the information on the fate and behaviour of NMs comes from studies of fresh water (reviews, Handy *et al.*, 2008a; Ju-Nam & Lead, 2008; Klaine *et al.*, 2008) and several important concepts have emerged which are relevant to fisheries (Handy *et al.*, 2011a): (1) NPs form emulsions and dispersions in liquids, they do not usually form *solutions* in the context of traditional aqueous chemistry; (2) NPs tend to form aggregates (*e.g.* particles sticking to each other) or agglomerates (*e.g.* particles loosely joined together or sometimes tangled up with natural organic matter), and this colloid chemistry will greatly influence the environmental fate and bioavailability of the NM; (3) colloid chemistry is strongly influenced by abiotic factors of water chemistry. The most important aspects here are water pH, the presence of divalent ions and ionic strength, although water temperature and the presence/type of natural organic matter in the water could also be important (Handy *et al.*, 2008a, c). The interactions between individual NPs typically involve weak forces, such as Borne repulsion, diffuse double layer potential and Van der Waals attraction (see Handy *et al.*, 2008a). These forces are described in DLVO theory

(DLVO from the names of the original authors of the theory; Derjaguin & Landau, 1941; Verwey and Overbeek in Verwey *et al.*, 1948), which details the features of particle interactions for spherical particles (Handy *et al.*, 2008a).

This colloid chemistry is likely to have some very important implications for the exposure of aquatic species. For example, very small increases in salinity above that of fresh water (*e.g.* 2.5 parts per thousand) can cause particle aggregation and large decreases in the particle number (or mass concentration) in the water (Stolpe & Hassellöv, 2007). Thus, particles may be rapidly removed from the water column on entry into sea water (Klaine *et al.*, 2008). In fresh water, the presence of organic matter such as humic and fulvic acids can stabilize NPs in the water column (Lead & Wilkinson, 2006). Thus, one might expect the low conductivity, peaty, soft waters of Scotland to support particle dispersions. Alternatively, the hard, chalky waters of Southern England may not. Of course, these generalizations will have exceptions. Notably, the hydrology of the water system itself may be important. The flow rate, mixing and agitation of the water will alter aggregation and precipitation processes. In still waters, one may expect deposition onto thin films (*e.g.* rocks and pebbles on the river bed) or in the dead spaces between gravel and sand (Hassellöv & von der Kammer, 2008). This could present microhabitats important to fish embryos and larvae, as well as shellfish, with higher concentrations of NMs than anticipated.

ECOTOXICITY TO FISHES AND SHELLFISH

The ecotoxicity of manufactured NMs to wildlife including fishes and invertebrates has been extensively reviewed (reviews, Handy *et al.*, 2008a; Kahru & Savolainen, 2010; Klaine *et al.*, 2008; Moore, 2006; Pérez *et al.*, 2009), and the following section is abridged from a more detailed review on fishes by Handy *et al.* (2011a). Much of the ecotoxicology research has focused on organisms that are used in regulatory toxicology, such as small freshwater invertebrates like *Daphnia magna*, and the simple end points used in such tests (*e.g.* survival, growth and reproduction) that are subsequently considered in environmental risk assessment (Crane *et al.*, 2008; Owen & Handy, 2007). Many of the experiments have used normal fresh water, and most of the physico-chemical issues like how pH, water hardness, presence of specific divalent ions like Ca, ionic strength, *etc.* alter ecotoxicity remain to be investigated for fishes and shellfish. It is not yet clear which of the many abiotic factors in NM chemistry will turn out to be the most important for ecotoxicity to fishes and shellfish.

Example data on the lethal concentrations (*e.g.* LC₅₀ values) of NPs in fishes and invertebrates are shown in Table II. The acute toxicity values are mostly in the mg or tens of mg l⁻¹ range, suggesting low acute toxicity for many materials. For example, the 96 h LC₅₀ values for several metal oxide NPs (Ag, Cu and Zn) in zebrafish, *Danio rerio* Hamilton, are between 1.5 and 1.79 mg l⁻¹ (Griffitt *et al.*, 2007; 2008; Zhu *et al.*, 2007). These values for metal NPs suggest that they are much less toxic than the typical µg l⁻¹ ranges reported for ordinary metal salts (Spry & Wiener, 1991). However, some metal NPs may dissolve to release free metal ions from the surface of the particle (dissolution, *e.g.* Ag NPs; Kittler *et al.*, 2010).

