Exposure Assessment and Inflammatory Response Among Workers Producing Calcium Carbonate Nanomaterials

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A dissertation
submitted in partial fulfillment of the
requirements for the degree of

Doctor of Philosophy

University of Washington
2013

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Program Authorized to Offer Degree:
Environmental and Occupational Health Sciences

University of Washington

Abstract

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Problem: Nanotechnology is one of the most rapidly growing fields of science and engineering, and its applications have expanded to numerous research and industrial sectors, from consumer products to medicine to energy. Nano-materials and nanotechnology promise substantial benefits. However, there are many uncertainties and concerns regarding human health and the environment. Numerous toxicological studies on animals and cells in vitro have demonstrated that nanomaterials could cause various adverse health effects, including

inflammation, oxidative stress, fibrosis and mutagenesis in the lungs, and cardiovascular and nervous system impairment.

Objectives: The overall objective of this study was to characterize particulate exposures in a calcium carbonate nanoparticle manufacturing facility, investigate possible respiratory and cardiovascular effects, and explore the plausibility than an inflammatory mechanism. The associations between exposure level and various health outcomes were investigated.

Methodology: Each job was characterized by mass, number and surface area concentration. Job classification was performed based on ranking of the exposure level and statistical models. Lung function tests, exhaled NO and blood pressure (BP) were measured before and after the workshift in the year of 2011. Inflammatory cytokines from induced sputum were measured cross-sectionally in the year of 2011. Data of lung function tests and blood pressure were collected cross-sectionally in the year of 2012. The associations between each exposure metric and health measures in 2012 were investigated. Only mass concentration was linked to both 2011 and 2012 health outcomes.

Results: The sampling and analytic methodology used in the study presents the potential to characterize nanoparticle exposure for a variety of operational processes. We found the highest mass exposure occurred at bagging job whereas the highest number and surface area concentration was found at modification. Modification is suspected to be the primary emission source. It is discovered nanoparticles in the size range of 20-300nm dominate in this workplace, which consists of 90-98% of particle counts in the respirable fraction. Based on the sampling results from 2012, there was a strong relationship between number

concentration in 5-25um range and the respirable mass concentration (r= 0.908); however, no such correlation was found between number concentration in nanoscale and respirable mass (r= 0.018). The deposited surface area in TB (r=0.66) and alveolar region (r=0.46) was modestly correlated with number concentration of particles in the nanoscale.

A reduced FEV1 and increased BP were consistently found among medium-mass exposure compared to low-mass exposure, however no statistical significance was found. When comparing the four exposure metrics, we found number concentration and surface area concentration in general produce effects in similar direction, however opposite to mass concentration. Such observation is consistent with the correlation among these exposure metrics.

Airway inflammatory responses presented a dose-response relationship using mass as exposure metric. The concentrations of IL1β (p=0.043) and IL8 (p=0.008) in sputum among high mass-exposure group were statistically greater than that in low-mass exposure group. It suggested the inflammatory responses were associated with mass concentration of inhaled nanoparticle particles, which are mainly made up by agglomerated form of nanoparticles. At current stage, with limited understanding of the toxicological perspective of nanoparticle, a complete exposure assessment in nanoparticle facility needs to be conducted in both bulk- and nano-form.

Dedication

I dedicate this dissertation to my beloved father and grandfather, who shaped an atypical personality and cultivated a free-minded Ling. Thank you for not setting the standards and rules, making me a happy and curious “boy” when I was young. I also want to extend my dedication to my dear husband, my co-pilot and fun partner. Thank you for your support and understanding while working on this project.

Acknowledgements

Chinese people have an old expression: “世有伯乐, 然后有千里马。千里马常有, 而伯乐不常”, which means “The horse connoisseur, Bo Le, must exist before the finest horses are recognized. Gifted horses are common but rare is the one who can recognize the talent.” I would like to give my special thanks to Dr. Daniel Frank, Dr. Peter Johnson and Dr. Philip Weinstein. Without your encouragement, I would not have the confidence to pursue such a challenging undertaking.

I also want to thank to my two dear hearted friends Eileen Kirkpatrick and Jessi Geier. Your genuine kindness makes Seattle feel like home. Thank you for helping me to get through the bad times and enjoy the good times!

Lastly and most importantly, I would like to offer my gratitude to my PhD committee members: Dr. Harvey Checkoway, Dr. Michael Yost, Dr. Sverre Vedal, Dr. Noah Seixas and Dr. Scott Emerson. Thank you for keeping me challenged and motivated; and thank you for placing a great model of a devoted scientist.

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Chapter 1 Introduction

Background and Context

Nanotechnology is a system of techniques and methods to manipulate matter at the nanoscale level (approximately 1 nm to 100 nm). It encompasses both an effort of advancing technology in its own right and a development of related innovative applications. Nanotechnology continues to expand in many areas of research and in the industrial sector, from consumer products to medicine to energy. It is one of the most rapidly growing fields of science and engineering.

Engineered nanoparticles, a subset of the nano-materials, are intentionally produced with specific properties [1]. The International Organization for Standardization (ISO) defined a nanoparticle as an object with all three external dimensions in the nanoscale [2, 3].Very often in the US, nanoparticles are broadly referred as microscopic particles with a nominal dimension (such as geometric, aerodynamic, mobility) smaller than about 100nm. There are various ways to classify nanoparticles. By composition, the diversity of nanoparticles include carbon-based (e.g., fullerenes), metal-based and metal oxides (e.g., gold, silver, titanium dioxide), and those of a biological nature (e.g., liposomes). By shape, there are spherical, polyhedral, multilegged and capsular nanoparticles [4]. By state, nanoparticles can be in the form of single (native) state, aggregation, agglomeration, or mixed states of the above.

Nano-materials and nanotechnology promise substantial benefits in the meanwhile, there are many uncertainties and concerns on human health and environment. Numerous toxicological studies on animals and cells in vitro demonstrated that nanomaterials could induce various

health effects, including inflammation, oxidative stress, fibrosis and mutagenesis in the lungs and cardiovascular and nervous system impairment [5-11]. Current thinking is that particles in the nanosized range may be potentially more toxic than larger particles of the same chemical component because they have greater potential to penetrate deeper lungs, cross various cellular barriers and translocate to different organs.

Many researchers argue that surface area and number concentration is a more relevant and meaningful metric [12-15]. It has been found that particles up to 50nm contribute to only 0.1% -10% of the total mass, but contribute to more than 90% of the total number concentration [16]. One study has shown nanoranged TiO_2 (20nm) induced a much greater level of pulmonary neutrophil cells than did fine TiO_2 (250nm) when both intratracheally at the same mass dose [17]. After surface area was expressed as the "dose", nanosized and fine TiO2 were fitted to the same dose-response curve. Number concentration is another exposure metric, in theory, provides an opportunity for comprehensive exposure assessment. However, precise quantification of number concentration requires specification of the size range. From a perspective of epidemiological studies, it is desirable to work with one integrated exposure metric than a size distribution.

Despite the rapid development of nanomaterials, little is known about the potential human health effects related to engineered nanoparticles. To our knowledge, there have been no epidemiological studies on engineered nanoparticle exposures. Several main challenges need to be addressed in the conduct of health studies related to nanoparticle exposure. These include identifying sufficiently large cohorts, exposure characterization technical barriers, and characterizing causal mechanisms for specific health endpoints [18]. One major

knowledge gap is the choice of exposure metric for nanoparticle exposure studies. Exposure-response relations are sensitive to the choice of exposure index; thus, research findings can be inconclusive if nonspecific inappropriate exposure indicators are used.

Scope and Objectives

The overall objective of this study is to characterize particulate exposure in a calcium carbonate nanoparticle manufacturing facility, investigate possible respiratory and cardiovascular effects, and explore plausible biological mechanisms that may explain associations between nanoparticles and observed health effects. The specific objectives are:

1) Characterize particle size, concentration (mass, number and surface area) and size distribution for each task location;
2) Construct potential exposure profiles for each job and job classification;
3) Investigate the correlation between various clinic and indicative health endpoints, including sputum biomarkers, Forced Expiratory Volume in 1 Second (FEV1), blood pressure, and exhaled nitric oxide, and exposure levels categorized by mass, number and surface area concentration;
4) Test methodologies for exposure assessment of nanoparticles in the context of an epidemiological study.

Overview of Dissertation

The first chapter of the dissertation consists of a review of background, current understanding and identification of gaps in current state of knowledge of nanoparticle exposures and potential health effects. The second chapter introduces the agent investigated, calcium

carbonate, and its production process. Personal and environmental exposure in respirable mass concentration is portrayed in detail. The third chapter describes number concentration and surface area concentration for each process. The association between health endpoints, including clinical outcomes and biomarkers, and exposure is examined in the next chapter. The methods of data collection and results are presented in each chapter, followed by a discussion of main findings, and limitations of the study. The last chapter will summarize and interpret the overall findings and recommend future research directions.

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Chapter 2 Exposure Characterization I

Abstract

In this study, we characterized nanoparticle airborne exposures in a calcium carbonate manufacturing facility in Shanxi Province in China. To estimate the potential exposure of workers, area and personal sampling were performed in the factory in 2011 and 2012. Geometric mean concentrations ranged from 7.24 to 3195.86 μg/m^3 for area sampling, and 44.69 to 10028.4 μg/ m^3 for personal sampling, respectively. There were substantial exposure variations between years and between jobs. One-way ANOVA model was used for job categorization. Particle characteristics, such as size, shape, chemical composition and degree of agglomeration were assessed by microscopic analysis.

Background

The potential for exposures to nanoparticles in workplaces, ambient air, water, the food chain and consumer products has increased in recent years, with occupational exposures perhaps being of highest intensity. Despite some toxicological evidence suggesting that nanoparticles may pose health risks, few studies have been conducted among exposed occupational groups, in large part due to the challenges posed in assessing nanoparticle exposures. The major routes of engineered nanoparticle exposure include inhalation, ingestion, dermal and ocular exposure [1]. Traditionally, measuring aerosol mass concentration by chemical composition is a standard and convenient procedure for occupational studies to describe exposure, estimate doses for intake and link to health outcomes of the respiratory system. Findings from recent toxicological studies indicate that particles <50nm contribute only 0.1%-10% of the total mass concentration [2], yet have a greater potential to induce inflammation per unit mass than

larger particles [3-6]. The choice of an appropriate dose metric in nanoparticle exposure assessment for epidemiological studies needs to be refined.

In an effort to improve understanding of occupational nanoparticle exposure, the University of Washington Department of Environmental and Occupational Health Sciences at the collaborated with the Beijing Municipal Institute of Labour Protection to facilitate exposure data collection. The main purposes of this study were to 1) characterize process-based environmental exposure and identify potential emission sources; 2) evaluate job-based personal exposure; 3) classify job exposure based on respirable mass concentration.

Introduction

This study was conducted in a facility that produces calcium carbonate nanoparticles, which are used in the production of auto chassis paints, adhesives, rubber and plastics. Annual production is approximately 20,000 metric tons. The main principle of synthesis follows as: CaO is hydrated with water to create a slurry of $Ca(OH)_2$; CO_2 is then pumped into a creator to generate $CaCO_3$ nanoparticles; the surface property of the particles is modified in the next step; multiple drying procedures are conducted to control the water content of the final product. The details of the technology are not reported at the request of the manufacturer; only the major production processes necessary for data interpretation are described in Figure 1. There are three identical manufacturing lines located in two workshops. The three lines share a common process path from hydration to mixing operations. The plant operates in three shifts: morning (8am-4pm), afternoon (4pm-12am), and evening (12am-8am). Crew membership in a shift does not change and the shifts rotated every three days. Drying job is an exception where workers from first workshop do not rotate with workers from the second workshop

and all drying workers in second workshop spent most of their time in drying room rather than staying on manufacturing line. Therefore we use “dyring1” and “drying2” to distinguish these workers who stay in workshop 1 and workshop. More detailed description of the jobs is shown by Appendix A.

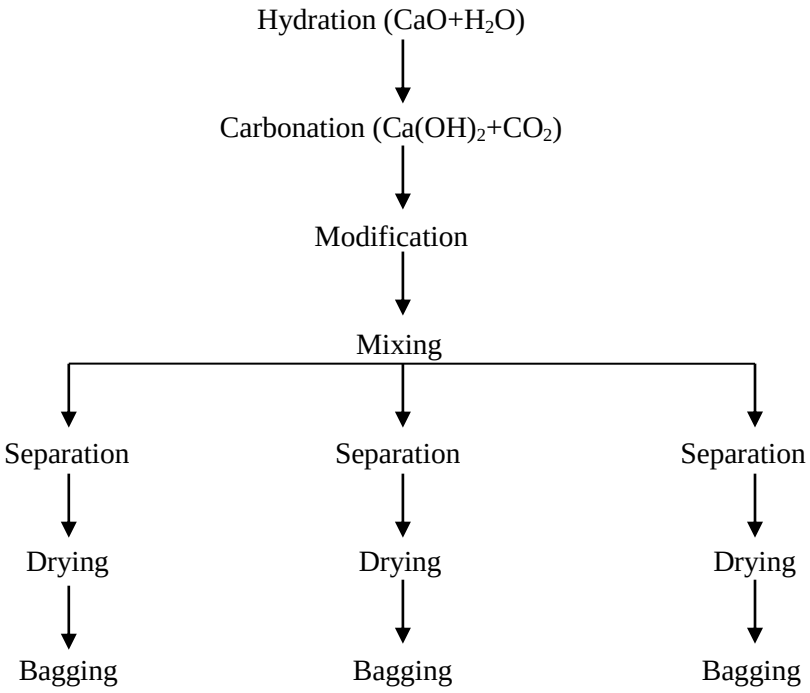

Figure 2.1 Calcium Carbonate Nanoparticle Manufacturing Processes

Methods

Sampling Frame

Different sampling zones were identified prior to sampling on the basis of job title and environmental similarity. A random selection of employees in each zone was then chosen for personal sampling. Area samplings were conducted at the location where workers spent most

of their time for a particular job. Mechanics and electricians work at different locations throughout the factory; therefore no area sampling was conducted for this kind of jobs.

Samples were chosen in a way to encompass nearly all jobs for personal sampling (at the breathing zone) and each manufacturing process for area sampling. For some jobs, employees were reluctant to carry pumps due to noise or inconvenience. This occurred more frequently in 2011 than 2012. Given the financial constraints, estimated concentration of each job is important in determining an overall number of samples to be collected. Jobs thought to have greater and more heterogeneous exposure were sampled more frequently. The number of samples for each job in a given year was presented in Table 2.1.

Sampling Time

Air sampling was conducted during two time periods, December 2011 and July 2012. All sampling activities occurred during day shifts from 8am - 4pm. In 2011, full-shift sampling with an average sampling duration of 6.6 hours was performed whereas in 2012, half-shift sampling with an average sampling duration of 3.1 hours was conducted due to time constraints.

Personal and Area Sampling

Samples were collected on 37-mm polytetrafluoroethylene (PTFE) filters with a pore size of 0.3 μm (SKC) attached to a GS-3 respirable dust cyclones with a 4.0 μm median cut-point at a 2.75 L/min flow rate (SKC). Respirable mass concentration was measured with Buck pumps (VSS-5, A.P. BUCK) in 2011 and Airchek pumps (224-PCXR7, SKC) in 2012 at a flow rate of 2.7-2.8 L/min. Gravimetric analysis was conducted to determine mass

concentration for each filter sample. Personal and area samples were analysed in the same manner.

Microscopy analysis

Additional respirable samples were collected using 37-mm polycarbonate filters with a 0.4 μm pore size (SKC) using identical pumps and cyclones as mass sampling. Sampling time varied between 20-120 minutes depending on the estimated exposure level to ensure adequate particle loading. The samples were coated with palladium and/or gold under vacuum conditions. A random section of each filter was cut and prepared for scanning electron microscope (SEM) analysis (S-4800, HITACHI). The samples collected in 2011 winter were analysed at magnifications from 5000 X to 10000 X. The elemental composition was evaluated by utilizing an energy dispersive X-ray spectrometer (EDX) fitted on the SEM.

Analysis

The geometric mean (GM) and geometric standard deviation (GSD) were computed for respirable mass ($\mu g/m^3$) for each job or process tested. One way ANOVA model was developed in order to understand underlying structure of the data and categorize job. It was assumed that workers who share common occupational experience have the same exposure level; therefore they were classified in the same exposure group. The natural log of mass concentration ($\mu g/m^3$, continuous) was analyzed as the response variable. Both 2011 and 2012 data, and both personal and area samples were built in the model. Variance component was extracted and compared to choose the grouping scheme with maximized between-group

and minimized with-in group variance. Data analysis was performed in the R package version 2.15.1.

Results

Exposure Level

In 2011, respirable mass concentration collected by area sampling ranged from 3.2 to 2304.5 $\mu g/m^3$ with a median exposure of 219.1$\mu g/m^3$. Personal exposures ranged from 20.5 to 3593.3$\mu g/m^3$ with a median exposure of 357.3$\mu g/m^3$. In 2012, for area sampling, the minimum and maximum concentration were 74.9 and 10028.4$\mu g/m^3$, with a median exposure of 256.0$\mu g/m^3$; for personal sampling, the minimum and maximum concentration were 99.6 and 10682.0$\mu g/m^3$, with a median exposure of 361.5$\mu g/m^3$.

