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Exposing the Exposures

Assessing occupational aerosol exposures and their possible health and toxicological effects

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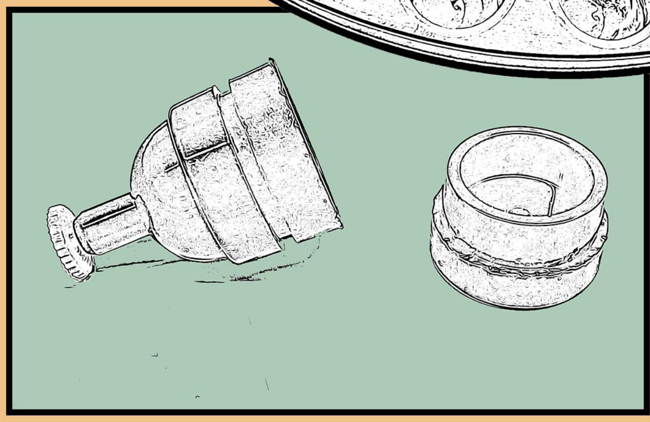
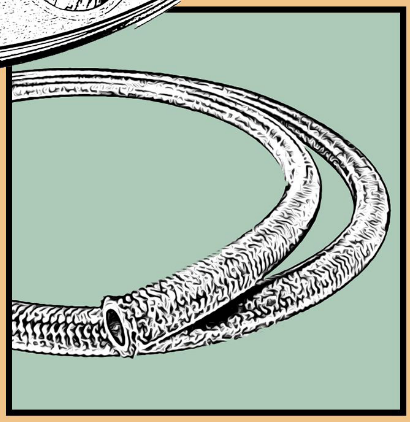
Exposing the Exposures

Assessing occupational aerosol exposures and their possible health and toxicological effects

KARIN LOVÉN
ERGONOMICS AND AEROSOL TECHNOLOGY | LTH | LUND UNIVERSITY





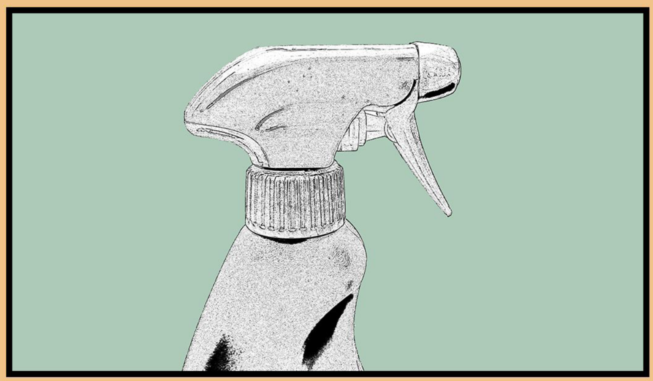


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Photo by: Kennet Ruona

I moved to Lund in the fall of 2009 to study at Lund University. In the spring of 2015, I received a Master's degree in Engineering Nanoscience. During my last year of studies, I discovered the field of aerosol science and developed an increasing interest. During the fall of 2015, I started as a PhD student in Aerosol Technology which, with its multidisciplinary focus, have been very interesting and fun, but at the same time challenging and demanding. My work during the last almost five years has finally resulted in this PhD thesis!

Karin Lovén

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DOCTORAL DISSERTATION

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To be defended at Stora Hörsalen, IKDC. Date: 15th of May 2020. Time: 09:15.

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Abstract

According to the Global Burden of Disease study, occupational exposures to particulates, gases and fumes were responsible for 0.36 million deaths and 8.8 million disability-adjusted life years globally in 2015. This thesis focuses on two occupational groups that are at risk for developing adverse health effects from the time they spend in their working environments: cleaning workers and workers in the nanotechnology industry. For both of these occupational groups, who are affected by the introduction of new products into their occupational environments, knowledge is needed about the present exposure situations, as well as evaluations of the potential health and toxicological effects of these exposures.

The overall aim of the research presented in this thesis was to provide a basis for the implementation of improvements of the working environments for these two occupational groups by addressing the research needs within them. Aerosol emissions and exposures present in the two occupational settings were characterized, and the potential health and toxicological effects that can possibly arise from these types of exposures were evaluated. Extensive methodological approaches were used, divided into the three overall categories of contextual information gathering, aerosol characterization, and health/toxicity assessment.

To assess the working environment of cleaning workers, with a specific focus on the possible aerosol exposure generated during spray use, an initial phone inquiry of cleaning companies (n = 20) with a following survey among professional cleaning workers (n = 225) were conducted. Characterizations of the spray aerosol were performed with both off-line and on-line methods. A human chamber exposure study was then conducted to evaluate acute health effects due to the use of different cleaning methods.

To assess the working environment in the nanotechnology industry, three different companies were studied. Contextual information was collected from the companies about the type of materials and processes they were handling. Workplace measurements were then conducted to characterize the current engineered nanomaterial (ENM) emission and exposure situations. Relevant toxicological assessments are also needed to evaluate the potential ENMs' hazards. A comparative study of the toxicity of a common ENM assessed with different cell exposure systems was therefore carried out. The more advanced Nano Aerosol Chamber for In Vitro Toxicity (NACIVT) system was compared with two traditionally submerged toxicological exposure systems to understand the similarities and differences between them.

Worker's exposures could be determined within both occupations. We showed that the use of cleaning sprays results in an aerosol exposure, but with a large variety between different products. Between 2.7 and 32.2% of the liquid leaving the bottle during spraying remained airborne and could constitute potential airway exposure. We also showed that the nose was the part of the respiratory system most affected by spray use, but that switching to a foaming application method greatly reduced both the aerosol concentration and the observed negative health effects.

At the nanotechnology companies, emissions and exposures were found from all three fibrous nanomaterials that were handled, foremost during the manual cleaning of the ENM production reactor and during the handling of both dry ENM powder and ENM-containing liquid. Comparing the different toxicological exposure systems revealed a higher sensitivity in the NACIVT system than the two traditionally submerged systems. This result encourages the use of more physiologically realistic cell exposure systems.

The knowledge generated by the thesis research can be used to promote safe working environments. Exposure data together with hazard/toxicity information can be used to provide improved risk assessments. The comparison and evaluation of measurement techniques can be used in recommendations to occupational hygienists. The identification of processes that cause exposure events can be used by companies to implement suitable mitigation systems. All this can be done to achieve healthier workplace conditions!

Key words Aerosol, occupational exposure, spray, engineered nanomaterial, air-liquid interface, toxicology

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LUND UNIVERSITY

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MADE IN SWEDEN

Nothing in life is to be feared, it is only to be understood.
Now is the time to understand more, so that we may fear less.
Marie Skłodowska Curie

Underskatta inte värdet av att göra Ingenting,
av att bara ströva omkring alldeles ensam,
lyssna på allt som man inte kan höra och bara ha det bra.
Nalle Puh (A.A. Milne)

Utan sång är livet bara nåt det inte borde vara!
Lennart Hellsing (från ’Den lyckliga osten’)

Table of Contents

Abstract

According to the Global Burden of Disease study, occupational exposures to particulates, gases and fumes were responsible for 0.36 million deaths and 8.8 million disability-adjusted life years globally in 2015. This thesis focuses on two occupational groups that are at risk for developing adverse health effects from the time they spend in their working environments: cleaning workers and workers in the nanotechnology industry. For both of these occupational groups, who are affected by the introduction of new products into their occupational environments, knowledge is needed about the present exposure situations, as well as evaluations of the potential health and toxicological effects of these exposures.

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Populärvetenskaplig sammanfattning

Hur länge tänker du jobba? Svaret på den frågan kan avgöras av vilka förutsättningar din arbetsmiljö ger dig. Ditt arbete kanske innehåller många fysiskt ansträngande arbetsuppgifter eller ställer stora krav under påfrestande förhållanden som leder till långvarig stress. Eller kanske påverkas du av något så grundläggande som luften du andas.

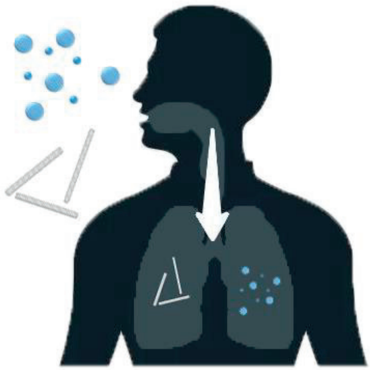
Luften består till största del av gaserna kväve och syre, men innehåller även luftburna partiklar. Går du längs med stranden kan luften innehålla saltpartiklar från havet och går du på en trafikerad väg, sotpartiklar från bilavgaser. Du kan välja var du promenerar, men din arbetsmiljö kan du inte lika lätt välja att inte vistas i och oavsett var du befinner dig kan du aldrig välja att inte andas. Vid införandet av nya produkter i arbetsmiljön kan det bildas luftburna partiklar och andra gaser än kväve och syre, tillsammans kallat *aerosoler*, som kan påverka din hälsa negativt. Du spenderar ungefär en tredjedel av ditt liv på jobbet och därför behöver nya produkter som introduceras i ditt arbete riskbedömas för att säkerställa att du inte blir sjuk av din arbetsmiljö.

Två yrkesgrupper med utmanande arbetsmiljöer är städare och anställda inom nanoteknologiindustrin. Arbetsmiljöerna för dessa grupper, med speciellt fokus på införandet av nya produkter, har undersökts i denna avhandling. Inom städbranschen har det länge funnits problem med hög fysisk belastning och utbredd användning av städkemikalier. På senare tid har man börjat använda städsprayer, visserligen med syftet att förenkla arbetet, men med följden att man samtidigt potentiellt skapar ett nytt problem med den aerosol som kan bildas. Inom nanoteknologiindustrin är ett stort problem snarare den osäkerhet som finns, både kring vilka typer av exponeringar som förekommer, vilka nya material som används och hur farliga dessa verkligen är om de andas in.

