Protecting the nanotechnology workforce: a new protocol for characterization of filter-captured nanomaterials from occupational exposure assessments

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ABSTRACT

The most significant hurdle holding back exposure science, risk assessment, and the development of safety guidelines for the nanotechnology workforce is the lack of validated analytical techniques that accurately identify and characterize engineered nanomaterials (ENMs) captured in occupational settings. The associated costs, time, and lack of standardization of existing methods make it impossible for industries to implement exposure assessment programs or comply with new or forthcoming recommended exposure limits for ENMs. This research team seeks to advance the state of the science by developing and testing a new protocol for analysis of ENMs on filters by: further developing a novel hyperspectral imaging (HSI) method for high-throughput screening; evolving best-known methods for direct visualization of filter-captured ENMs by developing and incorporating advanced techniques into the new protocol; and testing the new protocol on real-world samples obtained during occupational exposure scenarios.

Keywords: occupational health, electron microscopy, hyperspectral imaging, industrial hygiene, semiconductor manufacturing

1. INTRODUCTION

The nanotechnology workforce is growing, with an estimated total of 6 million in 2020, of which 2 million are projected to work in the U.S.[1] Risk assessment for nanotechnology workers is still in its infancy because occupational exposure assessment strategies and physiologic and health outcomes of occupational exposure to engineered nanomaterials (ENMs) have not yet been well-characterized or established. Due to the novel physical and chemical properties that emerge at the nanoscale, ENMs may be more toxic than their bulk counterparts,[2–5] particularly when considering inhaled aerosolized nanoparticles or nanoparticle (NP) penetration of skin. While assessment of the potential toxicity of NPs is at an early stage, the development of occupational health and safety programs, including hazard surveillance and risk management, is strongly recommended.

2. LIMITATIONS OF EXISTING PROTOCOLS

A fundamental hurdle currently holding back exposure science, risk assessment, and the development of safety guidelines for the nanotechnology workforce is the lack of validated analytical techniques that reliably and accurately identify and characterize ENMs captured in occupational settings. Traditional industrial hygiene sampling techniques and analytical methods, which rely on mass-based approaches, are not sufficient for assessing ENM exposures.[6] Current best-known methods (BKMs) for ENM exposure assessment are based on using air and surface sampling tools in tandem to characterize and quantify ENMs; these include real-time direct reading instruments (DRIs) to measure particle counts and filter-based methods for direct visualization techniques (off-line analysis by electron microscopy [EM] with energy-dispersive x-ray spectroscopy [EDS]).[7,8] BKMs for direct visualization of ENMs, based on those developed two decades ago for micron-sized asbestos,[9,10] are not appropriate for current real-world ENM exposures. Additionally, the associated costs, time, and lack of standardization and validation of methods make it impossible for industries to implement an occupational exposure assessment program for workers who handle ENMs, or attempt to comply with NIOSH's recommended exposure limits (RELs) for ENMs. Significant progress must be made regarding measurement methods for ENMs.[11] These refined and validated measurement methods must be cost-effective and have high practical utility in real and anticipated human exposure assessment scenarios. New protocols must measure key physicochemical properties that determine reactivity and toxicity of ENMs and deliver critical data that will inform nanotoxicology research.

3. OBJECTIVE

The goal of this collaboration is to rapidly advance exposure assessment methods for the nanotechnology workforce by developing and testing a new protocol for filter-based sample analysis. We are currently three years into the development of a novel hyperspectral imaging (HSI)

method for high-throughput, inexpensive screening of ENMs captured on filters. Industrially relevant metal oxide ENMs (SiO_2, silica; Al_2O_3, alumina; CeO_2, ceria) serve as the materials of interest for determining HSI's limit of detection (LOD) and screening threshold. We are also working to evolve and expand EM-based direct visualization BKMs to include physicochemical parameters relevant to ENM reactivity and toxicity. The new resulting protocol will be tested on real-world samples captured in occupational settings in semiconductor manufacturing.

4. CASE STUDY: NANOELECTRONICS INDUSTRY

4.1 Nanoparticle abrasives

The electronics industry was the first to embrace and upscale nanotechnology as it transitioned from the microscale around the turn of the 21st century. Within that industry, chemical mechanical planarization (CMP) has emerged as a critical process for health and safety evaluation due to the widespread use of ENMs and potential for occupational exposure. CMP is a polishing process employed repeatedly in integrated circuit fabrication to maintain local and global planarity of multiple dielectric and metal layers on the wafer. During CMP, the wafer is pressed facedown against a porous pad mounted on a rotating platen, with slurry flowing between the wafer and the pad. The slurry is a suspension of NPs in deionized water and a chemical mixture tailored for the surface it is intended to remove.[12] Based on the CMP processes and slurries currently in use, the NPs of interest include fumed or colloidal silica (SiO_2), alumina (Al_2O_3), and ceria (CeO_2). The ubiquitous nature of CMP processing, combined with current gaps in knowledge regarding workplace exposures, dramatically underscores the need to better evaluate occupational exposure of workers involved with this process.