For carbon-based NMs, initial reports suggested that C₆₀ fullerenes were toxic to fishes (Oberdörster, 2004; Zhu *et al.*, 2006; 2007), but subsequent experiments have attributed this toxicity to the solvents used (*e.g.* tetrahydrofuran, THF) rather than the C₆₀ itself (Henry *et al.*, 2007; Shinohara *et al.*, 2009). Invertebrate studies

TABLE II. Examples of acute toxicity estimates (LC₅₀) for nanomaterials in fish and invertebrates (modified from Handy *et al.*, 2008a and Handy *et al.*, 2011a with additions)

Nanomaterial (size)	LC ₅₀ (duration)	Species	Notes	References
Nano copper (80 nm) Dispersed by sonication	1.5 mg l ⁻¹ (48 h)	<i>Danio rerio</i>	Gill pathology was noted including a thickening of the primary filaments, but no change in branchial Na ⁺ K ⁺ -ATPase was observed. Genomic changes in the gill included upregulation of hypoxia inducible factor-1 (HIF-1) and stress proteins (HSP70)	Griffitt <i>et al.</i> (2007)
Ag NPs (20–30 nm) Dispersed by sonication with 0.5% sodium citrate	0.94 mg l ⁻¹ (48 h) 0.71 mg l ⁻¹ (48 h)	<i>D. rerio</i> (adults) <i>D. rerio</i> (juveniles)	Nano silver was at least two orders of magnitude less toxic than dissolved silver	Griffitt <i>et al.</i> (2008)
Ag NPs (35 and 100 nm) Prepared by sonication	1.25 mg l ⁻¹ (96 h) for 35 nm particles 1.36 mg l ⁻¹ (96 h) for 100 nm particles	<i>Pimephales promelas</i> (embryos)	The authors conducted tests with NPs prepared by stirring. Stirring was less toxic than material prepared by sonication. LC ₅₀ values for stirred media were 9.4 and 10.4 mg l ⁻¹ for the 35 and 100 nm NPs, respectively	Laban <i>et al.</i> (2010)
TiO ₂ NPs (10–20 nm) Dispersed in THF and filtered, but not sonicated	<10 mg l ⁻¹ (48 h)	<i>Daphnia magna</i>	Organisms exposed to 0.2–10 mg l ⁻¹ of TiO ₂ NPs for 48 h. Positive correlation between mortality and concentration. 100% mortality shown at 10 mg l ⁻¹	Lovem & Klaper (2006)
TiO ₂ NPs (10 nm) Dispersed by stirring for 30 min	500 mg l ⁻¹ (96 h)	<i>P. promelas</i>	The study also included LC ₅₀ measurement on invertebrates. TiO ₂ NPs were more toxic to the invertebrate species tested. LC ₅₀ values for <i>Ceriodaphnia dubia</i> and <i>Daphnia pulex</i> were 7.6 and 9.2 mg l ⁻¹ , respectively.	Hall <i>et al.</i> (2009)