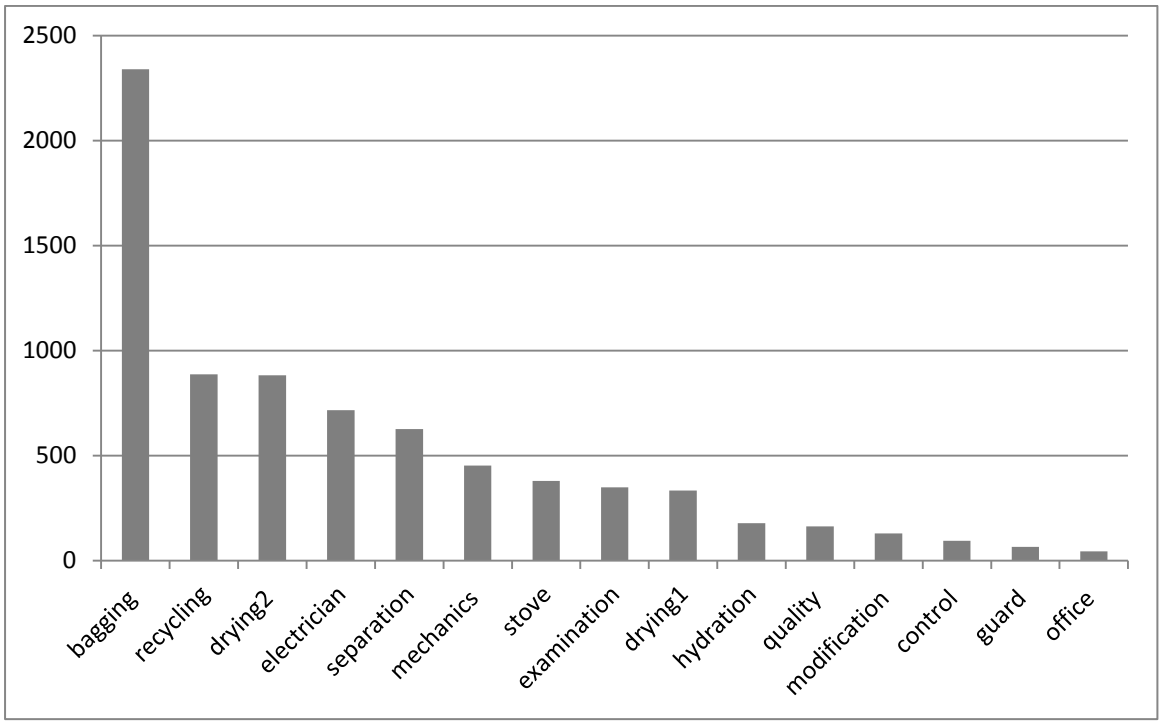

Figure 1.2 Personal and Area, 2011 and 2012 Samples Combined GM of Respirable Mass Concentration (ug/m3) by Job

Since June2012, the factory started to upgrade the manufacturing system by sealing the machines to prevent the release of particles. Our sampling occurred during this period of time. This change of working condition in the factory precludes direct comparison of concentrations sampled from 2011 with those from 2012. Other changed conditions related to seasonal factors might exist as well. Bagging workers have the highest respirable mass among all and the levels of exposure are much greater than other jobs, see Figure 2.2.

The concentrations of personal samples were consistently higher than area samples for job tasks such as bagging, drying and separation. These jobs require constant attention in which workers cannot leave their workstations once the manufacturing process started. Recycling tasks occurred 2-4 hours per 8- hour shift. Workers spend the rest of the shift in the drying machine room; therefore, the environmental concentration of the recycling room is much higher than the concentration collected by personal samples. The carbonation and modification processes are co-located: carbonation workers spent less than 1 hour (usually 30 minutes) per shift at this location and most of time stayed in the control room to monitor the carbonation process on computers. Therefore, we presented the area samples from the control room for carbonation workers in Table 2.1. In contrast, modification workers spent 3 hours or longer per shift at the modification process area. Electricians and mechanics were not assigned to a specific working area because they rotate through the entire factory. Jobs for guards, quality control, administrative and boiler workers are not located inside the factory workshops.

Table 2.1 Summary of Respirable Mass Concentration (GM and GSD, μg/m^3) by Process, Job or Location*

	2011 Personal		2011 Area		2012 Personal		2012 Area	
Job Location	**N**	**GM (GSD)**	**N**	**GM (GSD)**	**N**	**GM (GSD)**	**N**	**GM (GSD)**
Bagging	1	3593.28(NA)	4	697.66(6.93)	4	6023.21(1.72)	2	3195.86(5.04)
Drying1	1	1013.73(NA)			1	246.54(NA)	2	222.92(1.22)
Drying2	1	1509.17(NA)	1	965.10(NA)	2	1010.63(1.27)	2	562.04(1.01)
Separation	4	714.85(4.85)	3	703.30(2.79)	5	664.62(1.56)	2	348.89(8.45)
Hot stove	1	433.18(NA)	1	514.12(NA)	2	320.34(1.04)	1	341.27(NA)
Recycling	2	302.98(2.17)	1	1521.36(NA)	2	1033.41(1.40)	1	3264.69(NA)
Quality Control	1	357.34(NA)	1	52.87(NA)	2	138.82(1.60)	1	315.48(NA)
Modification	1	108.40(NA)	1	30.54(NA)	2	300.70(1.30)	1	122.73(NA)
Control	4	44.69 (1.71)	1	53.96 (NA)	3	152.89(1.13)	3	192.34(2.64)
Hydration			1	146.73(NA)	1	261.63(NA)	1	149.69(NA)
Examination					2	254.79(1.75)	1	656.48(NA)
Drying machine			1	223.76(NA)			1	2648.29(NA)
Mixing			1	159.18(NA)			1	181.37(NA)
Office			2	6.24(2.61)	1	193.53(NA)	2	145.78(1.64)
Mechanics	1	716.49(NA)			3	388.60(3.07)		
Boiler	1	402.64(NA)			1	185.51(NA)		
Electrician					3	716.12(1.63)		
Guard room			1	7.24(NA)	2	172.27(1.40)	1	84.52(NA)

*Samples were taken from both workshops. *Job categories without values were not measured. Mechanics and Electricians did not have a fixed working location; therefore no area samples were taken for these two jobs.

Job classification based on Mass

Jobs were classified into three job categories, high, medium and low mass exposure and the descriptive statistics is provided for each group (see Table 2.2). The grouping scheme relies on the between- and within-group variation (See Figure 2.3 and Table 2.3).

Table 2.2 Summary of the Job Classification Based on Respirable Mass

Grouping	Sample No.	GM (GSD)	Jobs
High	11	2338.6 (3.7)	Bagging
Medium	45	579.0(2.2)	drying2, mechanics, separation, recycling, drying1,examination, hot stove, boiler, electrician
Low	33	97.2(3.1)	quality control, carbonation (control) worker , office, modification, guard, kiln, group leader, pretreat

Table 2.3 Analysis of Variance of the Three Mass Exposure Groups

Source	SS	Df	MS	F	Prob>F
Between Groups	105.3	2	52.6	52.73	<0.001
Within Groups	85.9	86	1.00		

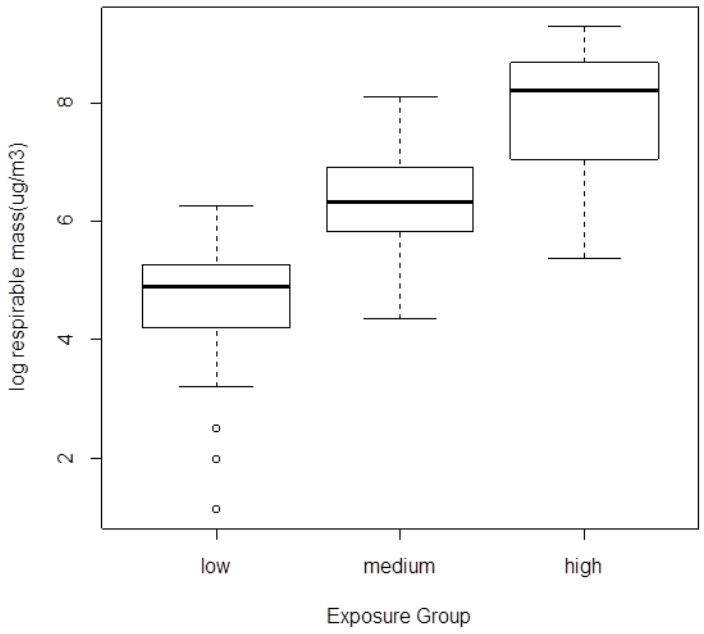

Figure 2.3 Boxplot of Three Mass Exposure Groups

Particle Shape

Microscopy of the samples from 2011 provided evidence that single and agglomerated nanomaterial is emitted during the drying process (Figure 2.4). Compared with the drying process, nanomaterial exposure for the bagging job (Figure 2.5) was in a more agglomerated form than individual particles. This pattern has been seen repeatedly. The particles were cube-like structures with a single particle size in the range of 30-100nm. The agglomerates can be as large as more than 10 um. As determined by SEM-EDX analysis, the particles were purely calcium carbonate. Traces of gold and palladium were due to the coating material (Figure 2.6).

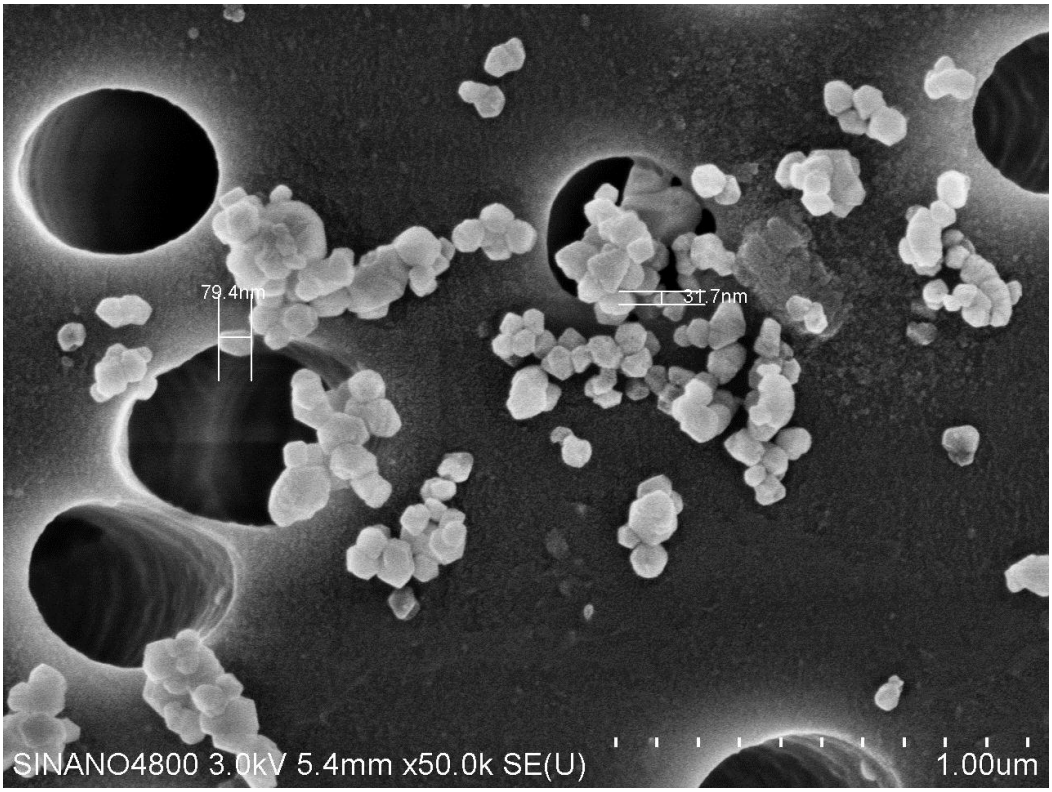

Figure 2.4 SEM of Particles Collected at Drying Process (95 mins, manufacturing line 1)

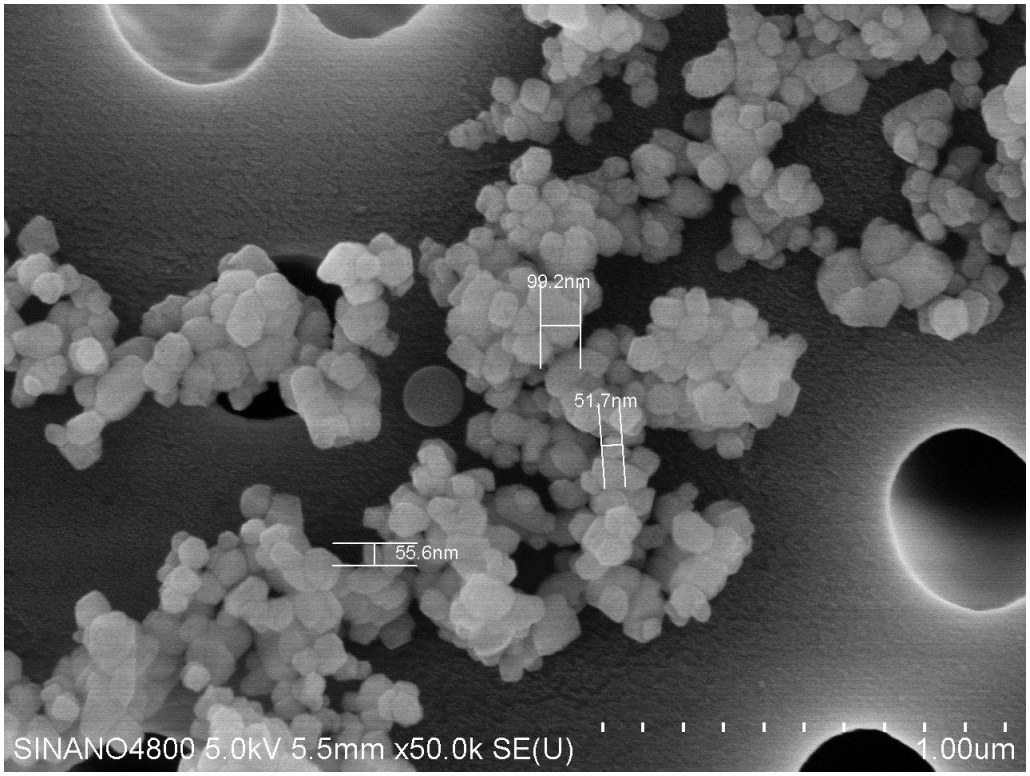

Figure 2.5 SEM of Particles Collected at Bagging Process (30 mins, manufacturing line 1)

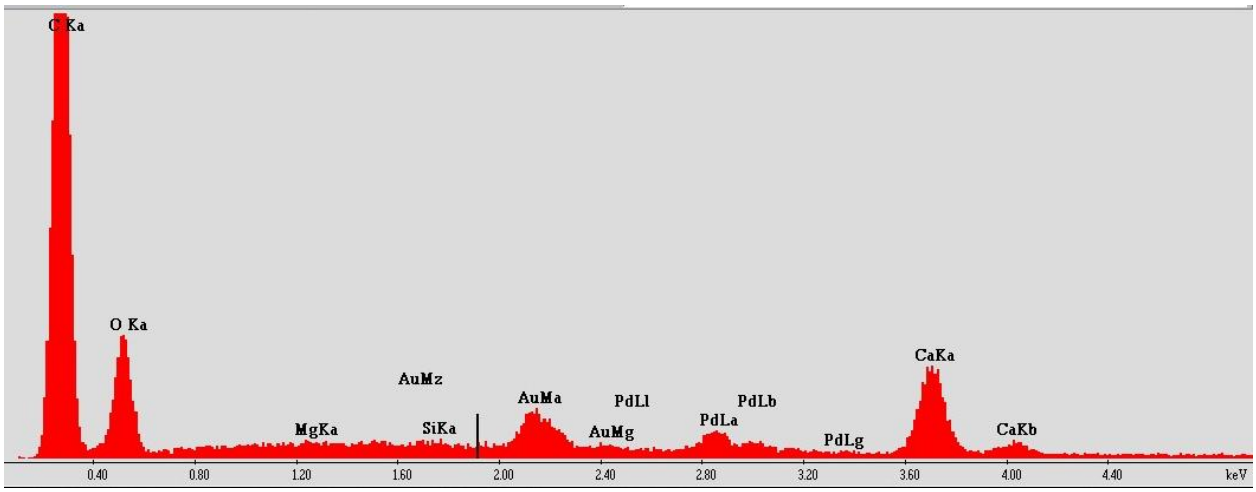

Figure 2.6 Particle Chemical Compositions

Discussion

The American Conference of Governmental Industrial Hygienists (ACGIH) suggests that threshold limit values (TLVs) for insoluable or poorly soluble, not otherwise specified (PNOS) respirable particles should be <3000 μg/m^3. Jobs such as bagging and recycling have potential exposures exceeding the standard, and could possible pose harm to exposed workers. Several previous studies investigated nanomaterial mass-based exposure in research or pilot manufacturing facilities [7-12]. Most of the nanomaterials were produced on a relatively small scale and in a controlled environment. Also, the technology and materials for creating nanoparticles differ, which makes direct comparisons difficult. Lee investigated exposure in seven carbon nanotube workplaces and concluded the exposure concentrations of total suspended particulate matter ranged from 7.8 to 320.8 μg/m^3 for personal samplings and 12.6 to 187.3 μg/m^3 for environmental samplings. A related study found the mass concentration (area and personal samples) for nanosized TiO_2 ranged from 100 to 4990 μg/m^3

whereas for the nanosized silver concentrations ranged from 0.02 to 1.18 μg/m^3 [7, 8]. NIOSH conducted field research on nanoparticles at 12 sites. Among studies where mass concentrations were reported, two had relatively high exposure levels. One occurred during Manganese (3600 ug/m^3) and Silver (6700 ug/m^3) reactor cleanout processes without local exhaust ventilation. For the other, an exposure level of 46000 (Fe) ug/$m^3$was generated inside the spray enclosure when producing silica-iron nanomaterial [11, 12]. Some of these studies reported similar mass concentrations compared to our study; however, all of their samples were collected on open-faced filter cassettes with relatively short sampling duration.

In this study, we utilized a filter-based approach to collect particles for gravimetric and microscopic analysis. Even though samplers could not be employed to distinguish nano-scale and micro-scale particles, the filter-based approach remains one of the most convenient methods for personal sampling. This approach is also useful to collect particles for off-line imaging analysis. We confirmed through the SEM analysis that agglomerates of the manufacturing nanoparticles were found in the environment. It is hypothesized single nanoparticles are more harmful than agglomerated nanoparticles as it has greater potential to penetrate deeper lungs and translocate to different organs. However, currently there is not sufficient evidence to support this. Some toxicological studies found no significant difference of toxicity between nanosized and agglomerates of particles in rat bioassays [13-15]. One thing needs to be emphasized is the agglomerates are made of single nanoparticles in a different state, and they are not equivalent to bulk version of the chemical. In fact, it is possible for these coagulated particles to become deagglomerated inside the body.

