A BRIEF REVIEW OF ENVIRONMENTAL ISSUES RELATED TO THE USE AND PRODUCTION OF NANOMATERIALS BY THE FOREST PRODUCTS INDUSTRY

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EXECUTIVE SUMMARY

Nanomaterials (NMs) or nanoscale materials are typically defined as materials with at least one dimension between 1 and 100 nm. NMs typically have unique characteristics when compared to bulk materials of the same composition, in part due to their exceptionally high surface area to volume ratios. NMs play an important role in papermaking, with up to half of the world's annual production of paper estimated to contain nanoparticulate fillers. The primary use of nanoscale materials in paper production today is in retention and drainage systems and coating formulations. The use and manufacture of NMs is growing and is expected to impact the forest products industry through both increased use of nanomaterials in the manufacturing process and as a manufacturing end product. For this reason, it is important to understand the environmental fate and effects of NMs.

At the current time, there appears to be nearly universal agreement that little is known about either the magnitude of releases of NMs to the environment or the fate and transport of those materials once they enter the environment. Research addressing these issues is hampered by the lack of universal agreement on terminology, standardization of appropriate test methodologies, and the difficulty in differentiating between manufactured and naturally occurring nanomaterials in the environment. However, most studies seem to support the conclusions that 1) in aquatic environments, most nanomaterials will aggregate and ultimately settle into the sediments; 2) in the atmosphere, the residence time of nanomaterials is short due to rapid diffusion to and deposition on surfaces, coagulation with larger particles, and evaporation of semi-volatile components; and 3) almost nothing is known about the behavior of manufactured nanomaterials in soil and sediment.

With respect to the toxicity of NMs, recent reviews have concluded that toxicity mechanisms for most NMs are not completely understood, although a number of potential mechanisms have been identified. These include the disruption of membranes or membrane potential, oxidation of proteins, genotoxicity, interruption of energy transduction, formation of reactive oxygen species, and release of toxic constituents from the nanomaterials themselves.

Many NMs have been shown to be bactericidal, a property that has been exploited for some uses (e.g., silver NMs in bandages and clothing). Studies suggest that NMs are not acutely lethal to aquatic invertebrates even at high concentrations, but that there may still be cause for concern since NMs have been shown to accumulate within invertebrate body compartments. In addition, there is little information available on sublethal effects, and tests have been conducted on only a few species. Carbon-based NMs appear to have few, if any, adverse effects on fish even at fairly high concentrations, however metallic and metal oxide NMs appear more likely to be toxic. There is almost no information available on the potential adverse effects of NMs on terrestrial organisms.

There has, until fairly recently, been little regulation of nanotechnology or nanomaterials in the US or other parts of the world. In the US, both EPA and FDA have started to address this issue, thus, it seems likely that the number and complexity of regulations governing the use and release of nanomaterials will increase in the future.

Nanomaterials are already widely used in pulp and paper production, and it seems likely that use and production of nanomaterials by the forest products industry will increase. Thus, industry will be faced with increasing requests to address a broad range of issues, concerns, and regulatory requirements related to the use of nanomaterials. For this reason, the rapidly evolving field of NM research warrants continuing attention.

A BRIEF REVIEW OF ENVIRONMENTAL ISSUES RELATED TO THE USE AND PRODUCTION OF NANOMATERIALS BY THE FOREST PRODUCTS INDUSTRY

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1.0 INTRODUCTION

Nanotechnology is defined by Los Alamos National Laboratory as "the creation of functional materials, devices, and systems through control of matter on the nanometer (1 to 100+ nm) length scale and the exploitation of novel properties and phenomena developed at that scale[1]." Nanomaterials (NMs) or nanoscale materials are typically defined as materials with at least one dimension between 1 and 100 nm. Nanomaterials can be further categorized according to the number of external dimensions that fall on the nanoscale, with, for example, nanoparticles (NPs) having three external dimensions in the nanoscale and nanofibers and nanotubes having two external dimensions in the nanoscale. Unfortunately, definitions seem to have evolved with the development of the field and are not always used consistently in the literature. In particular, the term "nanoparticle" seems often to be used interchangeably with "nanomaterial," in part because the vast majority of NMs are indeed NPs, and in part because some authors use the term "particle" as a generic term meaning "a very small piece or part." In this report, the specific definitions given in this paragraph will generally be followed. However, when a specific citation is given, the usage of the cited work has been retained.

Handy et al. (2008) suggest that, for discussions of environmental fate and ecotoxicity, NMs should be considered to have a distribution of particle sizes, including some primary particles larger than 100 nm or larger aggregates of nanoparticles of a few hundred nanometers diameter. In addition, Christian et al. (2008) note that definitions based on size alone do not adequately address other important aspects of NP characterization.

The small size of NMs gives them specific or enhanced physicochemical properties as compared to the same materials at the macroscale (Depledge et al. 2009). For example, bulk gold is inert. However, as 2-5 nm diameter NPs, gold is highly reactive (Depledge et al. 2009). NMs have exceptionally high surface area to volume ratios, which is one of the reasons for some of their unusual properties (Christian et al. 2008). According to Christian et al. (2008), even the simplest NP will have a surface chemistry that is distinctly different from that of the core material.

A distinction may be made among three types of NMs: naturally occurring, unintentional anthropogenic by-products, and engineered (Thomas and Sayre 2005). Naturally occurring NMs have existed in the environment throughout Earth's history. As noted by Handy et al. (2008), NPs have been found in 10,000 year old glacial ice cores, and estimates indicate about one billion metric tons per year of atmospheric dust are produced globally, millions of tons of which are natural NPs. Natural geological sources of NPs include physicochemical weathering, the formation of new minerals (e.g., in soils), and volcanic activity (Handy et al. 2008). In aquatic environments, natural colloids contain nanoscale materials such as humic substances, protein and polysaccharide exudates from microbes, metal oxides, viruses, and some bacteria (Klaine et al. 2008). Fullerenes (60-carbon atom hollow spheres also known as buckyballs) have been detected in geological materials nearly two billion years old, carbon nanotubes (CNTs) have been found in materials extracted from oil wells, and both have been found in 10,000-year-old ice cores (Nowack and Bucheli (2007).