TABLE II. Continued

Nanomaterial (size)	LC ₅₀ (duration)	Species	Notes	References
A range of metal NPs	1.5–162 mg l ⁻¹ (48 h)	<i>D. magna</i>	The LC ₅₀ values for mortality were 1.5 mg l ⁻¹ (ZnO NPs), and the highest value was 162 mg l ⁻¹ for Al ₂ O ₃ NPs. The Al ₂ O ₃ NPs were more toxic than the equivalent bulk material	Zhu <i>et al.</i> (2009)
C ₆₀ fullerenes Dispersed by stirring in water for a minimum of 2 months, 10–200 nm aggregates formed	>2.5 mg l ⁻¹ (6 days)	<i>D. magna</i> and <i>Hyalella azteca</i>	In <i>D. magna</i> , a decreased offspring production occurred over the 21 days, and ca. 40% mortality was observed by day 6 of exposure to 2.5 mg l ⁻¹ C ₆₀ . For <i>H. azteca</i> , no mortality was observed at concentrations up to 7 mg l ⁻¹ for 96 h.	Oberdörster <i>et al.</i> (2006)
C ₆₀ (aggregates 10–100 nm) Dispersed by THF	<0.5 mg l ⁻¹ (18 h)	<i>P. promelas</i> (adults)	0.5 mg l ⁻¹ C ₆₀ dispersed in THF caused 100% mortality within 18 h. When dispersions were made by stirring (no THF), 1 mg l ⁻¹ C ₆₀ did not lead to any mortality within 48 h	Zhu <i>et al.</i> (2006)

HSP70, heat-shock protein 70; NP, nanoparticle; THF, tetrahydrofuran.

have shown that lethal dose estimates for C₆₀ are also dependent on the method of preparation of the test material, with differences between stirred and unstirred media, and effects of sonication (see Handy *et al.*, 2008a for comparisons), which have the potential to change particle size distributions in the media and therefore bioavailability. The limited data also suggest that acute lethal concentrations for invertebrates are at the mg or tens of mg level (Table II). There appear to be no lethal concentration estimates for CNTs with fishes. Notably, most of the lethality information is on the test species used for regulatory ecotoxicology, rather than species relevant to aquaculture.

The biological fate [absorption, distribution, metabolism, excretion (ADME)] of NMs in fishes has been reviewed (Handy *et al.*, 2008c). For traditional chemicals, target organs are often identified by measuring the contaminant of interest in the tissues. This is problematic for NMs because reproducible, reliable methods for detecting NMs in tissues are still under development. For metal-based NPs, it may be possible to measure total metal concentrations in tissues (*e.g.* tissue Ti levels for rainbow trout *Oncorhynchus mykiss* (Walbaum 1792) exposed to TiO₂ NPs; Federici *et al.*, 2007), but methodology may require extensive modifications. Laborious electron microscopy is also often the only reliable way to confirm the presence of intact NMs in the tissues.

For NMs added to the water, the gills of aquatic organisms would be directly exposed. The translocation of intact NMs, or NPs, across the gills has yet to be unequivocally demonstrated. However, coherent anti-Stokes Raman scattering (CARS) microscopy indicates that some metal NPs may be located both on and inside gill epithelial cells of fishes (Johnston *et al.*, 2010). Gill injury from MNs has been observed. For example, the exposure to SWCNT increased the ventilation rate of *O. mykiss* and the gill irritation caused some secretion of mucus with gill pathology (Smith *et al.*, 2007). Damage to the gill of *D. rerio* exposed to 1.5 mg l⁻¹ nano Cu for 48 h was characterised by proliferation of epithelial cells and oedema (Griffitt *et al.*, 2007). These effects on the gill are also well known for many other chemicals (Mallat, 1985), but there may also be some nano-related gill injury. For example, SWCNTs cause an uncharacteristic hyperplasia of the epithelial cells in the gill of trout (Smith *et al.*, 2007). Interestingly, the gill injuries observed with NMs do not necessarily cause major haematological disturbances. Several authors have reported normal haematology without evidence of red cell swelling or changes in plasma Na⁺ (Federici *et al.*, 2007; Smith *et al.*, 2007). There are some significant knowledge gaps in our understanding of the respiratory effects of NMs, with only a few materials and species being tested. Studies of the effects of water chemistry (pH, hardness, dissolved oxygen, *etc.*) on ecotoxicity have yet to be completed.