Background exposure assessment has been recommended by many nanoparticle guidelines as it is necessary to separate the influence of the incidental particles from those intentionally made. One popular approach is to estimate background concentration prior to work [16-18]. However, this approach is not applicable to our study. The rotational shiftwork runs continuously, 24 hours per day, 7 days per week. It is challenging to measure background levels. Incidental nanoparticles could be generated from a variety of sources, including pumps, combustion, welding operation, and forklift trucks. Even if there were times that the manufacturing processes stopped due to a breakdown or scheduled maintenance, the background activities usually are suspended as well, or in some cases may became more intense (such as welding), and therefore would not be representative of the normal operational background.

There are some limitations to our project that deserve mentioning. Aerosol concentrations in workplaces are rarely uniform or constant. For many jobs and locations, we had a limited number of air samples, which adds uncertainty to our exposure assessment. In addition, the variation between 2011 and 2012 could not be fully characterized and explained. More measurements are needed to better quantify workers' exposures and confirm if a seasonal trend in exposure exists. At the time of sampling, bagging workers wore dual cartridge half face dust masks, and some other workers wore medical masks on a volunteer basis. Additional work would be required to assess the effects of personal protective equipment and the potential for exposure through skin absorption. Most current research including the present study have been focused on exposure via inhalation. A quantitative measurement of dermal exposure might be needed to estimate overall doses of nanoparticle exposure.

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Appendix A

Description of Each Job or Task

Job title	Job description	Location
Office	Administrative workers	Outside workshop in office building
Guard	Employees work in guardshack	Outside workshop
Boiler	Employees work in boiler room, where water is heated	Outside workshop in its own building
Quality control	Employees examine final products	Outside workshop in its own building
Pretreat	Employees crush big stone into smaller pieces	Outside workshop on a wide open ground
Kiln	Employees work at kiln producing quicklime through calcination	Outside workshop on a wide open ground
Hydration	Employees work near hydration pool to create slurry of quicklime	inside workshop 1
Softwater	Employees who are in charge of creating soft water	inside workshop 1
Carbonation	process where calcium carbonate nanoparticles are generated	inside workshop 1
Modification	process where surface area of the particles are modified	inside workshop 1
Mixing	process where modified particles are hydrated	inside workshop 1
Control room	Computer control room where carbonation workers stay	in a room inside workshop 1
Examination	Employees examine semi-final products	In a room connected workshop 1 and 2
Separation	Employee who stay at process where 50% water content is removed	Inside workshop 1 and 2
Drying	Employee who stay at process where the rest of water is removed	Inside workshop 1 and 2
Hot stove	Employees who in charge of hot stove where heated air is generated	Inside workshop 1
Bagging	Employees who pack the particles	in a room inside workshop 1 and 2
Recycling	Employees who recycle the particles in a wet process	in a room inside workshop 2

Chapter 3 Exposure Characterization II

Abstract

In the present study, we characterized nanoparticle airborne exposures in a calcium carbonate manufacturing facility in Shanxi Province PRC. To estimate the potential exposure of workers, real-time environmental monitoring of number concentration and lung deposited surface area concentration was performed in the summer of 2012. Number concentrations in the size range of 20-300 nm were calculated for each process. It was found that three locations where modification, carbonation and mixing processes are located have the highest number concentrations. Number concentration in the size range of 0.02-5 um and respirable mass were uncorrelated (r =0.009). Surface area concentration deposited in the alveolar region was higher than that in the TB region across all sampling processes. Surface area concentration deposited in the TB region was moderately correlated with number concentration in the nano-fraction (r= 0.66). Size number distributions for all jobs were presented.

Background

When assessing a monodisperse system of particles, a single exposure metric might be sufficient to describe the magnitude of exposure. The relationship between mass, surface area and number concentration of the particles becomes a matter of multiplying or dividing a geometric dimension, which is rather straightforward mathematically. However when particles of many sizes occur together, particularly where the spectrum of size crosses a wide range, the connection among the three becomes ambiguous. One relevant question is "which exposure metric is more closely associated with a health outcome concerned." Mass concentration is the most widely used exposure index. However, the inhalable and respirable

dust possibly can be negligible for nanoparticles that are created in a relatively controlled environment [1]. Therefore mass alone is insufficient to portray the exposure profile of nanoparticles. In contrast, particle number is largely determined by small particles in a natural environment. The divergence of counts between microscopic and larger particles can be further amplified in an engineering nanoparticle facility. Quantification of the number concentration along with an expanded size range counts including the nanoscale, in theory, provides an opportunity for a more comprehensive exposure assessment, as investigators could possibly measure number concentration at any size range to facilitate their research needs. Acknowledged challenges relate to variability in the exposure assessment equipment. In the past, studies to evaluate exposure have utilized sophisticated, often bulky and costly instruments that are not suitable for routine monitoring[2-4]. In addition, from an epidemiologic perspective, it is desirable to work with one integrated exposure/dose metric, rather than a size distribution. Leading toxicological experts contend that the inherent large surface area of nanoparticles is an essential property of nanoparticles, and therefore is an important extric[5-7]. Due to the limited volume of research and scarce data available on this topic area, it remains unclear as to the most appropriate exposure and dose metric for the endpoint of interest.

Introduction

There is an urgent need to identify reliable and standard monitoring devices and measurement strategies for nanomaterials. The Nanoparticle Emission Assessment Technique (NEAT) developed by NIOSH has been tested for exposure evaluation in a number of field studies. The approach used by NEAT combines portable on-line particle counters with filer-based air sampling and microscopic analysis [8-9]. NEAT has been put forward as a feasible and

effective method for nanomaterial detection and quantification [10]. The exposure assessment conducted of our study followed similar procedures, by monitoring both number and mass concentrations for each job and work task. An optical particle counting instrument facilitated size distribution characterization of the particles, while the size, shape, agglomeration and elemental composition were assessed by SEM. We also measured the lung deposited surface area concentration by using a direct-reading instrument. The purpose of this chapter is to: 1) describe the environmental exposure of each process by number and surface area concentrations; 2) construct potential job-based exposure profile for each job/task in order for the health implication; and 3) promote understanding of the metrics suitable for nanoparticles exposure research.

Methods

Airborne particle number concentrations were measured during 2012 summer with two direct reading instruments, Optical Particle Counter (OPC, Model 8220, TSI) and Ultrafine Particle Counter (UPC, Model 8525, TSI). The OPC provides differential number concentration in 6 size channels: 0.3-0.5 μm, 0.5-1 μm, 1-3 μm, 3-5 μm, 5-10 μm, and >10 μm. The device was programed to record 10-second average number concentration with a sampling flow rate of 2.8L/min. According to the manual, the OPC measures the particles in 0.3-0.5 μm size range at a 50% counting efficiency and the counting efficiency reaches 100% by size of 0.45 μm. The UPC measures total number concentration in the size range of 0.02 to 1 μm. This instrument was programed to record 10-second average number concentration with a sampling flow rate of 100 cm^3/min. Measurements of lung deposited surface area were performed with an on-line Nanoparticle Aerosol Monitor 9000 (NAM, AeroTrak 9000, TSI). This instrument provides the surface area equivalent dose of particles in the size range of 10

to 1000 nanometers and was programed to record 10-second average surface concentration with a sampling flow rate of 2.5L/min. The tubes connected to the three instruments were placed side-by-side close to the breathing zone region at each job location and sampled simultaneously for 30 minutes. The deposition mode of NAM can only be set to TB (Tracheobronchial) or AL (alveolar), therefore TB and AL mode were each sampled for 15 mins consecutively. Only one sample of number and surface area concentration was collected for each location due to time constraint.

Analysis

To adjust for the counting efficiency of OPC, the number concentration in the size range between 0.3-0.5 μm was calculated from the equation (3.1). The estimating procedure for the constant of 0.8125 is provided in appendix A.

$$c_{0.3-0.5} *= \frac{C_{0.3-0.5\ measuerd}}{0.8125} \tag{3.1}$$

Due to limitations of the instruments, we were unable to obtain the number concentration in the nano range directly. By subtracting or adding the numbers measured by the two instruments, we estimated the number concentration of particles in different size ranges for each process, including the surrogate nano fraction (0.02-0.3 μm) and surrogate respirable fraction (0.02-5 μm). Equations (3.2 -3.4) were used to calculate number concentration in various size ranges, each for 10 seconds. These numbers were averaged over the entire sampling duration (30 minutes).

$$c_{0.02-0.3} = c_{0.02-1} - c_{0.3-0.5} * - c_{0.5-1} \tag{3.2}$$

$$c_{0.02-5} = c_{0.02-1} + c_{1-3} + c_{3-5} \tag{3.3}$$

$$c_{5-25} = c_{5-10} + c_{10-25} \tag{3.4}$$

The deposited surface area concentrations measured by the NAM (AeroTrak 9000) over the monitoring period was time-averaged. Pearson's correlations were computed between number concentration in various ranges and surface area deposited in TB and Alveolar regions.

The OPC divided the particle sizes above the 300 nm range into discrete intervals. However, the choice of the intervals is somewhat arbitrary. Also, the structural information of aerosol size inside each bin is lost. To avoid such complications, one could define a size distribution function $n_N(Dp)$ (um^{-1} m^{-3}) as the number concentration (m^{-3}) of particles having diameters in the range Dp to Dp+dDp. Then the total number of particles is just

$$N=\int_0^\infty n_N(Dp)\, dDp \qquad (m^{-3})$$

By employing a function $n_N(Dp)$, we can assume the number concentration is a continuous function of the diameter. For each job, we created such a size distribution. Multiple algorithms were fitted to the size distribution data to generate functions using diameter as an independent variable and normalized number concentration as a dependent variable. Goodness-of-fit statistics was used for comparison to select the best fitted model for each job. Model selection and data processing was performed in MATLAB (MathWorks, Inc) version 2012a.

Results

Number concentration

A summary of the number concentration for particles in different size range including nanoscale between 20 nm and 300nm is shown in Table 3.1. The highest number

concentration in the nanoscale occurred at the modification process, which is almost 10 times as high as the background. Carbonation, modification and mixing were the three processes where the nanoparticles were immediately created, which had the top three nanoscale number concentrations. The nanoparticles were thought to be emitted from the modification process, as the number concentration was dramatically larger than that measured in the other locations. The carbonation process is co-located with modification process and the mixing process is relatively close to the modification process. It is possible that some elevated exposure levels in these processes were due to transport of particles from modification process. The bagging and drying machine room in a second workshop generated the highest number concentration of larger particles. For each workshop, bagging had a greater exposure for larger particles than the drying and separation processes. It can be noted that number concentration of nanoparticles becomes smaller, whereas number concentration of larger particles becomes higher along with progression along the manufacturing processes. This suggests a possible increasing agglomeration as we progress down the manufacturing line from the process step where the nanoparticles are created.

Particles in the nano fraction (0.02-0.3 um) dominate the number concentration in the size range of 0.02-1um and 0.02-5 um. In fact, there are too few particles in the size range of 1-5 um compared to the nanoscaled particles, which leads to similarity of the concentration in 0.02-1 um and 0.02-5 um. It is found there were three levels of nanoparticle emission. The highest exposure of nanoparticles occurred at the modification process, which was identified to be the primary emission point of origin. A second level of exposure happened at very specific operations or with certain work practice. Such a release of nanoparticles tended to be intermittent and presented as a temporary peak, such as shown by separation and drying in Figure 3.1. In addition, nanoparticles could originate from other work activities such as

combustion, forklift emissions and welding. The concentration of nanoparticles generated from such an emission source was relatively low compared to first and second level exposure (data not shown in the graph). A spatial pattern was observed, in that the number concentration in 0.02 -0.3 size range for drying, separation, and bagging where the exposure level was relatively constant varied with distance to the emission source, modification.

Table 3.1 Summary of 30-Min-Averaged Number Concentration in Four Size Ranges for Each Job Location and Fraction of Number Concentration in 0.02-0.3 um Size Range to Number Concentration in 0.02-5um Size Range

Sampling Location	**C0.02-0.3** 10^10 particles/m^3	**C0.02-1** 10^10 particles/m^3	**C0.02-5** 10^10 particles/m^3	**C5-25** 10^6 particles/m^3	**C0.02-0.3 / C0.02-5**
Modification	7.9	8.0	8.0	0.1	98.4%
Bagging_2_1*	3.6	3.6	3.6	15.2	98.3%
Mixing	2.8	3.0	3.0	0.3	95.6%
Carbonation	2.7	2.9	2.9	0.2	96.1%
Separation_2_1	2.7	2.7	2.7	11.7	98.2%
Drying_2	2.4	2.5	2.5	10.7	96.0%
Drying_1	1.9	1.9	1.9	3.4	97.4%
Examination	1.8	1.9	1.9	6.5	94.7%
Separation_1_1	1.5	1.6	1.6	0.9	93.1%
Control room	1.4	1.5	1.5	0.6	94.7%
Bagging_1 _1	1.4	1.4	1.4	9.4	99.3%
Quality control	1.3	1.4	1.4	0.5	92.9%
Office	1.3	1.4	1.4	0.3	91.3%
Hydration	0.9	1.0	1.0	1.4	89.7%
Ambient	0.8	0.9	0.9	0.4	86.6%

*The first number represents workshop, the second number represents manufacturing line.

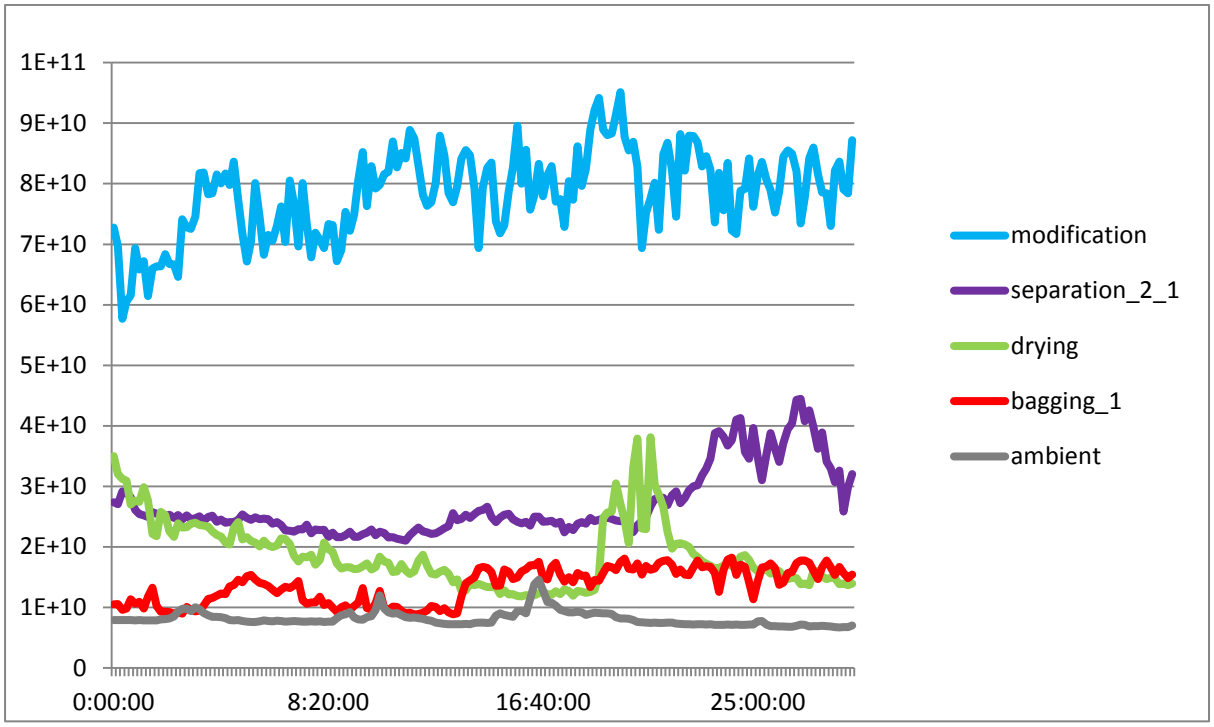

Figure 3.1 Time Series of Number Concentration between 20 and 300nm ($1/m^3$)

Surface Area Concentration

Surface area deposited in the Alveolar region is consistently higher than in the TB regions for all sampling locations. The distributions of the two surface area concentrations are not identical, as shown by Figure 3.2, due to the variation of the size number distribution among jobs. However, they are correlated with a correlation coefficient of 0.87.

