Personal Exposure to Engineered Nanoparticles

09/01/2007 – 08/31/2010
5 K01 OH009255

FINAL PROGRESS REPORT

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List of Terms and Abbreviations

AIHce - American Industrial Hygiene conference and exhibition
APS – aerodynamic particle sizer
ARD – Arizona road dust
CNT – carbon nanotube
CPC – condensation particle counter
CPM – capillary pore membrane
DC2000CE – diffusion charger model number 2000CE
DOI – digital object identifier
EDS – energy dispersive spectrometry
GM – geometric mean
GSD – geometric standard deviation
ICP-AES – inductively coupled argon plasma atomic emission spectroscopy
IH – industrial hygiene
LEV – local exhaust ventilation
LOD – limit of detection
MCE – mixed cellulose ester
NIOSH – National Institute for Occupational Safety and Health
NMD – number median diameter
OEL – occupational exposure limit
OPC – optical particle counter
P/B – process-to-background ratio
PVC – polyvinyl chloride
PTFE – polytetrafluoroethylene
R_{DC/ref_a} – ratio of the output from the DC2000CE to that from the SMPS
SEM – scanning electron microscope
SMPS – scanning mobility particle sizer
TEM – transmission electron microscopy

Abstract

Worldwide production of engineered nanoparticles is expected to grow from 2,000 metric tons to 50,000 metric tons over the next decade. New industrial processes must be introduced into the workplace to accommodate this growth. Although studies have shown some nanoparticles to be toxic, methods to assess exposure do not exist. However, knowledge of personal exposure may be particularly important for such small particles because their concentration tends to decay rapidly with distance from a source. The immediate objective of this K01 Career Development Award was to allow Dr. Thomas Peters to make a successful transition to an independent investigator in the field of occupational and environmental health, with emphasis on protecting the health of workers from exposure to aerosols.

In the research component of this Award, laboratory studies were conducted under Aim 1 to develop and evaluate methods to assess personal exposure to nanoparticles. We developed a method to assess rapid fluctuations in nanoparticle concentrations with direct-read instruments and a filter-based method to quantitatively collect nanoparticles for subsequent analysis by electron microscopy. Under Aim 2 field studies, we used these methods to investigate the extent to which workers are exposed to engineered nanoparticles in facilities that produce and handle them. We found that workplace exposure to engineered nanomaterials can be highly variable by worker, by activity and/or by area of a facility. Handling nanomaterials was found to be associated with particles that were respirable but not nano-sized. Further, incidental nanoparticles were generated by hot processes and unrelated to nanomaterial handling. Samples of particles collected from workplaces were analyzed by electron microscopy with energy dispersive X-ray detection under Aim 3. These analyses allowed us to classify particles within the workplace by size, composition, and morphology. These data are critical in evaluating hazards of working with nanomaterials and controlling sources.

Our field measurements have shown the inherent complexity of assessing nanoparticles in the workplace. We have identified new processes required to produce and handle engineered nanomaterials that pose a significant challenge to environmental health and safety. These processes represent a small number of many that will be required in the burgeoning field of nanotechnology. We have shown that substantial exposures to nanoparticles may occur in environments where respirable mass concentrations are low. Consequently, the industrial hygiene sampling paradigm of comparing mass measurements with mass-based OELs has severe limitations when applied to engineered nanomaterials.

This research component was complemented by a vigorous career development plan, which included: (1) formal training in ; (2) regular meetings with the sponsors of this award; (3) participation in group meetings and departmental seminars; (4) presenting results at scientific meetings; and (5) publishing results in peer-reviewed journals. The multidisciplinary team of sponsors has played an active and critical role in both the research and career development components of this K01 Award.

SECTION 1

Highlights/Significant Findings

Aim 1. Establish methods to assess personal exposure to nanoparticles. The highlights of the work conducted for this aim are as follows:

- We developed a method to assess rapid fluctuations in nanoparticle concentrations by relating data from two hand-held, direct-read instruments (a condensation particle counter, CPC, and an optical particle counter, OPC) to workplace activities. Laboratory tests were conducted with several engineered nanomaterials to refine data handling procedures from these instruments to estimate number concentrations of particles smaller than 100 nm. Importantly, this method allows us to determine specific activities that result in exposures to engineered nanomaterials in workplaces. It also provides information on the size of the particles produced by these activities.
- Laboratory tests were conducted with determine the surface collection efficiency of filters appropriate for analysis by electron microscopy. This work is a critical step in the development of a quantitative, filter-based personal sampling method for assessing personal exposure to engineered nanomaterials.
- We evaluated a small, battery-powered, direct-read instrument (diffusion charger)for use in measuring short-term personal exposure to nanoparticles. We found that measurements made with this instrument in workplaces with mixed-mode aerosols are not specific for nanoparticles. It also outputs spurious measurements when moved, which limits its use as a personal monitor.

Aim 2. Characterize worker exposure to nanoparticles. The research conducted under this Aim resulted in the following significant findings:

- Through three field studies, we found that workplace exposure to engineered nanomaterials can be highly variable by worker, by activity and/or by area of a facility. Further, handling nanomaterials was found to be associated with particles that were respirable but not nano-sized. And incidental nanoparticles were generated by hot processes in the workplace. These field studies provided excellent test environments for vetting Aim 1 methods.
- Extremely high nanoparticle but negligible respirable mass concentrations were observed in worker's breathing zones in a facility that manufactures polytetrafluoroethylene apparel. This work underscores the importance of evaluating hot processes in a workplace by means other than traditional mass concentration measurements.
- Sanding a nanocomposite—epoxy containing carbon nanotubes—was observed to result in exposure to respirable particles that have unique morphology. In contrast, weighing bulk carbon nanotubes results in negligible exposures.
- A biosafety cabinet was found to be an effective local exhaust ventilation method to control exposures to particles during sanding nanocomposites.

Aim 3. Apportion worker exposure to nanoparticles by source. Advanced single-particle analysis by electron microscopy was applied to characterize samples of particles collected in Aim 2 field studies. These analyses yielded significant findings as follows:

- This work is the first to classify airborne particles by type (engineered or background) and size in a nanomaterial manufacturing facility.
- The results unequivocally demonstrate that airborne nanomaterials can be larger than nano-sized particles but still have nanostructure that might make them workplace hazards.
- They also demonstrate that nanoparticles may be background aerosols unrelated to manufacturing.

Translation of Findings

This work developed several methods to assess airborne nanomaterials in the workplace. The direct-read methods are easily adaptable by industrial hygienists in assessing workplace hazards. They have been incorporated into guidance documents for safe handling of nanomaterials.

Our findings have spurred specific changes in the workplaces. Partnering companies have implemented or improved ventilation in areas where engineered nanomaterial exposures were observed in our studies.

We evaluated specific local exhaust ventilation that is most effective in the control of exposure to particles when working with engineered nanomaterials. This information is now available to industrial hygienists responsible for controlling workplace hazards.

Outcomes/Relevance/Impact

Our field measurements have shown the inherent complexity of assessing nanoparticles in the workplace. We have identified new processes required to produce and handle engineered nanomaterials that pose a significant challenge to environmental health and safety. These processes represent a small number of many that will be required in the burgeoning field of nanotechnology.

We have shown that substantial exposures to nanoparticles may occur in environments where respirable mass concentrations are low. Consequently, the IH sampling paradigm of comparing mass measurements with mass-based OELs has severe limitations when applied to engineered nanomaterials.