För att ta reda på hur det faktiskt ser ut med sprayanvändningen i den svenska städbranschen genomförde vi en enkätundersökning bland 225 professionella städare som visade att 77 % utnyttjar städsprayer under arbetstid och att nästan hälften av dem upplever någon typ av andnings- eller ögonsymptom under denna användning. Undersökningen visade också att ökad sprayanvändning ökade de upplevda symptomen. För att studera aerosolbildningen vid nyttjande av städsprayer genomförde vi ett antal aerosolmätningar. Till att börja med visade vi att användningen av städsprayer väsentligt ökar den luftburna halten av kemikalier, men också att det kan skilja mycket mellan olika produkter. Mätningarna visade att mellan 3 och 30 % av kemikalierna som sprayas ut från flaskan, beroende på vilken produkt som används, stannar i luften och kan andas in av städaren.

Vi genomförde även en laboratoriestudie där en grupp frivilliga professionella städare och en grupp icke-städare rekryterades för att undersöka hälsoeffekter vid användning av olika städmetoder, både med och utan kemikalier.

Resultaten visade att både självupplevda symptom från näsa och ögon samt mätbara effekter på näsan och ögonen kunde observeras vid sprayanvändning. Vi visade dock att det går att minska både aerosolkoncentration i luften och användarnas upplevda hälsoeffekter på flera enkla sätt. En rekommendation är självfallet att städa helt kemikaliefritt, vilket också görs på flera ställen redan idag, med endast förfuktade mikrofiberdukar. Behövs det kemikalier rekommenderar vi istället andra appliceringsmetoder än spray. I våra studier visade sig användningen av skummunstycken vara effektivt för att minska både luftkoncentrationen och hälsoeffekterna.



Inom nanoteknologiindustrin har tidigare ett urval av processer där tillverkning eller användning sker av ett antal nya nanomaterial undersökts för att kartlägga eventuella yrkesmässiga exponeringar. Dataunderlaget är dock fortfarande bristfälligt, framförallt kring nanofibermaterial. Risken med dessa material är att deras likhet med asbestsfibrer kan orsaka allvarliga hälsoeffekter som till exempel lungsäckscancer. För att undersöka hur exponeringen för olika fiberformiga nanomaterial ser ut i den svenska nanoteknologiindustrin genomförde vi ett antal arbetsplatsmätningar på olika företag. Dessa mätningar visade att vi i luften kunde detektera och identifiera alla typer av fiberformiga nanomaterial som hanterades på företagen. Ett antal mätmetoder användes och utvärderades under dessa mätningar. Vi visade att en kombination av filterprover med olika typer av efterföljande kemisk analys samt direktvisande mätningar av olika partikelegenskaper är avgörande för att få en komplett bild av exponeringssituationen, trots att det kan vara dyrt och tidskrävande. Dock finns det för vissa material, till exempel kolinnehållande material som grafen, snabbare och billigare metoder att använda och dessa visade sig fungera bra under våra studier.

För att kunna bedöma hur nanomaterial ska hanteras krävs kunskap om hur skadligt det är att exponeras för dessa material. Eftersom den yrkesmässiga exponeringen fortfarande inte är fullständigt kartlagd, samt att det finns en stor osäkerhet kring hur farliga materialen är, vore det oetiskt att genomföra studier med frivilliga försökspersoner.

Istället används ofta försöksdjur för att studera vilka effekter som kan uppstå. Men för att minska onödig användning av försöksdjur, samt för att undersöka de underliggande toxikologiska mekanismerna bakom effekterna, utförs experiment med cellkulturer istället.

Traditionellt görs detta med celler som är täckta med en tillväxtvätska i vilken nanopartiklarna blandas. Detta liknar dock inte den verkliga exponeringssituationen i våra lungor där nanopartiklarna är luftburna när de kommer i kontakt med cellytan. Av denna anledning har celler med ett luft-vätske-gränsskikt (air-liquid interface, ALI) börjat användas för toxikologiska studier. Men för att kunna exponera celler med ALI behövs speciella system som klarar av att hantera aerosoler och ett av dessa är det så kallade NACIVT-systemet (Nano Aerosol Chamber for *In Vitro* Toxicity).

I vår studie använde vi NACIVT och jämförde med traditionella system för att undersöka eventuella skillnader mellan effekter på cellerna. Vi fann att NACIVT-systemet var mer känsligt för låga doser av nanopartikelexponering än de traditionella systemen och att det framöver är angeläget att använda dessa mer fysiologiskt realistiska system för att utvärdera skadligheten från luftburna nanomaterial.

Den nya kunskap denna avhandling har genererat kan användas för att främja en god och säker arbetsmiljö. Information kring de olika exponeringssituationerna och potentiella hälsoeffekterna kan användas för att ta fram bättre riskbedömningar. Jämförelser och utvärderingar av olika mätmetoder kan användas i rekommendationer till yrkeshygieniker eller för utveckling av vidare forskning inom området. Identifiering av vilka processer som kan ge upphov till arbetsmiljömässiga exponeringar kan användas av företagen för att införa lämpliga åtgärder. Allt för att säkerställa hälsosamma arbetsmiljöer!

Popular science summary

How long are you planning to work? The answer to this question can be determined by the conditions of your occupational environment. Your work may be physically strenuous or require that you be highly focused in stressful situations. Or you just may be affected by something so basic as the air you breathe.

Air is mainly comprised by nitrogen and oxygen gases, but it also contains airborne particles. When you walk along the beach you inhale salt particles from the sea. And when you walk on a well-trafficked road you inhale soot particles from car exhaust. You can choose where you take your walks, but you can't as easily choose your occupational environment. And regardless of where you are, you can never choose not to breathe. When introducing new products into occupational environments, airborne particles and gases other than nitrogen and oxygen can be generated. Together, they are called *aerosols*, and they can affect your health negatively. You spend about one third of your life at work and the introduction of new products should thus be assessed in terms of the risks. This needs to be done in order to ensure that you will not fall ill as a result of your occupational environment.

Two groups with challenging occupational environments are cleaning workers and workers in the nanotechnology industry. The research presented in this thesis has investigated the occupational environments of these groups, with a specific focus on the introduction of new products. In the cleaning occupation, high physical workloads and extensive use of cleaning chemicals have been problems for a long time. In recent years, the use of sprays has been introduced to facilitate the work, but with the negative consequence of creating a problem due to the nature of the aerosols that are generated. In the nanotechnology industry, the problems are rather the uncertainty about the types of exposures that are present, the types of new materials that are being used, and how dangerous these really are if inhaled.

To determine the actual extent of cleaning spray usage in the Swedish cleaning occupation, we conducted a survey of 225 professional cleaning workers. It showed that 77% of the workers use cleaning sprays at work, and that almost half of them experience some type of airway or eye symptoms in the process. The survey also showed that increased use of cleaning sprays increased the experience of the symptoms. To study the aerosol generation when using cleaning sprays, we conducted a few different aerosol measurements. To start with, we showed that the use of cleaning sprays greatly increases the airborne concentration of chemicals, but that it can also vary a lot between products. The measurements showed that between 3 and 30% of the chemicals sprayed from the bottles remain airborne, depending on the type of product that is used, and can continue to be inhaled by the workers.

We also conducted a laboratory study where a group of volunteer professional cleaning workers and a group of non-cleaning workers were recruited to investigate health effects during the use of different cleaning methods, both with and without chemicals. The results showed that both self-assessed symptoms from the nose and eyes and the measured effects on the nose and eyes were observed during spray use. We showed, however, that it is possible to decrease both the aerosol concentration in the air and the health effects on the user by making several easy changes. The first recommendation would be, of course, to clean without any chemicals at all. This is already a practice in several places today where only premoistened microfiber cloths are used. If chemicals are necessary, we recommend the use of application methods other than spray. In our studies, the use of foaming nozzles was shown to be effective in reducing both the aerosol concentration and the negative health effects.

In the nanotechnology industry, a selection of processes or handling tasks have previously been investigated to find possible exposure situations. Knowledge, however, is still lacking, especially in terms of nanofiber materials. The risk with these materials is that they are similar to asbestos fibers, which means that they can have severe health effects resulting, for example, in lung cancer. We conducted several workplace measurements at different companies to investigate the exposure situations of different fiber-shaped nanomaterials in the Swedish nanotechnology industry. With these measurements we could show that all types of fiber-shaped nanomaterials that were handled at the companies could be detected and identified in the air. A number of measurement methods were used and evaluated. We showed that a combination of filter samples followed by chemical analysis, and direct reading measurements of different particle properties are crucial to getting the complete picture of the exposure situations, even though this can be expensive and time consuming. However, for some materials – for example carbon-containing materials such as graphene – faster and cheaper methods are available and were proven to work well in our studies.

To be able to assess how different nanomaterials should be handled, we need more knowledge about how dangerous it is to be exposed to these materials. Since the occupational exposure levels have not been completely determined, and due to the great uncertainty that exists about how dangerous the materials are, it would be unethical to conduct studies with human volunteers. Animals are often used instead to study the effects exposures can have. However, to reduce the unnecessary use of animals, and to investigate the underlying toxicological mechanisms behind the effects, experiments using cell cultures are performed. Traditionally, this is done by adding nanoparticles to cells covered with a growth liquid. But this does not resemble the actual exposure situation in our lungs where the nanoparticles are airborne when they come into contact with the cell surface of the lungs.