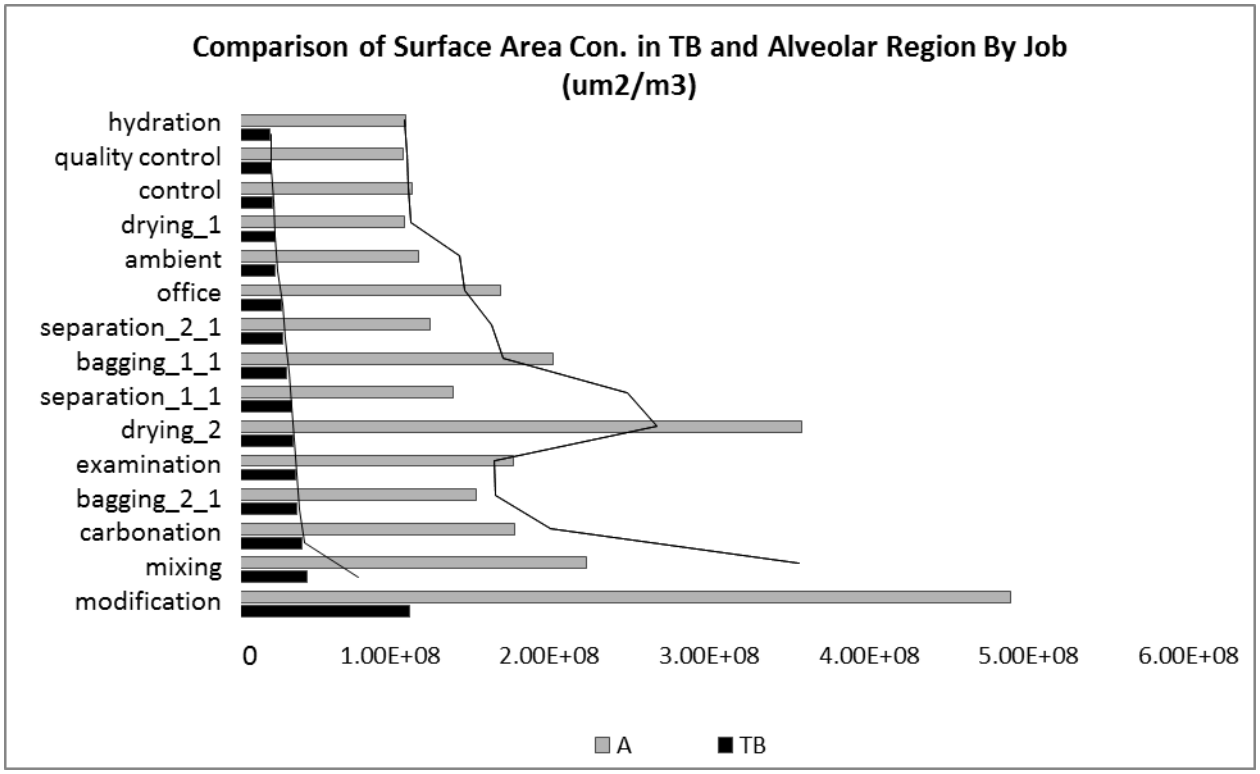

Figure 3.2 Comparison of Lung Deposited Surface Area Concentrations in the TB and Alveolar by Job with Trend Line Showing Two Surface Areas Do Not Agree Entirely. The Figure Was Presented by Ranking of Relative Magnitude According to Surface Area Deposited in the TB Region

Number concentration vs Mass Concentration

There is no linear relationship between respirable mass and particle number concentration in nano-fraction (r =0.0183), nor is there a correlation between respirable mass and number concentration in the respirable fraction (r =0.0090). The correlation coefficient of the respirable mass and number concentration in the range of 5-25 um is 0.9078, referred to Table 3.2 It is also illustrated by Figure 3.3 and 3.4 that number concentration in 0.02-0.3 um size range does not correspond to respirable mass

Table 3.2 Comparison of Number Concentrations with GM of 2012 Area Respirable Mass

Sampling location	**C0.02-0.3** 10^10 particles/m^3	**C0.02-0.3** 10^10 particles/m^3	**C5-25** 10^6 particles/m^3	**respirable mass** (ug/m3)
Modification	7.9	8.0	0.1	122.7
Mixing	2.8	3.0	0.3	181.4
Bagging*	2.5	2.5	12.3	3195.9
Drying_2	2.4	2.5	10.7	2648.3
Separation*	2.1	2.2	6.3	348.9
Drying_1	1.9	1.9	3.4	354.0
Examination	1.8	1.9	6.5	656.5
Control room	1.4	1.5	0.6	192.3
Quality control	1.3	1.4	0.5	315.5
Office	1.3	1.4	0.3	145.8
Hydration	0.9	1.0	1.4	149.7
Ambient	0.8	0.9	0.4	84.5

*average of the both workshop data

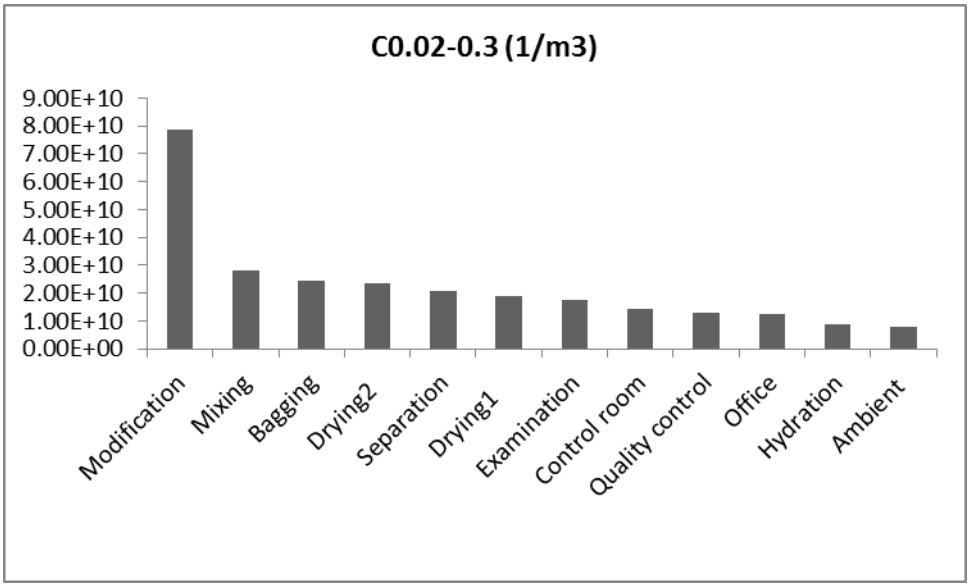

Figure 3.3 Number Concentration (1/m3) in Nano Fraction by Job in 2012

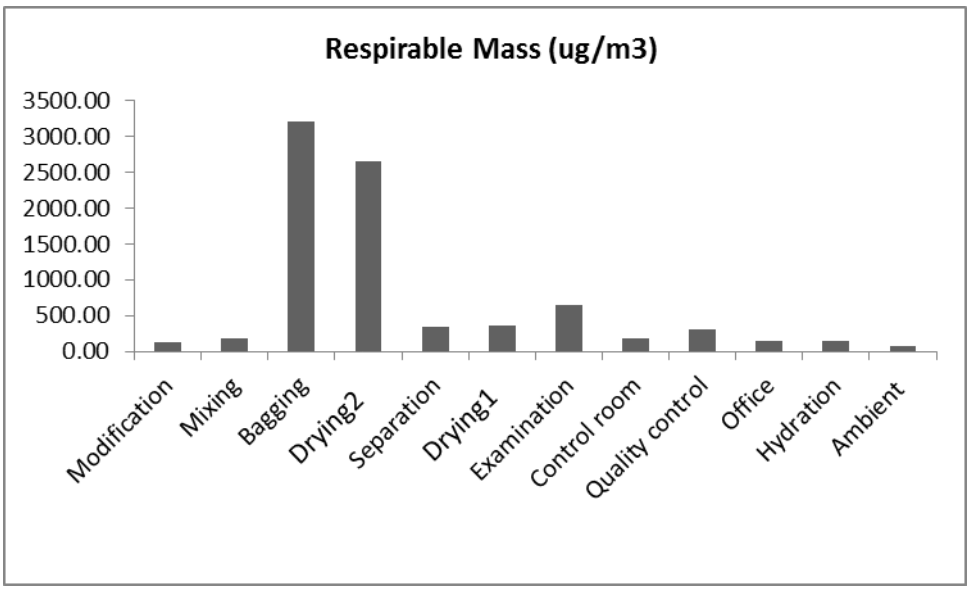

Figure 3.4 Environmental Respirable Mass Concentration (ug/m3) by Job in 2012

Number concentration vs Surface Area concentration

The OPC and UPC monitored number concentrations for 30- min duration and the lung deposited surface area concentrations in TB and Alveolar mode could not be monitored at the same time because the device was set to measure TB region for the first 15 mins and set to alveolar region for the second 15 mins. When we analysed the data, measurements of

number concentrations were split into two comparable15-min intervals, referred to Figure 3.5. The data are summarized in Table 3.3, with the first row of each job representing the first 15-min segment and the second row showing the second 15-min segment.

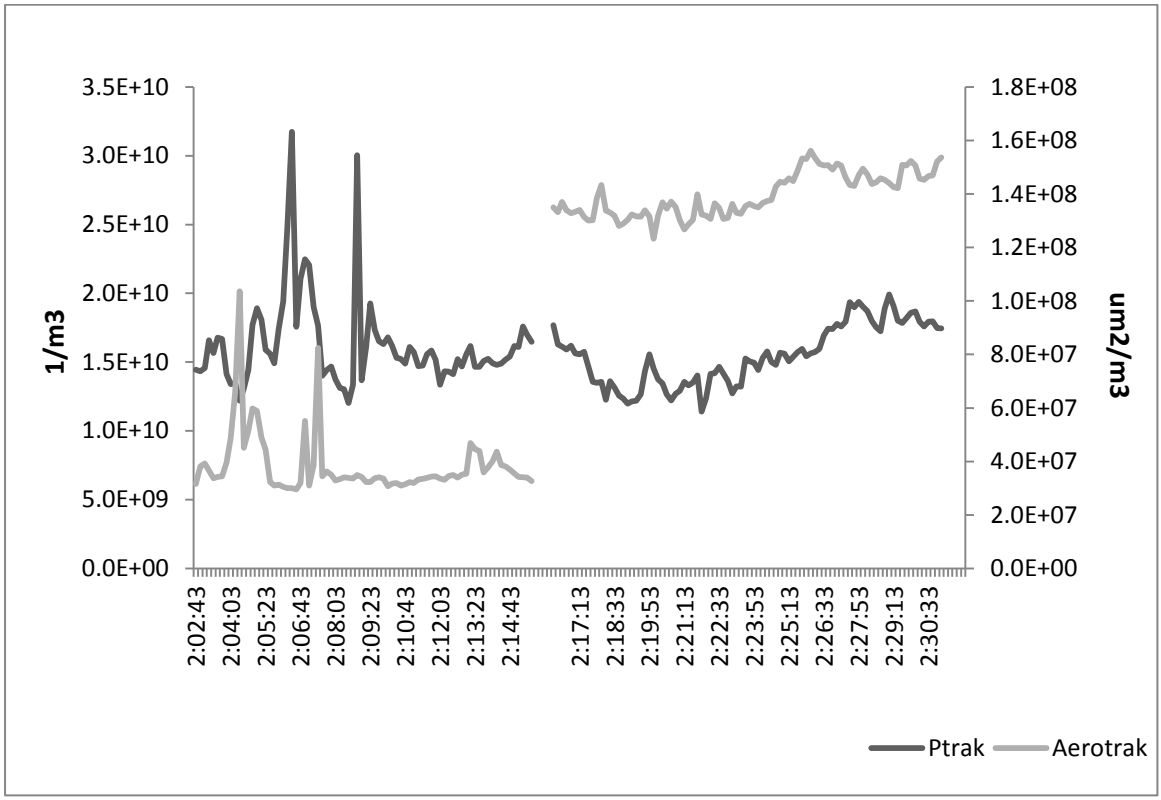

Figure 3.5 Time Series of Number Concentration Measured by Ptrak and Surface Area Concentration Measured by Aerotrak 9000 at Separation Process in Workshop 1

Table 3.3 Comparison of 15-Min-Averaged Surface Area Concentrations in the TB or the Alveolar Region with Respective 15-Min-Averaged Number Concentration

Sampling Location	TB/A 10^8 um^2/m^3	C0.02-0.3 10^10 particles/m^3	C0.02-1 10^10 particles/m^3	C1-5 10^6 particles/m^3	C5-25 10^6 particles/m^3
Modification	1.1	7.5	7.6	6.7	0.1
	4.9	8.2	8.3	6.8	0.1
Bagging_1_1	0.4	1.1	1.1	32.2	13.8
	2.0	1.6	1.6	19.7	6.0
Bagging_2_1	0.4	3.8	3.8	65.8	19.7
	1.5	3.3	3.4	44.6	10.5
Mixing	0.5	2.5	2.6	6.4	0.2
	2.2	3.1	3.2	7.1	0.4
Carbonation	0.4	3.0	3.2	4.9	0.3
	1.8	2.5	2.6	4.6	0.2
Drying_2	0.4	1.9	2.0	49.8	9.6
	3.6	2.7	2.8	66.2	11.7
Separation_1_1	0.4	1.5	1.6	7.0	0.8
	1.4	1.4	1.6	7.9	1.0
Examination	0.4	1.7	1.8	46.7	5.9
	1.8	1.8	1.9	55.4	7.2
Drying_1	0.3	2.1	2.1	14.8	3.5
	1.1	1.7	1.8	12.8	3.2
Separation_2_1	0.3	2.4	2.4	93.8	15.3
	1.3	3.0	3.1	51.8	8.6
Control	0.3	1.4	1.5	9.1	0.6
	1.1	1.5	1.6	7.6	0.6
Office	0.3	1.1	1.2	16.8	0.3
	1.7	1.4	1.5	17.3	0.3
Quality control	0.3	1.3	1.4	12.1	0.5
	1.1	1.3	1.4	9.1	0.5
Hydration	0.3	1.0	1.1	8.6	1.2
	1.1	0.8	0.9	9.6	1.7
Ambient	0.3	0.8	1.0	15.0	0.4
	1.2	0.8	0.9	14.6	0.4

Correlation analyses were conducted between surface area concentration and number concentration in different size ranges. As indicated in Table 3.4, surface area concentration deposited in the TB region are moderately correlated with number concentrations in the nano-sized fraction (r=0.66), but not with number concentration s of the larger particles (r = [0.17-0.24]). The correlation between surface area in the alveolar region and number concentration is relatively constant across different size ranges (≈0.5). Pairwise relationship matrix between surface area concentrations in the alveolar and TB region, number concentration in various size ranges and respirable mass was presented in Appendix B.

Table 3.4 Correlation Analysis between Surface Area Concentrations and Number Concentration*

Correlation	C0.02-0.3	C0.02-1	C1-C5	C5-C25	C1-25
TB	0.66	0.67	0.17	0.24	0.19
A	0.46	0.47	0.52	0.51	0.53

* Modification job was excluded from the analysis due to its high influence on correlation.

Number Size Distribution

Number size distribution presents a variation across processes. However, some manufacturing activities produce quite similar pattern of particle size distribution. For example, the shape of the distribution is almost identical between quality control and separation in workshop 1. The entire distribution of quality control seems shifted to a lower level compared to separation task. It is estimated from the mathematical models shown by Figure 3.6 that nanoparticle counts between 0.02-0.3 um dominate the number concentrations of particles between 0.02-25 um. In addition, number concentration in size range of 0.02-0.1 um (real nano fraction) make up a great proportion of that in 0.02-0.3 um range. Therefore the number concentration in 0.02-0.3 um has the potential to be a good proxy for the number concentration between 1-100 nm.

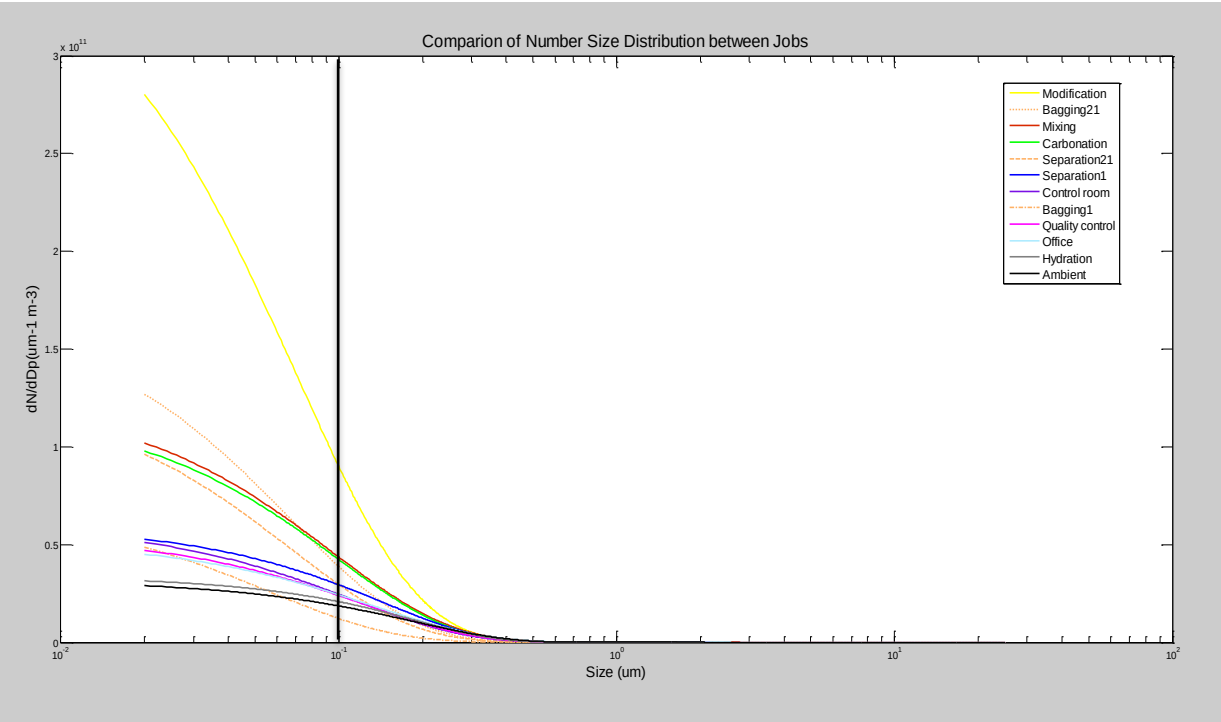

Figure 3.6 Number Size Distributions by Jobs with the X-axis in Log10 Scale

Job classification based on Number and Surface Area

Three types of jobs are presented in the factory: 1) jobs with almost all tasks accomplished in one location such as administrative or separation; 2) jobs where workers spend time at 2-3 fixed locations, such as quality control and carbonation; and 3) jobs where the tasks of the job vary greatly from day to day and the exposure level fluctuates such as electricians and mechanics. Personal sampling was conducted for mass concentrations, which made exposure characterization of the type 2 and type 3 jobs possible. In contrast, only area sampling of the number and surface area concentrations were performed for each process. Therefore, it is sensible to calculate the job-based number and surface area concentrations of a worker over all the tasks within that job. For example, modification workers spent 30% time at the modification process, 40% time at mixing and 30% time in the control room. The exposure profile for this kind of job (2nd type) should be computed on the basis of the weight of time

spent at each process location. (See Appendix C for details.) However, the exposure characterization of type 3 jobs remains a challenge. Exposure categorization is based on the ranking of job-based number and surface area exposure level referred to Table 3.5 for illustration of grouping. Number concentration is ranked based on their magnitude with those fall in the 30 billion range rank the highest, those in the 20 billion range in the next and so on. Modification has significantly higher number and surface area concentration compared to the other jobs, therefore it is logical to set itself as one exposure group. However, there are not enough modification workers to conduct analysis for epidemiology study; therefore modification was collapsed with the second job category. A comparison of job classification based on different exposure metrics is shown in Table 3.6.