4.2 Toxicity of metal oxide nanoparticles

The team seeks to develop exposure assessment methods that yield characterization data of toxicological significance for ENMs captured in occupational settings. This characterization will include the most likely determinants of toxicity, focusing on elemental composition in the 'bulk' and on the surface of the ENM, specific surface area (as determined from size and shape data), and, where applicable, the valence state of the metals. Most toxicology studies have focused on the effect of a single type of NP; however, the potential for mixed ENM exposures in an industrial setting may exist for certain industries or job tasks. Exposure assessment data suggests that exposures occurring at the testing location often consist of mixed aggregates and/or agglomerates.[13–19] It is suspected that particle count and specific surface area are among the most important determinants of toxicity,[2–4,20–22] but other physicochemical parameters may also influence biological activities.[2–4,23–28] Several physicochemical properties have been implicated as leading determinants of toxicity for different classes of ENMs, namely: size[2–4] or fiber length,[22] surface area,[3,4,20,21] surface coating,[2,23,24,26] crystal phase,[4] and agglomeration state.[25]

4.3 Exposure assessment

Exposure assessments conducted with semiconductor workers have shown that certain workers may be exposed to ENMs via inhalation or cutaneous (skin) contact during various tasks.[13–18] Since 2011, through a combination of on-site field-based sampling and advanced laboratory-based metrology, the research team has adapted and developed methods for air and surface sampling to characterize and quantify ENMs using real-time DRIs and filter-based methods for direct visualization techniques.[13–18] The team's exposure assessment research has focused on the processes associated with the CMP tools located at a semiconductor R&D facility where wafer fabrication takes place and ancillary spaces associated with CMP including on-site wastewater treatment, and material and waste storage areas.[13–19] While current work practices include engineering controls and use of personal protective equipment, their effectiveness in controlling exposure to NPs during CMP requires further investigation. The sampling approach for airborne exposures has been described[13,16–18] with parameters presented in **Table 1**. Air monitoring methodology draws from the framework proposed for distinguishing ENMs from incidental sources and determining number concentration,[29,30] applying the Nanoparticle Emission Assessment Technique (NEAT) 2.0, which emphasizes filter-based personal breathing zone (PBZ) and task area sampling, in tandem with DRIs.[7,8,31]

5. ADVANCING THE STATE OF THE SCIENCE

Current measurement methods and direct visualization techniques for ENMs are based on existing, historical protocols for micron-sized asbestos (NIOSH 7402[9]). No standard has emerged for evaluating the appropriateness, accuracy, reliability, and reproducibility of the various

Metric/Characteristic	Instrument/Technology	Size Range	Limitations
Mass concentration	Filter-based	Varies	Mass-based; not real-time
Size range; number concentration	Optical particle counter	300nm – 50,000nm	Sjze-range; non-specific
Number concentration	Condensation particle counter	10nm – 1000nm	Not size-resolved; non-specific
Size; concentration by size	Scanning mobility particle sizer	10nm – 420nm	Non-specific
Size, shape, morphology, elemental composition	SEM/TEM with EDS	<10nm – 1000nm	Not real-time
Shape; elemental composition; concentration	Hyperspectral imaging system*	>128nm^2 pixels	Not real-time

Table 1. Complementary exposure assessment methods used for air sampling and direct visualization of ENMs.

asbestos-adapted protocols for studying ENMs and agglomerates. The NIOSH 7402 method uses transmission electron microscopy (TEM) for direct visualization of fibers and particles. While TEM is considered the gold standard for ENM characterization, it is costly, resource-intensive, and low-throughput (**Fig. 1a**). Specimens are manually prepared, and analysis requires highly skilled microscopists. These considerations, in addition to the limited number of available commercial labs with such capacity, make this modality impractical for high-throughput industrial occupational exposure assessment. In reality, many samples obtained in workplace settings may not contain ENMs in appreciable number, making routine TEM a time-intensive overkill; therefore, in routine air sampling (even for high priority tasks), it would be ideal to have a less expensive, faster screening modality available. If ENMs of interest were identified at that stage, the same sample could then move to more intensive analysis.