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[1] http://www.lanl.gov/mst/nano/definition.html

Unintentional anthropogenic by-products include wear and corrosion products and waste and combustion products. Wear and corrosion products include things like NMs from tire and brake wear, metals from bearing wear, metal oxides from roof run-off, and additives from lubricants (Christian et al. 2008). Waste and combustion products include things like combustion by-products (soot), tar leachates in plumes, fly ash, and fine dust (Christian et al. 2008).

Engineered or manufactured nanoscale materials are those intentionally produced in laboratories or industrial settings, either by reducing larger samples of the material to nanoscale (top down) or assembling smaller scale subunits through chemical synthesis (bottom up) (Thomas and Sayre 2005). Klaine et al. (2008) identify five different classes of manufactured NMs:

- Carbon nanotubes (CNTs) and related materials
- Metal-containing materials (e.g. metal oxides)
- Semi-conductor nanocrystals (quantum dots)
- Zero-valent metals (e.g. iron, silver, gold)
- Dendrimers (polymers)

A CNT is a cylindrical derivative of a fullerene. The diameter and size are determined by the synthesis conditions. CNTs have excellent thermal end electrical conductivity (Klaine et al. 2008). The first CNTs were multi-walled and composed of concentric cylinders, but single-walled CNTs (SWCNTs) can also be produced. SWCNTs are graphite sheets rolled into tubes and may be capped on either end by half-fullerene domes (Tsuji et al. 2006). SWCNTs have a strength-to-weight ratio 460 times that of steel (Klaine et al. 2008). CNTs and their derivatives are used in plastics, catalysts, battery and fuel cell electrodes, supercapacitors, water purification systems, orthopedic implants, conductive coatings, adhesives and composites, sensors, and components in the electronics, aircraft, aerospace, and automobile industries (Klaine et al. 2008).

Metal and metal oxide NPs are commonly produced by grinding bulk materials and are widely used in a variety of applications. Titanium dioxide (TiO_2) and zinc oxide (ZnO) are used extensively in sunscreens and cosmetics and as bottle coatings (Klaine et al. 2008). TiO_2 is used as a photocatalyst in solar cells and a pigment and opacifier in paints and coatings (Klaine et al. 2008). Cerium dioxide (CeO_2) is used as a combustion catalyst in diesel fuels as well as in solar cells, gas sensors, and oxygen pumps (Klaine et al. 2008).

Semiconductor nanocrystals, which are also known as quantum dots, display narrow fluorescence or absorption bands because of quantum constraints imposed on electrons by the size of the material (Wiesner et al. 2006). They have a reactive core surrounded by a shell that protects against oxidation and enhances the photoluminescence yield (Klaine et al. 2008). The main use for quantum dots thus far has been in medical imaging and targeted therapeutics, but they are beginning to see use in solar cells, photovoltaics, security inks, photonics, and telecommunications (Klaine et al. 2008).

Zero-valent metals are typically made by the reduction of solutions of metal salts. Nanoparticulate zero-valent iron has been used to remove nitrates from water, sediments, and soils via reduction to ammonia and can also be used to detoxify organochlorine pesticides and polychlorinated biphenyls via reductive dechlorination (Klaine et al. 2008). Nanoparticulate silver is widely used in consumer products such as wound dressings, textiles, air filters, toothpaste, baby products, vacuum cleaners, and washing machines (Klaine et al. 2008). Colloidal gold has medical applications and can also be used in flexible conducting inks or films and as a catalyst (Klaine et al. 2008).

Dendrimers are multi-functional polymers whose size, topology, flexibility, and molecular weight can be controlled (Klaine et al. 2008). Applications for dendrimers include macrocapsules, nanolatex, colored glasses, chemical sensors, modified electrodes, DNA transfecting agents, therapeutic agents for prion diseases, hydrogels, drug delivery, and DNA chips[2] (Klaine et al. 2008).

1.1 Nanomaterials in the Forest Products Industry

Nanotechnology already plays an important role in papermaking. In his book, *Applied Nanotechnology: The Conversion of Research Results to Products*, Jeremy Ramsden reported that as much as half of the world's annual production of paper (0.02% - 0.2% of the total mass) contains nanoparticles (Ramsden 2010). Hubbe (2005, as quoted by Steward et al. 2006) stated that "the use of nanoparticles by papermakers is arguably the world's most important present application of nanotechnology…" The primary use of nanoscale materials in paper production thus far has been in retention and drainage systems and coating formulations.

The growth of nanotechnology is expected to impact the Forest Products Industry in two ways. First, as noted in the Vision and Technology Roadmap for Nanotechnology for the Forest Products Industry, "[e]merging nanotechnologies offer the potential to develop entirely new approaches for producing engineered wood and fiber-based materials" (Energetics, Inc. 2005). Second, nanotechnologies "enable the development of a wide range of new or enhanced wood-based materials and products that offer cost-effective substitutes for non-renewable materials used in the manufacture of metallic, plastic, or ceramic products" (Energetics, Inc. 2005). Indeed, participants in a workshop on nanotechnology and the forest products industry, which was sponsored by the American Forest & Paper Association, TAPPI, USDA Forest Service, and US Department of Energy, concluded that "[n]anotechnology could transform the forest products industry in virtually all aspects…" (Energetics, Inc. 2005). Potential uses for nanotechnology in forest products identified in the workshop include:

- Manufacturing intelligent products with nanosensors for measuring forces, loads, moisture levels, temperature, et cetera.
- Using NMs as building blocks of products with substantially enhanced properties.
- Applying NM coatings for improving surface qualities in order to make existing products more effective.
- Constructing lighter-weight products using less material and with fewer energy requirements.

According to the Roadmap, participants in the workshop identified five areas for research and development that they believed would have the greatest impact on the forest products industry:

- Polymer Composites and Nanoreinforced Materials—Combining wood-based materials with nanoscale materials to develop new or improved composite materials with unique multifunctional properties.
- Self-Assembly and Biomimetics—Using the natural systems of woody plants as either the source of inspiration or the template for developing or manipulating unique nano-, micro-, and macro-scale polymer composites via biomimicry and/or direct assembly of molecules.
- Cell Wall Nanostructure—Manipulating the cell wall nanostructure of woody plants in order to modify or enhance their physical properties and create wood and wood fibers with superior manufacturability or end-use performance.