Our findings also stress that further development of quantitative filter-based collection coupled with single-particle analysis methods are critically needed to assess hazards posed by working with engineered nanomaterials in workplace. The type of detailed information from these analyses is essential for the design of toxicity tests that are environmentally relevant and is necessary to select appropriate strategies for routine monitoring, controlling exposures, and establishing appropriate OELs.

SECTION 2

Scientific Report

Background and Specific Aims

Worldwide production of engineered nanoparticles (diameter <100 nm), particles intentionally produced to enhance commercial products, is expected to grow from a current level of 2,000 metric tons to 50,000 metric tons over the next decade. New industrial processes must be introduced to meet this demand, which increases the likelihood that workers will be exposed to engineered nanoparticles through either production or handling. Environmental studies have associated exposure to incidental nanoparticles, generated as byproducts of a process, with adverse cardiorespiratory health outcomes. Our previous work has shown that incidental nanoparticles are common in the workplace and often 100 to 1000 times greater than ambient nanoparticle concentrations.

The research supported by this K01 Award contributes to the body of knowledge on the extent to which workers are exposured to nanoparticles in facilities that produce and handle engineered nanoparticles. The objective of the laboratory studies in this research plan was to establish methods to assess nanoparticles in the workplace. These methods were then taken into the field to characterize exposures to engineered nanoparticles in facilities that produce and handle them.

Our central hypothesis of this K01 Award was that exposure to nanoparticles shows great between-worker (spatial) and within-worker (temporal) variability. The rationale for this project was that this variability in exposure will have important implications in the development and progression of adverse health outcomes, which are related to my long-term career goal of preventing illness associated with exposure to occupational and environmental particles.

The specific aims for this work were:
Aim 1. Establish methods to assess personal exposure to nanoparticles. New methods are needed to assess occupational exposures to nanoparticles. Laboratory tests were conducted to develop and evaluate several new methods that enable exposures to be assessed on time scales of one minute, eight hours, and one month.

Aim 2. Characterize worker exposures to nanoparticles. The methods established through the work in Aim 1 were used to assess exposures in a series of three field studies. These studies helped us to determine that occupational exposures to nanoparticles occur frequently and that they are highly influenced by tasks and work practices.

Aim 3. Apportion worker exposure to nanoparticles by source. We hypothesized that exposures vary substantially by nanoparticle size, morphology, and composition depending on their source. We used electron microscopy to analyze field samples collected for Aim 2 to determine that airborne nanoparticle exposures are often dominated by “incidental” rather than “engineered” nanoparticles.

Aim 1. Establish methods to assess personal exposure to nanoparticles

The necessary first step before embarking on the field studies outlined in Aim 2 was to evaluate the performance of methods to assess personal exposure to nanoparticles. These methods included direct-reading instruments to assess rapid fluctuations in exposure. They also included a method to collect particles over 8 hours with subsequent analysis by electron microscopy. A third method based on passive sampling was originally proposed but not ultimately pursued because of excessive times required to collect a sufficient number of particles for analysis by microscopy.

This section is divided into a series of experiments that address the development and evaluation of various methods. Each section has resulted in a manuscript that is in various stages of publication.

Activity Monitoring with Direct-Read Instruments to Detect Nanoparticle Exposures

We used two hand-held, battery-powered, direct-read instruments to assess nanoparticle exposures. Here we showed how a CPC and an OPC could be combined with activity monitoring to identify sources of nanoparticles in the workplace. This work was published in the Joural of Occupational and Environmental Hygiene (DOI: 10.1080/15459620802590058).

Methods. Two handheld, direct-reading, battery-operated instruments were used to obtained a time series of very fine particle number (<300 nm), respirable mass, and total mass concentration. We then related to activities within the area of extensive material handling. Airborne concentrations were derived from two direct-reading instruments: a CPC (Model 3007, TSI Inc., Shoreview, MN) that measures particle number concentration from 10 nm to 1000 nm; and an OPC (PDM-1108, Grimm, Ainring, Germany) that measures particle number concentration by size in 15 channels from 300 nm to 20 µm. Very fine particle number, respirable mass, and total mass concentration were estimated from the data collected from these instruments.

Although an imperfect measure of nanoparticle number concentration because N includes particles from 100 nm to 300 nm, we chose this method because the CPC and OPC are relatively affordable within industrial hygiene budgets. Their compact size and battery operation also allows them to be easily transported throughout the facility. Other instruments like a scanning mobility particle sizer would have provided better size resolution but are considerably more expensive and not easily transportable. Additionally, the combined particle size information provided by the CPC and OPC instruments spanned a range of 10 nm to 20 µm which represented the range of particle sizes expected.

An example of the use of this method in a facility that handles nanomaterials is provided in **FIG 1**, which depicts instrument response along with activities in a workplace. Worker activities in the this workplace (loading a hopper and replacing collection bags in a rotary calciner room) were unrelated to the very fine particle number concentrations (**FIG 1a**). In contrast, worker activities coincided with the respirable and total mass concentrations (**FIG 1b**). Similar results were obtained for the other four activity-based monitoring events.

Our findings from activity-based monitoring were consistent with those derived from filter-based sampling. The fact that the number concentrations of very fine particles were unrelated to worker activity suggests that the nanoparticles present in the rotary calciner area were incidental to production of the nanomaterial. The further finding that respirable and total mass concentration were related to worker activity suggest that the particles associated with nanomaterial production were quite large. Although respirable mass concentration includes all particles that collect on a filter <4 µm, it is dominated by the largest particles because mass is dependent on particle diameter cubed. From these results, we can infer that the airborne engineered nanomaterials were larger than nanosize in this area. The activity-based monitoring provided us with information about the work environment that was unavailable from microscopy-based analysis: time-series data revealed that handling was the primary source of airborne nanomaterial.

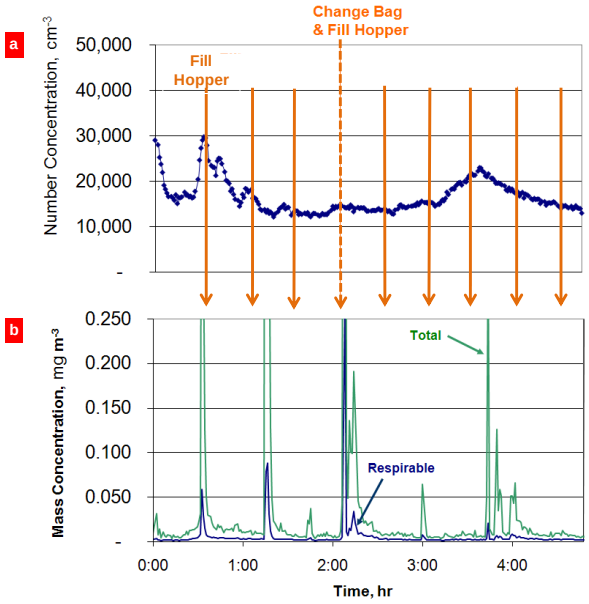

FIG 1 Sample results from real-time, activity-based monitoring. Worker production activities are identified with arrows and labeled at the top of the figure. **a**, very fine particle number concentrations (<300 nm) were not related to worker activities indicating the presence of other sources of small particles within the facility. **b**, respirable and total mass concentrations were strongly related to changing a nanomaterial collection bag and filling the hopper.

Use of a condensation particle counter and optical particle counter to assess the number concentration of engineered nanoparticles.