This is why cells with an air-liquid interface have been introduced for these types of studies. But to be able to expose cells at the air-liquid interface, special systems that can handle aerosols are needed. In our research, we used the Nano Aerosol Chamber for *In Vitro* Toxicity (NACIVT) system and compared it to traditional systems to investigate the differences in the effects on the cells. We showed that the NACIVT system was more sensitive to low doses of nanoparticle exposure than the traditional systems. Further on, it will be crucial to use more physiologically realistic systems such as this one to evaluate the hazards of airborne nanomaterials.

The new knowledge generated through the research presented in this thesis can be used to promote safe working environments. Information about different exposure situations and potential health effects can be used to provide improved risk assessments. Comparisons and evaluations of different measurement techniques can be used to come up with recommendations for occupational hygienists or for continued research in the area. The identification of processes that cause exposures can be used by companies to implement suitable control systems. All this can be done to ensure healthier workplaces!

Papers included in this thesis

Paper I **Karin Lovén**, Christina Isaxon, Aneta Wierzbicka, Anders Gudmundsson. **Characterization of airborne particles from cleaning sprays and their corresponding respiratory deposition fractions.** *Journal of Occupational and Environmental Hygiene* **2019**, 16(9): 656-667. DOI: 10.1080/15459624.2019.1643466

Paper II **Karin Lovén**, Anders Gudmundsson, Eva Assarsson, Monica Kåredal, Aneta Wierzbicka, Camilla Dahlqvist, Catarina Nordander, Yiyi Xu, Jörn Nielsen, Christina Isaxon. **Effects of cleaning sprays on eyes, airways, and ergonomic load in a human chamber exposure study.** *Submitted to a scientific journal and under review.*

Paper III Christina Isaxon, **Karin Lovén**, Linus Ludvigsson, Sudhakar Sivakumar, Anders Gudmundsson, Maria E. Messing, Joakim Pagels, Maria Hedmer. **Workplace emissions and exposures during semiconductor nanowire production, post-production, and maintenance work.** *Annals of Work Exposures and Health* **2020**, 64(1): 38-54. DOI: 10.1093/annweh/wxz088

Paper IV **Karin Lovén**, Sara M. Franzén, Christina Isaxon, Maria E. Messing, Johan Martinsson, Anders Gudmundsson, Joakim Pagels, Maria Hedmer. **Emissions and exposures of graphene nanomaterials, titanium dioxide nanofibers and nanoparticles during down-stream industrial handling.** *Submitted to a scientific journal and under review.*

Paper V **Karin Lovén**, Julia Dobric, Deniz Bölükbas, Monica Kåredal, Darcy E. Wagner, Jenny Rissler, Christina Isaxon. **Toxicological effects of zinc oxide nanoparticle exposure and the bias of the exposure system: an *in vitro* comparison between air-liquid interface and submerged exposure systems.** *Submitted to a scientific journal.*

Author's contribution to the papers

Paper I I had a major role in designing the study. I conducted the measurements, analyzed and interpreted the data, as well as produced the figures for publication. I was also the main contributor in the writing of the paper and in the peer-review process.

Paper II I had a major role in the preparation and conduction of the survey as well as the planning of the exposure study. I was a major contributor in the team that carried out the exposures. I analyzed and interpreted most of the data mainly related to the aerosol measurements as well as the medical data (except for ergonomic data), and I produced the figures for publication. I was also the main contributor in writing the paper and in the peer-review process.

Paper III I actively participated in designing the experimental setup and conducting the measurements. I analyzed and interpreted parts of the data mainly related to the on-line measurements, and I produced many of the figures for publication. I also had a major role in the writing of the paper and in the peer-review process.

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Paper V I was responsible for designing the study. I conducted parts of the experiments and supervised the remaining experimental work as well as the analysis of the data, in part as the main supervisor of Julia Dobric, a Master Thesis student. I was responsible for interpreting the results and I was also the main contributor in writing the paper.

Scientific conference presentations (oral or poster)

K. Lovén, C. Isaxon, J. Nielsen, G. Wieslander, A. Wierzbicka, A. Gudmundsson. Aerosols from cleaning sprays: Characterization and human chamber exposure study. *Nordic Society for Aerosol Research (NOSA)*, Aarhus, Denmark, **2016**.

K. Lovén, C. Isaxon, J. Nielsen, G. Wieslander, A. Wierzbicka, A. Gudmundsson. Cleaning sprays: Aerosol characterization and human exposure. *4th Workplace and Indoor Aerosols Conference*, Barcelona, Spain, **2016**.

K. Lovén, M. Hedmer, J. Pagels, A. Gudmundsson. Real-time exposure measurements of carbon nanotubes using an aethalometer. *International Conference on Health & Safety Issues related to Nanomaterials, Nanosafe*, Grenoble, France, **2016**.

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List of abbreviations and symbols

AER	Air Exchange Rate
ALI	Air-Liquid Interface
APS	Aerodynamic Particle Sizer
As	Arsenic
Au	Gold
BC	Black Carbon
BSI	British Standards Institution
BUT	Break-Up Time
BZ	Background Zone
CMD	Count Median Diameter
CNT	Carbon Nanotube
COPD	Chronic Obstructive Pulmonary Disease
CPC	Condensation Particle Counter
CRP	C-Reactive Protein
Cu	Copper
DALY	Disability-Adjusted Life Years
DMA	Differential Mobility Analyzer
DMS	Fast Aerosol Mobility Size Spectrometer
EC	Elemental Carbon
EDX	Energy Dispersive X-ray spectroscopy
ENM	Engineered Nanomaterial
EZ	Emission Zone
FEV_1	Forced Expiratory Volume in one second
FVC	Forced Vital Capacity
Ga	Gallium
GaAs	Gallium arsenide
Hb	Hemoglobin
IL	Interleukin

ISP-MS	Inductively Coupled Plasma Mass Spectrometry
LDH	Lactate Dehydrogenase
LDSA	Lung Deposited Surface Area
MCP	Monocyte Chemotactic Protein
MMD_{ae}	Mass Median Aerodynamic Diameter
MPPD	Multi-Path Particle Dosimetry
NACIVT	Nano Aerosol Chamber for *In Vitro* Toxicity
NaCl	Sodium Chloride
NP	Nanoparticle
OECD	Organisation for Economic Cooperation and Development
PBZ	Personal Breathing Zone
PID	Photo Ionization Detection
PIXE	Particle Induced X-ray Emission
PNIF	Peak Nasal Inspiratory Flow
PPE	Personal Protective Equipment
RH	Relative Humidity
SA	Supply Air
SEM	Scanning Electron Microscopy
sEMG	Surface Electromyography
Si	Silicon
SMPS	Scanning Mobility Particle Sizer
Ti	Titanium
TiO_2	Titanium dioxide
TNF-α	Tumor Necrosis Factor
UV	Ultraviolet
VAS	Visual Analogue Scale
VOC	Volatile Organic Compound
WST	Water-Soluble Tetrazolium
ZnO	Zinc Oxide

Motivation, Aim and Objectives

Motivation

We, as a population, are getting older and living longer. This means that we most likely need to work for a longer period of time. Due to this, there is an extra demand on employers to provide occupational environments with sustainable working conditions for all their workers, from young to old. In some of these environments, it will be challenging to continue working problem-free. One such challenge is the air we breathe that contains particles and gases, that is, *aerosols*, and the adverse health effects they may cause if inhaled. The introduction of new products into occupational environments can generate harmful aerosols, regardless of if the product has a low or high technological level.

Cleaning workers make up a large occupational group, both in Sweden and worldwide, in which low-tech spray products have been introduced. This is also an occupation dominated by women (SCB 2017). They are subjected to several different risk factors affecting their ability to have a long and healthy working life. One such risk factor is handling a range of chemical cleaning products. This usage can lead to both skin problems (Garza et al. 2015; Singgih et al. 1986) and inhalation-related problems, such as asthma and nasal symptoms (Garza et al. 2015; Gonzalez et al. 2014; Quirce and Barranco 2010). To facilitate the application of these cleaning products, the use of sprays was proposed. However, a recently published Norwegian study showed that cleaning, including with sprays, can lead to a large decrease in lung function, especially for women (Svanes et al. 2018).

The introduction of more high-tech products are found in the rapidly growing nanotechnology industry (Roco et al. 2011), where many new materials and particles are increasingly being produced and used. These materials can have new and sometimes unknown properties, both regarding the utilization of the materials, but also regarding the possible health effects they may cause. The definition of nanomaterials recommended by the European Commission (2011) is: “Materials that have at least one dimension that is smaller than 100 nm for 50% or more of the particles constituting the material.” Nanomaterials can have different morphologies, such as being spherical or fiber-shaped. Nanofibers are of special concern since if inhaled, they can have the same or worse adverse effects as asbestos (Donaldson et al. 2010), which can cause fibrosis and different types of lung cancers (e.g., mesothelioma) (Luus 2007).

Workers in the nanotechnology industry are among the first during development and production to have contact with potential health hazards from these materials, and their exposure is often higher than for the general population.

For both of these occupational groups that are affected by the introduction of new products into their occupational environments, knowledge is needed about the present exposure situations, as well as evaluations of the potential health and toxicological effects of these exposures. All of this is necessary to be able to improve occupational environments and to provide correct risk management strategies so that healthier workplace conditions can be achieved.