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[2] a microchip with attached DNA probes from known sources that are used to identify DNA from samples being tested

- Nanotechnology in Sensors, Processing, and Process Control—Using non-obtrusive, nanoscale sensors for monitoring and control during wood and wood-based materials processing, to provide data on product performance and environmental conditions during end use service, and to impart multifunctional capabilities to products.
- Analytical Methods for Nanostructure Characterization—Adapting existing analytical tools or creating new tools (chemical, mechanical, electrical, optical, magnetic) that accurately and reproducibly measure and characterize the complex nanoscale architecture and composition of wood and wood-based lignocellulosic materials.

2.0 NANOMATERIALS IN THE ENVIRONMENT

With respect to releases of manufactured NMs to the environment, according to an EPA White Paper (EPA 2007):

> As more products containing NMs are developed, there is greater potential for environmental exposure. Potential NM release sources include direct and/or indirect releases to the environment from the manufacture and processing of NMs, releases from oil refining processes, chemical and material manufacturing processes, chemical clean-up activities including the remediation of contaminated sites, releases of NMs incorporated into materials used to fabricate products for consumer use including pharmaceutical products, and releases resulting from the use and disposal of consumer products containing nanoscale materials (e.g., disposal of screen monitors, computer boards, automobile tires, clothing and cosmetics).

However, at the current time, there appears to be nearly universal agreement that little is known about the magnitude of releases of NMs to the environment or the fate and transport of those materials once they enter the environment (e.g. EPA 2007, Klaine et al. 2008, Nowack and Bucheli 2007, NSET 2010, RCEP 2008). As noted by Nowack and Bucheli (2007), "[d]ata on the current use and production of NP are sparse and often conflicting."

As an industry that both uses and potentially produces nanomaterials, it is important for the forest products industry to understand what is currently known about nanomaterials in the environment. Understanding what is currently known will allow the industry to respond to questions and concerns about its use of nanotechnology and nanomaterials and also to assess and effectively mitigate potential risks. Understanding existing knowledge gaps will allow the industry to focus on and support research that is directly relevant to its concerns. This section of the report addresses several key components for assessing the potential impacts of nanomaterials in the environment: environmental fate, characterization and quantification of manufactured nanomaterials in the environment, and the effects of exposure to nanomaterials.

2.1 Environmental Fate

According to Handy et al. (2008), the behavior of manufactured NMs in the environment is likely to be complex, affected by:

- Aggregation chemistry and the ability of manufactured NPs to form stable dispersions in liquids such as water;
- Changes in aggregation chemistry due to differences in particle shape, size, surface area and surface charge;
- Adsorption of manufactured NPs on surfaces, including the exterior surfaces of organisms;

- The effect of other abiotic factors on all the above, including the influence of changing environmental pH, salinity (or ionic strength), water hardness, and the presence of natural organic matter.

Christian et al. (2008) report that the properties of NPs differ "remarkably" from those of small molecules and that they should be considered more like complex mixtures. Because small molecules attach to the surface of NPs and exchange with other small molecules already in place, Christian et al. (2008) concluded that a careful consideration of NP chemistry is the key to predicting the fate of NPs in the environment. Christian et al. (2008) also make a clear distinction between the core of a NM and the surface, which likely have entirely different chemistries. They note that while the core chemistry is generally responsible for the material properties important to the commercial application of the NM, the fate and environmental behavior of the material may well be dominated by surface chemistry.

Due to the small size of NPs, it might be assumed that they would form stable dispersions because they are strongly affected by Brownian motion (Christian et al. 2008). However, such assumptions fail to take into account the high surface energy of NPs, which means that collisions between NPs may result in aggregation and subsequent precipitation (Christian et al. 2008). It should be noted, though, that NP dispersions can be stabilized if the surface layers are coated with counter ions or if a relatively long molecule is tethered to the surface of the NP, providing a steric barrier to aggregation (Christian et al. 2008).

Several researchers have suggested that knowledge gained over the years with naturally occurring NMs can serve as a guide for predicting the behavior of manufactured NMs (e.g. Christian et al. 2008, Klaine et al. 2008, Nowack and Bucheli 2007). For example, naturally occurring colloids include components in the nanosize range and have been widely studied.

2.1.1 Aquatic Fate

As noted earlier, NMs may be expected to act like colloids and, for this reason, consideration of colloid behavior in the aquatic environment may provide insight regarding the behavior of NMs. In aquatic environments, natural colloids include three major types of compounds: inorganic matter (e.g. clay minerals, metal oxides), humic substances, and large biopolymers (e.g. polysaccharides, peptidoglycans) (Nowack and Bucheli 2007). Colloidal fate and behavior are dominated by collision, which is driven primarily by Brownian motion and gravitational settling, with subsequent aggregation, then sedimentation (e.g. Klaine et al. 2008, Nowack and Bucheli 2007, Lead & Wilkinson 2006). Fibrillar components (e.g. polysaccharides) interact with each other and smaller colloids via bridging mechanisms to enhance aggregation (Lead and Wilkinson 2006). Humic substances mainly react with the surface of inorganic colloids and form nanoscale coatings on solid phases (Klaine et al. 2008, Lead and Wilkinson 2006). Although humic substance coatings may stabilize surface charges and reduce the tendency to aggregate, fibrillar bridging, along with the impact of factors such as pH, calcium ion concentration, and the presence of other types of natural colloids, ultimately leads to aggregation and settling in most environments (e.g. Klaine et al. 2008, Lead and Wilkinson 2006).

Trace elements (e.g. metals), nutrients, and organic pollutants all bind to colloids (Lead and Wilkinson 2006). Indeed, colloids are believed to control, to a large extent, the transport of pollutants in many compartments of the environment (Lead and Wilkinson 2006). In the "colloidal pumping model," pollutants bind preferentially to small colloids, which eventually aggregate into particles that are large enough to settle, with sediments becoming the final environmental sink for those pollutants (Lead and Wilkinson 2006).

Fate and behavior of manufactured NMs is likely to be determined primarily by their interactions with naturally occurring colloidal material, meaning that their fate, too, will be dominated by aggregation and subsequent sedimentation (Klaine et al. 2008). Thus, sediments are expected to be the ultimate sink for most manufactured NMs entering waterbodies. However, Klaine et al. (2008) also note that NMs may be released to the environment along with other substances that will reduce the tendency to aggregate. For example, in wastewaters, NMs may be released along with significant quantities of household and industrial detergents or coated with proteins as a result of interactions with microorganisms, either of which would reduce aggregation and increase the time NMs spend in the water column (Klaine et al. 2008).