The work described in the previous section uses subtraction (referred to as the 'count difference method) of OPC data from CPC data to estimate 'very fine' (<300 nm) particle number concentrations as a proxy for nanoparticle (<100 nm) concentrations. However, the resulting count of particles <300 nm can be used as an additional channel of count data in addition to those obtained from the OPC to determine size distributions from which particles <100 nm may then be estimated. To test the efficacy of this approach, estimates of number concentration determined using a CPC and OPC were compared to those from SMPS for engineered nanoparticles. This work was published in the Journal of Occupational and Environmental Hygiene (DOI: 10.1080/15459624.2010.496072).

Methods. Two "size-distribution" methods, weighted-average and log-probit, were applied to reproduce particle size distributions from OPC and CPC data and were then evaluated relative to their ability to accurately estimate the nanoparticle number concentrations. Various engineered nanoparticles were used to create test aerosols, including titanium dioxide (TiO_2), silicon dioxide

(SiO_2), and iron oxide (Fe_2O_3). These materials were chosen because of their different refractive indices and therefore may be measured differently by the OPC.

Results. The count difference method was able to estimate very fine particle number concentrations with an error between 10.9 to 58.4%. In estimating nanoparticle number concentrations using the size-distribution methods, the log-probit method resulted in the lowest percent errors that ranged from –42% to 1023%. Percent error was lower than the instrument manufacturer's indicated level of accuracy when the test aerosol refractive index was similar to that used for OPC calibration standards. Accuracy could be increased if there was an increase in the size resolution for number concentrations measured by the CPC of very fine particles and mitigation of optical effects.

Nanoparticle collection efficiency to the surface of capillary pore membrane (CPM) filters.

The primary objective of this work is to experimentally determine surface collection efficiency of CPM filters at flow rates appropriate for personal sampling. The experimental setup also allowed for easy measurement of overall collection efficiency simultaneously with surface measurements. The secondary objective was to compare experimentally determined filter collection efficiencies to those estimated from theory. The completion of these objectives is an important step in the development of a reliable filter-based personal sampling method for assessing exposures to airborne nanoparticles. This work was published in the Journal of Aerosol Science (DOI:10.1016/ j.jaerosci. 2010.04.007).

Methods. A ball valve was used to manually divert a potassium chloride aerosol to pass through either a filter or a bypass line. In the filter line, a filter was placed in a conductive polypropylene cassette (Model 225-321, SKC inc., Eighty Four, PA), modified to accommodate two o-rings for securing the filter without a support pad. Filters were held above a Po-210 neutralizer for several seconds per side of the filter to eliminate static charge before they were placed in the filter cassettes. A manometer was used to ensure that there was no appreciable increase in pressure drop across the filter, which would indicate filter clogging. A scanning mobility particle sizer (SMPS, Model 5.402, GRIMM Technologies Inc., Douglasville, PA) was used to measure the particle concentrations by size upstream (bypass line) and downstream (filter line) of the filter. A secondary pump was used to supplement the 0.3 Lpm flow from the SMPS to achieve the test air flows of 1 Lpm and 5 Lpm. Surface and overall collection efficiencies of 25 mm capillary pore membrane filters with 0.29 μm and 0.72 μm pore diameters were measured for potassium chloride particles 9 nm to 528 nm in diameter at face velocities of 3.7 cm/s and 18.4 cm/s.

Results. Results for one filter pore size and air velocity are presented in **FIG 2** with an image from microscopy shown in **FIG 3**. These results are representative for other pore sizes and air velocities. For nanoparticles, collection efficiencies overall were substantially higher than those to the filter surface, indicating that deposition occurs to a large extent inside the filter pores. Observed collection efficiencies compared reasonably well with existing theories for surface collection and very well for overall collection. Surface collection efficiencies were well below 100% for the range of particle sizes tested and must be considered when estimating airborne concentrations from microscopic measurements. With knowledge of surface collection

efficiency, size-specific correction factors can be created to estimate airborne particle concentrations from SEM-analyzed filter surface loading.

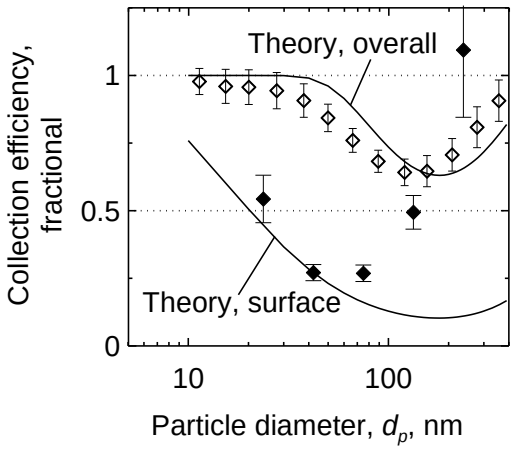

FIG 2. Particle collection efficiency by size at V_0 = 3.7 cm/s (Q = 1 Lpm) and D_0 = 0.72 µm. Filled symbols indicate surface collection efficiency (n = 3, 1σ); open symbols indicate overall collection efficiency (n = 9, 1σ).

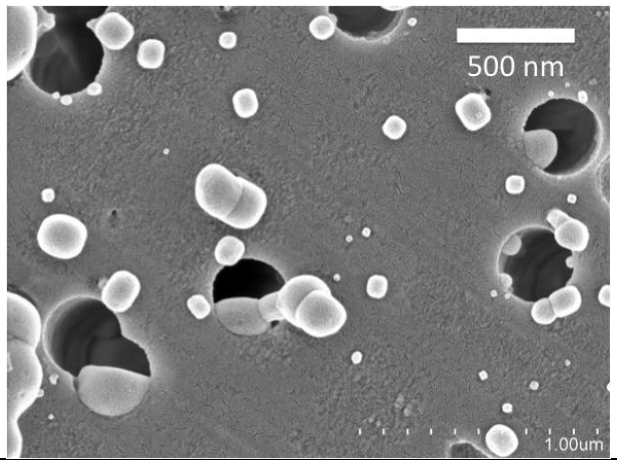

FIG 3. SEM micrograph of filter surface, with pores and deposited particles, for experimental conditions of V_0 = 3.7 cm/s (Q = 1 Lpm) and D_0 = 0.29 µm.

Evaluation of a diffusion charger for use as a personal sampler.

This work evaluated a diffusion charger for its ability to measure rapid fluctuations in workplace nanoparticles. The DC2000CE was selected because its small size (17.1 cm by 6.3 cm by 12.7 cm), light weight (1.6 kg), and battery powered operation make it amenable to assessment of personal exposures. This work is being prepared for submission to the Journal of Occupational and Environmental Hygiene.

Comparison of Response to Reference Instruments. The accuracy of the DC2000CE to measure particle surface area concentrations was evaluated for monodispersed and polydispersed test aerosols. For monodispered test aerosols, a pneumatic nebulizer (Model AirLife, Cardinal Health, McGaw Park, IL) was used to nebulize an ammonium fluorescein solution. The resulting polydispersed aerosol was then directed into a chamber to mix the aerosol, a desiccant dryer to dry the aerosol, and then an electrostatic classifier (Model 3071, TSI, Inc., Shoreview, MN) to select a specific particle sizes.

Polydispersed aerosols included exhaust from a propylene torch (Model MAP-Pro, Worthington Cylinders, Columbus, OH), a burning incense stick, and a diesel generator (Model DG6LE, RedHawk Equipment, Columbus, OH). A coarse polydispersed aerosol composed of Arizona Road Dust (ARD) (ISO Medium, Powder Technology Incorporated, Burnsville, MN) was generated with a fluidized bed aerosol generator (Model 3400, TSI Inc., Shoreview, MN). Tests

were conducted with one source (unimodal, polydisperesed aerosols) or multiple sources (multimodal, polydispersed aerosols).

