Nanocellulose – Evaluation of the full spectrum of workplace health and safety

A. Eastlake[*] A. Rudie[**] and C. Geraci[***]

[*]National Institute for Occupational Safety and Health, Cincinnati, Ohio, aeastlake@cdc.gov
[**]United States Forest Service, Forest Products Laboratory, Madison, Wisconsin, arudie@fs.fed.us
[***] National Institute for Occupational Safety and Health, Cincinnati, Ohio, cgeraci@cdc.gov

ABSTRACT

In partnership with the Forest Products Laboratory, the National Institute for Occupational Safety and Health has conducted two exposure characterization studies to characterize potential exposure to cellulose nanocrystals (CNC) and cellulose nanofibrils (CNF). In order to increase the ability to detect and identify the cellulose nanomaterials, approximately half the sodium counterions in these materials were replaced with cesium. Analyzing the filter-based air samples for cesium indicated that nanocellulose is being aerosolized during product centrifugation, handling of dry product, and during the production and manipulation of nanocellulose polymer composites. Additional sampling has indicated that the use of engineering controls serves to decrease the potential for exposure during the handling of dry product.

Keywords: nanocellulose, exposure assessment, cellulose nanocrystals, cellulose nanofibrils, worker exposure

1 INTRODUCTION

Nanotechnologies promise enhanced benefits to society by improving a wide variety of products and industries, including energy, medical, construction, coatings, materials, electronics, and optics. By 2015, approximately 2.5 trillion in manufactured goods will involve nanotechnology (1). Products are being enhanced with nanomaterials to benefit from the unique nanoscale properties such as increased strength, electrical conductivity, thermal resistance, and increased chemical reactivity. As particle size decreases, a greater proportion of surface area is available, which can affect surface reactivity and toxicological properties. This may lead to a different biological activity compared with larger particles of the same material. Cautious risk management strategies should be enacted to provide a safe and healthy environment for those working with these materials. The total number of workers involved in nanotechnology is increasing annually, with estimates projecting 6 million workers employed worldwide by 2020 and 2 million jobs in the United States (2).

Nanocellulose is gaining prominence as a nano structured material with production volumes forecasted to reach 780 tons per annum by 2017 (3). Nanotechnology has the potential to play a significant role in the future of a variety of industries. The unique mechanical, optical, thermal, and surface properties, and advantages of light-weight, renewable, biodegradable, and biocompatible materials, nanocellulose could have application in composites (bioplastics and reinforced polymers), porous materials (filter media, insulation, and packaging), energy (batteries and super-capacitors), photonic devices, membranes, pharmaceuticals, biomedical devices, and coatings.

New technologies are often applied prior to obtaining critical knowledge about the risks to the workers, consumers, or the environment and nanocellulose is no exception (4). Exposure to nanocellulose can occur through inhalation, ingestion or dermal routes. The number of toxicity studies performed and published on nanocellulose is limited. It is also important to consider that different types of cellulose nanomaterials may have different levels of toxicity. The rigid rod form of cellulose nanocrystals for example, could pose different health hazards than the more flexible, string-like form of cellulose nanofibrils. Mouse macrophages and human monocyte-derived macrophages were found to have no evidence of inflammation or cytotoxic effects following actue exposure to microfibrillated cellulose (5). De Lima *et al*. determined that nanofibers derived from different plants could have different effects; brown cotton and curaua cellulose nanofibers caused breaks in genetic material and were genotoxic in animal cells (human lymphocytes and mouse fibroblasts) (6). Clift *et al*., in comparing the response to multi-walled carbon nanotubes, observed that cotton cellulose nanofibers elicited a significantly ($p < 0.05$) lower inflammatory and cytotoxic response (7).

Biopersistence of nanomaterials has been made evident from animal studies that show impaired clearance of particles from the lungs of rats and mice (8, 9). The few *in vitro* and *in vivo* experimental studies that have been performed using respirable cellulose have indicated that pulmonary inflammation and lung biopersistence occurs (10-13).