Table 3.5 Presentation of Grouping for Number Concentration

Job	C0.02-0.3 10^10 particles/m^3	Groups
modification	3.9	1
bagging	2.5	2
drying2	2.4	2
separation	2.1	2
recycling	2.0	2
drying1	1.9	3
examination	1.8	3
welding	1.8	3
control	1.4	3
quality control	1.3	3
softwater	1.3	3
office	1.3	3
hydration	0.9	4
guard	0.8	4
kiln	0.8	4

Table 3.6 Job Classification Based on Different Exposure Metrics

Grouping	Number	Surface Area_TB	Surface Area_AL	Mass
High	modification, bagging, drying2, separation, recycling	modification, bagging, examination,	modification, drying2, recycling	bagging
Medium	drying1, quality, softwater, examination, welding, control, office	drying2, softwater, recycling, office, separation, welding	examination, office, welding, separation, bagging, softwater	drying1, drying2, mechanics, examination, welding, separation, recycling, hot stove, boiler, electrician
Low	hydration, guard, kiln, pretreat	hydration, guard, quality, kiln, Pretreat, drying1, control	hydration, guard, quality, kiln, pretreat, drying1, control	hydration, guard, kiln, pretreat, quality, softwater, control, office, modification

Discussion

The sampling and analytic methodology presented shows promise for characterizing nanoparticle exposures for a variety of operational processes. The highest number concentration of nanoparticles occurred in the modification process, which was identified as the primary emission origin for nanoparticles. A second level of exposure happened at very specific operations or with certain work practices. The spike shown in Figure 3.5 represents a form of nanoparticle's release. In addition, nanoparticles may originate from other work activities. The measured nanoparticle concentrations stem from a complex mixture of the multiple emission sources. A spatial pattern was observed that the number concentration in

nano-size range varied with distance to the emission source. The elevated exposure of mixing and carbonation process was likely due to transport of particles through the atmosphere It can be noted that the number concentrations of nanoparticles occur especially elevated at early stages of manufacturing process, and that larger particles are more likely to be generated from later processes due to agglomeration.

Nanoparticle counts dominate in the workplace, which consists of 90-98% of the respirable number concentration. It would not make a significant difference in terms of job ranking, to choose an exposure metric based on number concentration in either the 0.02-0.3, 0.02-1, or 0.02-5 um size class. Alternatively, there is a strong relationship between number concentration in the 5-25um range and respirable mass concentration; however, no such correlation was found between number concentrations in the nanoscale 0.02-0.3 um size class and the respirable mass. The deposited surface area in the TB and alveolar region is moderately correlated with number concentration in nanoscale.

To our knowledge, there is no standard methodology for exposure assessment of nanomaterials. The challenges are due to limited exposure data and a lack of suitable sampling instruments. There is an urgent need to identify a practical strategy to effectively evaluate workplace nanoparticle exposure. The primary objective of this research is generating quantitative exposure information to serve the needs for epidemiological study. In this study, we demonstrate that respirable mass concentration is not always in close agreement with number concentration in nanoscale because of the negligible contribution of nanoparticles to the total mass. Therefore, when monitoring nanoparticles in an engineered nanomaterial facility, respirable mass concentration alone does not provide a complete picture

of exposure. A comprehensive exposure assessment and characterization of nanoparticles needs to be conducted in both bulk- and nano-form. Despite efforts that were made to place the monitoring instruments in close proximity to the workers' routine job and to apply activity weighted adjustment, these process-and job-based concentrations should not be considered sufficiently representative or equivalent to full-shift personal exposures. Novel sensing technologies integrated with handy devices might offer promises to measure the personal number and surface area exposure in future. Direct reading instruments provide an opportunity to quickly identify emission sources, and they are portable and relatively convenient to use. However, they currently are unable for distinguishing incidental nanoparticles from engineered nanoparticles. It is crucial to have off-line analysis as a supplement for the direct reading devices to provide a complete characterization. SEM analysis revealed that a mixture of both single and agglomerated nanoparticles are presented in the air, depending on the stage of the work process It is possible that agglomerates may contribute to a great number of larger particles detected Further measurements are needed to clarify the reliability of the direct reading instruments when responding to agglomerates and aggregates. As shown by Figure 3.5, the Aerotrak 9000 seems a little more sensitive and respond more quickly to the change of background concentration than Ptrak.

Another limitation is the short amount of time that was available to sample in the factory. It is possible that considerable variability occurred in number and surface area concentrations between runs and days. Unfortunately, our limited data could not capture much information on changes in nanoparticle levels due to varying manufacturing activities, ventilation and ambient air (background) concentrations. This also created uncertainty in the exposure

categorization. Further measurements would be necessary to confirm our exposure assessment results and to assess the potential for misclassification.

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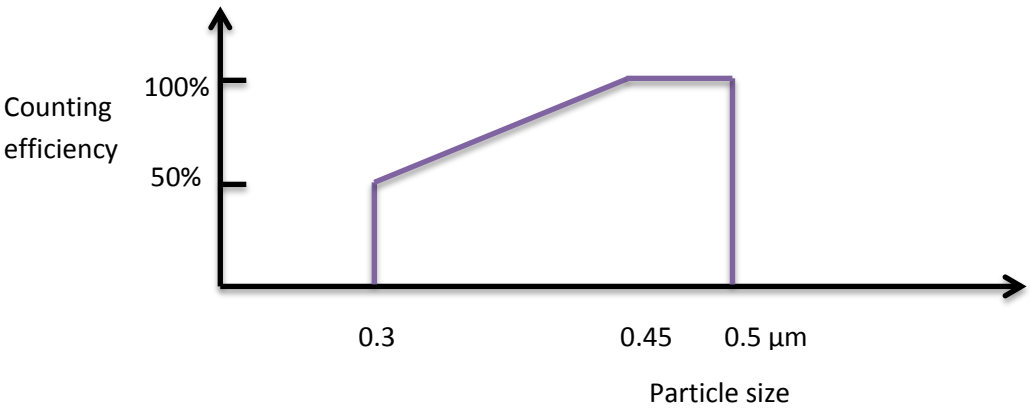
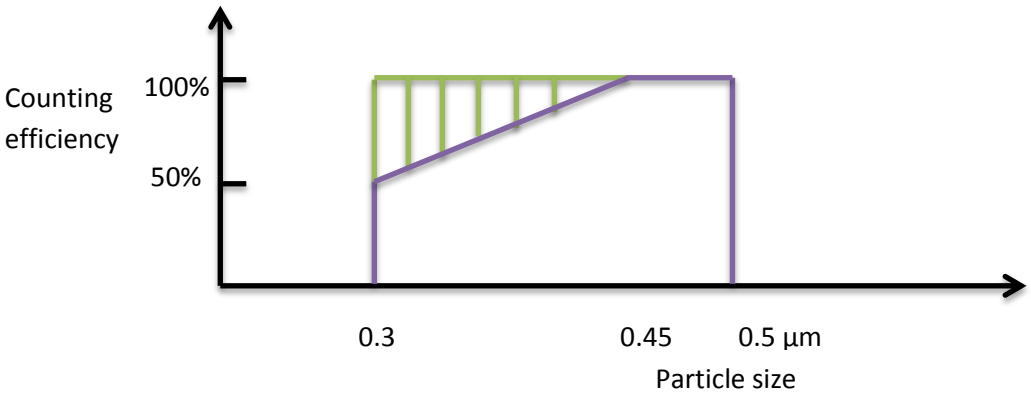
Appendix A



According to the manual, the OPC measures the 0.3-0.5 size range at a 50% counting efficiency and the counting efficiency reaches 100% by size of 0.45 µm. Assuming the increase of counting efficiency from 0.3 to 0.45 µm follows a linear trend, the total area of the figure above could be used to describe the number concentration "X" in the size range of 0.3-0.5 µm measured by the OPC.



However, what we are interested in is the number concentration if the counting efficiency were 100 across the entire interval. Assuming the particles were distributed evenly within this bin size, the adjusted number concentration could be calculated by the following equations

Total area of the rectangle =(0.5-0.3) *100% = 0.2

Total area of the triangle (shadow area) =50 %*(0.45-0.35)*0.5= 0.025

Percentage of non-shadow area included as part of total area= (0.2-0.025)/0.2 = 0.875

Adjusted number Con. = X / 0.875

Appendix B

Correlation analysis between surface area concentration and number concentrations with all data

	C0.02-0.3	C0.02-1	C1-C5	C5-C25	C1-25	C0.02-5	C0.02-25
TB	0.930	0.933	-0.128	-0.103	-0.126	0.933	0.933
A	0.83	0.84	0.15	0.12	0.146	0.84	0.84

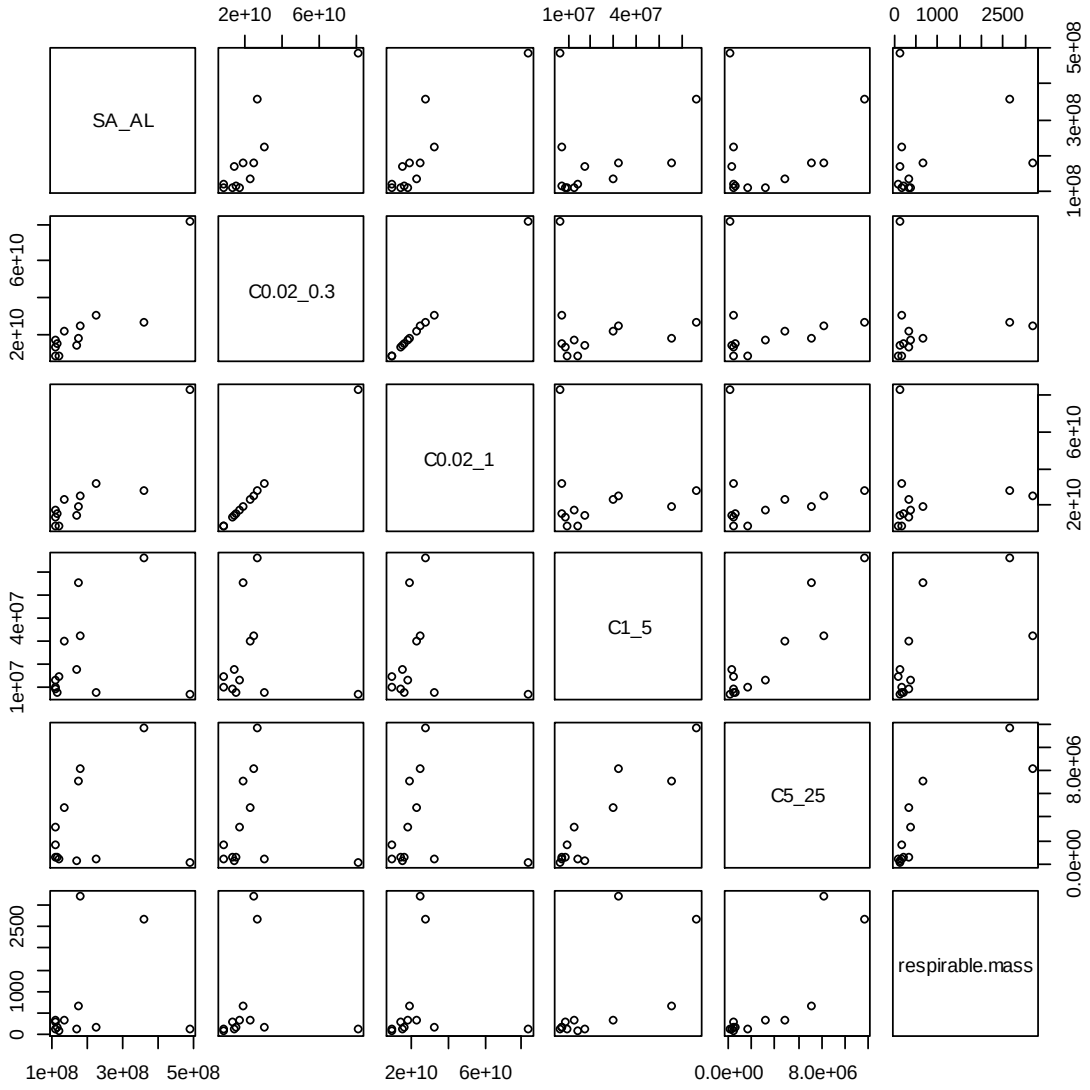


Figure 1 Pairwise relationship matrix between surface area concentration in the AL region, number concentration in various size ranges and respirable mass with all data

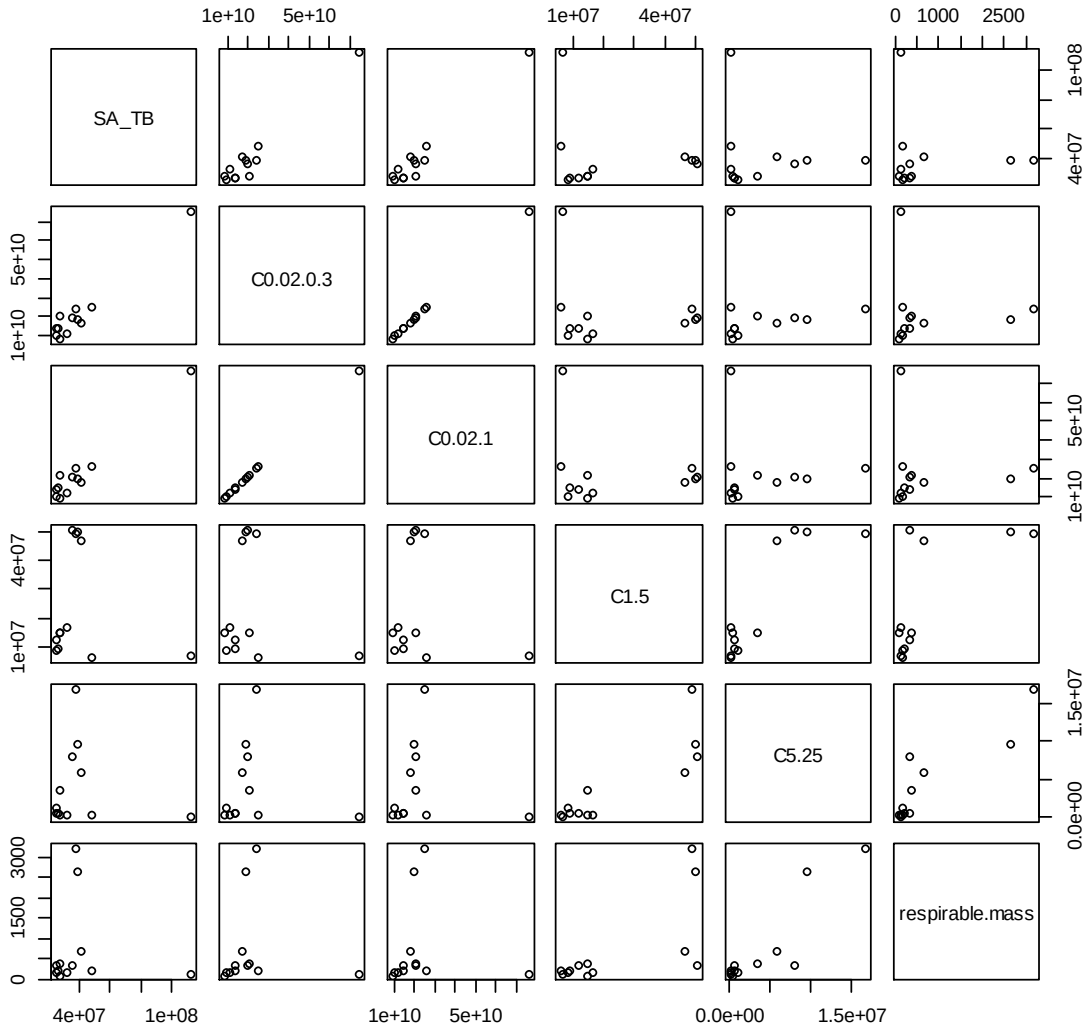


Figure 2 Pairwise relationship matrix between surface area concentration in the TB region, number concentration in various size ranges and respirable mass with all data

Correlation analysis between surface area concentration and number concentrations without modification job

	C0.02-0.3	C0.02-1	C1-C5	C5-C25	C1-25	C0.02-5	C0.02-25
TB	0.662	0.673	0.174	0.237	0.191	0.673	0.673
A	0.463	0.474	0.523	0.505	0.525	0.475	0.475