These aerosols were measured with the DC2000CE and two reference instruments: the scanning mobility particle sizer (SMPS) and the aerodynamic particle sizer (APS). The ratio of the active surface area concentration measured by the DC2000CE divided by the active surface area concentration measured by the reference instruments (R_{DC/ref_a}) was calculated. This ratio is expected to be near unity if the DC2000CE is measuring aerosols similarly to the reference instruments.

The value of R_{DC/ref_a} was substantially less than 1 for all test aerosols, indicating that the DC2000CE is biased low compared to the reference instrument. This ratio ranged in value from 0.18 to 0.32 for monodispersed aerosols. The results for unimodal, polydispersed aerosols are provided in the table below. The ratio was greatest for the propylene torch (R_{DC/ref_a} = 0.61), which was the test aerosol composed of the smallest particles (number median diameter, NMD = 22 nm). It was least for the ARD (R_{DC/ref_a} = 0.61), which was composed of the largest particles (NMD = 978 nm).

Table 1: Active surface area concentration measured by the DC2000CE compared to active surface area concentration measured by reference equipment for unimodal polydispersed aerosols.

Aerosol	NMD (nm)	GSD	Number Concentration (particle cm^{-3})	DC2000CE Surface Area Concentration (mm^2 m^{-3})	Reference Surface Area Concentration (mm^2 m^{-3})	R_{DC/ref_a}
Propylene Torch	22	1.53	144,171	133	220	0.61
Diesel	122	1.48	62,491	560	2,426	0.23
Incense	128	1.69	46,366	581	2,358	0.25
ARD	978	1.50	108	4	30	0.13

The DC2000CE responds to nano-, fine, and coarse particles. Removal of fine and coarse particles prior to measurement is needed if it is desired to detect nanoparticles separately from other airborne particles. The response of the DC2000CE compared to reference instruments varies by particle size and is not described by adjustments from theory. Thus, a calibration by size is needed to estimate actual surface area concentration from DC2000CE measurements.

Determination of Maximum Measureable Surface Area Concentration. Varying amounts of diesel exhaust was injected into the mixing chamber to produce ten test aerosols with similar size distributions but varying concentrations that ranged from 0 mm^2 m^{-3} to 50,000 mm^2 m^{-3} as measured by the reference SMPS. This aerosol was measured with the DC2000CE and the SMPS simultaneously. The DC2000CE was able to measure concentrations with similar accuracy up to approximately 2,500 mm^2 m^{-3} (**FIG 4**) or 2.5 times the maximum concentration stated in the user's manual (1000 mm^2 m^{-3}). The relationship between the DC response and the surface area concentration measured with the reference instrument was linear ($y=0.15x+1.74$; R^2

= 0.99) for concentrations less than 2,500 mm^2 m^{-3}. This relationship was no longer linear at concentrations greater than this value.

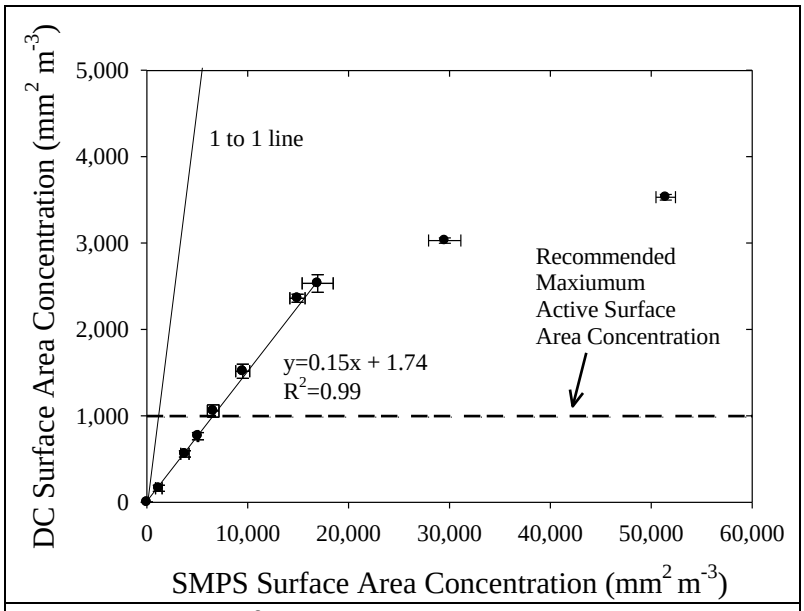

FIG 4. Active surface area concentrations measured with the DC2000CE compared to that measured with the SMPS for diesel exhaust. Error bars represent one standard deviation.

Evaluation of Physical Limitations. The influence of orientation and movement on the DC2000CE response was evaluated by placing a zero filter (8016245, TSI Inc., Shoreview, MN) placed on the inlet. The DC2000CE was set to log every 10 seconds and placed in its neutral position (on feet) for one minute to allow the response to zero. Then every two minutes the instrument was moved to a different orientation as follows with the instrument on its: 1) feet (normal mode of operation); 2) back where the mode switches are located; 3) top (unit upside down); 4) right (inlet) side; and 5) left (window) side. The orientation of the instrument was changed with two movements: a gentle rolling motion without the DC2000CE losing contact with the table surface; and abrupt motion with the DC2000CE picked up approximately two inches off the table and then quickly placed back on the table in a new orientation. All tests were conducted in triplicate.

As shown in **FIG 5(top and middle plots)**, surface area concentrations were observed substantially above the manufacturer-reported minimum active surface area concentration measureable by the DC2000CE (10 mm^2 m^{-3}) as reported by the manufacturer. The greatest concentrations (rolling movement; 400 mm^2 m^{-3}) were observed when the instrument was rolled to the window side. In all cases, concentrations returned to below 10 mm^2 m^{-3} after approximately 30 seconds.

Another test was conducted to evaluate the influence of vibration on instrument response. The DC2000CE was placed on a vibrating surface with a zero filter on the inlet. The vibrating surface used for these tests was a blower (Model 4C129, Dayton, Chicago, IL) that was set to 20% of motor RPM. The DC2000CE was set to log every 10 seconds throughout the following sequence: a one minute zeroing period; blower on for two minutes; blower off for one minute. This sequence was repeated in triplicate. The average root mean square vibration was 2.82 m s^{-2} in the x-direction, 3.25 m s^{-2} in the y-direction, and 1.87 m s^{-2} in the z direction as measured with a human vibration monitor (Model HVM100, Larson Davis, Depew, NY). The influence of vibration on a DC2000CE is shown in **FIG 5 (bottom plot)**. When the DC2000CE was vibrating, the results were greater than two times the minimum resolution of the instrument (10 m^2 m^{-3}).

We attribute deviations from zero response to the sensitivity of electronic components required to detect particles. The values observed in these tests are comparable to measurements observed by our group in various workplace environments. Moreover, the movements tested here are similar to those required to move an instrument from one location to another in a workplace. They would be common if the DC is incorporated into a backpack configuration. Consequently, we recommend that a DC that is less sensitive to movement is needed to successfully develop the pDC/DB.