Although the health risks of inhaling nanocellulose have not been well studied, several occupational exposure limits (OEL) have been established for bulk cellulose particles based on gravimetric analysis. The OSHA permissible exposure limits are 15 mg/m^3 for total dust and 5 mg/m^3 for respirable dust, both expressed as time-weighted averages. The NIOSH recommended exposure limits (RELs) are 10 mg/m^3 and 5 mg/m^3 as a respirable fraction, both as a time-weighted average. These limits are primarily based on the potential for irritation to eyes, skin, or mucous membranes.

A respirable mass-based REL for bulk cellulose exposure provides a benchmark for judging exposures, but caution must be used because of the potential for health effects not related to the bulk material. Based on studies of other engineered nanomaterials, there is a potential for increased toxicity from exposure to nanocellulose compared to the bulk cellulose product. For example, NIOSH is concerned that other poorly soluble, low-toxicity nanoparticles may have health effects similar to those observed for titanium dioxide. Ultrafine titanium dioxide particles (< 100 nm), were observed to be more carcinogenic and inflammogenic on a mass basis than fine titanium dioxide (14). Therefore, a mass-based bulk cellulose REL may not be sufficient to protect workers against nanomaterials that can behave differently than the larger bulk solid particles. Further kinetic and toxicological research is necessary to understand the toxicological nature and potential health effects as a result of chronic exposure to nanocellulose.

2 METHODS

As part of its nanotechnology research agenda, NIOSH created a field studies team to assess workplace processes, materials, and control technologies associated with nanotechnology and conduct on-site assessments of potential occupational exposure to a variety of nanomaterials. The team was tasked with expanding knowledge of the research, production, and use of engineered nanomaterials through the establishment of collaborative partnerships with public- and private-sector producers and users. These partnerships provide opportunities for on-site investigations that enable NIOSH to observe and better understand the variety of processes used in nanomaterial workplaces and to determine whether and in what concentrations these processes release nanoparticles.

The NIOSH Nanotechnology field study team (NFST) workplace assessment technique can be applied by practicing industrial hygienists to identify nanoparticle emissions and characterize exposures. It enables a quantitative evaluation of processes and tasks in the workplace where releases of engineered nanomaterials may occur. The NFST uses several sampling approaches simultaneously with the goal of obtaining qualitative and quantitative particle metrics, including the number, concentration, size, shape, degree of agglomeration, and mass concentration of elemental constituents. Measurements are also collected to assess the effectiveness of engineering control systems. The sampling approach includes time-integrated, filter-based air samples, direct instrument readings, and surface sampling (when appropriate).

Filter-based air samples are collected during specific tasks and processes to determine the possible presence and quantity of a nanomaterial, as well as in non-production areas to determine background concentrations. Full-shift samples are also collected to determine a worker's cumulative exposure. Personal breathing zone (PBZ) samples are collected as close as possible to the subject's breathing zone (e.g., the lapel of a lab coat), while area samples are collected outside, but close to the evaluated process. Area samples are collected to provide an indication of fugitive process emissions and potential occupational exposures. Task-based exposures are assessed with short-term samples to identify work practices that can contribute significantly to overall exposure patterns and to prioritize control strategies.

As the core component of the exposure assessment strategy, time-integrated air samples are collected both for elemental mass and for electron microscopy analysis. This holistic approach to air sampling provides a confident estimate of the existence of nanoparticles, even in the absence of a cellulose-specific validated sampling and analytical method. Nanoparticles contribute little to the collected overall mass, and therefore electron microscopy, being more sensitive, may identify the existence of nanomaterials where mass analysis cannot.

Direct reading instruments (DRIs) are also used to provide supplemental data on emissions and concentration trends. This information can then be used to obtain a better understanding of engineering control efficacy and work practices. The NFST uses a combination of DRIs including condensation particle counters, optical particle counters and sizers (OPS), and dust monitors to characterize a broad range of aerosol particles. Instrument selection is in part based on portability and availability to practicing industrial hygienists. All DRIs currently in use by the NFST operate as aerosol photometers. These instruments pass a collected aerosol through an illuminated field in a known volume of air and then detect the total light scattered by all particles in that volume. Together, these instruments provide an indication of the concentration of particles ranging from 10 nanometers to greater than 15 micrometers. The OPS and dust monitor are capable of differentiating particle (or mass) concentrations by size. However, none of the DRIs currently in use by the NFST is material specific.