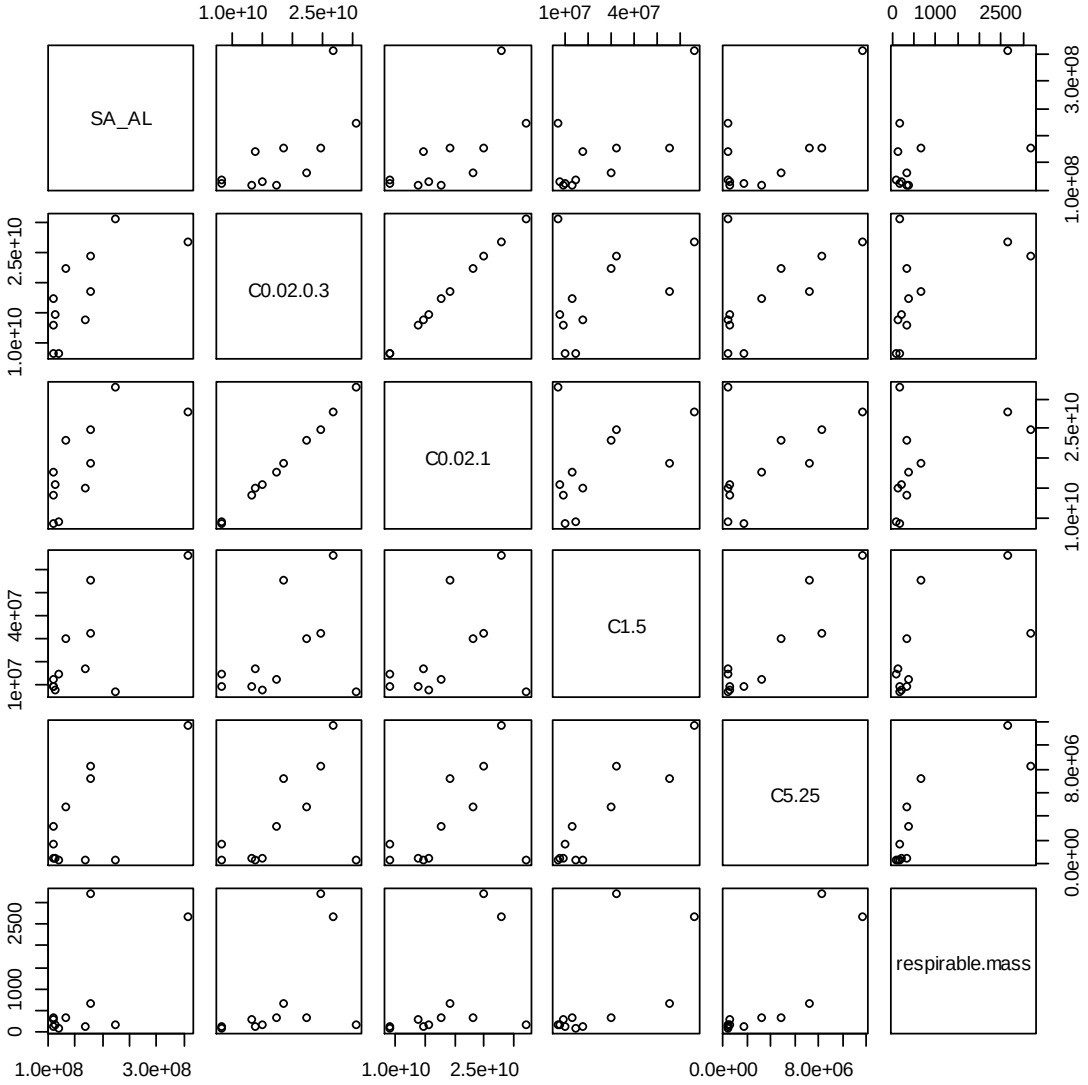


Figure 3 Pairwise relationship matrix between surface area concentration in the AL region, number concentration in various size ranges and respirable mass excluding modification job

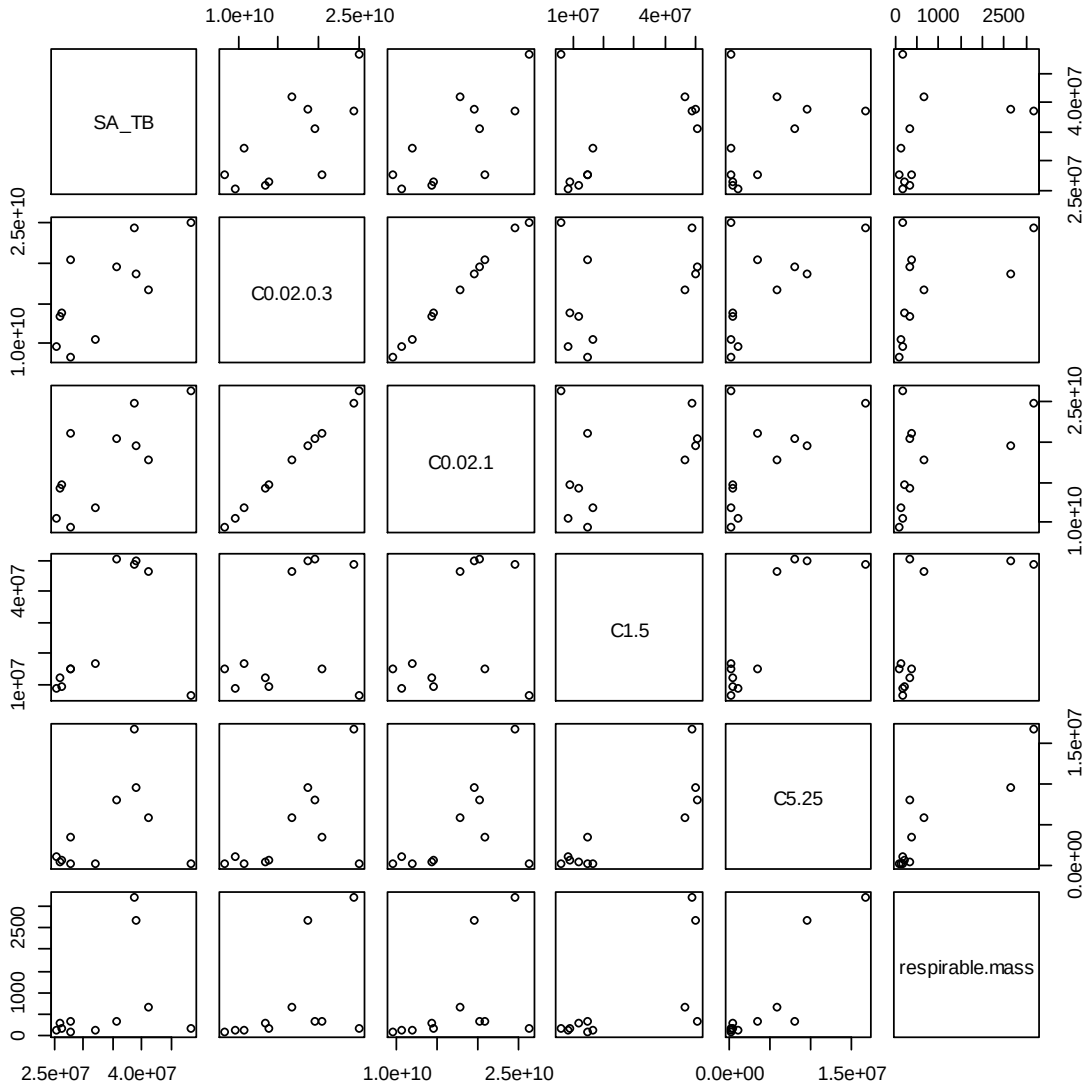


Figure 4 Pairwise relationship matrix between surface area concentration in the TB region, number concentration in various size ranges and respirable mass excluding modification job

Appendix C

Some jobs were composed of one or more tasks. Each task could last few minutes or up to several hours. We illustrated examples below showing how to create job-based number concentrations adjusted by the time spent at each process. The same method applies to job-based surface area concentration.

Modification		Time Activities Pattern	Number Concentration (0.02-0.3)	Calculation Process	Adjusted Concentration
Primary Location	Mixing	40%	2.82E+10	2.82E+10*0.4+	3.91E+10
Secondary Location	Modification	30%	7.85E+10	7.85E+10*0.3+	
Third Location	Control room	30%	1.43E+10	1.43E+10*0.3	
Quality Control		Time Activities Pattern	Number Concentration (0.02-0.3)	Calculation Process	Adjusted Concentration
Primary Location	Quality control	90%	1.31E+10	1.31E+10*0.9+	1.43E+10
Secondary Location	Bagging	10%	2.46E+10	2.46E+10*0.1	
Third Location					
Carbonation		Time Activities Pattern	Number Concentration (0.02-0.3)	Calculation Process	Adjusted Concentration
Primary Location	Control room	95%	1.31E+10	1.31E+10*0.95+	1.38E+10
Secondary Location	Carbonation	5%	2.74E+10	2.74E+10*0.05	
Third Location					
Recycling		Time Activities Pattern	Number Concentration (0.02-0.3)	Calculation Process	Adjusted Concentration
Primary Location	Drying machine room	62.5%	2.37E+10	2.37E+10*0.625+	1.97E+10
Secondary Location	Recycling	37.5%	1.31E+10	1.31E+10*0.375	
Third Location					

Chapter 4 Assessment of Cardio-Respiratory Effects

Abstract

Emerging toxicological studies have demonstrated that exposure to Engineered Nano-Particles (ENPs) could induce pulmonary toxicity in animals. Little is known about the potential health effects in humans. The aim of this epidemiological study was to evaluate whether inhalation exposure to engineered nanoparticle is associated with cardio-respiratory effects. Study subjects were recruited from a calcium carbonate manufacturing facility in Shanxi Province in China. In 2011, subjects participated in repeated measurements of FEV1, BP, and exhaled NO before and after the work shift. In addition, several pro-inflammatory mediators were analyzed from induced sputum bio-samples. In 2012, cross-sectional measurements of FEV1 and BP were obtained. Associations between exposure and various health outcomes were examined. It was observed that workers in the high mass-exposure group had 8 folds of IL8 levels (p=0.008), and 3 folds of IL1β levels (p=0.043) compared to the workers in the low-mass exposure group. The elevation of IL1β and IL8 followed a dose-response pattern with increasing exposure. The medium mass-exposure group had lower FEV1 and greater systolic and diastolic blood pressure than the low mass-exposure group however this effect did not reach to statistical significance. The present study suggests a pulmonary inflammatory effect of inhaled nanoparticles in humans.

Background

Scientists and engineers continuously discover new applications products utilizing nanotechology and nanomaterials. Such fast growing nano-related activities and developments have been expanded into almost every industry and touched a wide range of

commercial and consumer products. There is thus the possibility that is a substantial amount of potential for nanoparticles to enter the environment [1, 2]. In parallel to these developments there has been an expressed concern over the unknown health risks associated with accidental contact and occupational exposure to nanoparticles.

Toxicological studies have observed pulmonary fibrosis and granulomas in mice or rats within days of intratracheal instillation of single-walled carbon nanotubes (SWCNTs) [3-5]. Increasing evidence suggests that multi-walled carbon nanotubes (MWCNTs) could cause or exacerbate airway allergic reactions in mice [6-8]. It was found that SWCNT-induced pulmonary toxicity was associated with cardiovascular effects related to mitochondrial oxidative modifications and accelerated atheroma formation in a mouse model [9].

Despite the emerging evidence of nanotoxicity in vivo and in vitro studies, little is known about the potential health effects related to engineered nanoparticles in humans. It is widely acknowledged that long-term exposure to elevated PM2.5 could increase risk for ischemic heart disease mortality [10-12] and ischemic heart disease events were associated with PM2.5 exposure, even as short as several hours[13-15]. Inhalation of ambient ultrafine particles has been associated with a decrease in peak expiratory flow (PEF) and an increase in cough and feeling ill during the day [16]. Penttinen found that particle concentrations (especially UFP) were negatively associated with the daily PEF deviations of adult asthmatics [17]. However, there is far less evidence to support adverse health effects associated with ultrafine particle compared with fine particles inhalation.

Introduction

The objectives of this study were to evaluate respiratory and cardiovascular effects associated with short-term and long-term nanoparticle inhalation exposure in a manufacturing facility. Epidemiologic studies are not inherently aimed at studying biological mechanisms, yet generate information to aid in understanding potential pathological links between inhaled particles and relevant health endpoints. Potential mechanistic pathways are still not completely understood, although there is greatest support for inflammation and the oxidative stress[18, 19]. Cytokines are regulators of cell responses to cell stressors such as infection and environmental pollutant agents. There is evidence that exposure to ambient particulate matter is associated with elevated circulating C-reactive protein (CRP), fibrinogen, or white blood cell counts [20-23], IL-1β[24] and IL-6[25,26]. A key feature in the pathogenesis of asthma and COPD is chronic airway inflammation, characterized by the presence of the mediators in the airways [27, 28]. Analysis of induced sputum is a non-invasive approach to understanding disease processes in inflammatory airway disease. It has been found that non-allergic occupational asthma is mediated by a number of cytokines, including IL-1, IL-6, IL-8 and TNF-α [29-31]. MMPs comprise a group of biologically active enzymes, including MMP-2 and MMP-9 that have the ability to degrade a broad range of extracellular matrix proteins including elastin, collagen and fibronectin [32, 33]. By degrading components of the basement membrane, MMP-9 may promote inflammation by facilitating the movement of inflammatory cells into the airway lumen and bronchial wall. It could also directly injure airway cartilage [34-36]. MMP-9 was implicated in causing airway damage in several chronic inflammatory lung disorders including asthma [37, 38], COPD [39, 40], idiopathic pulmonary fibrosis [41, 42] and bronchiectasis [43, 44]. Measurement of exhaled nitric oxide

is another quantitative, non-invasive method of measuring airway inflammation. It provides complementary information of assessing airway disease.

The objective of present study was to examine a prior hypothesis that inhalation of nanoparticles is associated with a reduction of FEV1 and an increased blood pressure; and that such changes may be accompanied by up-regulation of proinflammatory cytokines in the lungs. We also explored the relationship among different inflammatory biomarkers.

Methods

Study Population

The health study was conducted between 2011- 2012. A hundred thirty-eight workers were recruited from the factory. The number of employees working at the factory was 166 by the end of January 2012 so the overall participation rate is about 83%. However, due to time and logistic constraints, not all recruited subjects joined in every health assessment. Repeated measures of FEV1 and exhaled NO and sputum induction procedures require more participation effort and resources, which limited the number of participants for each outcome. The study was approved by the Human Subjects Review Committee of University of Washington and Shanxi Medical University and informed consent was administered. All participants completed a questionnaire giving information about their sex, age, height, weight, smoking status, education level, work and medical history.

Exposure Assessment

Air monitoring was conducted in a nanoparticle manufacturing factory in 2011 winter and 2012 summer. Each job was categorized into three job categories (high, medium and low) based on measured concentrations of respirable mass, submicron-sized (20-300nm) number concentration, and lung deposited surface area (TB & AL). Refer to chapters 2 and 3 for detailed information. Only the mass metric was used to assess health outcomes measured in the year of 2011. In the 2012 study year, four exposure metrics were compared when evaluating health outcomes.

Health Measurements

Lung function tests and blood pressure measures were conducted both in 2011 and 2012. In 2011, FEV1 and blood pressure were measured before and after work. In 2012 only the cross-sectional measurements of FEV1 and blood pressure were taken. Spirometric measurements of FEV1 were performed with the MicroDL spirometer (Micro Direct Inc). At least 3 and up to 6 measurements were obtained on each subject. These results were evaluated for reproducibility and the highest acceptable values for FEV1 were used for subsequent analyses. Blood pressure was measured with an automatic blood pressure monitor (Omron HEM 6111). These measurements were taken by two field staff with the same training.

Pre- and post- shift nitric oxide concentrations in the exhaled breath were measured with a direct reading instrument (NIOX MINO, Aerocrine AB) for each subject. Inflammatory markers were exanimated in 2011. All sputum samples were induced by inhalation of 3% saline solution. Hypertonic saline was nebulized by means of an ultrasonic nebulizer and was

inhaled for 5-minute periods for up to 30 minutes. Every 5 minutes after the start of nebulization, patients were asked to rinse their mouth and throat carefully, to discharge saliva, and to try to cough sputum into a container. The sample was weighted, diluted with 4 times the volume of 0.1% dithiothreitol. The sputum sample was centrifuged at 3000 r/min for 10 minutes, and the supernatant was collected and stored for analysis. The concentrations of sputum biomarkers were detected using commercially available sandwich enzyme immunoassays (R&D Systems and Beijing Biolead Biology Sci&Tech Co,.Ltd) according to the manufacturers' recommended protocols. The sputum supernatants were assayed in duplicate.

Analysis

Cross-shift Health Outcomes

The cross-shift changes of FEV1, BP and exhaled NO were calculated for each subject and the statistical summary were presented as mean±SD and median and interquartile range for comparison between exposure groups. The differences of these measures between exposure groups were tested by using Kruskal-Wallis non-parametric test.

Cross-sectional Health Outcomes

Linear regression techniques were applied to examine the effect of nanoparticle exposure on FEV1and BP. This includes the average of repeated measured FEV1 and BP collected in 2011 and cross-sectional data in 2012. We calculated a predicted FEV1 value for each subject by using a standardized equation based on height, gender and age generated from a Hong Kong reference population [45]. This predicted FEV1 was included in the regression model as an independent variable. These models also controlled for available individual confounders,

including work duration (year), ever smoking status (yes, no). Cytokine levels are expressed as median (interquartile ranges). The correlation between sputum biomarkers and exposure status was investigated using linear regression model with natural logarithm transformation of the response variable. The model also adjusted for potential confounders including age (year), gender (male, female), work duration (year) and current smoking status (yes, no). Each biomarker was evaluated one at a time. Similar analysis was conducted for cross-sectional eNO effect. We used current smoking status in biomarker models in consideration of the level of cytokines might be more related to recent smoking activity. In fact, only two subjects changed smoking status over the past years. Education was not included in the model as it did not contribute to the model based on likelihood ratio tests. Non-parametric Spearman's rank ρ and Kendall's rank τ were calculated in an attempt to estimate correlation among sputum biomarkers. The data obtained were analysed with R package (version 2.15.1).

Results

Summary of Study Population

In the year of 2011, thirty-nine subjects completed both pre- and post-shift lung function tests and produced data that met our criteria for inclusion. The acceptance of FEV1 followed the two criteria 1) the ratio of the difference and average is less than 10% for the two morning measures and 2) the ratio of the difference and average is less than 10% for the two afternoon measures. Sixty-three subjects completed cross-shift blood pressure examinations and 61 subjects completed cross-shift exhaled NO tests. Sixty-eight employees participated in the sputum induction process and 66 sputum samples were successfully collected. In 2012, 85 subjects produced valid FEV1 measures and 102 subjects participated in the blood pressure

examination. Description of the study design is shown by Table 4.1 and the demographic characteristics of the study population for major outcome measures are outlined in Table 4.2.