From the view point of environmental risk assessment, information on dietary uptake rates, the trophic transfer of NMs and the chronic effects of NMs on growth are of interest. Ramsden *et al.* (2009) report no effects on the growth of *O. mykiss* with inclusions up to 100 mg kg⁻¹ TiO₂ NPs, but also report subtle biochemical disturbances to oxidative stress markers, Na⁺K⁺-ATPase and electrolytes. Ramsden *et al.* (2009) argued that these effects were broadly similar to those of other trace metals such as Cu and Cd (Handy *et al.*, 2005). Mesocosm studies have shown the transfer of NMs from the water to sediment surfaces, and into aquatic food chains (Bradford *et al.*, 2009; Ferry *et al.*, 2009), suggesting that fishes are likely to receive dietary NM exposure in the field via the food chain. However, it is still very early

on in the research, and detailed studies confirming the amounts and mechanisms of NM uptake across the gut of aquatic species are needed.

Studies on sub-lethal effects with manufactured NMs (Table III) have been conducted reporting effects on a range of body systems, and life stages. In most cases, it has not been technically feasible to measure the NMs levels in the tissues, so some caution in data interpretation is needed. Most of the research has been conducted over short time scales of a few days, and chronic sub-lethal effects information is lacking. However, it is clear that NMs do have some adverse effects on fishes and invertebrates (Table III). Much of the existing data on body systems effects on aquatic species come from animals that are large enough to dissect, such as *O. mykiss*, largemouth bass, *Micropterus salmoides* (Forbes, 1884) and carp species (Federici *et al.*, 2007; Handy *et al.*, 2008c; Oberdörster, 2004; Oberdörster *et al.*, 2006; Smith *et al.*, 2007). Details of how NMs are carried in the blood or other body fluids of aquatic organisms have yet to be elucidated, but it is generally agreed that the colloidal behaviours of NPs in the circulation will be a key factor (Handy *et al.*, 2008c). The saline condition of body fluids may cause NMs to aggregate or agglomerate, suggesting that they may adhere to blood cells. NMs also have a high capacity for adsorbing macromolecules from the external media (*e.g.* albumins and other small macromolecules forming a 'protein corona' on the surface of the NMs; Hellstrand *et al.*, 2009). The effects of this chemistry on blood functions, such as clotting and immunity, are unknown.

The liver, or equivalent organ (hepatopancreas), is a central compartment in the handling of metals, and the metabolism of organic chemicals. The ability of the liver to metabolize or excrete NMs is discussed elsewhere (Handy *et al.*, 2008c). Some liver pathology has been reported in fishes. Histological lesions consistent with fatty change (*e.g.* changes in intracellular storage patterns and lipidosis) have been found in livers of *O. mykiss*, as well as hepatocytes with condensed nuclei (Federici *et al.*, 2007; Smith *et al.*, 2007). Carp livers showed necrotic and apoptotic cells after aqueous exposure to 100 and 200 mg l⁻¹ TiO₂NPs for 20 days (Hao *et al.*, 2009). However, with data only from a few studies, much more research is needed. Similar arguments apply to renal physiology (see discussion in Handy *et al.*, 2008c). At least one study suggests that infusion of TiO₂ NPs into *O. mykiss* has no effect on glomerular filtration rate (GFR), although NP deposits were suspected in the haematopoietic cells of the kidney (Scown *et al.*, 2009).

Evidence that NMs can cause inflammation and immune responses has been obtained from respiratory exposure studies on mammals (Dobrovolskaia & McNeil, 2007; Nel *et al.*, 2006; Warheit *et al.*, 2004). One particular concern is for high-aspect ratio materials such as CNTs, which cannot be easily engulfed by immune cells. This frustrated phagocytosis causes a prolonged respiratory burst with the inevitable release of reactive oxygen species (ROS), and further inflammation (Brown *et al.*, 2007). It is clear that immunological effects are a major mechanism of toxicity for NMs, but information on these responses in fishes and shellfish is currently limited. Histological evidence suggests that the spleen of fishes is able to manage NP exposure so that circulating blood cells, including immune cells, remain relatively normal (Handy *et al.*, 2011a). Recent work on aquatic invertebrates (Galloway *et al.*, 2010; Ringwood *et al.*, 2010) shows that the potential immunotoxic effects of NPs are not restricted to vertebrate animals. However, in general, there is a lack of inclusion of immunological end points in the ecotoxicity literature and more research is needed.