3 RESULTS

CNC and CNF present unique challenges for sampling and analysis of environmental samples. A validated analytical method for cellulose nanomaterials does not exist; therefore, electron microscopy provides the only practical strategy for confirmation of nanocellulose. However, filter preparation for electron microscopy can present analytical complications. For example, dissolving a mixed-cellulose ester filter for analysis by transmission electron microscopy would be damaging to any nanocellulose that had collected on the filter.

The NIOSH NFST has partnered with U.S. Department of Agriculture, Forest Service, Forest Products Laboratory (FPL) to evaluate the potential for occupational exposure to nanocellulose during various production processes. FPL aims to support the emerging market for plant-derived renewable nanomaterials like nanocellulose. Processes currently in use at the pilot plant consist of scaled-up versions of a reaction that was first publicized in 1949 (15-18). The CNCs generated are roughly 5 nanometers (nm) in diameter and 200 nm long. CNFs are 10-30 nm in diameter and >1000 nm long. Based on the chemistry of the materials, the products can be uniquely tagged by exchanging sodium ions with an alternative alkali metal. To increase the ability of NFST to detect and identify nanocellulose, FPL agreed to tag both products (CNC and CNF) with cesium for use during evaluation of certain tasks.

To date, two separate site evaluations have been performed. During the first, performed in 2012, four separate processes in the production of CNC were observed: CNC production (digestion and neutralization); membrane filtration; centrifugation of product slurry; and removal of dried product from a freeze dryer. The CNC production and membrane filtration processes did not contain cesium-tagged product and therefore cesium samples were not collected.

Sample	Location	Filter	Time (Minutes)	Air Volume (m3)	Conc TWA (ug/m3)
Centrifuge	PBZ	Open-face	176	0.348	0.00083
Centrifuge - Inside	Area	Open-face	152	0.301	0.06138
Centrifuge - Outside	Area	Open-face	152	0.302	0.00082
Centrifuge - Inside	Area	DRI - OPS	168	0.168	0.04499
Hallway	Background	Open-face	260	0.517	0.00059
Hallway	Background	DRI - OPS	260	0.260	0.00020

Table 1. Summary of cesium detected from the centrifuge of CNC suspensions.

Task-based samples collected during operation of a centrifuge located inside a partially enclosed cabinet indicated aerosolization of CNC product due to elevated levels of cesium found in source, area, PBZ, and background samples (19). Source, area, and PBZ samples collected during removal of product from the freeze dryer also indicated that dried product was becoming airborne during handling. At this time, the freeze dryer was located in an open area with no local exhaust ventilation.

Elemental analysis (cesium) results for the filter-based air samples indicated that CNCs were being aerosolized during both removal of product from the freeze dryer/trays and centrifugation of product (Tables 1 and 2). Because of a process improvement, FPL no longer uses the centrifuge as product clarification is now accomplished using a cartridge filter. The dispersal of particles into the air was due mainly to the design of the centrifuge used and is not common to all centrifuges.

In September 2013, the NFST again partnered with FPL to evaluate additional tasks and processes. Five different processes were evaluated that included the following: pretreatment of wood pulp by oxidation with TEMPO, homogenization, incorporation and modification of a cesium-tagged CNC composite, and two methods for breaking up plant cell walls into the fibrils, using a Sprout Waldron atmospheric disk refiner, and a Masuko Grinder. The CNF pretreatment, refining and homogenization processes did not contain cesium-tagged product and therefore cesium samples were not collected.