Table 4.1 Population Size of Health Outcomes

	FEV1	BP	Exhaled NO	Sputum
2011	Cross-shift (N=39)	Cross-shift (N=63)	Cross-shift (N=61)	Cross-sectional (N=66)
2012	Cross-sectional (N=85)	Cross-sectional (N=102)		

Table 4.2 Basic Characteristics of the Study Subjects for Outcome Measures

Characteristics	2011 Biomarker (N=66)	2012 FEV1 (N=85)	2012 BP (N=102)
Age (till end of 2011)	39.2±9.1*	39.4±8.7	39.4±9.2
BMI	22.9±2.5	23.3±2.5	23.3±2.7
Working duration	6.0±3.6	5.8±3.5	5.7±3.5
Male	55%	64%	61%
Smoker	11%	39%	36%
Education			
College	12%	7%	8%
High school	52%	59%	59%
Middle school	35%	27%	26%
Lower than middle school	2%	7%	7%

Effects of Short-term Exposure (Based on Mass Concentration)

1. Cross-shift Change of FEV1

On average, the level of FEV1 increased slightly after an 8-hour work shift for workers in the low and high exposure group, however, this increasing trend is diminished among the

medium exposure group. The difference of the FEV1 change over 8 working hours between these groups is not statistically significant (p=0.469).

Table 4.3 Statistical Summary of Cross-shift Change of FEV1

FEV1 change (PM-AM)	High (N=4)	Medium Exposure(N=22)	Low Exposure (N=13)
Mean ± SD	0.070±0.18	-0.002±0.25	0.069±0.25
Median (IQR)	0.070(-0.105,0.246)	-0.030(-0.213,0.083)	0.020(-0.065,0.16)

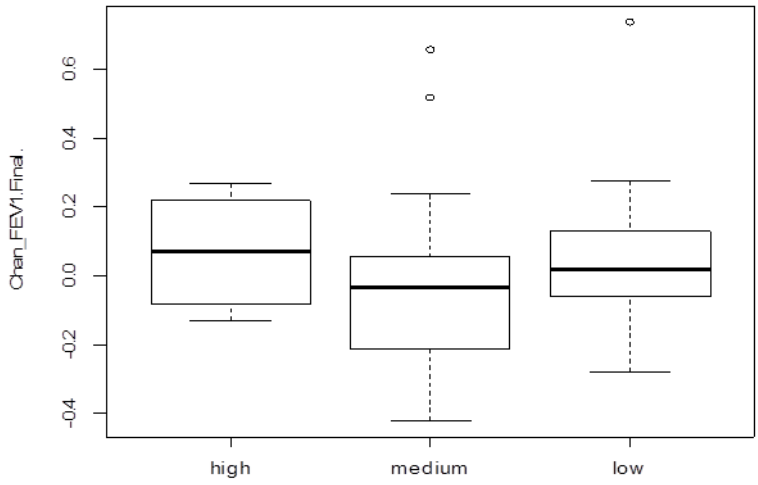


Figure 4.1 Cross-shift Change of FEV1 among the Three Exposure Groups

2. Cross-shift Change of BP

Comparing different groups, there seems no systemic trend of cross-shift changes in systolic (p=0.557) and diastolic blood pressure (p=0.377).

Table 4.4 Statistical Summary of Cross-shift Change of Blood Pressure

BP change (PM-AM)		High Exposure(N=7)	Medium Exposure (N=29)	Low Exposure (N=27)
SBP	Mean ± SD	-2.7±10.0	-1.4±11.4	1.2±18.1
	Median (IQR)	2 (-12,6)	0 (-7.5,6)	2 (-5,11)
DBP	Mean ± SD	-0.1±11.6	-2±7.8	1.0±9.6
	Median (IQR)	1 (-9,8)	-2 (-8,3.5)	2 (-4,7)

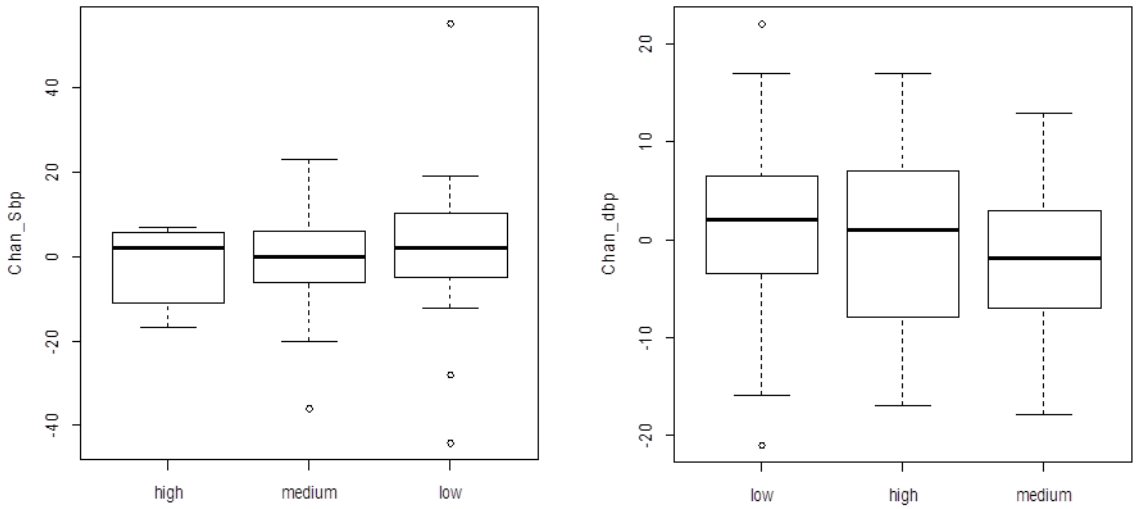


Figure 4.2 Cross-shift Change of Blood Pressure among the Three Exposure Groups

3. Cross-shift Change of eNO

On average, the level of exhaled NO dropped for all exposure groups after an 8 hour working day. However, no clear and meaningful differences have shown among exposure groups (p=0.880).

Table 4.5 Statistical Summary of Cross-shift Change of Exhaled NO

eNO (PM-AM)	High Exposure(N=7)	Medium Exposure (N=27)	Low Exposure (N=27)
Mean ± SD	-1.9±3.3	-1.9±3.2	-1.3±5.9
Median (IQR)	-1 (-6,2)	-3 (-5,1)	-1 (-4,2)

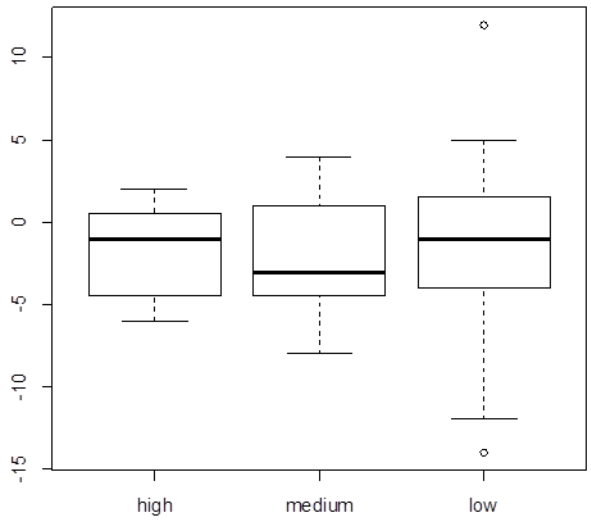


Figure 4.3 Cross-shift Change of eNO among the Three Exposure Groups

Inflammatory Response (Based on Mass Concentration)

Median concentrations of IL1β, IL8 and IL6 in sputum were greater in high exposure group compared to the other two groups. In contrast, the median concentration of TNFa in the high exposure group was lower than that of medium and low exposure groups, see Table 4.6.

Table 4.6 Median and Interquartile Range of Sputum Biomarkers

	High Exposure (N=5)		Medium Exposure (N=22)		Low Exposure (N=39)	
	Median	IQR	Median	IQR	Median	IQR
IL1β (pg/ml)	102	(40,147)	35	(18,65)	28	(15,56)
IL8 (pg/ml)	914	(433,1946)	135	(40,533)	136	(42,428)
IL6 (pg/ml)	4	(2,8)	2	(2,4)	3	(2,3)
TNFa (pg/ml)	9	(8,19)	28	(16,53)	28	(14,48)
MMp9 (ng/ml)	27	(21,121)	28	(21,48)	32	(22,45)

On average, levels of cytokines including IL1β, IL8, IL6, and MMp9 increased with mass concentration. The levels of IL1β and IL8 among high exposure group were significantly greater than for the low exposure group. The results have shown that workers in the high mass-exposure group had 8 times folds of IL8 levels (p=0.008), and 3 times folds of IL1β levels (p=0.043) compared to the corresponding measures in the low mass-exposure group, referred to Table 4.7 and Table 4.8. The elevation of IL1β and IL8 also presented a dose-response pattern with increasing exposure, referred to Figure 4.4. The effect of inhaled subject particles is similar or greater than the effect of smoking in terms of raising cytokine levels.

Table 4.7 Model Estimated Parameters and 95% Confidence Interval for IL1β

Mass, IL1β, N=66	Exp(β)	95% CI	P value
Group_high (5)	3.087	1.035, 9.206	0.043*
Group_medium (22)	1.464	0.764, 2.805	0.245
Age	1.004	0.966, 1.044	0.825
Current Smoker	1.363	0.483, 3.845	0.552
Male	0.676	0.355, 1.286	0.228
Working duration	1.063	0.964, 1.172	0.216

Table 4.8 Model Estimated Parameters and 95% Confidence Interval for IL8

Mass, IL8, N=66	Exp(β)	95% CI	P value
Group_high (5)	8.251	1.786, 38.126	0.008*
Group_medium (22)	1.319	0.531, 3.277	0.546
Age	1.016	0.962, 1.072	0.569
Current Smoker	1.689	0.395, 7.219	0.473
Male	0.638	0.259, 1.571	0.322
Working duration	1.057	0.922, 1.212	0.422

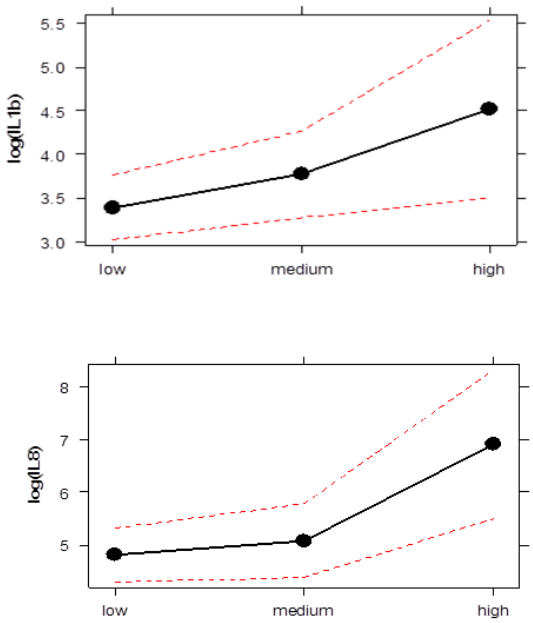


Figure 4.4 Comparisons of Effect Plots for IL1β and IL8 among Groups to Show a Similar Dose-response Trend

Table 4.9 Model Estimated Parameters and 95% Confidence Interval for IL6

Mass, IL6, N=66	Exp(β)	95% CI	P value
Group_high (5)	1.678	0.954, 2.951	0.071
Group_medium (22)	1.299	0.928, 1.817	0.124
Age	1.001	0.981, 1.021	0.952
Current Smoker	1.502	0.879, 2.566	0.134
Male	0.836	0.600, 1.166	0.287
Working duration	1.011	0.961, 1.063	0.672

Table 4.10 Model Estimated Parameters and 95% Confidence Interval for TNFa

Mass, TNFa, N=66	Exp(β)	95% CI	P value
Group_high (5)	0.427	0.171, 1.067	0.068
Group_medium(22)	1.054	0.612, 1.817	0.847
Age	1.009	0.977, 1.042	0.577
Current Smoker	1.007	0.422, 2.399	0.988
Male	1.044	0.609, 1.788	0.875
Working duration	0.988	0.911, 1.072	0.774

Table 4.11 Model Estimated Parameters and 95% Confidence Interval for MMp9

Mass, MMp9, N=66	Exp(β)	95% CI	P value
Group_high(5)	1.515	0.471, 4.874	0.480
Group_medium(22)	1.529	0.763, 3.064	0.226
Age	1.005	0.964, 1.047	0.827
Current Smoker	1.326	0.438, 4.020	0.612
Male	0.678	0.341, 1.349	0.263
Working duration	1.039	0.936, 1.153	0.466

IL1β plays a major role in controlling the inflammatory response therefore is considered as a priori here. A reasonably strong correlation (ρ=0.77, p <0.001) between IL1β and IL8 has been exhibited. IL6 and IL8 also present a similar positive correlation (ρ=0.73, p <0.001), see Table 4.12 and Figure 4.5.This suggested a possible cross-talk among these interleukins. And it also might reflect processes that have a global impact on a host of inflammatory and oxidative pathways, causing many components of these mechanisms to be affected together.

Table 4.12 Non-parametric Correlation Coefficient between Sputum Biomarkers

Correlation	Spearman's rank ρ	Kendall's rank τ
IL1β vs IL8	0.77	0.59
IL1β vs IL6	0.46	0.32
IL6 vs IL8	0.73	0.56
IL1β vs TNFa	-0.05	-0.03
IL1β vs MMp9	0.24	0.15
TNFa vs MMp9	-0.006	0.006

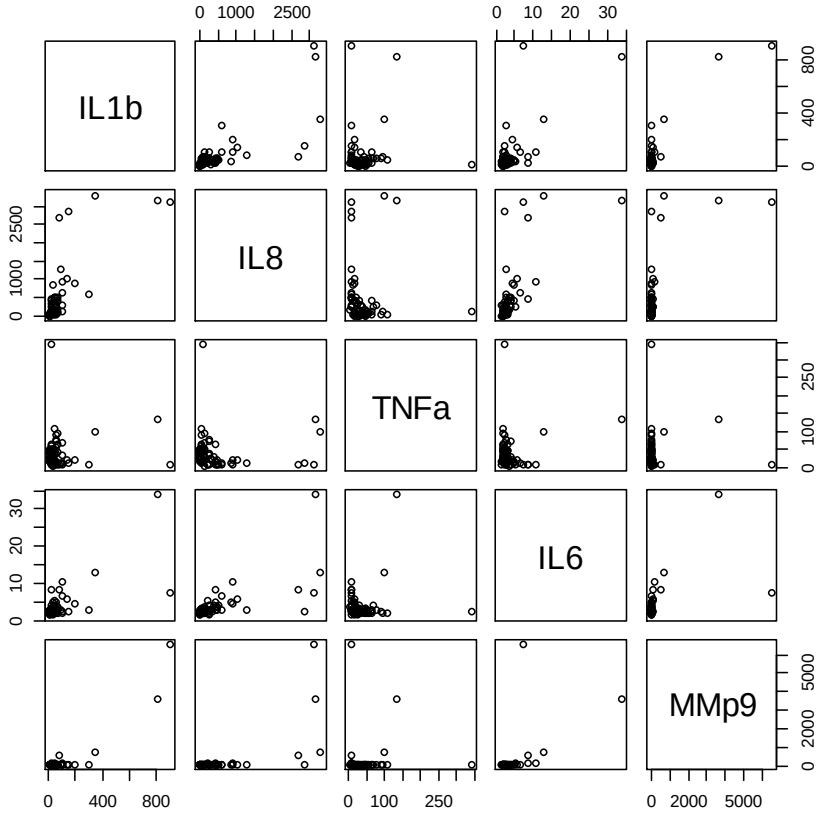


Figure 4.5 Pairwise Relationship Matrix between Sputum Biomarkers

Cross-sectional Effects

1. 2011 Average FEV1 of AM-PM measurements

We calculated the average of the pre- and post-shift FEV1 as cross-sectional FEV1 measures taken in 2011. It was discovered that this average FEV1 is declined along with the increase of mass concentration of the inhaled nanoparticles. However such does-response trend is not statistically significant, see Table 4.13.

Table 4.13 Model Estimated Parameters and 95% Confidence Interval for 2011 FEV1 (L) using Mass Concentration as the Exposure Metric

Mass, N=39	β	95% CI	P value
Group_high (4)	-0.192	-0.607, 0.223	0.353
Group_medium (22)	-0.166	-0.419, 0.087	0.191
Ever smoker	-0.183	-0.497, 0.131	0.245
Working duration	-0.0001	-0.033, 0.032	0.994
Predicted FEV1	0.746	0.521, 0.971	<0.000

2. 2012 Cross-sectional FEV1

Medium exposure group had a reduced FEV1 compared to the low exposure group, which was similar to observed in the 2011 data. When using number and surface area concentration as the exposure metric in the regression model, FEV1 levels were increased with exposure levels. However, none of these estimates are statistically significant, see Table 4.14.

Table 4.14 Comparison of Model Estimated Exposure Parameters and 95% Confidence Interval for 2012 FEV1 (L) using Four Exposure Metrics

FEV1,N=62	Groups	β	95% CI	P value
Mass	Group_high (9)	0.047	-0.318, 0.413	0.796
	Group_medium (15)	-0.153	-0.466, 0.160	0.333
Number	Group_high (21)	0.104	-0.264, 0.473	0.573
	Group_medium(29)	0.019	-0.329, 0.366	0.915
TB	Group_high (12)	0.083	-0.264, 0.431	0.632
	Group_medium(29)	0.164	-0.111, 0.439	0.238
AL	Group_high (4)	0.049	-0.474, 0.573	0.852
	Group_medium (37)	0.150	-0.112, 0.411	0.256

*Adjusted for smoking, work duration, predicted FEV1

3. 2011 Blood Pressure

We calculated the average of the pre- and post-shift blood pressure as cross-sectional blood pressure measures taken in the year of 2011. Averaged systolic pressure was associated with an increase of mass concentration of the inhaled particles. Compared to the low exposure group, the increase of systolic pressure among the medium group is approaching statistical significance, see Table 4.15. The increase of diastolic blood pressure was only seen among the medium exposure group compared to low exposure group, see Table 4.16.