TABLE III. Examples of sub-lethal effects nanomaterials on fish and invertebrates (modified from Handy *et al.*, 2008a and Handy *et al.*, 2011a with additions)

Nanomaterial/ characteristics	Concentration and exposure time	Exposure method	Species	Toxic effects	References
Single-walled carbon nanotubes (SWCNTs); <i>ca.</i> 1–2 nm diameter, 5–30 µm length, suspended in SDS and sonicated	0.1–0.5 mg l ⁻¹ for 10 days	Waterborne exposure	<i>Oncorhynchus mykiss</i> (juveniles)	Respiratory distress evidenced by increased ventilation rates and mucus production. Gill pathologies observed in exposed fish, and vascular injury in the brain	Smith <i>et al.</i> (2007)
SWCNT coated with lysophosphatidyl choline	0–20 mg l ⁻¹ for up to 96 h	Waterborne exposure	<i>Daphnia magna</i>	<i>Daphnia</i> ingested the particles and may use the lysophosphatidylcholine coating as a food source. 100% mortality at the 20 mg l ⁻¹ dose	Roberts <i>et al.</i> (2007)
C ₆₀ , 10–20 nm dissolved in THF then diluted and filtered	260 µg l ⁻¹ C ₆₀ for 60 min	Waterborne exposure	<i>D. magna</i>	C ₆₀ caused some changes in heart rate; changes in locomotor behaviours were observed	Lovern <i>et al.</i> (2007)
C ₆₀ fullerenes, 10–200 nm aggregates formed by stirring in ultrapure water for at least 2 months	0.5 mg l ⁻¹ for 96 h	Waterborne exposure	<i>Pimephales promelas</i> (adult males)	Downregulation in expression of the peroxisomal membrane protein PMP70	Oberdorster <i>et al.</i> (2006)
C ₆₀ , C ₇₀ and C ₆₀ (OH) ₂₄ fullerenes suspended in DMSO. No sizes given	100–500 µg l ⁻¹ for C ₆₀ and C ₇₀ , 500–5000 µg l ⁻¹ for C ₆₀ (OH) ₂₄	Waterborne exposure of dechlorinated embryos; exposed at 24 hpf until 96 hpf	<i>Danio rerio</i> (embryos)	Delayed development and abnormalities in embryos exposed to C ₆₀ and C ₇₀ . Functionalized C ₆₀ (OH) ₂₄ was significantly less toxic causing similar injuries at 2500 µg l ⁻¹ , compared to 200 µg l ⁻¹ for the same injuries with other fullerenes	Usenko <i>et al.</i> (2007)

TABLE III. Continued

Nanomaterial/ characteristics	Concentration and exposure time	Exposure method	Species	Toxic effects	References
TiO ₂ NPs. Primary particle size 24 nm, suspended in ultrapure water and sonicated	0, 0.1, 0.5 or 1.0 mg l ⁻¹ for 14 days	Waterborne exposure	<i>O. mykiss</i> (juveniles)	Gill pathology with inhibition of branchial Na ⁺ K ⁺ -ATPase was observed in the TiO ₂ NP treatments. Glutathione depletion occurred in the liver with necrotic hepatocytes also evident. Disturbances to Zn and Cu status in several tissues, especially the brain	Federici <i>et al.</i> (2007)
TiO ₂ NPs. Primary particle size 50 nm, suspended in ultrapure water and sonicated	0, 10, 50, 100 or 200 mg l ⁻¹ for 20 days	Waterborne exposure	<i>Cyprinus carpio</i>	Dose-dependent increase in respiratory distress. Alterations of hepatic superoxide dismutase, catalase and peroxidase activity implicated oxidative stress as a toxic mechanism	Hao <i>et al.</i> (2009)
TiO ₂ NPs. Primary particle size 24 nm, suspended in ultrapure water and sonicated	0, 10 or 100 mg kg ⁻¹ TiO ₂ NPs diets for 8 weeks followed by 2 weeks recovery on the control diet	Dietary exposure	<i>O. mykiss</i> (adults)	Ti accumulation occurred in several organs with the brain not clearing Ti after the exposure period. 50% inhibition of brain Na ⁺ K ⁺ -ATPase activity was observed during the exposure	Ramsden <i>et al.</i> (2009)
TiO ₂ NPs (32 nm), specific surface area 46.3 m ² g ⁻¹ . Sonicated in distilled water prior to addition of sediments	Up to 2 g kg ⁻¹ in the sediment for 10 days	Marine sediment	<i>Arenicola marina</i> (adults)	No effect on burrowing time, but a decline in the number of casts. DNA damage and a decline in neutral red retention were observed in coelomocytes	Galloway <i>et al.</i> (2010)