	Sample	Location	Filter	Time (Minutes)	Air Volume (m3)	Conc TWA (ug/m3)
Open Area General Ventilation	Freeze Dryer	PBZ	Open-face	58	0.115	0.00006
	Freeze Dryer	Source	Open-face	25	0.050	0.00001
	Freeze Dryer	Area	Open-face	25	0.050	0.00003
	Freeze Dryer - DT	Area	DRI - DT	25	0.075	0.00015
	Freeze Dryer - OPS	Area	DRI - OPS	25	0.025	0.00013
Isolated Room HEPA LEV	Freeze Dryer - Table	Area	Open-face	62	0.184	0.00000*
	Freeze Dryer - Table	Area	DRI - DT	167	0.501	ND
	Freeze Dryer - Table	Area	DRI - OPS	167	0.167	ND
	Storage Area	Background	Open-face	179	0.534	0.00000*
	Storage Area - DT	Background	DRI - DT	181	0.534	ND

* Sample results are between the level of detection and level of quanititation

Table 2. Summary of cesium detected during freeze dryer transfer of CNC (open area) and CNF (isolated room).

In addition, the NFST also evaluated the removal of dried cesium-tagged CNF from the freeze dryer. The cellulose fibrils in the freeze dried form are entangled in a foam-like structure and it was suspected they would not be swept up in air currents as extensively as are the cellulose nano-crystals. Since the previous evaluation, the facility had also moved the freeze dryer to its own room, thereby isolating any potential fugitive emissions during product handling. In addition, the facility had started using local exhaust ventilation (LEV) that passes exhaust through a HEPA filter and recirculates it into the same room. This ventilation is turned on prior to removal of product from the freeze dryer and turned off after all product has been transferred, sealed, and clean up activities have been completed. Samples collected in 2013 indicated a decrease in the detected concentration of cesium during the removal of dried product from the freeze dryer thus indicating that the CNF particles are not as readily dispersed into the air. DRI data (not shown) indicated that when air filtration was started, the airborne particle count dropped significantly, indicating that the LEV with HEPA filtration is functioning to remove particulates from the air (Table 2).

Elemental analysis (cesium) results for the filter-based air samples indicate that cesium-tagged product was being aerosolized during the production, cutting, and milling of a CNC containing composite. Current consensus indicates that materials in a solid or composite form are unlikely to pose an exposure potential unless product modifications are performed that release the encased nanomaterials. Individual task-based samples collected during the production of of a cesium-tagged CNC composite indicate that there is an increased potential for employee exposure during production of the composite, and the cutting and the milling of the polymer. The increased amount of cesium present in the employee's PBZ sample indicates that the modification of nanocellulose composites is likely to release nanocellulose product into the operators PBZ and the surrounding areas.

Sample	Location	Filter	Time (Minutes)	Air Volume (m3)	Conc TWA (ug/m3)
Composites, Milling, & Cutting	PBZ	Open-face	97	0.283	0.0117
Extruder	Source	Open-face	28	0.084	0.0012
Extruder	Area	Open-face	28	0.084	0.0001
Cutting	Area	Open-face	19	0.057	0.0000
Milling	Area	Open-face	36	0.108	0.0000
Composites	Area	DRI - DT	97	0.291	0.0000
Composites	Area	DRI - OPS	97	0.097	0.00011*
Office Space	Background	Open-face	104	0.311	0.0000
Office Space	Background	DRI - DT	104	0.312	0.00004*

* Sample results are between the level of detection and level of quanititation

Table 3. Summary of cesium detected from the extruder, cutting and milling processes.

4 CONCLUSIONS

Currently there are no occupational exposure limits specific to engineered cellulose nanomaterials. As with many nanomaterials, the size and surface area of the cellulose nanoparticles may be a critical factor with respect to toxicological risks and biological effects. Therefore, it is good practice to keep exposures to new and uncharacterized materials as low as possible. Tagging the nanocellulose products with cesium proved to be informative for understanding potential occupational exposures in the absence of a cellulose-specific validated sampling and analytical method. It should be noted that no applicable occupational exposure limits for cesium were exceeded by any of the air samples. As the amount of cesium used in this study may only account for a small fraction of the total CNCs present in the actual workplace, it is best practice to keep exposures as low as possible.