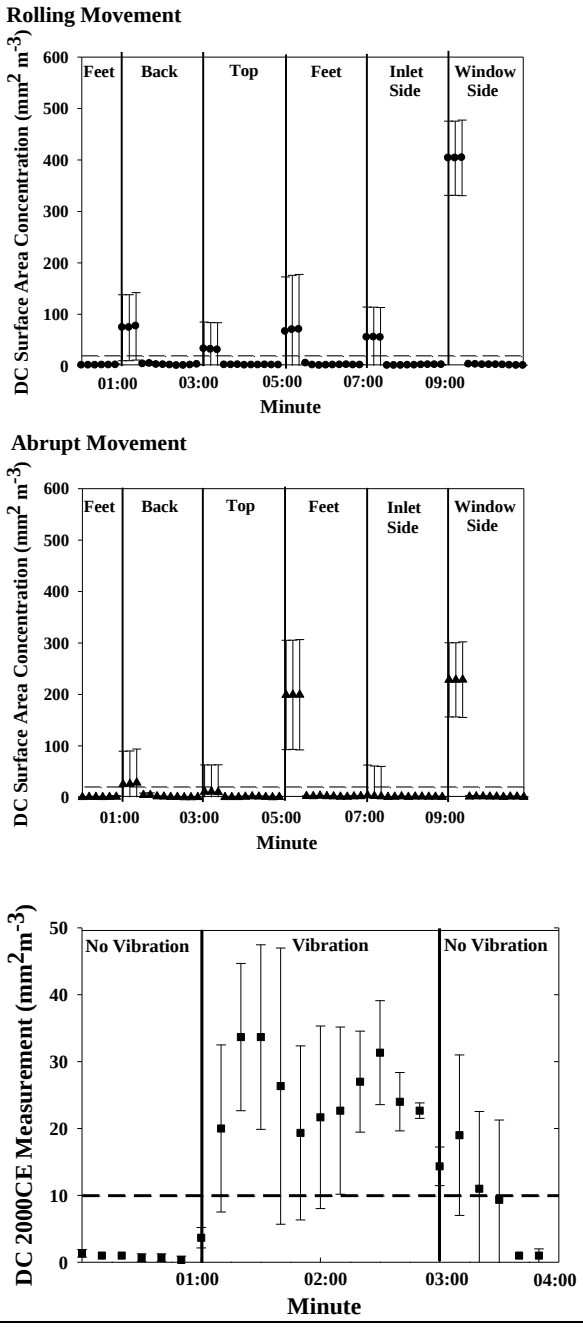

FIG 5. Influence of instrument motion on surface area concentrations measured with the DC2000CE: rolling motion (top); abrupt motion (middle); and vibration (bottom). Dotted line represents the manufacturer's reported minimum measureable concentration (10 mm^2 m^{-3}). Error bars represent one standard deviation.

Aim 2. Characterize worker exposure to nanoparticles

For this aim, we hypothesized that personal exposure to nanoparticles are highly variable among workers who handle and produce them. We tested this hypothesis by assessing the variability of worker exposure with direct-read monitors and active-filter samplers that were established in Aim 1. We used mixed models where appropriate to relate exposures to job and work area, while controlling for between and within subject variability. Work task and practice were incorporated into the analysis of short-term exposures through video-based exposure monitoring. These measurements were conducted in a series of three field experiments.

Airborne particles in a facility that produces lithium titanate nanomaterial.

The approximately 60,000-ft^2 facility where this work was performed produces lithium titanate metal oxide nanomaterial. This nanomaterial is noted by the company for its high surface area and superior performance in fuel cells. The material was produced in six primary work areas: wet mill; spray dryer; rotary calciner; an open area with a powder sifting hood and a stationary calciner; an area with new equipment installation; and a loading dock. Generally, the areas of the facility were open to each other with partial walls in some places. This work was published in the Journal of Occupational and Environmental Hygiene (DOI: 10.1080/15459620802590058).

In each area, we collected three respirable dust samples on consecutive days on polyvinyl chloride (PVC) filters (SKC Inc. Eighty Four, PA) for gravimetric analysis. At each location, duplicate respirable dust samples were collected on mixed cellulose ester (MCE) filters (SKC Inc. Eighty Four, Pa) for analysis by electron microscopy (discussed under Aim 3). PVC filters were weighed according to NIOSH Method 0600 to determine the mass concentration of the respirable dust. These filters were then analyzed by inductively coupled argon plasma atomic emission spectroscopy (ICP-AES) for titanium and lithium according to NIOSH method 7300.

As shown in Table 2, exposures to the nanomaterial being produced in this facility were highly dependent on the work area and limited mainly to the rotary calciner area. This information provided evidence to support the hypothesis that exposures to nanomaterials are often highly variable by location in a workplace.

Further measurements were made in the Rotary Calciner area with a CPC and OPC (**FIG 1**). The mass concentrations and not the particle number concentrations were associated with handling the nanomaterial. This finding is consistent with the fact that the nanomaterial was associated with large (primarily supermicrometer) particles. Moreover, the direct-read instruments provided valuable temporal information that the airborne nanomaterial is highly temporally variable. This information is critical to developing strategies for controlling exposures in this workplace. Described in the next section (Aim 3), further analysis by electron microscopy was conducted on these samples to determine the source of particles collected.

TABLE 2. Respirable mass concentration and percent of respirable mass concentration lithium titanate by location

Area / Location	Respirable Mass Concentration, mg m^{-3} Mean ± Std. Dev.	Percent Lithium Titanate
Rotary Calciner / Bagging	0.118 ± 0.023 *	39% ± 11%
Rotary Calciner / Hopper	0.035 ± 0.006	<10% **
Spray Dryer ***	--	--
Wet Mill	0.026 ± 0.007	<LOD
Powder Sifting Hood	0.039 ± 0.016	<4% **
Loading Dock	0.036 ± 0.015	<LOD
Calciner	0.028 ± 0.012	<LOD
Outdoors	<LOD	<LOD

* Duncan multiple range test identified mean as statistically different from other means.
** One or more sample below LOD for titanium; value reported based on sample(s) >LOD.
*** No samples available because spray dryer was not operational.

Airborne nanoparticle concentrations in the manufacturing of polytetrafluoroethylene (PTFE) apparel

One form of waterproof, breathable apparel is manufactured from polytetrafluoroethylene (PTFE) membrane laminated fabric, using a specific process to seal seams that have been sewn with traditional techniques. The sealing process involves applying waterproof tape to the seam by feeding the seam through two rollers while applying hot air (600°C). This study addressed the potential for exposure to incidental nanoparticles from this sealing process, by characterizing airborne particles in a facility that produces over 1,000 lightweight PTFE rain jackets per day. This work is under review for publication in the Journal of Occupational and Environmental Hygiene.

Methods. The facility was divided into three main areas: office area, sewing area, and sealing area. The office area housed management, administration, and engineering staff and was physically partitioned from the other areas. Jobs performed in the sewing and sealing areas to create a rain jacket included: 1) traditional sewing of cut pieces of fabric (sewing area); 2) sealing of sewn seams (sealing area); 3) quality control (sealing area); 4) reheating overlapping seams (sealing area); and 5) bundling jackets for shipping (sewing area). Aerosol concentrations throughout the facility were mapped, breathing zone concentrations were measured, and hoods used to ventilate the seam sealing operation were evaluated. Mixed models with covariates identified through video exposure monitoring were used to identify determinants of exposure.

Results. Particle number concentrations were greater in the sealing area than in the sewing area (**FIG 6**). The GM of the particle number concentrations in the sewing area (67,000 particles cm^{-3}) was over three times greater than that in the office area (12,100 particles cm^{-3}). The GM of the particle number concentrations in the sealing area (188,000 particles cm^{-3}) was more than a magnitude greater than the office area. Respirable mass concentrations in the sewing and sealing

areas ranged from 0.001-0.007 mg m^{-3} with the GM concentration (0.002 mg m^{-3}) in the sealing area slightly greater than that in the sewing area (0.001 mg m^{-3}).