Another aspect of aggregation behavior is the potential tendency of NMs to accumulate in “unstirred surface layers (USLs)” because of slower flows around those USLs or viscous properties of the USL (Handy et al. 2008). While Handy et al. (2008) apply this concept primarily to the potential accumulation of NMs along the surfaces of aquatic organisms, they also note that similar phenomena might apply to solid-liquid and air-water interfaces in the environment, leading to accumulation of NMs at those interfaces. For example, they suggest that NMs might be trapped and concentrated in ocean surface microlayers, which have a different chemical composition than surrounding seawater (e.g. hydrophobic organic compounds of natural and anthropogenic origin are concentrated at the air-water interface).

2.1.2 Atmospheric Fate

The atmospheric fate of NPs has been widely studied, although NPs are more typically identified as “ultrafine particles” in atmospheric science research (Biswas and Wu 2005). NPs may be emitted directly into the atmosphere, but others are formed through nucleation and condensation processes when emissions of hot supersaturated vapors cool to ambient temperatures or chemical reactions take place within the atmosphere (Navarro et al. 2008). Soot, for example, is defined by Nowack and Bucheli (2007) as NPs produced in re-condensation processes during incomplete combustion.

NPs have a short residence time in the atmosphere because of their rapid diffusion to and deposition on surfaces, coagulation with larger particles, and evaporation of their semi-volatile components (Biswas and Wu 2005). However, obviously, if NPs attach to larger particles, their fate will be determined by that of the larger particle. According to Nowack and Bucheli (2007), even if NMs are released into the atmosphere, they will eventually all end up in soil or water.

2.1.3 Fate in Soil

In their recent review, Klaine et al. (2008) report that very little is known about the transport of NMs through soils. They note that soils provide a large and reactive sink for NMs, and the identification of manufactured NMs in soil represents “a significant analytical challenge.” There are, as yet, no published partition coefficients for NM contaminants in soil, although data suggest that with respect to NPs, surface coatings are important determinants of mobility and may enhance transport (Klaine et al. 2008).

Darlington et al. (2009) conducted laboratory studies using soil columns and aluminum NPs. They found that in solutions that mimic surface water conditions, the NPs rapidly formed micron-sized agglomerates that did not flow through the soil columns and thus, in the environment, would not be likely to move through the soil into ground water. The researchers speculated that such aggregation might also reduce bioavailability. However, they also noted that, depending on the surface charge of the NPs and the soil of the receiving environment, NPs could remain unaggregated and thus have greater transport potential and bioavailability.

2.1.4 Characterizing and Quantifying Manufactured Nanomaterials in the Environment

At the current time, there is a distinct lack of methodology for detecting and measuring the concentration or speciation of manufactured NMs in the environment (Klaine et al. 2008). In a 2007 review, Nowack and Bucheli reported that “the development of analytical methods for engineered NPs is still in its infancy." Even if appropriate methodology was available, researchers are faced with the problem of measuring trace levels against a high background of natural colloids (Klaine et al. 2008). For example, Klaine et al. (2008) note that simple box models, with many simplifying assumptions, estimate that concentrations of the most common manufactured NPs expected to be present in natural waters are in the range of 1 – 10 µg/L, while natural NP concentrations may approach 100 µg/L in water and be even higher in sediments.

In addition, it has also been suggested that mass, which is the commonly used metric for quantifying particulate matter in the environment, is not necessarily sufficient for NPs (SCENIHR 2007). The Scientific Committee on Emerging and Newly-Identified Health Risks of the European Union feels that number concentration (i.e. number of particles in a specific volume) and surface area may be more appropriate parameters (SCENIHR 2007).

2.2 Effects of Exposure to Nanomaterials

Handy et al. (2008) estimate that, considering atmospheric dust alone, millions of tons of natural NPs are produced every year. Comparing that against production estimates for manufactured NMs, which are a few thousand tons per year of each major type of material, it is clear that exposure to natural NPs vastly outweighs anthropogenic production (Handy et al. 2008). Similarly, many biological processes typically operate at the nanoscale, with, among other things, proteins, peptides, DNA/RNA, ATP, and viruses all being nanosized (Handy et al. 2008).

However, even though many forms of natural NMs exist in the environment and organisms on Earth have evolved in the presence of these NMs, the effects of exposure to manufactured NMs are unknown and thus of concern to some (Handy et al. 2008). Certain natural NMs, such as those found in volcanic dust, can be toxic to living organisms under some circumstances and so might some manufactured NMs (Handy et al. 2008). Furthermore, many natural NMs are short-lived in the environment, either disappearing through dissolution or becoming larger through particle growth or aggregation, while some manufactured NMs may persist in the environment (Handy et al. 2008). Finally, although natural NMs may exist in a variety of structures, it is uncertain whether or not some manufactured NMs might exist as structures that are not found in nature and are toxic (Handy et al. 2008).

According to Buffle (2006), the main impacts of NMs on ecosystems will likely fall into one of three categories: (1) direct effects on microorganisms; (2) changes in the bioavailability of toxic or vital compounds that may react with NMs; or (3) possible changes in environmental microstructures (although this is unlikely except in local areas of high discharge of NMs).

2.2.1 Uptake of NMs

Numerous laboratory studies have demonstrated that NMs are readily taken up by a variety of mammalian cell types via endocytosis or phagocytosis (Nowack and Bucheli 2007). In a recent review, Nowack and Bucheli (2007) describe several studies in which NMs, including CNTs and nanosized ZnO and CeO_2, were taken up by or preferentially adsorbed to the outside of bacteria and protozoa.

However, Klaine et al. (2008) note that quantitative research on uptake and accumulation of NMs by whole organisms is scarce. If NMs are present in the environment, organisms will likely incorporate them within their bodies, mainly through ingestion and subsequent translocation within the body (Klaine et al. 2008). In addition to ingestion, aquatic organisms may absorb NMs across epithelial boundaries (e.g. gills, body wall) (Nowack and Bucheli 2007). Nowack and Bucheli (2007) identified a study in which latex NPs were taken up by the eggs and adults of fish (Medaka), where the NPs were concentrated in gills and intestine, but also detected in brain, testis, liver and blood. In other studies, CNTs coated with lipids (Nowack and Bucheli 2007) and fluorescent carboxylated NPs (Klaine et al. 2008) were readily taken up by *Daphnia magna*.