REFERENCES

1. Wilson W. Project on Emerging Nanotechnologies. consumer Products Inventory. 2013.
2. Mihail C, Mirkin, A., Hersam, M. Nanotechnology research directions for societal needs in 2020: retrospective and outlook. World Tecnology Evaluation Center (WTEC) Panel Report, National Science Foundation. 2010.
3. Future Markets I. The Global Market for Nanocellulose to 2017. Future Markets, Inc.: 2012.
4. Howard J, Murashov V. National nanotechnology partnership to protect workers. Journal of Nanoparticle Research. 2009;11(7):1673-83.
5. Vartiainen J, Pöhler T, Sirola K, Pylkkänen L, Alenius H, Hokkinen J, et al. Health and environmental safety aspects of friction grinding and spray drying of microfibrillated cellulose. Cellulose. 2011;18(3):775-86.
6. de Lima R, Feitosa LO, Maruyama CR, Barga MA, Yamawaki PC, Vieira IJ, et al. Evaluation of the genotoxicity of cellulose nanofibers. International journal of nanomedicine. 2012;7:3555.
7. Clift MJ, Foster EJ, Vanhecke D, Studer D, Wick P, Gehr P, et al. Investigating the interaction of cellulose nanofibers derived from cotton with a sophisticated 3D human lung cell coculture. Biomacromolecules. 2011;12(10):3666-73.
8. Mercer RR, Scabilloni J, Wang L, Kisin E, Murray AR, Schwegler-Berry D, et al. Alteration of deposition pattern and pulmonary response as a result of improved dispersion of aspirated single-walled carbon nanotubes in a mouse model. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2008;294(1):L87-L97.
9. Pauluhn J. Multi-walled carbon nanotubes (Baytubes): approach for derivation of occupational exposure limit. Regulatory toxicology and pharmacology: RTP. 2010;57(1):78.
10. Cullen R, Searl A, Miller B, Davis J, Jones A. Pulmonary and intraperitoneal inflammation induced by cellulose fibres. Journal of Applied Toxicology. 2000;20(1):49-60.
11. Muhle H, Ernst H, Bellmann B. Investigation of the durability of cellulose fibres in rat lungs. Annals of Occupational Hygiene. 1997;41:184-8.
12. Tátrai E, Brozik M, Adamis Z, Merétey K, Ungváry G. In vivo pulmonary toxicity of cellulose in rats. Journal of Applied Toxicology. 1996;16(2):129-35.
13. Warheit D, Snajdr S, Hartsky M, Frame S. Two-week inhalation study in rats with cellulose fibers. Advances in the prevention of occupational respiratory diseases Eds Chiyotani K, Hosoda Y, Aizawa Y. 1998:579-82.
14. NIOSH. Current Intelligence Bulletin 63: Occupational exposure to titanium dioxide. Cincinnati, OH: US Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Pub Num 2011-160, 2011 Apr: 1-119. 2011;63.
15. Ranby BG. Aqueous colloidal solutions of cellulose micelles. Acta Chem. Scan.; 1949. p. 649-50.
16. Immergut E, Rånby B. Heterogeneous acid hydrolysis of native cellulose fibers. Industrial & Engineering Chemistry. 1956;48(7):1183-9.
17. Reiner R, Rudie A. Process scale-up of cellulose nanocrystal production to 25 kg per batch at the Forest Products Laboratory, In *Production and Applications of Cellulose Nanomaterials*, Eds. Postek M, Moon R, Rudie A, Bilodeau M, TAPPI, Peachtree Corners, GA. 2013:21-4.
18. Reiner R, Rudie A. Pilot plant scale-up of TEMPO-pretreated cellulose nanofibrils, In *Production and Applications of Cellulose Nanomaterials*, Eds. Postek M, Moon R, Rudie A, Bilodeau M, TAPPI, Peachtree Corners, GA. 2013:177-8.
19. Martinez K, Eastlake A, Rudie A, Geraci C. Occupational Exposure Characterization during the Manufacture of Cellulose Nanomaterials, In *Production and Applications of Cellulose Nanomaterials*, Eds. Postek M, Moon R, Rudie A, Bilodeau M, TAPPI, Peachtree Corners, GA. 2013:61-4.