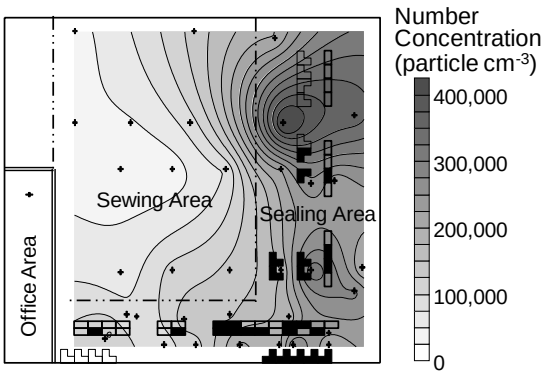

FIG 6. Map of particle number concentrations in the sewing and sealing areas. Crosses indicate sample locations, and shaded work stations indicate stations that were in use during mapping.

The particles exiting the final discharge of the facility's ventilation system were dominated by nanoparticles (number median diameter = 245 nm; geometric standard deviation of 1.39). The breathing zone particle number concentrations of the workers who sealed the sewn seams were highly variable and significantly greater when sealing seams than when conducting other tasks ($p<0.0001$). The sealing workers' breathing zone concentrations ranged from 147,000 particles cm^{-3} to 798,000 particles cm^{-3}, and their seam responsibility significantly influenced their breathing zone concentrations ($p=0.03$). The finding that particle number concentrations were approximately equal outside the hood and inside the local exhaust duct indicated poor effectiveness of the canopy hoods used to ventilate sealing operations.

Characterization and control of airborne particles emitted during production of epoxy / carbon nanotube nanocomposites.

This work characterized airborne particles that were generated from the weighing of bulk, multi-wall carbon nanotubes (CNTs) and the manual sanding of epoxy test samples reinforced with CNTs. It also evaluated the effectiveness of three local exhaust ventilation (LEV) conditions (no LEV, custom fume hood, and biosafety cabinet) for control of particles generated during sanding of CNT-epoxy nanocomposites. This work has been accepted for publication in the Journal of Occupational Hygiene.

Methods. This study was conducted in a facility that produced test samples composed of epoxy reinforced with CNTs. The test samples were rectangular sticks of CNT-epoxy with dimensions of 12.5 x 1.3 x 0.5 cm that were used to evaluate the effect of formulation variables on the properties of the nanocomposites. To produce the test samples, bulk multi-wall CNTs with 10-50 nm outer diameter and 1-20 µm length (Baytubes, Bayer Material Science LLC, Pittsburg, PA) were weighed and mixed with epoxy. This mixture was poured into a mold designed to produce four test samples at a time and baked in an oven for several hours. Then the hardened nanocomposite test samples were broken apart manually, and each one was manually sanded to remove excess material until final dimensions are achieved.

Airborne particle number and respirable mass concentrations were measured with two direct-read instruments: a CPC and an OPC as reported under Aim 1 above. The morphology of representative airborne particles was observed by transmission electron microscopy (TEM) on a copper TEM grid (300 mesh with carbon type-b film, 01813-F, Ted Pella, Inc., Redding, CA)

affixed with carbon tape onto the center of the face of a polycarbonate membrane filter (E0055-MB, SPI Supplies, West Chester, PA). This filter was then mounted in an open-face conductive filter cassette (25 mm, 225-3-23, SKC Inc., Eighty Four, PA). Airflow was pulled through the sampler with a personal sampling pump (Buck Basic-5, A.P. Buck Inc., Orlando, FL) at 1 L/min. The copper grids were analyzed under TEM (JEOL JEM-1230, Peabody, MA) to characterize the size and morphology of a representative subset of the collected airborne particles.

Airborne concentrations were measured during two processes: weighing bulk CNTs and sanding epoxy nanocomposite test sticks. To simulate weighing, 600 mg of the bulk CNTs were transferred by scooping material between two 50 mL beakers for three five-minute intervals at a rate of ~ 1 scoop/sec. Each scoop contained approximately 50 mg of CNT material. To study the sanding process, an operator manually sanded epoxy test sticks that contained 2% by weight CNTs with sandpaper (220 grit, model 20240, 3M, St Paul, MN).

Aerosol concentrations were measured for 15-30 min in two locations (adjacent to the sanding process and in the operator's breathing zone). For sanding, source and breathing zone measurements were taken under three LEV conditions (no LEV, a custom fume hood, and a biological safety cabinet). The ratios of the geometric mean (GM) concentrations measured during the process to that measured in the background (P/B ratios) were used as indices of the impact of the process and the LEVs on observed concentrations.

Results. Processing CNT-epoxy nanocomposites materials released respirable size airborne particles (P/B ratio: weighing = 1.79; sanding = 5.90) but generally no nanoparticles (P/B ratio ~1). As shown in **FIG 7**, respirable mass concentrations in the operator's breathing zone were lower when sanding was performed in the biological safety cabinet (GM = 0.20 µg m^{-3}) compared to those with no LEV (GM = 2.68 µg m^{-3}) or those when sanding was performed inside the fume hood (GM = 21.4 µg m^{-3}; p-value < 0.0001). The poor performance of the custom fume hood used in this study may have been exacerbated by its lack of a front sash and rear baffles and its low face velocity (0.39 m/sec).

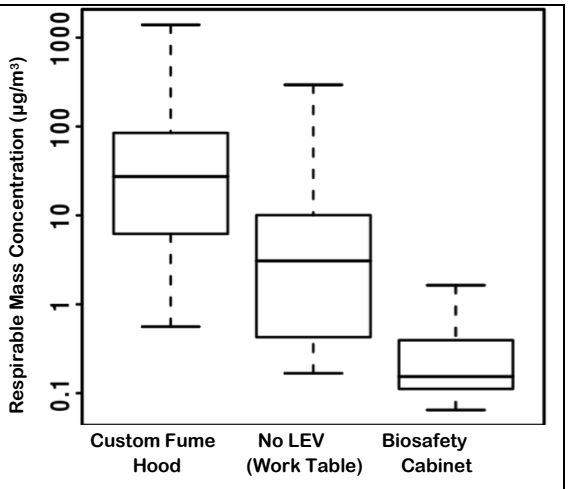

FIG 7. Box-and-whisker plot of log-transformed respirable mass concentrations. Box parameters represent median, lower and upper quartiles. Whiskers represent sample min and max.

Aim 3. Apportion worker exposure to nanoparticles by source

The working hypothesis for this aim was that nanoparticle exposures vary substantially by size, morphology, and composition depending on their source. We analyzed the field samples collected for Aim 2 using electron microscopy with energy dispersive X-ray analysis.

Analysis of samples from the manufacture of lithium titanate.

The facility and locations where samples were collected is described under Aim 2 above. The MCE filters from this study were analyzed by TEM **(**Hitachi H-7000 TEM) and SEM **(**Hitachi S-4800 SEM**)** in order to assess the morphology and composition of the particles by size. MCE filters were collapsed with a mixture of 50% deionized water, 35% dimethylformamide, and 15% glacial acetic acid, etched using a plasma asher, and coated with a carbon layer. A square section of the collapsed filter was placed specimen side up on 3-mm TEM grids and placed in a Jaffe washer filled with dimethylformamide for 2 hrs. We examined TEM images to classify particles by shape: *(1)* large 200-nm to 10-µm spheres; *(2)* irregularly shaped particles of varying size; and *(3)* submicrometer particle chains. For each shape class, we then identified the range of particle size by TEM, the general surface morphology by SEM, and chemical composition by TEM with energy dispersive spectrometry (EDS). Specialized software was used to acquire elemental maps of selected particles.