Aim and objectives

The overall aim of the research presented in this thesis was to provide a basis for the implementation of improvements of the working environments for different occupational groups by addressing the research needs within them. The specific objectives were:

- To develop a measuring methodology to determine the aerosol emission and exposure characteristics present in the cleaning occupational environment (**Papers I and II**).
- To assess and apply measuring methodologies used for engineered nanoparticle exposure and emission characterizations, and evaluate the different work processes regarding emissions in the nanotechnology industry (**Papers III and IV**).
- To evaluate the potential health and toxicological effects caused by different occupational exposures, and compare the effects of different exposure conditions and toxicological methodologies (**Papers II and V**).

Introduction

What's in the air?

Except for the more romantic answer of love, the most common scientific answer to that question is probably oxygen – the compound that humans need to survive. Nitrogen, which is in fact the largest component of air, could also be a possible answer. Another answer could be aerosols. Today, aerosols are quite often associated with emissions from cars or industries, but some aerosols are naturally occurring and can be formed and emitted from, for example, the forest or from the sea (Janson et al. 2001; Vignati et al. 2010). Exposure to some types of aerosols can be damaging to human health. Aerosols such as combustion-generated particles and cigarette smoke can cause several adverse health effects including respiratory problems such as asthma, chronic obstructive pulmonary disease (COPD) and lung cancer (Das 2003; Kennedy 2007).

Other problematic aerosols of concern are occupational aerosols. These are aerosols generated during different work processes in a variety of occupational environments. They can be of concern for the workers present in these environments for long periods of time, several hours per day up to a whole working shift, during a whole working life. Even though smoking has been established as a major risk factor for developing COPD, occupational aerosol exposure – including organic and inorganic dust, plastic chemicals and combustion pollutants from transportation – have been identified as risk factors associated with COPD in non-smokers (Salvi and Barnes 2009). As much as 31% of the population-attributed fraction of COPD could possibly be associated with occupational exposure. The Global Burden of Disease study has also estimated that in 2015, occupational exposures to particulates, gases and fumes were responsible for 0.36 million deaths and 8.8 million disability-adjusted life years (DALYs) globally (Forouzanfar et al. 2016).

To increase the knowledge of aerosols and their effects on human health, the Lancet Commission recently recommended a few research focuses including a better understanding of the diseases caused by chemical pollutants, and knowledge of the health effects associated with new and emerging pollutants (Landrigan et al. 2018).

Which occupational groups are at risk for exposure?

Some well-known groups that can be at risk for developing adverse health effects as a result of their occupational exposures are mine workers exposed to stone dust (Souza et al. 2017), farmers exposed to organic dust (Melbostad and Eduard 2001) truck drivers exposed to diesel exhaust (Cantrell and Watts Jr 1997), and hair dressers exposed to bleaching powder (Nilsson et al. 2016). Another large occupational group at risk are cleaning workers. Their use of a wide range of cleaning products, often containing irritants or disinfectants, have been associated with respiratory problems including the development of occupational asthma (Vizcaya et al. 2011; Walters et al. 2018; Weinmann et al. 2017). Using sprays to apply these cleaning products is a common practice; however, this application method can increase the exposure of chemicals to the respiratory system from the aerosols generated during use. A few epidemiological studies (S. J. Lee et al. 2014; Nielsen and Bach 1999; Zock et al. 2001) have associated the specific use of cleaning sprays with a higher occurrence of respiratory symptoms. An additional risk factor prevalent among professional cleaning workers is the high physical workload present during their daily work with strenuous postures, heavy lifting, and repetitive movements (Unge et al. 2007). Pain in the neck and upper extremities is very common among cleaning workers, and they also run an increased risk of disability retirement (Jensen et al. 2016).

A quite different occupational group, with a more unclear exposure and effect situation, includes workers in the nanotechnology industry. A major concern for this group is the production and handling of a wide range of new engineered nanomaterials (ENMs), consisting of different compounds and with different morphologies (Ding et al. 2017). These can be materials with three nano-scale dimensions such as spherical particles, materials with two nano-scale dimensions such as wires or fibers, or materials with one nano-scale dimension such as planes or platelets. Due to their nano-scale they present different properties than their equivalent bulk materials (Jiang et al. 2009; Kim et al. 2019). The exposure situations of some of these ENMs during some work processes have been characterized previously (Basinas et al. 2018; Debia et al. 2016) and have shown that manual activities, such as handling powders, spraying and equipment maintenance, are likely to cause particle exposures. The effects of these types of exposures are not well known; however, a few studies involving workers who handle ENMs have been conducted and have shown increases in some inflammatory (Glass et al. 2017) and oxidative stress biomarkers (Wu et al. 2019). Animal and cell studies, though, are more extensive and have revealed that ENMs can induce several adverse effects including oxidative stress, inflammation and fibrosis (Srivastava et al. 2015).

How can occupational environments be improved?

A general idea for improving the occupational environments is to apply what is referred to as a *hierarchy of controls* (NIOSH 2015), see **Figure 1**. The first step is to remove the chemical, material or method used that can constitute a risk for the worker. The second step is to replace the chemical, material or method used with a suitable safer alternative. The third step is to decrease the possible exposures by use of engineering controls such as encapsulations of the work processes and use of process ventilations or other air extracting mechanisms. The fourth step is to apply administrative controls such as changing the way the work is performed and the fifth and final step is to use personal protective equipment (PPE). The basis of this hierarchy is that the first step is the most effective in protecting workers and the final step is the least effective.

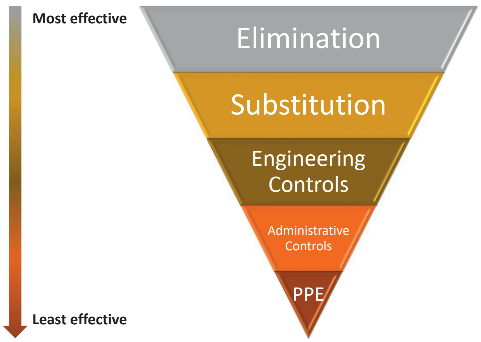

Figure 1. The five steps of the hierarchy of controls that should be applied to improve occupational environments. Figure adapted from (NIOSH 2015).

An additional approach is to apply the *precautionary principle*, which is a strategy stating that precautionary measures should be taken, or the activity should not be carried out at all if the consequences could be serious and are subjected to scientific uncertainties (Stefansson 2019). This principle can be used in both of the occupations focused on in this thesis, but it applies foremost to the nanotechnology industry, where a special emphasis on caution and reviewing new nanotechnology innovations should be taken into consideration before wide spread use is established (Spruit 2017).

Performing full and complete workplace *risk analyses* are also an important part in the effort towards improving occupational environments. Occupational risk can be defined as the product of the probability and the consequence of a hazardous event or phenomenon (risk = probability x consequence). The probability can be interpreted as the extent of the worker exposure, consisting of the concentration and time exposed, while the consequence can be interpreted as the health effect or the toxicity caused by the exposure.

How can the exposures and health effects be studied?

The simple answer to this question is that there is no simple answer. A multidisciplinary approach, including several different types of studies, are needed to get the full picture of the sequence of events required for an aerosol emission source to induce human health effects, as seen in **Figure 2**. Aerosol studies are needed to measure the air concentrations generated from an aerosol emission source, as well as determine the actual human exposure. Epidemiological studies are used to find the statistical link between different source concentrations and various health effects. Clinical studies are used to find the actual causality between a specific exposure and a specific effect, and toxicological studies are needed to understand the mechanisms behind the initiation of a certain effect from a certain exposure, and at what dose levels these effects arise. **Figure 2** presents an overview of how the various studies are connected to the sequence of events required for an aerosol emission source to induce human health effects.

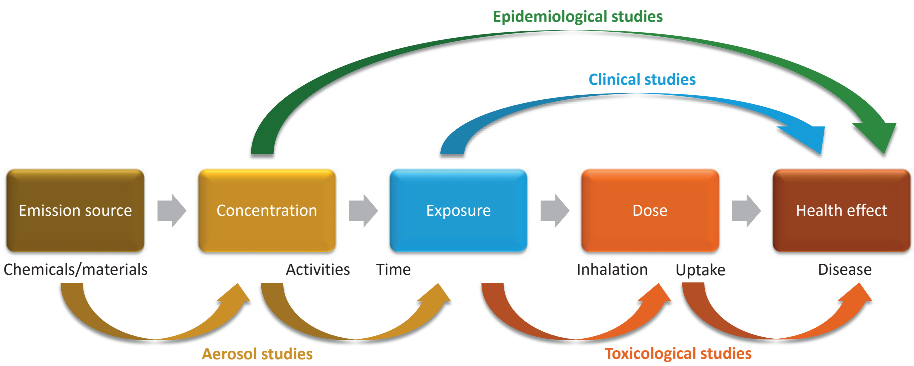

Figure 2. The sequence of events required for an aerosol emission source to induce human health effects, and the different types of studies needed to examine the connection between them.

Epidemiological studies involving the association between aerosol exposure and human health effects often lay the ground for, and are used as motivation for other more detailed types of studies (Pope 2000).

Aerosol studies can be performed with a variety of instruments and methods to measure emission concentrations and human exposure. The type of chemicals or materials that are present in the occupational environment can be determined with, for example, off-line methods such as filter sampling. The specific activities that are of special concern and the concentrations they generate can be determined with, for instance, on-line methods such as particle counters in combination with observation logbooks. Measuring what you are interested in can, however, be most challenging when you are trying to identify something specific in a complex and dynamic background aerosol.

Clinical studies, such as human chamber exposure studies, can be conducted with the help of different medical methods (e.g., lung function measurements) depending on the type of effects that are of interest. How large fraction of the airborne particles that is deposited in different parts of the lung after inhalation can, for example, be modeled for different conditions by applying the multiple-path particle dosimetry (MPPD) model (Netherlands National Institute for Public Health and the Environment (RIVM) 2002). Possible input parameters are measured particle characteristics and breathing frequencies.