Navarro et al. (2008) discuss the possibility that manufactured NMs might bioaccumulate in higher trophic level organisms. They note that some NMs, like quantum dots, are taken up by bacteria and due to their long residence time in cells may be transferred through food webs. They also point out that carbon-based NPs are reported to partition into cell membranes, which could lead to “significant bioaccumulation” (Navarro et al. 2008).

2.2.2 Toxicity of NMs

In their review, Klaine et al. (2008) report that toxicity mechanisms for most NMs have not yet been completely elucidated. Potential mechanisms include disruption of membranes or membrane potential, oxidation of proteins, genotoxicity, interruption of energy transduction, formation of reactive oxygen species, and release of toxic constituents (e.g., release of silver ions from silver NPs or cadmium from quantum dots) (Klaine et al. 2008).

Nowack and Bucheli (2007) point out that interaction between NMs and toxic organic contaminants may act to amplify or reduce the toxicity of those organic contaminants. In the environment, NMs may adsorb or absorb pollutants, reducing the free concentration and thus the toxicity. On the other hand, if organic contaminant-NM complexes are preferentially taken up by organisms, toxicity may be enhanced. Also, if the bioavailability of the organic contaminant is reduced by its association with the NM, toxicity may be reduced.

In their recent review, Nowack and Bucheli (2007) identified a number of studies in which NMs, including nanosized silver, TiO_2, SiO_2 and ZnO, as well as CNT, were shown to be toxic to bacteria and protozoa (Nowack and Bucheli 2007). The review of Klaine et al. (2008) provides a list of NMs that have been shown to be bactericidal to a greater or lesser degree, including fullerenes, single- and multi-walled CNTs, quantum dots, silver, gold, magnetite, TiO_2, MgO, CeO_2, ZnO, and SiO_2.

Klaine et al. (2008) reviewed studies of NM toxicity to freshwater invertebrates. They noted that most studies have used crustaceans as test organisms and that *D. magna* was the most commonly used crustacean. Acute toxicity tests using *D. magna* indicate that the lethality of tested NMs is relatively low, but that there may still be cause for concern since NPs have been shown to accumulate within invertebrate body compartments, and there is still little information available on sublethal effects or on a wider range of invertebrates (Klaine et al. 2008).

Interpretation of NM toxicology studies conducted on fish is complicated by potential confounding effects of the solvents used in many cases to prepare test suspensions (see Section 2.2.3). Klaine et al. (2008) reviewed studies of NM toxicity to freshwater fish. This summary is taken from that review, but limited to studies in which no solvents were used to prepare NM suspensions. Carbon NMs appear to have few if any adverse effects on fish even at fairly high (several hundred mg/L) concentrations. Metallic and metal oxide NMs, on the other hand, appear more likely to be toxic, with, for example, nanocopper having a 48-hr LC_{50} value of 1.5 mg/L in zebrafish.

In their review, Klaine et al. (2008) conclude that there is very little information available to assess the environmental risk of manufactured NMs to terrestrial ecosystems. They report finding only two published reports on the toxicity of NMs performed in soil, both of which used fullerenes. In both studies, fullerenes appeared to have little or no effect on soil microorganisms.

2.2.3 Studying NM Toxicity

Toxicology testing using NMs is difficult. In general, NMs are insoluble and susceptible to aggregation, thus likely to settle out of solution rapidly (Klaine et al. 2008). This makes it difficult to quantify actual exposures and ensure a consistent exposure level for the duration of the test. In addition, many NMs tend to form large aggregates, which makes them micro- rather than nanoparticles, and it is not clear whether these aggregates have the same toxic potential or bioavailability as dispersed NMs (Kaline et al. 2008).

Handy et al. (2008) identify several key issues that must be dealt with when assessing the potential ecotoxicity of manufactured NMs:

- The fate and behavior, and thus toxicity, of manufactured NMs are likely to be different in seawater than in freshwater.
- Ecotoxicity in freshwater is likely to be influenced by the presence of natural organic matter (which can coat NMs), changes in pH, and the presence of cations such as Ca^{+2} (which can affect surface charge and thus aggregation tendency).
- Environmental exposure will not be homogeneous. Adsorption and aggregation phenomena may result in local areas of higher concentrations of NMs, e.g. in sediments, on biofilms, or in microsurface layers.
- Aggregation may lead to the deposition of materials, including NMs, on organisms in the aquatic environment, as in the aggregation of materials from the water onto benthic organisms (Handy et al. 2008). NMs could also be adsorbed to the exterior surface of an organism, which could result in different types of toxicity than those associated with internal (e.g. dietary, inhalation) exposures (Handy et al. 2008).
- Uptake into the organisms will depend on aggregation of NMs on exterior surfaces of organisms and the behavior of NMs in body fluids.
- Toxicity may be a function of particle size and shape, and there may be inherent differences in toxicity of NMs and micron scale materials.

Another difficulty in the study of potential effects of NMs on living organisms is the lack of information on and inability to chemically characterize NMs (Handy et al. 2008). Handy et al. (2008) note that information is lacking on particle size distributions, surface charge, surface area, surface reactivity, and impurities likely to be present in NMs that may be released into the environment.

Crane et al. (2008) evaluated the usefulness of traditional ecotoxicity test methods in assessing the ecotoxicity of NMs. They concluded that, in general, standard methods are appropriate. However, they note that when conducting toxicity tests with NMs, considerable uncertainty is associated with the way in which the test material is dosed into, and maintained and measured in, the test medium. They also recommend that the concentration of NMs in ecotoxicity tests should be expressed as number of particles or surface area per volume and not just as mass per volume.

There has been some controversy over some studies of the toxicity of carbon NMs. As described by Klaine et al. (2008) in their recent review, many of the early published ecotoxicity studies on CNTs and fullerenes used the solvent tetrahydrofuran (THF) in the preparation of suspensions of NMs. Subsequent studies have demonstrated that even after filtration and evaporation, THF, a known neurotoxin, and its oxidation products remain trapped between particles. In some cases, the adverse effects reported in studies using THF were not observed when suspensions were prepared using only stirring or sonication. The issue remains somewhat murky, however, as in some cases, suspensions of fullerenes prepared by sonication did have toxic effects (Klaine et al. 2008).