TABLE III. Continued

Nanomaterial/ characteristics	Concentration and exposure time	Exposure method	Species	Toxic effects	References
Ag NPs. Primary particle size 81 nm, suspended in ultrapure water and sonicated	63, 129 or 300 $\mu\text{g l}^{-1}$ overnight exposure	Aqueous exposure	<i>Perca fluviatilis</i> (adults)	Ag NPs had no effect on basal metabolic rate (BMR), but the critical oxygen tension required to meet basal metabolism (P_{crit}) increased significantly from 4.8 to 9.2 kPa in the 300 $\mu\text{g l}^{-1}$ Ag NP treatment, indicating a respiratory disturbance	Billberg <i>et al.</i> (2010)
Ag NPs (15 nm) suspended in a citrate buffer	Up to 1.6 $\mu\text{g l}^{-1}$ for 48 h	Waterborne exposure	<i>Crassostrea</i> <i>virginica</i> (adults and embryos)	Concentration-dependent decline in the normal development of embryos, with increased metallothionein production. Adult oysters showed changes in loss of lysosomal stability in the hepatopancreas	Ringwood <i>et al.</i> (2010)
Gold-citrate NPs (13 nm) dispersed method not reported	750 $\mu\text{g l}^{-1}$ for 24 h	Waterborne exposure	<i>Mytilus edulis</i> (adults)	Increased catalase activity in the digestive gland and mantle. 2D gels of extract proteins suggested ubiquitination in the digestive gland and gill	Tedesco <i>et al.</i> (2008)

2D, two dimension; DMSO, dimethyl sulphoxide; NP, nanoparticle; SDS, sodium dodecyl sulphate; THF, tetrahydrofuran.

There are also few investigations of the neurotoxicity of NMs to aquatic species. An early study reported lipid peroxidation in the brains of juvenile *M. salmoides* exposed to C₆₀ (Oberdörster, 2004), but this toxicity has since been attributed to the THF solvent used (Henry *et al.*, 2007; Shinohara *et al.*, 2009). However, subtle biochemical changes have been reported in brains of fishes in several other studies on *O. mykiss*, including disturbances to brain Cu and Zn levels (Federici *et al.*, 2007; Smith *et al.*, 2007) and inhibition of whole brain Na⁺K⁺-ATPase (Ramsden *et al.*, 2009). For many traditional chemicals, the neurological and endocrine effects of pollutants manifest as changes in animal behaviour (review, Scott & Sloman, 2004). It remains to be seen if this is also the case for NMs.

SENSITIVITY OF EARLY LIFE STAGES SUCH AS FISH EMBRYOS

This topic is discussed in more detail elsewhere (Handy *et al.*, 2011a). Briefly, the sensitivity of early life stages, and reproductive effects that influence the number or quality of gametes, is particularly relevant to the aquaculture industry where broodstock are used to produce the next generation of fish for production. The detrimental effects of pollutants and poor water quality on the early life stages of fishes are well known (Eddy & Talbot, 1985; Finn, 2007), and great lengths are taken to ensure water quality in aquaculture for these early life stages. There is some evidence that very high mg l⁻¹ concentrations of NMs are acutely toxic to fish embryos in the laboratory (*e.g.* *D. rerio*, zebra fish embryos, Zhu *et al.*, 2008). In the absence of the protective chorion, NMs can be toxic. Usenko *et al.* (2007) exposed dechorionated *D. rerio* embryos to C₆₀, C₇₀ or C₆₀(OH)₂₄ and reported LC₅₀ values of around 200 µg l⁻¹ for C₆₀ and C₇₀, and 4000 µg l⁻¹ for C₆₀(OH)₂₄.