Table 4.15 Model Estimated Parameters and 95% Confidence Interval for 2011 Systolic Blood Pressure (mmHg) using Mass Concentration as the Exposure Metric

SBP, Mass, N=63	β	95% CI	P value
Group_high (7)	1.473	-15.577, 18.523	0.863
Group_medium (29)	9.207	-0.759, 19.174	0.069
Age	0.213	-0.315, 0.742	0.422
Ever smoker	5.469	-8.276, 19.213	0.429
Male	-3.21	-14.015, 7.595	0.554
BMI	3.405	1.443, 5.367	0.001

Table 4.16 Model Estimated Parameters and 95% Confidence Interval for 2011 Diastolic Blood Pressure (mmHg) using Mass Concentration as the Exposure Metric

DBP, Mass, N=63	β	95% CI	P value
Group_high (7)	-1.559	-12.120, 9.002	0.769
Group_medium (29)	4.533	-1.641, 10.706	0.147
Age	0.038	-0.290, 0.365	0.819
Ever smoker	3.720	-4.794, 12.234	0.385
Male	-0.301	-6.994, 6.393	0.929
BMI	2.219	1.003, 3.434	0.001

4. 2012 Blood Pressure

Using mass concentration as the exposure metric, both systolic and diastolic blood pressure was positively associated with exposure level. Consistent with 2011 blood pressure results,

the effect of medium exposure group is more pronounced compared to the high exposure group. However, such increase trend is not statistically significant. When using number and surface area concentration as the exposure metric, exposure to inhaled nanoparticles is associated with lower blood pressure, as shown by Table 4.17 and 4.18.

Table 4.17 Comparison of Model Estimated Exposure Parameters and 95% Confidence Interval for 2012 Systolic Blood Pressure (mmHg) using Four Exposure Metrics

SBP,N=79	Groups	β	95% CI	P value
Mass	Group_high (9)	1.491	-9.722, 12.704	0.792
	Group_medium (22)	2.390	-5.290, 10.070	0.537
Number	Group_high(28)	-8.599	-17.487, 0.289	0.058
	Group_medium(35)	-13.655	-22.508, -4.802	0.003
TB	Group_high(13)	-2.620	-9.231, 3.992	0.432
	Group_medium(38)	-4.625	-9.457, 0.206	0.060
AL	Group_high(6)	-10.037	-24.164, 4.089	0.161
	Group_medium(45)	-7.001	-13.837, -0.165	0.045

*Adjusted for age, smoking, gender and BMI

Table 4.18 Comparison of Model Estimated Exposure Parameters and 95% Confidence Interval for 2012 Diastolic Blood Pressure (mmHg) using Four Exposure Metrics

DBP,N=79	Groups	B	95% CI	P value
Mass	Group_high (9)	0.592	-6.930, 8.115	0.876
	Group_medium (22)	1.433	-3.719, 6.585	0.581
Number	Group_high (28)	-4.270	-10.384, 1.843	0.168
	Group_medium(35)	-7.071	-13.161, -0.982	0.023
TB	Group_high (13)	-5.221	-14.994, 4.552	0.290
	Group_medium(38)	-7.994	-15.136, -0.852	0.029
AL	Group_high (6)	-2.698	-12.263, 6.867	0.576
	Group_medium (45)	-4.256	-8.885, 0.372	0.071

*Adjusted for age, smoking, gender and BMI

5. 2011 Exhaled NO

We also calculated the average of AM and PM exhaled NO. There were no statistically significant association between the mass concentration and exhaled NO, as shown by Table 4.19.

Table 4.19 Model Estimated Parameters and 95% Confidence Interval for 2011 Exhaled NO using Mass Concentration as the Exposure Metric

eNO, Mass, N=61	Exp(β)	95% CI	P value
Group_high (7)	1.033	0.694, 1.536	0.872
Group_medium (27)	0.920	0.717, 0.180	0.505
Age	1.001	0.985, 1.018	0.903
Current smoker	0.768	0.552, 1.648	0.115
Male	1.264	0.970, 1.068	0.082
Working duration	1.008	0.969, 1.049	0.677

Discussion

In this study, we demonstrated that exposure to manufactured $CaCO_3$ nanoparticles could possibly induce pulmonary inflammatory response. The high mass exposure group has 8 times of IL8 (p=0.008) and 3 times IL1β (p=0.043) compared to the low mass exposure group. An apparent dose-response trend has been displayed between IL1β and IL8 concentrations in sputum and mass concentration. These findings may have important physiological relevance. The mass exposure metric used in this study is respirable mass, which is associated with the number concentration in large-sized particles. These relatively large particles are agglomerates of nanoparticles. In another word, agglomerates of nanoparticles might have inflammatory effects. It is possible for these particles to be deagglomerated in lungs. If that is the case, the number concentration (0.02-0.3 um) and surface area concentration (0.01-1um) would underestimate the level of exposure. It is known that IL1 is produced by a variety of cells, including stimulated monocytes and macrophages.

It plays a central role in the cytokine network [46], and regulates inflammatory process and contributes to atherosclerosis [47-49]. IL6 is secreted by B cells, T cells and macrophages and plays an important role in host defence activation and proliferation of T cells and induction of monocyte differentiation to macrophages [50-51]. IL8 is a chemokine that is potent chemo-attractant for neutrophils. It regulates the adhesion of neutrophils and manipulates accumulation of leukocytes at sites of inflammation [52-53]. Despite knowledge and understanding of the mechanisms leading to pulmonary disease after exposure to air pollutants, the role of inflammatory cytokines in this process remains limited. Inflammatory responses in sputum have been found induced. There is an increasing appreciation that the balance among these mediators is often disrupted in chronic diseases. Emerging evidence has shown that the levels of IL6 and IL8 in sputum or bronchoalveolar lavage fluid are significantly raised among COPD and asthma patients [54-56]. Among COPD patients, such elevation of cytokine level becomes more prominent during exacerbations [57]. Many of these cytokines are overlapping and multi-functional. It is not surprising to see them correlated and up-regulated simultaneously (IL8 vs IL6, IL1β vs IL8). However, we did not find statistically significant increase of eNO associated with increase of mass exposure. There are two possible reasons. Nitric oxide is formed in the airway when L-arginine is oxidized to L-citrulline catalyzed by three isoenzymes of NO synthase.[58,59]. However.
the inflammatory makers we tested might be related to NF-Kb pathway, a different mechanism [60]. In addition, variances in exhaled nitric oxide output could be attributed to salivary nitric oxide formation due to high or low nitrate/nitrite diets [61]. Even though efforts were made to collect exhaled NO before breakfast and dinner, it is still possible that subjects had breakfast before measurements.

A repeated measurement study design conducted in 2011 allows us to examine the response of short-term exposure and compare cross-sectional effects between the groups at same time. No significant differences in cross-shift changes of blood pressure and exhaled NO were found between groups. FEV1 was reduced among subjects in medium mass-exposure group after 8-hr shift whereas a slight increase of FEV1 occurred among low and high mass-exposure group, however such difference did not reach statistical significance. In general, the risk of acute effect after short-term exposure of these subject nanoparticles is relatively low. After all, calcium carbonate is considered one of the most inert chemicals. As FEV1 and BP fluctuate over the day or week, using the average of AM and PM has an advantage as morning and afternoon FEV1 and BP data were measured almost at the same time for every subject. From the averages of the pre- and post-shift measures, we are able to examine these health outcomes cross-sectionally. It was found that the decline of FEV1 is associated with the increase of mass concentration of the inhaled nanoparticles. The medium mass-exposure group tends to have higher systolic and diastolic blood pressure than lower mass-exposure group. The increase of SBP is approaching statistical significance. This response pattern was replicated in the 2012 cross-sectional study. The medium mass-exposure group has lower FEV1 and higher SBP and DBP compared to low mass-exposure group. A seemingly unusual result is that the medium mass-exposure group has consistently stronger cross-sectional effects than the high mass-exposure group. A careful examination of the characteristics of the exposure reveals that the high mass-exposure group was solely constituted by the bagging job. The sample size is admittedly small. Moreover, bagging is a relatively labor-intensive job. It is also possible that the factory deliberately assigns this job to healthy workers. Undeniably, our model is not able to adjust for such confounding impacts if it ever exists.

Our hypothesis is exposure to engineered nanoparticles is associated with adverse cardiopulmonary effects. The major challenge in conducting this epidemiologic study is the nanoparticle exposure characterization. Currently, there are no devices for directly measuring the breathing zone concentration of nanoparticles, and no national or international consensus standards on exposure assessment methods for engineered nanoparticles in the workplace. Personal exposure of respirable mass concentration for each job was evaluated using a sampler instrument however we were not able to monitor personal exposure of a size-discriminating number and surface area concentration. Instead, online monitoring devices were used to obtain area samples as a surrogate to personal exposure for analysis. We combined area measurements of exposure concentrations in multiple tasks where subjects have been exposed with questionnaire information about time-activity patterns to estimate personal exposures. Still, there remains a problem for exposure assessment of number and surface area concentration to jobs without fixed location such as electricians or mechanics. To make meaningful comparisons among all exposure metrics, we excluded such jobs from our analysis. As stated in the previous chapter that the intervention program occurred right before the 2012 exposure assessment, which could potentially affect the exposure profile for jobs. It is necessary to point out the inference of number and surface area concentration as exposure metric in disease models should be interpreted carefully. In our study, mass and number/surface area concentration might vary in accuracy due to discrepancy between area and personal samples. We also expect there exists a difference between number and surface area concentration in terms of measurement precision as the number concentration in nanoscale were calculated from two measuring devices. Until the development of size-selective personal devices monitoring various exposure metrics specifically for nanoparticles, the comparison and choice of exposure metrics will remain a critical challenge.

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Chapter 5 Conclusions

Summary of Findings

In this study, we experimented with exposure assessment in a nanoparticle manufacturing facility. The sampling and analytic methodology used in the study presents the potential to characterize nanoparticle exposure for a variety of operational processes. We discovered nanoparticles in the size range of 20-300nm dominate in this workplace, which consists of 90-98% of particle counts in the respirable fraction. Based on the sampling results from 2012, there was a strong relationship between number concentration in 5-25um range and the respirable mass concentration; however, no such correlation was found between number concentration in nanoscale and respirable mass. The deposited surface area in TB and Alveolar region was modestly correlated with number concentration in the nanoscale particles. We demonstrated that some jobs (such as modification and softwater) have greater number concentration accompanied by a relatively low mass concentration and vice versa.

We found there are three levels of nanoparticle emission. The highest exposure of nanoparticles occurred at the modification process, which was identified to be the primary emission point of origin. A second level of exposure happened at very specific operations or with certain work practice. Such a release of nanoparticles tended to be intermittent. In addition, nanoparticles could originate from other work activities such as combustion, forklift emissions and welding. The concentration of nanoparticles generated from such an emission source was relatively low. A continuous monitoring should be considered to capture the peak exposure as well as average exposure for each process in future studies and both should be included in the risk assessment frame.

Aerosol concentrations in workplaces rarely remain uniform and constant. We saw a change in mass concentration between 2011 winter and 2012 summer. Some of this change might be due to an intervention program and some of it was related to a seasonal change. Considerable variation in exposure was observed between runs and days. We were able to characterize such variance in mass concentration as multiple samples were collected for many jobs in both years. However, such analysis was not conducted for number and surface area concentration as we had a limited number of measures. This added uncertainty to our exposure assessment and our job classification based on number and surface area concentration.

Exposure to nanoparticles quantified by mass was discovered to be associated with significant response of pulmonary inflammation. It is very likely detected sputum cytokines were produced by active alveolar macrophages and certain types of white blood cells. The role of these mediators is to initiate damage repair. However, excessive expression could exacerbate or perpetuate injury. Studies have shown continuous exposure to inhaled particles could cause persistent inflammation in lungs, which appears to be a key factor in the development of both silicosis and asbestosis [1]. At current stage, it is unclear whether these triggered cytokines are involved in pathogenic processes among subject workers.

We did not observe acute effects of FEV1, blood pressure and exhaled NO after 8-hour inhalation of nanoparticles. The subject particles we studied are considered one of the least harmful chemicals. That might explain why we don’t see adverse health effects after short-term exposure. . An increase of blood pressure and decrease of FEV1 among the medium mass-exposure group was discovered compared to low mass-exposure group. However, such

effects did not reach statistically significant. It is possible that our sample size is still limited or there might be no cross-sectional effects of BP and FEV1.

When comparing the four exposure metrics, we found number concentration and surface area concentration in general produce effects in similar direction, however opposite to mass concentration. Such relationship might have been revealed in correlation analyses, in which 1) two surface areas are correlated well (r=0.87); 2) number concentration in size range of 0.02-0.3 um is correlated with surface area concentration deposited in the TB region (r=0.66); 3) Number concentration in the size range of 0.02-5 um and respirable mass is linearly independent (r =0.009). In our study, we found inflammatory response in sputum is associated with mass concentration. Such association is largely due to agglomerated nanoparticles. As number and surface area only measure the particles size up to 0.3 and 1 um, respectively, these two exposure metrics will significantly underestimate the exposure level if agglomerated nanoparticle have similar effects of single nanoparticles. At current stage, with limited understanding of the toxicological perspective of each kind of nanoparticle, a complete exposure assessment in nanoparticle facility needs to be conducted in both bulk- and nano-form.

Current Challenges

There is always a distance between the ideal and the reality. Even though many efforts have been exhausted to characterize worker's exposure to nanoparticles; the exposure assessment is rather crude for the purpose of occupational epidemiology studies. The primary objective of this research is generating quantitative exposure information in regards to nanoparticles, so

the ideal metrics would be direct measurements of nanoparticles (<100nm) in mass, number and surface area concentration from a personal sampling device. Therefore, meaningful comparisons could be made between these metrics and health outcomes. Since such samplers have not been invented, respirable mass was used as a primary exposure metric for disease models. Environmental samples of number and surface area concentration were used as a surrogate for personal samples. Consequently, the comparison of the inferences generated with four exposure metrics in disease models should be interpreted with caution as they are not entirely comparable. However, our study also discovered that it is possible agglomerated form of nanoparticles might induce adverse effects as well. It remains unclear what the most appropriate exposure/dose metric for the endpoint of interest. This issue would not be solved immediately, and may await development of novel monitoring devices.

In occupational epidemical studies, it is necessary to have sufficient sample size and statistical power to find differences between exposure groups. However, many of the nanotechology-related businesses are start-up companies and university research activities. The average size of the exposed workforce therefore is relatively small [2]. In this study, the modification job has a 10 fold higher nanoparticle number concentration compared to the background, however, only two workers are exposed to conditions at this job. It is not realistic to make this job as an independent number-exposure group so we have to combine modification with other jobs. If the effects are hypothesized to be associated with number or surface area concentration, this known misclassification might bias our results toward to null. We need to either increase the study population or refine our exposure characterization in order to reach statistical significance. Each consideration is adequately challenging in its own right.

Background exposure assessment has been recommended by many nanoparticle guidelines as it is necessary to separate the influence of the incidental particles from those intentionally made. One proposed approach is to estimate background concentration prior to work, in order to be subtracted from the total concentrations during manufacturing processes. However, such a strategy is not applicable and practical for our study which has rotational shiftwork that runs 24-hrs continuously. This method also raised another issue, whether it is necessary to distinguish nanoparticles from multiple sources from an epidemiologic perspective. After all, the health outcome of subjects presumably are a consequence of summing effects from exposure to all types of inhaled nanoparticles.

Future Studies

The respiratory system possesses a number of important mechanisms to minimize injury induced by inhaled toxicants. Investigation of pulmonary inflammation using induced sputum has been used scientific research [3]. A key feature in the pathogenesis of asthma and COPD is chronic airway inflammation, characterized by the presence of inflammatory cells and the mediators in the airways. Studies found leukocytes distribution in induced sputum is different among allergic asthma, COPD and healthy population. Increasing evidence showed a pattern that significant higher percentage of eosinophils presenting in the sputum with atopic asthma compared with higher number of neutrophil percentage in the COPD patients [4-6], which negatively correlates with forced expiratory volume in the one second [7]. Moreover, a number of studies found non-eosinophilic asthma is associated with increased neutrophils [8-9], which is likely to be the underlying mechanism for occupational asthma [10]. In the light of this new knowledge, future studies could investigate a mediator panel in sputum together

with cell counts and activation of macrophages, neutrophils and epithelial cells for a better understanding of the disease process. A temporal cycle of these biomarkers might exist, and multiple measures and follow-up over time might capture short-term and long-term fluctuation trends. The scope of the next phase is also to address additional endpoints that are necessary to gain understanding of the hazard potentials

Furthermore, robust characterisation of nanoparticles is an essential prerequisite for any investigation of health related studies. As suggested from this study, the nature of agglomeration might influence the deposition of nanoparticles, which in turns affect the interpretation of the study results. This is an urgent need to understand the agglomeration and de-agglomeration process of nanoparticles inside the facility. It is necessary to ascertain whether the larger particles observed at bagging are related to this specific process, or represents single particles that evolved and agglomerated during the transportation from early process stages. Many studies have revealed the potential influence of surface charge, crystal structure, shape and hydroscopic properties on interactions between nanoparticles and tissues. To correlate properties of the nanoparticle to their toxicity potential and ensure that the results are reproducible and meaningful, accurate and thorough characterisation is the key.

The major source of "error" for this study is the fact that a single day's exposure measurement does not serve as an adequate measure of the cumulative exposure. There are seasonal differences, as well as day-to-day and worker-to-worker variations. Robustness of estimation and inferences should be one of the concerns. Future exposure study should emphasize sufficiently repeated measures on different workers and same workers within one

job. Such design provides a direct assessment of variation and measurement error, which could contribute to account for uncertainties in the estimated exposure.

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