2.3 Ecological Risk Assessment for NMs

At the conclusion of a recent review article on the ecotoxicity of manufactured NMs, Navarro et al. (2008) noted that evaluation of the risks posed by manufactured NMs to the environment involves a comparison of environmental concentrations to those that are toxic to organisms. They then pointed out that both types of data are lacking and reported that that they were unable to identify any available estimates of environmental concentrations of manufactured NMs (Navarro et al. 2008). According to the authors, the important unanswered questions include those about:

- the relevant manufactured NP concentrations in the different aquatic, terrestrial, and aerial environments;
- the physical and chemical characterization of manufactured NPs and exposure routes;
- the mechanisms allowing manufactured NPs to pass through cellular membranes and cell walls;
- the specific properties that are related to manufactured NPs' toxic effects; and
- the mechanism underlying manufactured NPs' trophic transfers (Navarro et al. 2008).

Many researchers and reviewers have focused on the behavior of specific manufactured NMs, but mostly in laboratory settings. For example, Christian et al. (2008) discuss, in detail, work such as the surface coating of NPs, CNT, and fullerenes by naturally occurring organic matter, the effects of humic acids and cations on nanoparticle aggregation and disaggregation, and the effects of pH and ionic strength on the behavior of individual NMs. However, others focus on a larger picture and point out that the mass and particle number of manufactured NMs are dwarfed by the mass and particle number of naturally occurring NMs (e.g. Klaine et al. 2008). It is not clear, at this time, whether, in most circumstances, any effects of manufactured NMs can be distinguished against the existing background of naturally occurring NMs.

Handy et al. (2008), in the introduction to a special issue of the journal *Ecotoxicology* devoted to nanomaterials, pointed out that the literature on the ecotoxicology of NMs is still emerging and "at this early stage most ecotoxicological studies have been observational or 'proof of principle' experiments." They also note that "[t]here are many gaps in our knowledge on the ecotoxicity of NPs" (Handy et al. 2008). Without better data on ecotoxicology, meaningful ecological risk assessments for NMs will be difficult to conduct.

3.0 REGULATING NANOMATERIALS

There has, until fairly recently, been little regulation of nanotechnology or nanomaterials in the US or other parts of the world. In 2009, a consortium of research institutions from the US and Europe issued a briefing paper titled "Regulating Nanomaterials: A Transatlantic Agenda" (Falkner et al. 2009). In the paper, the group called for, among other things, stronger international regulatory cooperation and increased mandatory reporting requirements for nanomaterial use in commercial products. In May 2010, the US Government Accountability Office (GAO) issued a report titled "Nanomaterials Are Widely Used in Commerce, but EPA Faces Challenges in Regulating Risk" (GAO 2010). The GAO encouraged EPA to move forward with plans to further regulate nanomaterials.

In the past year, EPA and FDA have both taken steps to increase regulation of nanomaterials. In September, 2010, EPA issued significant new use rules (SNURs) for multi- and single-walled carbon nanotubes under the Toxic Substances Control Act (TSCA) (EPA 2010). In June 2011, FDA issued for public comment a "Draft Guidance for Industry; Considering Whether an FDA-Regulated Product Involves the Application of Nanotechnology" (FDA 2011). Also in June 2011, EPA issued for public comment a document describing potential approaches for obtaining additional information on nanomaterials that may be contained in registered pesticide products (EPA 2011).

Thus, it seems likely that the number and complexity of regulations governing the use and release of nanomaterials will increase in the future.

4.0 CONCLUSIONS

Nanomaterials are already widely used in pulp and paper production, and all indications are that developing nanotechnology will greatly increase the use and production of nanomaterials by the forest products industry. At the current time, with respect to the environment, the primary area of concern for the industry would seem to be nanomaterials used in retention and drainage aids and coating formulations entering the wastewater treatment system and subsequently being discharged into the environment in effluent. However, based on limited existing research into the aquatic fate of nanomaterials and more extensive research on the behavior of naturally occurring aquatic colloids, it appears likely that any nanomaterials entering the wastewater treatment system would rapidly aggregate and be incorporated into colloids of primarily natural origin – especially since some of the nanomaterials in question (e.g. kaolin) are themselves natural products. Thus, these materials may no longer be nano-scale at the point of discharge.

However, if, as seems likely, the forest products industry embraces future opportunities offered by nanotechnology, both in the use and production of nanomaterials, the industry will be faced with a broader range of issues, concerns, regulatory requirements, and greater demands for ecological risk assessments for industry use of nanomaterials. For example, as noted in a recent editorial about cellulosic nanotechnology in *TAPPI Journal*, “[a]s cellulosic nano-enabled consumer products begin to become a reality, it is increasingly important that our forest products community develop and agree upon national and international materials standards for cellulosic nanomaterials, as well as generate all pertinent science-based environmental, health, and safety information.”

At this time, questions far outweigh answers about the effects of manufactured MNs on ecosystems. This is a rapidly evolving field of research that warrants monitoring, especially given that manufactured NMs are already being used and produced by the forest products industry, and it seems that this is likely to increase in the future.

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APPENDIX 1

NANOCELLULOSE

1.0 INTRODUCTION

Nanocellulose has a wide range of potential uses, including:

- food additive for thickening and calorie reduction
- thickener in cosmetics, paint, ink, and latex adhesives and sealants
- stabilizer for dispersions, emulsions, and suspension
- pharmaceutical manufacturing (e.g. tablet binder, precipitation control agent)
- flocculants and retention aids
- coating papers used for, e.g., printing and food packaging
- the production of highly porous and strong insulation and packaging materials
- production of bioactive membranes and filters
- as a filler for enhancement of paper and board strength
- manufacturing of renewable and biodegradable composites
- high strength spun fibers and textiles

A survey of published literature reveals that most of the research on nanocellulose structures has focused on microfibrillated cellulose (MFC) (also called nanofibrillated cellulose) and nanocrystalline cellulose (NCC) (also called cellulose nanocrystals or cellulose whiskers).