5.1 Development of a new screening modality

Hyperspectral imaging (HSI) is an existing technique that has recently been applied to the analysis of ENMs.[32] The research team and NIOSH are the only known groups working to develop novel methods for HSI analysis of ENMs captured on filter media from occupational settings. HSI is a direct visualization technique that combines HSI with advanced optics to collect characteristic spectral data of visible and near infrared (VNIR) light reflected by samples that can be used to identify materials of interest via mapping algorithms.[32–36] Currently, at the nanoscale, these mapping methods are used only *semi*-quantitatively;[37] this data has not been used to directly estimate the physical parameters critically relevant to occupational exposure assessment, such as the airborne NP concentration. The team intends to develop HSI as a fully quantitative method, capable of estimating the average air concentration of a specific NP in filter captured air samples. The tool would then provide exposure quantitation *specific to the NP of interest*, a capacity missing from the current toolkit for ENMs (**Table 1, green**). Based on protocol development already underway, this new tool represents a potential breakthrough in high-throughput ENM screening, which would dramatically cut the costs and time associated with occupational exposure assessment. This project will transform the outdated asbestos method into a new higher-throughput, affordable, field-ready protocol needed to conduct ENM-specific exposure assessment and protect workers (**Fig. 1b**).

5.2 Expand and evolve BKMs for direct visualization using EM and other methods

While existing BKMs using TEM/EDS allow for direct visualization of ENMs, they are inadequate and impractical in terms of ENM characterization for occupational exposure assessment. Therefore, we will use the asbestos protocol as a starting point only, as we will adapt portions related to the BKM for handling filters for analysis and the development of appropriate search and counting algorithms. The existing methods also fail to exploit nanoscale characterization techniques that would yield key physicochemical data of toxicological significance. Therefore, our new ENM protocol will include different specific terms than the asbestos algorithm. Our new protocol will focus on key determinants of ENM toxicity: composition, shape, agglomeration, specific surface area, and surface coating/chemistry. The new protocol will incorporate HSI screening, followed by advanced analytical methods appropriate for further ENM characterization, where indicated (**Fig. 1b**).

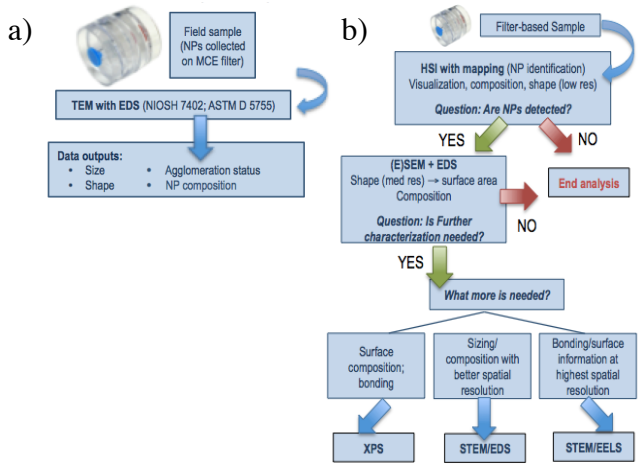

Figure 1. Protocols for analysis of filter-captured nanoparticles. a) Existing protocol; b) new protocol.

5.3 Protocol testing on real-world samples

It is critical to test the new protocol on real-world samples. The team will conduct occupational exposure assessments as for high-priority tasks, as previously described. Filter-based samples will be collected in parallel in the field during CMP-related tasks, enabling side-by-side analysis of data obtained through both the standard method and new protocol.[13–18,38] Regardless of whether a sample meets the threshold to continue beyond the HSI checkpoint, each field sample will be run through the new protocol to exhaustion to evaluate its efficacy and provide maximum data for comparison to the conventional approach.

6. FUTURE DIRECTIONS

This project will transform exposure assessment for the nanotechnology workforce at a crucial tipping point as the use of ENMs in manufacturing is accelerating. Our new protocol will rapidly advance the state of the science for exposure assessment, provide critical data to nanotoxicologists, and illuminate a comprehensive risk assessment picture in order to protect workers in real time. Use of the new protocol, when implemented in the field, thus has the potential to impact not only the thousands of workers in our facility, but also the hundreds of thousands of workers employed in manufacturing facilities. Exposure assessment

findings also inform better design principles in constructing and managing facilities that use or produce ENMs. . In this way, this initiative embodies NIOSH's Prevention through Design (PtD) initiative. Furthermore, by applying research findings and methods directly to field-based exposure assessment in real-time with industrial partners, this initiative also aligns with the spirit of NIOSH's Research to Practice (r2p) initiative by transferring and translating knowledge, interventions, and technologies directly into effective prevention practices in the workplace.

With the successful development of a new protocol, the occupational health community will have access to a streamlined set of assessment protocols, including a rapid screening method for routine exposure assessment, which will include criteria for determining if and when a more intensive direct visualization protocol should be applied to a particular sample for further analysis. Additionally, the new protocol will deliver key physicochemical characterization data of toxicological significance that will underpin comprehensive risk assessment for human health.

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DISCLAIMER

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of CDC-NIOSH. The authors declare no conflict of interest.

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