Effects on the development of fish embryos are also suggested. Nelson *et al.* (2010) used transgenic *D. rerio* to explore the effects of nano silica on the genes involved in development, and differential expressions of some genes between controls and treatments were observed. Usenko *et al.* (2007) observed morphological abnormalities in *D. rerio* embryos exposed to 200 µg l⁻¹ C₆₀ or C₇₀ fullerenes, and embryonic development was also delayed by 12–20 h. C₆₀ exposure also resulted in pericardial and yolk sac oedema. However, all the data on early life stages are on test species that are models for ecotoxicological research, and species relevant to aquaculture (such as salmonids) have not been investigated.

IMPLICATIONS FOR FISH POPULATIONS

The risk of NMs to fishes and shellfish populations, like other chemicals, will depend on the level of exposure and whether population level end points such as survival, growth and reproductive success are affected. However, with the huge diversity of chemistries and structures of NMs, any assessment of population effects would need to be done on a case-by-case basis for individual materials, or at best several NMs with very similar properties. The large difference between the mg l⁻¹ concentrations required for acute lethal toxicity (Table II), and the predicted ng l⁻¹ to µg l⁻¹ range in surface waters (Gottschalk *et al.*, 2009), suggests that overt effects on wildlife are unlikely. Of course, there is always the possibility of accidental releases (*e.g.* tanker spillage), as with other chemicals. Many of the sub-lethal effects that

have been reported (Table III) are also in the low mg l^{-1} range, but the data set is limited with exposures lasting only a few days or a couple of weeks. Chronic exposure data over longer time scales and at much lower concentrations are needed to support environmental risk assessment. The data set on adult fishes is not large enough to make any generalisation about the ability of adults to produce gametes or the quality of the resulting offspring. The fact that body systems of adult fishes do suffer biochemical disturbances and organ pathologies suggests that inevitably the fishes would have less energy for reproduction, but the scientific community has insufficient data to numerically estimate population level effects of NMs such as recruitment.

PRODUCT SURVEILLANCE AND ENVIRONMENTAL MONITORING SCHEMES FOR NANOMATERIALS

Responsible development and use of nanotechnology by the fisheries and aquaculture industries should also involve active engagement in the development of surveillance schemes to monitor the environmental impact of their nano-containing products, and also for any unforeseen hazards relating to product usage or the life cycle of the materials. Surveillance schemes would also demonstrate to the public that the industry is being responsible. For environmental monitoring and food safety, there is currently a technical barrier. Methods of detection are needed for NMs in complex environmental samples, and to detect the materials in the edible parts of fishes. There are three major technical challenges in relation to measurement: (1) detecting manufactured NMs against the vast amount of natural NM already in the environment (see Handy *et al.*, 2008b on the latter) and in animal feeds, (2) measuring the presence of particles inside tissues of wildlife as part of environmental monitoring, or in the edible muscle of fish as part of food surveillance, and (3) increasing the detection limits of current measurement techniques.

The advantages and disadvantages of various measurement techniques for NMs are outlined in Handy *et al.* (2008a). Most of these methods involve very specialist expertise and/or equipment and are not routine methods, even in the fish biology research laboratory. There is also a consensus view amongst scientists that, while these technical problems remain, several independent methods should be used to verify the presence of the NMs. This might involve electron microscopic studies to confirm primary particle size and shape, dynamic light scattering measurements to confirm particle size distributions in liquid samples, measurements of the zeta potential to estimate particle charge as well as mass concentration measurements (see review, Handy *et al.*, 2011b, on practical methods for ecotoxicology). In addition, most of the methods for measuring colloidal NPs have detection limits at around 1 mg l^{-1} , and with predicted environmental concentrations potentially as low as ng l^{-1} , there is a measurement gap of some 6 orders of magnitude. The methods also need to be routine to facilitate the long-term monitoring of such low levels. This is a huge technical challenge, and it is likely that the fisheries and aquaculture industries will need to work closely with scientific instrument manufacturers to close this gap. There is also a need to focus effort on measurements in sea water, as much of the current research uses fresh water or other low ionic strength media.