Microfibrillated cellulose is typically manufactured by repeatedly refining, homogenizing, crushing, and/or grinding wood pulp. The outer layer of the cellulose fibers is stripped away, exposing the underlying microfibril bundles. MFC is composed primarily of individualized cellulose microfibrils, which are long and flexible with diameters in the range of 10-40 nm and lengths generally in the micrometer scale (Siqueira et al. 2010, Siro and Plackett 2010). MFC presents a web-like structure due to the high density of hydroxyl groups on the microfibril surfaces, which interact and lead to agglomeration (Siqueira et al. 2010, Siro and Plackett 2010). Often, MFC suspensions are described as consisting of cellulose nanofibers and nanofiber bundles along with some larger fiber fragments and unfibrillated fibers (Siro and Plackett 2010).

Nanocrystalline cellulose is typically produced by acid treatment of cellulose fibers. The acid preferentially hydrolyzes the amorphous regions of cellulose, releasing the crystalline regions in the form of rod-like nanocrystals (Habibi et al. 2010, Peng et al. 2011). NCC prepared from wood typically has diameters in the range of 3-5 nm and lengths of 100-300 nm (Peng et al. 2011). The behavior of NCC in aqueous solution is affected by the type of acid used in its preparation. For example, if hydrochloric acid is used in the hydrolysis, the resultant crystals do not readily disperse and tend to form agglomerates (Habibi et al. 2010). If sulfuric acid is used, the resultant crystals readily disperse in water (Habibi et al. 2010).

2.0 ENVIRONMENTAL FATE AND EXPECTED CONCENTRATIONS OF NANOCELLULOSE

In a laboratory test of biodegradability under aerobic conditions (test not specified, probably OECD 310), NCC was 42% degraded after 28 days of incubation with bacterial cultures (O'Connor 2011).

Kovacs et al. (2010) calculated a worst-case scenario concentration of NCC in kraft mill effluent based on the complete spillage of 1 ton, an estimate of one day's production of NCC from a "first generation NCC production facility." They assumed that the NCC production line/facility would be a part of an existing kraft pulp mill and that all wastewater would enter the pulp mill treatment system. They also assumed that there would be no loss of NCC through the treatment system. The worst-case estimate of NCC concentration in mill effluent was 0.024 g/L, which, for the average kraft mill in Canada, would yield a short-term NCC concentration in receiving waters near the discharge point of 0.24 mg/L.

Kovacs et al. (2010) used the same assumptions to calculate worst-case effluent and receiving water concentrations associated with a continuous loss of 1% of the NCC produced. Those concentrations were 0.18 mg/L and 1.8 ug/L for effluent and receiving waters, respectively.

3.0 NANOCELLULOSE TOXICITY

Cellulose itself is not known or expected to present any adverse environmental or health risks under any realistic exposure scenarios. However, since it has been shown that nanomaterials have different characteristics than the parent material simply due to the change in scale, it cannot be assumed that risk assessments conducted with cellulose will be valid for nanocellulose. There has, as yet, been only limited research into the potential environmental and health effects of nanocellulose.

3.1 Ecotoxicity Assessments of Nanocellulose

Kovacs et al. (2010) evaluated the aquatic ecotoxicity of NCC produced from kraft pulp by sulfuric acid hydrolysis. The extensive battery of tests used in the study and the results are shown in Table 1, which is modified from Kovacs et al. (2010). The authors compared the results of their testing to the criteria developed by US EPA to categorize the toxicity of pesticides. Based on those criteria, they reported that NCC would be classified as practically non-toxic. Similarly, comparing the results to the criteria developed by US EPA to categorize the toxicity of new substances indicated that NCC would be categorized as a new material of low concern. The researchers concluded that NCC is not likely to harm aquatic organisms at the concentrations that might occur in receiving waters even under worst-case scenarios.

Vartiainen et al. (2011) attempted to use the ecotoxicity test ISO 6341, which is an assessment of the inhibition of the mobility of neonates of the aquatic invertebrate *Daphnia magna* Stratus, to evaluate MFC derived from never-dried ECG-bleached birch kraft pulp. However, the researchers discovered that the tendency of MFC to form agglomerations made it impossible to disperse the test sample in solution. The agglomerations both formed mechanical barriers to *D. magna* movement and entangled neonates. The authors concluded that ISO 6341 was inappropriate for the evaluation of substances such as MFC that cannot be evenly dispersed in the test solution.

Vartiainen et al. (2011) also evaluated the ecotoxicity of MFC using ISO/DIS 21338, a test that measures the inhibition of light emissions from *Vibrio fisheri*, a luminescent bacteria. They found that MFC was not acutely toxic to *V. fisheri* at environmentally relevant concentrations, with a No Observable Effect Concentration (NOEC) greater than 100 mg/L.

Table 1. Bioassays used to Determine the Toxic Potential of NCC by Kovacs et al. (2010)

Toxicity Test	**Assessment Endpoint**	**Measurement Endpoint**	**Results**[1]
Rainbow Trout	Acute lethality	96-hr LC50[2]	LC50 > 1 g/L
Daphnia Magna	Acute lethality	48-hr LC50[3]	LC50 > 1 g/L
Ceriodaphnia dubia	Acute lethality	48-hr LC50	LC50 > 1 g/L
Ceriodaphnia dubia	Chronic sublethal reproduction	7-day IC25[4]	IC25 > 1 g/L
Fathead minnow	Chronic sublethal reproduction	10-d IC25[5]	IC25 = 0.29 g/L
Vibrio fischeri (Microtox bacterial toxicity test)	Acute sublethal light inhibition	15-min IC25[6]	IC25 ≥ 0.46 g/L
Pseudokirchneriella subcapitata (Algal test)	Chronic sublethal growth inhibition	72-h IC25[7]	IC25 ≥ 0.12 g/L
Micro-crustacean test (ThamnoToxkit assay)	Acute lethality	24-h LC50[8]	LC50 ≥ 3.5 g/L
Cnidarian test (*Hydra attenuata* assay)	Acute sublethality indicated by morphology changes	96-h EC50[9] 96-h LC50	EC50 ≥ 0.06 g/L LC50 ≥ 0.36 g/L
Zebrafish (*Danio rerio*)	Acute sublethal embryo development	96-h LC50 96-h IC50[10]	LC50 > 6 g/L IC50 > 6 g/L
Rainbow trout hepatocyte assay	Acute cytotoxicity	48-h EC20[11] 48-h EC50[12]	EC20 = 34 mg/L EC50 = 245 mg/L