As shown in **FIG 8**, two particle classes were identified in TEM images (**A**;left): spheres; and chain agglomerates. The spheres contained titanium, hence they were identified as nanomaterial through single-particle EDS mapping (**A**;right). Chain agglomerates contained elements characteristic of welding fume (**B**). These findings allowed us to conclude that the airborne nanomaterial was substantially larger than nanosize. Further images from scanning electron microscopy (SEM) revealed that the morphology of these particles do have nanostructure (**C**).

This work is the first to classify airborne particles by type (engineered or background) and size in a nanomaterial manufacturing facility. It shifts the way one would approach protecting workers from inhalation to nanomaterials. The results unequivocally demonstrate that airborne nanomaterials can be larger than nano-sized particles but still have nanostructure that might make them workplace hazards. They also demonstrate that nanoparticles may be background aerosols unrelated to manufacturing. This type of detailed information is essential for the design of toxicity tests that are environmentally relevant and is necessary to select appropriate strategies for routine monitoring, controlling exposures, and establishing appropriate OELs.

Analysis of samples collected in processing epoxy / carbon nanotube nanocomposites.

The facility and locations where samples were collected is described under Aim 2 above. The morphology of representative airborne particles was observed by transmission electron microscopy (TEM, JEOL JEM-1230, Peabody, MA) to characterize the size and morphology of a representative subset of the collected airborne particles.

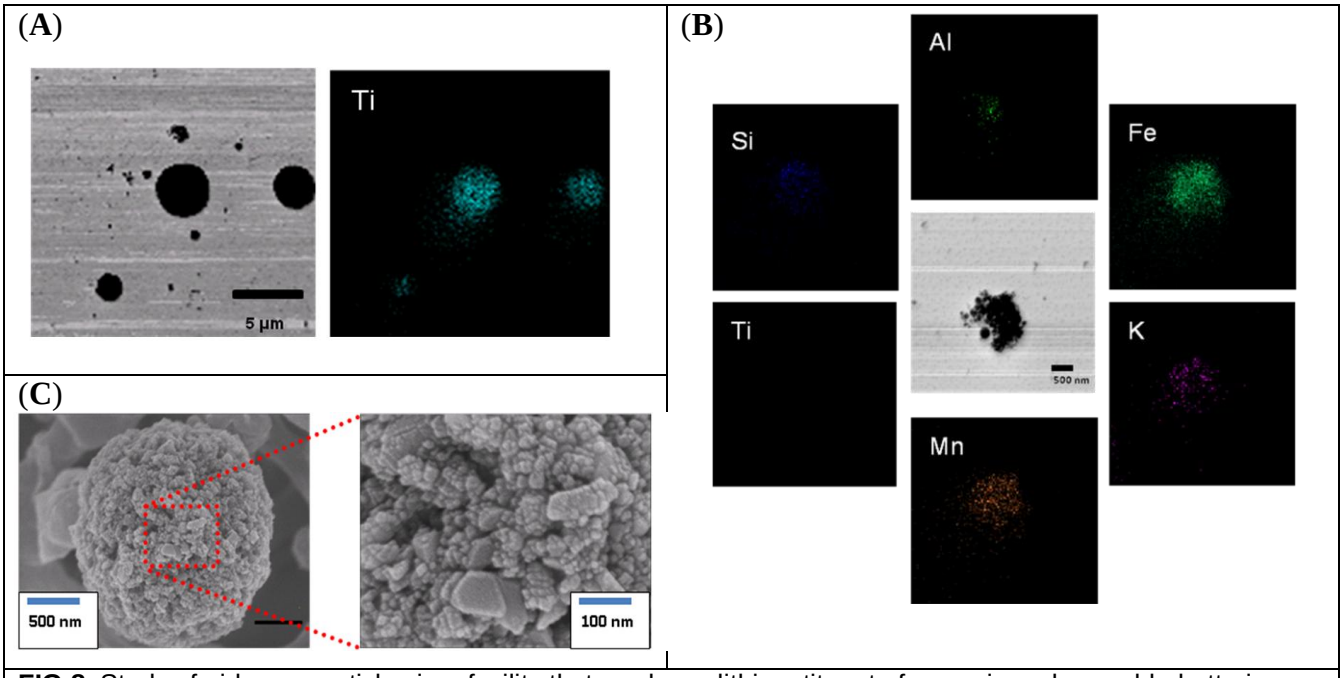

FIG 8. Study of airborne particles in a facility that produces lithium titanate for use in rechargeable batteries (Peters, Elzey et al. 2009). TEM image (**A**;left) allowed us to identify two morphologies, spheres and agglomerates. Spheres were classified as nanomaterial because only they contained Ti in TEM/EDX maps (**A**;right) and (**B**). SEM was used to that the spherical nanomaterial was composed of fused nanoparticles ranging from 10-80 nm (**C**).

Bulk CNTs artificially deposited onto TEM grids appeared as large bundles (>1 µm) containing many tangled nanotubes (**FIG 9**). The particles collected during sanding were predominantly large (>300 nm) and irregular in shape (a representative particle is shown in **FIG 10**). These particles commonly had protuberances that emerged from the perimeter of the particles. These protuberances had an outer diameter between 10 and 50 nm, which is consistent with that of the CNTs. No CNTs were observed free from composite material for the manual sanding studied in this work. It is, however, possible that free CNTs are generated and would be observed at higher concentrations or when mechanical sanding is performed.

As shown in **FIG 11**, X-Ray mapping was used to identify that the bulk CNTs contained iron and nickel within the CNTs. These metals were likely used as a catalyst during the formation of the CNTs and can serve as a signature to identify CNTs in unknown samples. The protuberances observed in the particles liberated from sanding epoxy nanocomposites did contain the signature catalyst showing that they were CNTs (**FIG 12**).

Toxicological studies have focused to date on the adverse health effects associated with exposure to bulk CNTs. Our findings suggest that inhalation exposure to bulk CNTs is likely to be low in activities such as weighing. In contrast, sanding does generate respirable particles that can enter a worker's breathing zone, and these particles were very different morphologically than bulk CNTs. Given the lack of toxicity data for this type of particles, it cannot be determined whether exposure to the sanding particles generated at the concentrations measured in this study presents a risk to workers' health. Precautions such as the use of personal protective equipment or

working within a hood that meets design specifications and standards or a biological safety cabinet should be taken until health effects are better understood.

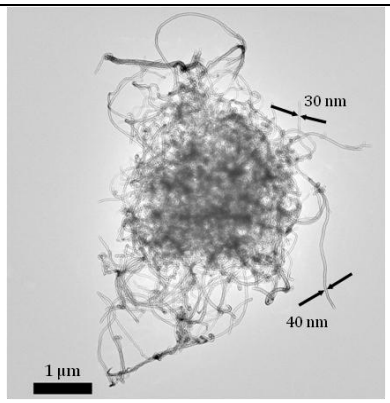

FIG 9. Bulk 10-50 nm outer diameter multi-wall CNTs with many tangled nanotubes.

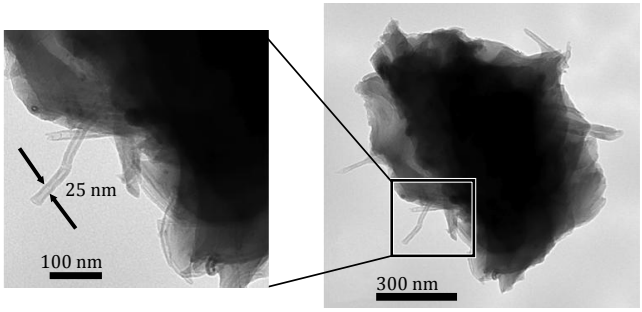

FIG 10. Sanding particle with detail of protruding fibers (TEM image).