Toxicological studies can be performed with a variety of methods, such as, with animals (*in vivo*) or cells (*in vitro*), depending on if it is the effects or the mechanisms behind the effects that are of interest. There is a large variability in these studies in terms of what type of animals, what type of cells, what kind of exposures, and what doses that are used. It can thus be challenging to perform and use them for risk analyses for humans.

Methodology

Evaluation methods for occupational environments

Overview

Two different occupational groups were studied in the thesis research: cleaning workers and workers in the nanotechnology industry. In the first group, the existing chemicals and materials are repeatedly and extensively used, and the type of application method used can be detrimental and cause long-term occupational exposures. In the second group, new ENMs with properties other than those of the bulk materials are being produced and used, and the production and handling processes can be of importance for the occupational exposure situations. These exposures can then potentially lead to adverse health effects.

Extensive and comprehensive methodological approaches were used to generate knowledge about the present exposure situations, as well as the potential health and toxicological effects in the two occupational groups focused on in this thesis. The methodologies are divided into the three overall categories of contextual information gathering, aerosol characterization, and health/toxicity assessment. **Table 1** shows the different methodologies used in the studies presented in the papers and the type of information that was retrieved. **Table 2** then shows the specific aerosol measurement techniques used in the studies presented in the papers. More details regarding the specific methods and the choice to use them follows in the coming sections.

Table 1. The comprehensive approach including the methodologies used in the five appended papers and the type of information that was retrieved.

Occupational group	Cleaning workers		Workers in the nanotechnology industry		
Paper	**I**	**II**	**III**	**IV**	**V**
Contextual information gathering	Type of cleaning spray products used by professional cleaning workers		Type of ENMs produced/used and the processes conducted by the workers		
		Self-reported eye and airway symptoms and physical pain experienced by professional cleaning workers			
Aerosol characterization (more details in Table 2)	Airborne particle characterization with off-line methods		Airborne particle characterization with off-line methods		
	Airborne particle characterization with on-line methods		Airborne particle characterization with on-line methods		
		Gas characterization with on-line methods	Surface contamination particle characterization		
Health/toxicity assessment	Respiratory deposition fractions	Self-assessed symptoms, physician-conducted medical assessments, ergonomic load measurements			Toxicological responses

Table 2. The different aerosol measurement techniques used in the five appended papers.

Occupational group	Cleaning workers		Workers in the nanotechnology industry				
Paper	**I**	**II**	**III**		**IV**		**V**
Off-line			**Study 1**	**Study 2**	**Study A**	**Study B**	
Paper-weighing method	X						
Filter sampling			X	X	X	X	X
Surface sampling			X	X		X	
On-line (stationary)							
APS (cm^{-3})	X	X	X	X	X	X	X
SMPS (cm^{-3})	X		X				X
DMS (cm^{-3})	X				X		
CPC (cm^{-3})		X	X	X	X	X	
VelociCalc (gas phase VOC, ppb)		X					
P-Trak (cm^{-3})			X	X	X	X	
DustTrak ($\mu g/m^3$)			X	X	X	X	
Aethalometer (μg BC/m^3)					X	X	
On-line (personal)							
SidePak ($\mu g/m^3$)		X		X			
Nanotracer (cm^{-3})			X	X			
Partector ($\mu m^2/cm^3$)					X	X	
Portable aethalometer (μg BC/m^3)					X	X	

Methods for evaluating the cleaning environment

To assess the working environment for cleaning workers, with a specific focus on the possible aerosol exposure generated during spray use, a first step was to collect information about the real work-life situation regarding spray use. An initial phone inquiry of cleaning companies (n = 20), and a survey among professional cleaning workers (n = 225) from ten different cleaning companies/organizations of different size (all in Sweden) were conducted. The questions were about the extent of cleaning spray usage during working hours, the surfaces the sprays were used on, and the products (including brands) that were used. Based on these answers, seven products were chosen from two different brands to go through aerosol characterization (to achieve high societal relevance of the study). The products included window, bathroom and universal sprays from both brands (**Paper I**).

Characterization of the spray aerosol is needed to understand what causes the manifestation of symptoms that are seen among cleaning workers. The aerosol gas phase has previously been measured (Bello et al. 2010; Medina-Ramon et al. 2005; Singer et al. 2006). Studies on the characterizations of the particle phase, on the other hand, are lacking but are necessary to understand the deposition in the respiratory system and the potentially adverse health effects these particles may have. To gain a comprehensive knowledge base, the first step of the aerosol characterization in our study included determining the amount of aerosol generated from the seven different cleaning sprays. A paper-weighing method was developed for these measurements. When the amount of the total aerosol generated (total airborne mass fraction) had been determined, more specific characterizations of the particle phase were conducted. These characterizations included measurements of the particle size distributions, initially conducted with both a scanning mobility particle sizer (SMPS) and an aerodynamic particle sizer (APS) to include the whole size range from nanometer to micrometer size particles. The characterizations also included experiments to investigate new particle formation (from reactions with ozone), performed with a fast aerosol mobility size spectrometer (DMS) to be able to capture the rapid changes. To relate these measurements to potential health effects, particle respiratory deposition fractions were calculated using the MPPD (v 3.04) model (Netherlands National Institute for Public Health and the Environment (RIVM) 2002).

The survey additionally included questions regarding perceived health-related symptoms (both eye and airway symptoms) and the experience of physical pain among the professional cleaning workers. A human chamber exposure study was performed to validate these results experimentally and try to get more causality-related information about the reason for the reported symptoms (**Paper II**).

The human chamber exposure study was performed in a 21.6 m^3 stainless steel exposure chamber with a floor area of 9 m^2 and with controlled environmental settings (temperature, relative humidity (RH), and air exchange rate (AER)).

The chamber had an AER of 0.9 h^{-1}, in accordance with typical residence rates, and was furnished with a toilet, a sink, a mirror and a shower corner with tile walls and glass doors to simulate a hotel bathroom. The aerosol concentrations and experienced symptoms were measured during three different cleaning exposure scenarios in order to compare different commonly used cleaning methods. The scenarios tested were:

- *Spray/cloth* – spraying the cleaning product onto the surfaces and wiping with microfiber cloths.
- *Foam/cloth* – cleaning product application by foam onto the microfiber cloths and wiping the surfaces with the cloths.
- *Water/cloth* – wiping the surfaces only with microfiber cloths.

All microfiber cloths were premoistened with clean water prior to use. We aimed to include only professional cleaning workers in the human chamber exposure study to capture how such cleaning work is actually performed in reality; however, this proved to be difficult due to challenging scheduling issues, both from the employers and from the individual cleaning workers. A comparative group of non-cleaning workers was therefore also recruited, which additionally made it possible to compare the two groups regarding experienced health effects. In total 19 participants were recruited for the study: 11 professional cleaning workers and 8 non-cleaning workers. Only women were recruited since this is a heavily female dominated occupational and it has been shown that the lungs of women are more sensitive to chemical exposure than men's (Svanes et al. 2018).

Each participant used one cleaning method during one day, for a total of three separate days with a few weeks in between the different exposure scenarios. During each exposure day, three 30-minute cleaning sessions were conducted in the exposure chamber with a 1.5-hour break between them. In one 30-minute cleaning session, each participant cleaned the bathroom inside the chamber eight times, for a total of 24 cleaned bathrooms in one day. This represents a normal working day for a hotel cleaning worker. **Figure 3** shows a simplified flow chart of the study design (a more detailed chart can be found in **Paper II**).

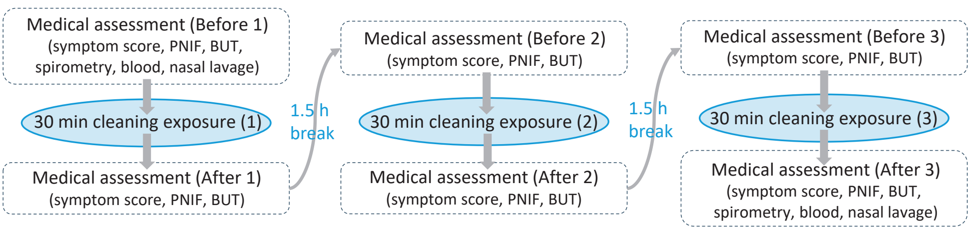

Figure 3. Flow chart of the study design during one exposure day of the human chamber exposure study.

The aerosol characterizations included both particle and gas stationary on-line measurements to monitor the room concentrations, as well as personal on-line particle measurements to determine the human exposure. To investigate the effect these exposures had on the eyes and airways, medical assessments were conducted before and after exposure. These included self-assessment symptom scores for an initial overview, and for more detailed studies of the effects, physician-conducted evaluations consisting of peak nasal inspiratory flow (PNIF), tear film break-up time (BUT) and spirometry measurements as well as biomarker analyses in blood and nasal lavage samples. The medical assessments were based on the types of reported symptoms found in previous studies (Garza et al. 2015; S. J. Lee et al. 2014; Nielsen and Bach 1999). The participant's pulse was also monitored continuously throughout the whole exposure day. The study was single-blinded, but in the opposite meaning compared to the traditional denotation: the participants knew the type of exposure (since they generated it themselves by using different cleaning methods), while the medical personnel did not. In addition to the aerosol characterizations and the medical assessments, measurements of the ergonomic load were also recorded to get the most comprehensive view possible of the occupational exposure situation for cleaning workers and the health effects caused thereof. This is important to take into account when considering interventions in the cleaning occupational environment.