[1] Most tests were repeated using several different batches of NCC. The results shown here, in most cases, represent the lowest reported or most representative LC50, IC50, or EC50.
[2] Concentration lethal to 50% of test organisms after 96 hours of exposure
[3] Concentration lethal to 50% of test organisms after 48 hours of exposure
[4] Concentration producing a 25% inhibition in measured endpoint after 7 days of exposure
[5] Concentration producing a 25% inhibition in measured endpoint after 10 days of exposure
[6] Concentration producing a 25% inhibition in measured endpoint after 15 minutes of exposure
[7] Concentration producing a 25% inhibition in measured endpoint after 72 hours of exposure
[8] Concentration lethal to 50% of test organisms after 24 hours of exposure
[9] Concentration producing an effect on 50% of test organisms after 96 hours of exposure
[10] Concentration producing a 25% inhibition in measured endpoint after 96 hours of exposure
[11] Concentration producing changes in cell morphology in 20% of cells after 48 hours of exposure
[12] Concentration producing changes in cell morphology in 50% of cells after 48 hours of exposure

3.2 *In Vitro* Assessments of Cytotoxicity and Genotoxicity

The most commonly raised concern about potential health effects of exposure to nanocellulose is that, because it is a fiber, it may behave similarly to asbestos when inhaled. Thus, several studies have focused on *in vitro* assessments of cytotoxicity and genotoxicity in order to compare outcomes with known effects of asbestos fibers.

Moreira et al. (2009) used several *in vitro* assays that have previously been used to demonstrate the genotoxic effects of asbestos fibers in order to assess genotoxicity and cell proliferation in response to exposure to bacterial cellulose (BC) nanofibers. The *Salmonella* reversion assay was used to assess mutagenicity. Chinese hamster ovary (CHO) and mouse embryo fibroblast cell suspensions were used to assess cell proliferation. Genotoxicity was assessed using the alkaline single cell gel assay (also called the comet assay) with CHO cells. The researchers reported that, under the testing conditions of their study, BC nanofibers were neither mutagenic nor genotoxic and cell proliferation was reduced.

Pitkanen et al. (2010) assessed the mutagenicity and cytotoxicity of both MFC made from birch pulp and NCC. Short-term toxicity (24-72 hour exposures) tests measured changes in total protein content in three different cell lines: human keratinocyte, human cervix carcinoma (MFC only), and mouse hepatoma. A sublethality test measured the inhibition of RNA synthesis in human cervix carcinoma cells. Mutagenicity was assessed using a Salmonella assay (Ames test) (MFC only). Neither MFC nor NCC showed any evidence of mutagenicity or cytoxicity in these assays.

Vartiainen et al. (2011) assessed the immunotoxicity of MFC derived from ECF-bleached birch kraft pulp using cells from a mouse macrophage cell line and human peripheral blood derived monocytes. Cell viability was determined following 24 hours of exposure to MFC. The production of cytokine and chemokine mRNA by both cell types was measured after 6 hours of exposure. Secretion of cytokine and chemokine protein by human cells was determined after 24 hours. The researchers found no evidence of inflammatory effects or cytotoxicity on either mouse or human cells.

Clift et al. (2011) used a 3D triple cell coculture model of the epithelial airway barrier in order to assess the cytotoxicity and inflammatory response and also the intracellular localization of NCC derived from cotton via sulfuric acid hydrolysis. The results were compared to those obtained with multiwalled carbon nanotubes (MWCNTs) and crocidolite asbestos fibers (CAFs). The triple cell coculture was created using human monocyte derived macrophages, dendritic cells, and human bronchial epithelial cells. Cytotoxicity was assessed by measuring leakage of lactate dehydrogenase from cells into the surrounding medium. Inflammatory response was assessed by measuring the cells' production of a pro-inflammatory cytokine and an inflammatory chemokine. Intracellular localization of fibers was determined using electron tomography.

Clift et al. (2011) reported that NCC fibers are localized within the cells differently than either MWCNTs or CAFs and that NCCs do not appear to induce the "frustrated phagocytosis" of macrophages, a phenomenon believed to play a role in the carcinogenicity of asbestos. The researchers also reported that NCC elicited a dose-dependent cytotoxicity and pro-inflammatory response, but noted that those responses were significantly lower than the responses elicited by both MWCNTs and CAFs.

3.3 Mammalian Toxicity Testing

In a rat acute oral toxicity test (OECD 425) using NCC, the LD50 was greater than the highest dose tested, which was 2000 mg/kg. NCC had no impact on survival or gross pathology (O'Connor 2011).

In a rabbit acute dermal irritation test (OECD 404), there were no signs of skin irritation following application of 0.5 grams NCC (O'Connor 2011).

In a rat acute inhalation toxicity study (OECD 403), the maximum airborne concentration of NCC that could be reached was 0.25 mg/L due to technical limitations of the aerosol generation systems. The LC50 was greater than 0.25 mg/L (O'Connor 2011).

4.0 WORKER EXPOSURE

Vartiainen et al. (2011) evaluated worker exposures to particulate matter associated with grinding and spray drying processes carried out during the production of MFC from bleached birch kraft pulp. They found that the concentration of particulate matter during these processes was generally not much different from background levels. The researchers also noted that MFC is highly reactive with water and "had a tendency towards instant agglomeration in ambient conditions," resulting in micron- rather than nanometer-sized particulate.

5.0 CONCLUSION

There is, as yet, very little published information on the environmental fate and effects or toxicity of nanocellulose products. Evidence suggests that nanocellulose, like cellulose fibers, is readily biodegradable, and concentrations of nanocellulose in the environment or in workplace atmospheres as a result of manufacturing-related releases are likely to be quite low. Thus far, nanocellulose has been found to have minimal effects in all test systems/organisms that have been studied.

In addition, it is important to note that given the observed behavior of most types of nanocellulose under ambient environmental conditions, nanocellulose is likely to have agglomerated into particulate matter that is classified as microscale rather than nanoscale, which significantly affects the behavior of these substances in the environment and also potential routes of exposure to living organisms.

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