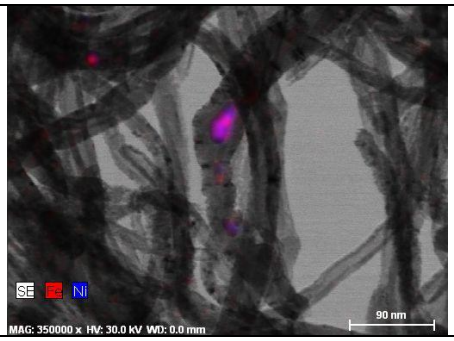

FIG 11. EDS X-Ray mapping to identify a signature to identify the CNT. Bulk CNTs contain nickel that was used as a catalyst in their production.

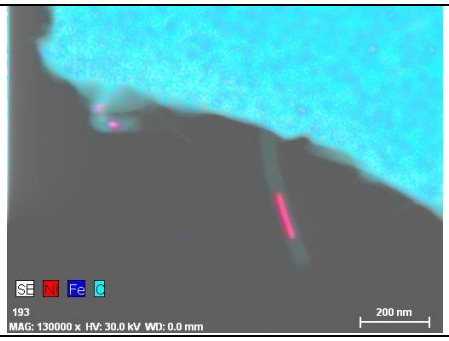

FIG 12. Protuberances in particles from sanding epoxy nanocomposite contain nickel signature.

Career Development

This K01 Award supported valuable training to further my career as an independent investigator. Importantly, I was promoted from Assistant to Associate Professor with tenure in June 2010. During the Award period, I devoted 50% of my time on career development and K01 research. I formally attended the following courses at the Iowa:

- Epidemiology II: Advanced Methods
- Genetics and Epidemiology
- Scanning Electron Microscopy and X-Ray Microanalysis

The epidemiology courses have helped me understand how my exposure assessment methods fit within a broader framework. The electron microscopy course was directly applied to performing the work under Aim 3.

The sponsors of this Award have provided invaluable mentoring in all aspects of my career from writing successful research grants to advising students in my laboratory. These sponsors have also been collaborators that actively work with my students and me to publish our research in quality peer-reviewed journals.

I have been actively engaged in numerous other training activities. I have attended and presented at numerous seminars on campus and national meetings. These meetings have provided many opportunities for learning, networking, and improving presentation skills. I submitted numerous grant applications during this period (two R01, two R21, and numerous smaller grant applications). One of the R21 applications was funded and started this fall 2010. This new work is based on K01 Award research. I plan to resubmit revised versions of one of the R01 applications.

Publications

Published or "in press" articles

Peters TM, Elzey S, Johnson R, Park H, Grassian V, Maher T, O'Shaughnessy T: [2009] Airborne monitoring to distinguish engineered nanomaterials from incidental particles for environmental health and safety. J. Occup. Envir. Hyg. 6(2):73-81. DOI: 10.1080/15459620802590058.

- This article presents the use of two methods to distinguish airborne engineered nanomaterials from other airborne particles in a facility that produces nano-structured lithium titanate.
- This work was applicable to all of the aims of this project. Relevant to Aim 1, the article describes two techniques that can be used to assess exposures to nanoparticles. Relevant to Aim 2, the use of these methods allowed us to characterize worker exposures to nanoparticles in the workplace. Relevant to Aim 3, we were able to further use advanced electron microscopy to apportion exposures to specific sources within the manufacturing facility.
- This article has won several awards: 2010 David Swift Memorial Award for "Best Aerosols Paper Published in Journal of Occupational and Environmental Hygiene"; 2009 Michigan Industrial Hygiene Society "Best Paper Applied to Industrial Hygiene Award".

Cyrs WD, Boysen DA, Casuccio G, Lersch T, Peters TM: [2010] Nanoparticle collection efficiency to the surface of capillary pore membrane filters. J. Aerosol Sci. 41:655-664. DOI:10.1016/j.jaerosci.2010.04.007.

- Surface and overall collection efficiencies of capillary pore membrane filters were measured for sub-micrometer particles.
- This work fulfilled the Aim 1 objective to develop a quantitative method to collect nanoparticles for subsequent analysis by electron microscopy.

Schmoll LH, Peters TM, O'Shaughnessy PT: [2010] Use of a condensation particle counter and an optical particle counter to assess the number concentration of engineered nanoparticles. J. Occup. Envir. Hyg. 7:535-545. DOI: 10.1080/15459624.2010.496072.

- This article summarizes the evaluation of several methods to process data derived from direct-read instruments for various engineered nanoparticles: TiO_2, SiO_2, and Fe_2O_3.
- It is directly related to fulfilling the objectives of methods development under Aim 1.

Vosburgh D, Boysen DA, Oleson JJ, Peters TM: [in press] Airborne nanoparticle concentrations in the manufacturing of polytetrafluoroethylene (PTFE) apparel. J. Occup. Envir. Hyg.

- This study addressed the potential for exposure to particulate matter from this sealing process, by characterizing airborne particles in a facility that produces over 1,000 lightweight PTFE rain jackets per day. Aerosol concentrations throughout the facility were mapped, breathing zone concentrations were measured, and hoods used to ventilate the seam sealing operation were evaluated.
- It is applicable to characterizing exposures in Aim 2.

Cena L, Peters TM: [in press] Characterization and control of airborne particles emitted during production of epoxy/carbon nanotube nanocomposites. J. Occup. Envir. Hyg.

- This work characterized airborne particles that were generated from the weighing of bulk, multi-wall CNTs and the manual sanding of epoxy test samples reinforced with CNTs. It also evaluated the effectiveness of three local exhaust ventilation conditions (no LEV, custom fume hood, and biosafety cabinet) for control of particles generated during sanding of CNT-epoxy nanocomposites.
- This work contributed to completion of the aim Aim 2 to characterize particle exposures where nanomaterials are handled. The samples collected at this facility and analyzed under Aim 3 helped to determine that sanding epoxy nanocopmposites generates particles with highly unique morphology.

Articles "in preparation"

Vosburgh D, Ku BK, Peters TM (in prep) Evaluation of a diffusion charger for its effectiveness in measuring workplace aerosols (PTFE) apparel. J. Occup. Envir. Hyg.

- This study evaluated a DC2000CE diffusion charger for its effectiveness to measure worker exposure to multiple types and sizes of aerosols.
- The work helped to show limitations of using a direct-read instrument for measurement of personal exposure to engineered nanoparticles (Aim 1).

Dissertations/Theses

Johnson R: [2007] "Airborne particles in the manufacturing and handling of nano-structured lithium titanate", MS Thesis, The University of Iowa.

Cyrs W: [2009] "Surface collection efficiency of polycarbonate membrane filters", MS Thesis, The University of Iowa.

Cena L: [expected spring 2011] "Methods for the industrial hygiene evaluation of carbon nanotubes", PhD Dissertation, The University of Iowa.

Vosburgh D. [expected fall 2010] "Personal exposure assessment of nanoparticles in workplace environments", PhD Dissertation, The University of Iowa.

Inclusion of Gender and Minority Study Projects

Not Applicable: we were not required to enroll human subjects to perform these studies.

Inclusion of Children

Not Applicable: this work did not involve human subjects.

Materials Available for Other Investigators

No other materials are immediately available to other researchers as part of this research.