Throughout the whole Cleaning Spray Project, a reference group including representatives from cleaning unions, trade organizations and product companies, were consulted on issues considering some design aspects of the project (e.g., the types of cleaning methods to include in the human chamber exposure study) in order to conduct the studies with a high level of societal relevance.

Methods for evaluating the nanotechnology environment

The specific focus in assessing the working environment in the nanotechnology industry was on the possible aerosol exposure generated during the production and handling of ENMs. The first step was to collect contextual information from the companies involved in the studies (**Papers III and IV**). The contextual information included the types of ENMs that were handled and the types of processes that were conducted. This is in accordance with the first (tier 1) of the three-step harmonized tiered approach suggested by the Organisation for Economic Cooperation and Development (OECD) as guidelines when conducting nanomaterial emission and exposure assessments (OECD 2015). If particle release into the workplace air cannot be excluded during tier 1, a basic exposure assessment with simple measuring instruments should be carried out (tier 2); and if increased particle concentrations of unknown origin over background can be found, an expert exposure assessment with more advanced instruments and analysis should be performed (tier 3). **Figure 4** shows a schematic flow of the tiers in the OECD guidelines.

The workplace assessments included in this thesis research followed this approach and the completion of tier 1 was followed by a tier 2 assessment with an on-site visit to the companies. These included an initial meeting with the employees, a walk-through of the facilities, discussions regarding the different processes (where and how they were performed), and a few simple measurements. Tier 3 expert exposure measurements were then conducted and these are presented in **Papers III and IV**.

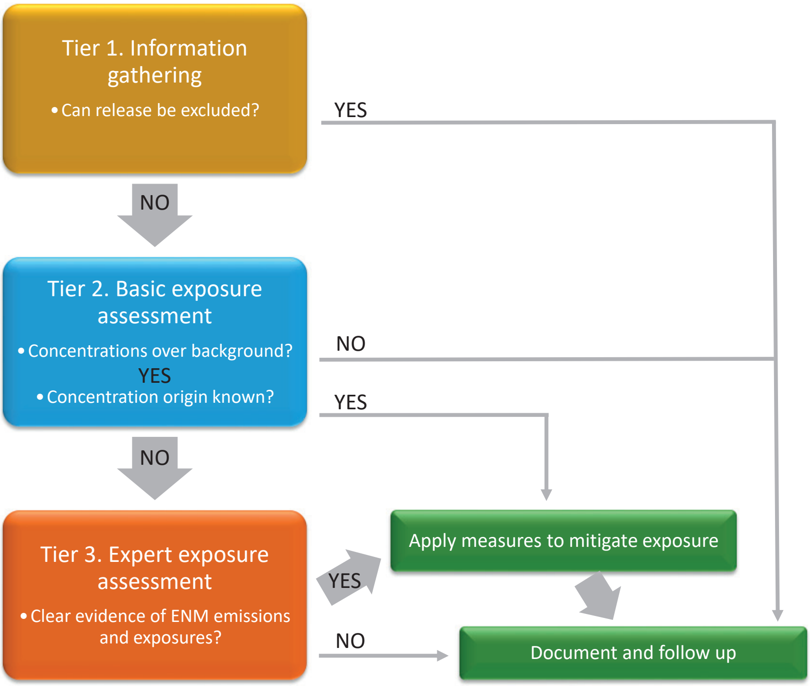

Figure 4. Schematic flow chart of the three-tiered approach included in the OECD guidelines for conducting nanomaterial emission and exposure assessments. Figure adapted from (OECD 2015).

Workplace emission and exposure measurements are needed to assess the real work-situation and to employ relevant safety measures to protect the workers from potentially hazardous materials, foremost with regard to airway exposures. A selection of ENMs and work processes have previously been identified as causing possible airway exposures (Debia et al. 2016) including work processes involving different types of carbon nanotubes (CNTs). Because the fiber-shaped nano-materials are of special concern, exposure characterizations of other unexplored fibrous nanomaterials are needed to complete the data pool. Metal nanowires and titanium dioxide (TiO_2) nanofibers are two fibrous nanomaterials for which occupational exposures have not been characterized previously. In addition, the lack of exposure data during down-stream use of ENMs has also been pointed out (Basinas et al. 2018), one example being during the down-stream use of graphene.

For our studies, emission and exposure measurements were carried out in several different laboratories at companies handling metal nanowires, TiO_2 nanofibers (and nanoparticles (NPs)), and graphene. A selection of work tasks were observed, which have been stated to be especially important in industries with a high degree of daily variability, such as the nanotechnology industry (Oberbek et al. 2019).

In order to really understand the exposure situations and establish a reliable knowledge base for comprehensive process characterizations, particle measurements were carried out in four different zones:

- *Emission zone (EZ)* – close (no more than a few centimeters) to the potential particle source.
- *Personal breathing zone (PBZ)* – close (no more than 30 cm) to the nose and mouth of the worker.
- *Background zone (BZ)* – far away (at least a few meters) from any potential particle source.
- *Supply air (SA)* – the incoming air from the ventilation system.

Stationary aerosol instruments and open-face filter cassettes were placed in the emission zone and the background zone to characterize the source emissions and particle background in the facility. Smaller, portable instruments were worn by the workers along with open-face filter cassettes to measure in the personal breathing zone and establish the worker exposure. Depending on the type of ENMs that were handled at the different companies, different instruments and analysis methods were used. Two stationary instruments were also placed at the supply air, to monitor the infiltration of particles from outdoors. In addition to the air sampling, surface samples were collected to evaluate the potential for secondary inhalation exposure through particle resuspension. The work processes, engineering controls, PPE, and the measurement setups were thoroughly documented with both logbooks and by photos.

Relevant toxicological assessments are needed to evaluate the potential hazards of new ENMs. The use of animals for inhalation exposure studies is probably the most representative method to study the resulting effects since human chamber exposure studies with these new ENMs would be unethical. However, to minimize unwarranted use of animals for initial screenings of possibly toxic exposures, as well as to study the underlying mechanisms resulting in toxic effects seen in *in vivo* studies, *in vitro* cell models are commonly applied. *In vitro* toxicity testing is traditionally conducted with submerged cell cultures, where the cells are covered with growth medium (left part of **Figure 5**) and the NPs are added into the medium.

For more realistic respiratory exposure toxicity evaluations, improved systems with cells at the air-liquid interface (ALI) can be used. These are cells grown on a semi-permeable membrane, where the cells are in contact with the growth medium from below (basal side) and with air from above (apical side) (right part of **Figure 5**). This more closely resembles the physiological conditions in the lungs. The NPs can then be deposited onto the cells from the aerosolized phase. For this purpose, several different types of ALI exposure systems have been introduced (Secondo et al. 2017; Upadhyay and Palmberg 2018). Most of these systems use diffusion to deposit the particles onto the cells, but to increase the deposition efficiency, electrostatic deposition can be used. One system that utilizes this technique is the Nano Aerosol Chamber for *In Vitro* Toxicity (NACIVT). The NACIVT system in comparison with two submerged cell exposure systems were used for toxicity assessments in **Paper V** in order to understand the similarities and differences between these exposure systems.

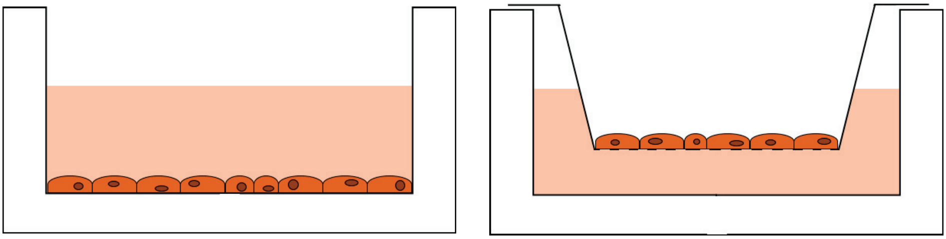

Figure 5. Schematic images of traditionally submerged cell cultures (left) and cell cultures at the ALI (right).

The exposure to zinc oxide (ZnO) NPs (as a model ENM) onto human alveolar epithelial cells (A549) at three different doses was tested in the NACIVT system and in two different submerged systems, *SUB(growth)* and *SUB(iso)*. In all three systems, the cells were grown in Transwell inserts on a semi-permeable membrane. In the NACIVT system, the cells were exposed at the ALI to the aerosolized NPs. In the SUB(growth) system, the cells were covered with growth medium on the apical side throughout the experiments and the NPs were added into the growth medium during exposure. In the SUB(iso) system, the cells were first allowed to grow at ALI conditions (the same way as the cells in the NACIVT), but during exposure, a low volume of isotonic solution (0.9% NaCl) containing the NPs was added to the cells. The low volume was motivated by the intention of resembling ALI as closely as possible (i.e., minimal liquid volume at the apical side of the cells). A more detailed description of the different exposures can be found in **Paper V**. The toxicological responses were recorded as level of metabolic activity, cytotoxicity and release of cytokines, which are typical endpoints investigated in similar studies (Chen et al. 2015; Mihai et al. 2015; Stoehr et al. 2015; Thongkam et al. 2017). They were compared in the three systems to evaluate the sensitivity of the different methods.

Aerosol measurement techniques

A variety of aerosol measurement techniques have been used in the studies included in this thesis to provide a comprehensive knowledge base of the aerosol emissions and exposures present in different occupational settings, as seen in **Table 2**. This section presents a short summary of these different techniques with additional details.

Off-line methods

Paper-weighing method

This is a setup developed to measure the total airborne mass fraction, that is, the total mass that remains airborne during spraying. The setup consists of three paper napkins (unfolded on top of each other), a can with a screw cap and a scale. The paper napkins are taped on the wall and one pulse of liquid is sprayed against them at a distance of 30 cm. The paper napkins (placed in the can to avoid evaporation) and the bottle are weighed before and after the spraying. **Figure 6** shows a typical spray bottle used for these measurements.