However, the immediate future for environmental monitoring of NMs is not completely bleak. There are proposals for environmental monitoring schemes based on detecting biological effects (see Handy *et al.*, 2011b for an example), rather than the NMs. In such schemes, a tiered approach using existing rapid toxicity screens is followed by more specific bioassays or biomarkers for individual sub-classes of contaminants. The good news is that there are a number of bioassays that are specific to particles, such as measuring the phagocytosis responses of macrophages, which could be adapted and incorporated into biomonitoring schemes (Handy *et al.*, 2011b). It may also be possible to develop a scheme based on some nano-specific pathologies in fishes (*e.g.* SWCNT pathology; Smith *et al.*, 2007).

For food safety, as with all chemical substances, there will be a requirement to monitor residue levels of NMs in the food. For fish, this should include not only mass concentration measurements (*e.g.* mg of MN kg⁻¹ of fillet), but also confirmation of the presence of nanoscale material (*e.g.* particles). Clearly, any method for routine use would need to be scientifically robust, economic, and cope with a high throughput of samples. The main approach to method development here has been towards extracting the NM from the fillet so that it can be measured in a liquid sample. Approaches could include using toluene extraction for hydrophobic NMs, and less aggressive acid digestion methods to recover intact metal NPs. For inorganic metal-containing NPs, substantially modified mass spectrometric methods or emission spectroscopic methods can be used to measure mass concentrations of total metals in fish (*e.g.* Ti; Federici *et al.*, 2007). However, this will only inform on the metal content, not the presence of particles.

CONCLUSIONS, RISKS AND BENEFITS

The hazards and risks associated with manufactured NMs, as with any new substance, should be balanced against the potential benefits (Owen & Handy, 2007). Currently, there are many potential applications of these novel materials in fisheries and aquaculture. Obvious areas for commercial development are the inclusion of nanoscale ingredients and use of nanotechnology in fish feeds, food packaging, the use of NMs in the construction of aquaculture systems and applications in fish health. These potential benefits to the fisheries and aquaculture industries are considerable, and the fish toxicity data so far suggest that NMs are not as hazardous as other chemicals already used in these industries. The predicted occupational health hazard to workers using nanotechnology products in fisheries and aquaculture would also appear to be within acceptable limits. Practitioners will most likely be using manufactured NMs in commercial products and goods, rather than raw materials containing free particles. The occupational health and safety risks for most fisheries personal in terms of NM exposure might therefore be low or similar to members of the public, given that manufactured NMs are already available in consumer goods. The use of NMs in the research laboratory is a practical proposition, and guidance on the safe handling of the materials is available for laboratory managers.

Ecotoxicity data so far suggest that manufactured NMs have low acute toxicity to aquatic species, and so immediate threats to aquaculture systems and fisheries may be very small. However, chronic studies are needed on low level exposure using realistic

environmental scenarios to determine the long-term impact of nanotechnology on the environment, and even this must be balanced against the benefits of nanotechnology in environmental remediation and water purification technology. The colloid chemistry and behaviour of NMs suggest that NMs in the water column are likely to be precipitated, and this will be particularly relevant to sediments underneath fish cages. However, this is not a new issue for the industry, and benthic environments under caged aquaculture systems are monitored for pollution and biodiversity (Carroll *et al.*, 2003). The hazard from NMs identified to date appears to be less than that for many other traditional chemicals (*e.g.* agrochemicals/pesticides) used in the aquaculture and fisheries industry. Public engagement will also be important to maintaining confidence in this new technology, especially with respect to food safety, and to the environment. Overall, the benefits of nanotechnology are worth pursuing in this sector and the hazard to wildlife should not be a barrier to innovative, responsible development of nanotechnology.

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