Figure 6. Typical spray bottle used for total airborne mass fraction measurements.

Filter sampling

The unintentional release of airborne ENMs at the nanotechnology companies was assessed by the following filter-based methods:

25-mm cellulose filters with a pore size of 0.45 µm (SKC Inc., USA) were used for open-face sampling in the filter cassettes (**Figure 7**) for mass concentration analysis of the total dust and the following metals: gallium (Ga), arsenic (As) (**Paper III**), and copper (Cu) (**Paper IV**). Total dust was determined gravimetrically, and the metal composition was measured with inductively coupled plasma mass spectrometry (ICP-MS, iCAP Q, Germany).

25-mm polycarbonate filters with a pore size of 0.4 µm (SKC Inc., USA) were used for open-face sampling in filter cassettes (**Figure 7**) for particle number concentration quantification and identification of ENMs with scanning electron microscopy (SEM) (**Papers III and IV**), as well as analysis of mass concentration of titanium (Ti) with particle-induced X-ray emission (PIXE) (**Paper IV**). In **Paper III**, the SEM analyses included energy dispersive X-ray spectroscopy (EDX) for elemental analysis.

25-mm quartz filters (SKC Inc., USA) were used for open-face sampling in filter cassettes (**Figure 7**) for mass concentration analysis of elemental carbon (EC) (**Paper IV**). Quantification of EC was conducted with thermal-optical analysis (OC/EC DRI Model 2001 Carbon Analyzer, USA).

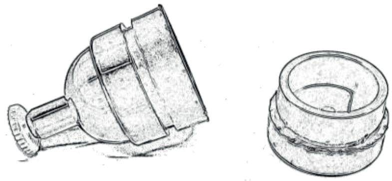

Figure 7. 25-mm filter cassette used for air filter sampling at the nanotechnology companies.

5-mm silicon wafers were used for the visualization and identification of ENMs after intentional aerosolization of the NPs for the toxicity studies, as well as primary particle size and aggregate morphology determination, with SEM.

Surface sampling

Tape samples, according to a method developed by Hedmer et al. (2015), were collected from different surfaces for assessment of the potential for secondary inhalation through resuspension of the ENMs deposited on workplace surfaces at the nanotechnology companies through analysis with SEM.

On-line methods

Aerodynamic Particle Sizer (APS)

The APS model 3321 (TSI Inc., USA) was used to measure the particle number concentration and the aerodynamic size distribution of particles in the size range 0.5-20 µm. The APS is an instrument that uses a time-of-flight particle sizing technique together with the light-scattering intensity produced from the particles interacting with a light source. The particles are accelerated using a narrowing nozzle and the time-of-flight is then measured downstream the nozzle between two lasers to determine the aerodynamic diameter. This results in a time resolution of seconds and a high size resolution.

Scanning Mobility Particle Sizer (SMPS)

The SMPS consisted of the Differential Mobility Analyzer (DMA) model 3075 and the Condensation Particle Counter (CPC) model 3010 (**Papers I and III**) or model 3775 (**Paper V**) (TSI Inc., USA). It was used to measure the particle number concentration and the electrical mobility size distribution of particles in the size range 0.01-0.7 µm. The SMPS is an electrical mobility instrument that uses a radioactive neutralizer to charge the particles, which are then sorted by scanning the voltage of the DMA. The particles with a specific electrical mobility are then counted by the CPC. This results in a time resolution of minutes, but with a high size resolution.

Fast Aerosol Mobility Size Spectrometer (DMS)

The DMS model 500 MkII (Cambustion, UK) was used to measure the particle number concentration and the mobility size distribution of particles in the size range 0.005-1 µm. The DMS is an electrical mobility instrument that uses electrometer detectors to measure the whole size distribution at once. This results in a time resolution of down to 0.1 seconds, but with a lower size resolution than the APS and SMPS.

Condensation Particle Counter (CPC)

The CPC models 3010 and 3775 (TSI Inc., USA) (only 3010 in **Paper II**) were used to measure the number concentration of particles from 0.007 µm up to a few µm. The CPC is an instrument that detects single particles using a heating and cooling system to condense butanol onto small particles in order for them to grow into sizes able to scatter light from a laser diode. The CPC can have a time resolution of seconds.

VelociCalc

The VelociCalc model 9565-P with the probe 986 (TSI Inc., USA) was used to measure the total volatile organic compound (VOC) gas phase concentration in the range 10-20,000 ppb. The VelociCalc uses a photo-ionization detection (PID) technique where an ultraviolet (UV) light source breaks down VOCs in air to ions. The PID then measures the charge of the ionized gas, reflecting the VOC concentration. The VelociCalc can have a time resolution of seconds.

P-Trak

The P-Trak model 8525 (TSI Inc., USA) was used to measure the number concentration of particles in the size range 0.02-1 µm. The P-Trak is an optical measuring instrument similar to the CPC, but hand-held. The particles are transported through a heating and cooling system where isopropyl alcohol is condensed onto the particles and they can then be detected by the laser light source. The P-Trak can have a time resolution of seconds.

DustTrak

The DustTrak models DRX 8533 and 8534 (TSI Inc., USA) (only 8534 in **Paper III**) were used to measure the particle mass concentration for the size fractions PM_1, $PM_{2.5}$, respirable, and PM_{10}. The DustTrak is an optical instrument based on a light-scattering technique where the particles pass by a laser diode and the light being scattered is focused onto a photodetector. This produces a voltage reflecting the mass concentration, calibrated with Arizona Test Dust. The DustTrak can have a time resolution of seconds.

Aethalometer

The Aethalometer model AE33 (Magee Scientific, USA) was used to measure the black carbon (BC) mass concentration. Particles are collected on a filter tape, and are illuminated by a multi-wavelength light source. Detectors measure the attenuation of light relative to an un-exposed reference point of the tape. The AE33 aethalometer can have a time resolution of seconds.

SidePak

The SidePak model AM510 (TSI Inc., USA) was used to measure the PM_{10} particle mass concentration. The SidePak is an optical instrument based on a light-scattering technique similar to the DustTrak. The SidePak is small enough to be used as a personal aerosol monitor and can have a time resolution of seconds.

Nanotracer

The Nanotracer (Philips Aerasense, The Netherlands) was used to determine the number concentration and average diameter of particles in the size range 0.01-0.3 µm. The Nanotracer is based on an electrical measuring technique where a unipolar charger charges the particles and the current carried by the particles is measured by electrometers. The Nanotracer is small enough to be used as a personal aerosol monitor and has a time resolution of 16 seconds.

Partector

The Partector (Naneos, Switzerland) was used to measure the lung deposited surface area (LDSA) concentration of particles in the size range 0.01-10 µm. The Partector is based on an electrical detection principle where a corona wire charges the particles and an electrometer measures this charge. The Partector is small enough to be used as a personal aerosol monitor and can have a time resolution of seconds.

Portable aethalometer

The portable Aethalometer model AE51 (AethLabs, USA) was used to measure the BC mass concentration. Particles are collected on a filter and the rate of change in absorption of transmitted light is measured. The AE51 aethalometer is small enough to be used as a personal aerosol monitor and can have a time resolution of seconds.

Medical assessments

A few different methods for medical evaluation were used to assess the human health effects from cleaning aerosol exposure in the thesis research (**Paper II**). What follows is a brief summary of the different methods with additional details.

Self-reported assessments

Self-assessment symptom scores, with visual analogue scales (VAS), were used to collect information about any health-related symptoms after aerosol exposure. Symptoms from the eyes, nose, throat (pharynx) and lower airways were registered.

Physician-conducted assessments

Peak Nasal Inspiratory Flow (PNIF)

PNIF measurements were performed to investigate nasal obstruction after aerosol exposure. A mask, connected to a PNIF meter (GM instruments, UK), was placed over the nose and mouth and the subject inhaled as much and as fast as possible through the nose. A low inertia indicator ring in the PNIF tube was moved by the air flow, and its position after inspiration indicated the maximum flow achieved (in liters per minute). **Figure 8** shows how the PNIF measurement is performed.

Figure 8. Demonstration of how a PNIF meter is used for assessment of nasal obstruction.

Tear film Break-Up Time (BUT)

BUT measurements were performed to evaluate tear film stability after aerosol exposure. An ocular microscope (Keeler TearScope®, Keeler Instruments, UK) was placed in front of one eye. The microscope has a grid of circles of light at equal distances from each other (**Figure 9**), and the time it took for the light to blur was used as the BUT (in seconds).

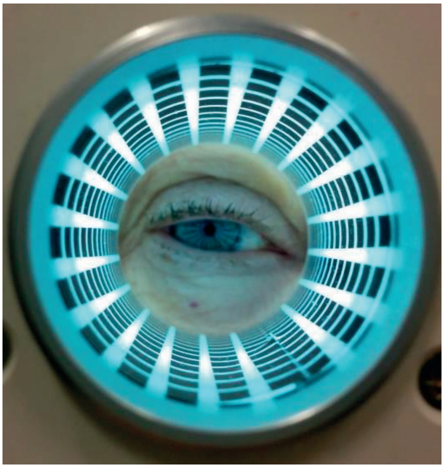

Figure 9. Demonstration of the light grid generated by a TearScope® ocular microscope, used for assessment of tear film stability.

Spirometry

Lung function tests were performed with spirometry (SPIRARE 3, Diagnostica, Norway) after aerosol exposure. Forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1) were recorded.

Biomarkers

Hemoglobin (Hb) levels, immune cell counts (neutrophils, eosinophils, basophils, lymphocytes, monocytes, total leukocytes) and C-reactive protein (CRP) levels were analyzed in blood samples. Interleukin 6 and 8 (IL-6, IL-8) levels were analyzed in both blood and nasal lavage samples.

Ergonomic assessments

The physical workload was recorded with: a) inclinometry to measure the postures and movements of the head, upper back and both upper arms; b) electrogoniometers to measure the postures and movements of the wrists; and c) surface electromyography (sEMG) to measure the muscular load in the shoulders and forearm muscles.

Toxicological methods

A few different methods for toxicological evaluation were used to assess the cell toxic effects from ENM aerosol exposure in the thesis research (**Paper V**). What follows is a short summary of the different methods with additional details.

Exposure methods

Nano Aerosol Chamber for In Vitro *Toxicity (NACIVT)*

The NACIVT is a system for aerosol exposure of cells at the ALI, where the cells are in contact with the growth medium from the basal side and the air from the apical side. The system combines an incubation environment with a well-controlled RH of 85% and temperature of 37 °C to simulate the physiological conditions, with a high efficiency electrostatic deposition of particles onto the cells. One important part of the system is the well plate (**Figure 10**) in which 24 inserts with cells can be simultaneously exposed to the same aerosol (Jeannet et al. 2015).

Figure 10. NACIVT chamber well plate for simultaneous aerosol exposure to 24 cell cultures at the ALI.

Submerged exposures

Two different submerged exposure systems were used for particle exposures in comparison with the NACIVT system. The first was a traditionally growth medium submerged system (SUB(growth)) where the cells were in contact with the growth medium from both the basal and apical sides from the point of seeding to exposure. The particles were added into the growth medium on the apical side of the cells at the time of exposure. In the second system (SUB(iso)), the cells were brought to the ALI state at the same time as the cells used in the NACIVT system, but at the time of exposure they were exposed to the particles suspended in a 0.9% NaCl isotonic solution on the apical side of the cells.

Toxicological responses

Viability assay

The cell viability, or more accurately the level of metabolic activity, was measured in the cells after particle exposure. The water-soluble tetrazolium (WST-1) assay (Roche diagnostics, Germany) was used for this purpose.

Cytotoxicity assay

The cytotoxic effects, or more specifically the necrosis of the cells, were measured in the basal growth medium after particle exposure. The lactate dehydrogenase (LDH) assay (Roche Diagnostics, Germany) was used for this purpose.

Cytokines

Release of the cytokines IL-6, IL-8, the tumor necrosis factor (TNF-α) and the monocyte chemotactic protein (MCP-1) were analyzed in the basal growth medium after particle cell exposure.

Results and Discussion

Evaluation of the cleaning occupational environment

Contextual information gathering

The survey among professional cleaning workers (n = 225) confirmed that the cleaning occupation is dominated by women: 73% of the professional cleaning workers that answered our survey were women. Furthermore, an extensive use of cleaning sprays (77%) was found among the workers, motivating continued research into this subject. Two brands in particular, one commercially available (C) and one only available to professional cleaning workers (P), were identified as the most common. The frequently used window, bathroom and universal sprays from both brands were chosen for further study (**Paper I**).

Among the workers that used sprays, almost half (48%) stated that they experienced one or more symptoms from the eyes and airways at least at some point during spray use; 28% stated that they experienced symptoms often or always during spray use. The most frequently reported symptoms were nasal. A dose-response relationship was also found between the use of cleaning sprays and the amount of experienced symptoms. Workers who used cleaning sprays had more than twice the risk of experiencing symptoms compared to workers who never used them. Workers who used sprays more than 5 times per day had a five times higher risk than the workers who only used sprays 1-5 times per week. When it comes to the use of PPE among the workers, 91% of the spray users use gloves while only 14% use respiratory protection.

Regarding the experience of physical pain, the vast majority (77%) of the cleaning workers stated that they do experience pain, regardless of if they are spray users or not. Their shoulders, neck, hands and wrists were the most frequently reported locations for the pain (**Paper II** and our Swedish report (Lovén et al. 2018)).

Aerosol characterization

The first step in the characterization of the aerosols emitted during cleaning was to determine the amount of total aerosol generated from the use of cleaning sprays (i.e., total airborne mass fraction). A new and easy-to-use paper-weighing method was developed to perform these types of measurements, since no established method for assessing the total airborne mass fraction was available. The newly developed measuring setup (partly developed during my Master Thesis research from 2015) was later also adopted and further developed by STAMI, the Norwegian National Institute of Occupational Health, and used for similar spray characterizations (STAMI 2017).

Among the seven different cleaning sprays included in our study, the total airborne mass fraction varied by about one order of magnitude. Between 2.7 and 32.2% of the liquid leaving the bottle during spraying remained airborne, see **Figure 11**. This constituted potential airway exposure. For two of the tested products, *Bathroom (normal) P* and *Bathroom (acidic) P*, the cleaning chemicals were provided as concentrates and were manually mixed with water to achieve the recommended concentration of 1%. The two spray bottles available for these products had adjustable nozzles (not just one fixed position like the rest of the tested product bottles had). Nozzle positions of 180° and 360° from a closed nozzle position were chosen to resemble the spray mist from *Bathroom C* and to facilitate comparisons. *Bathroom (normal) P* and *Bathroom (acidic) P* had the highest total airborne mass fractions; thus, the adjustability of the nozzles can be one underlying cause for the high total airborne mass fraction and the difference in reproducibility (indicated by the high standard deviations). Most of the products had a nozzle that created a full spray mist, while the *Universal* sprays created more of a foam. Since the *Universal* sprays had the lowest total airborne mass fraction, the foaming nozzles seem to reduce the aerosol exposure (**Paper I**).

An additional test was performed where the *Window C* bottle was tested with chemicals from the *Universal (stains) P* product (far left in **Figure 11**), and the *Universal (stains) P* bottle was tested with chemicals from the *Window C* product (far right in **Figure 11**). As seen from these results, the bottle, or more specifically the nozzle, seems to have a greater effect on the total airborne mass fraction than the chemical composition. These results are all in accordance with those obtained by STAMI, which also showed both that the highest total airborne mass fraction was generated by a bottle with an adjustable nozzle, and that the lowest was generated by a bottle with a foaming nozzle (STAMI 2017). In addition, the STAMI results showed that the same type of bottle generated similar total airborne mass fractions regardless of which cleaning chemicals that were used.

From the human chamber exposure study, it was further shown that using foaming nozzles instead of regular spraying nozzles, greatly reduces the airborne concentration of both particles and gases (**Figure 12**). A 7 times lower particle concentration and a 2.5 times lower VOC concentration could be seen when using a foaming nozzle instead of a spraying nozzle (**Paper II**).

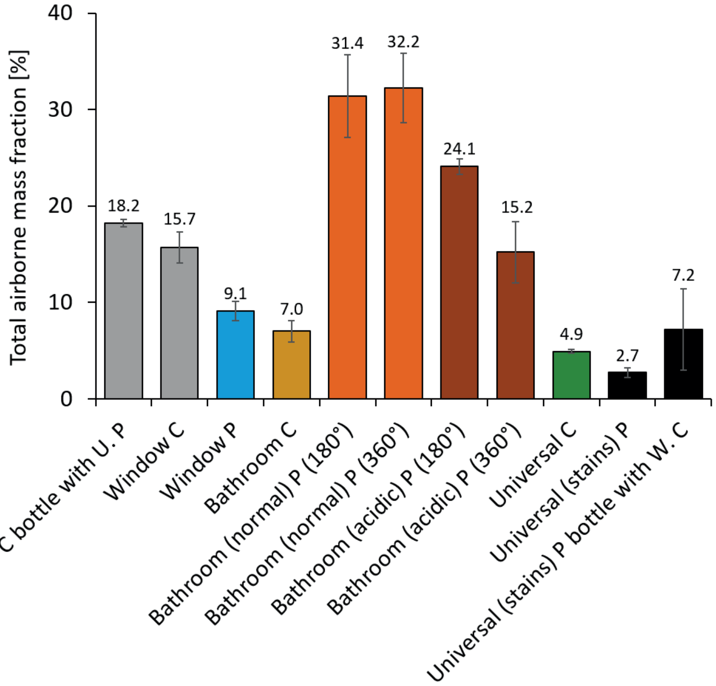

Figure 11. Total airborne mass fractions for the seven cleaning sprays tested. The degrees in parentheses show the nozzle positions of the two bottles with adjustable nozzles. U. P = Universal (stains) P chemicals, W. C = Window C chemicals. The average values are displayed in the graph and the error bars represent the standard deviation.

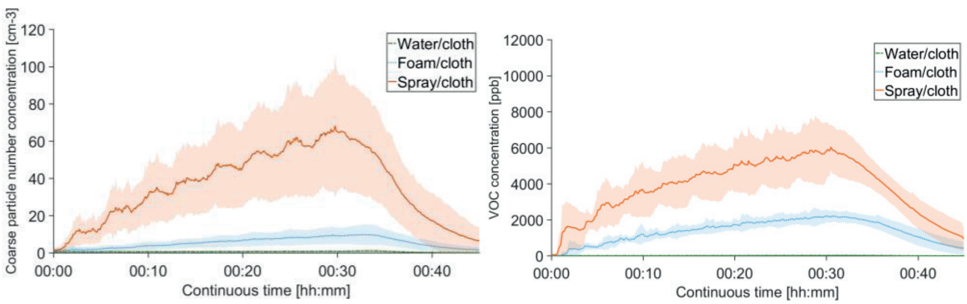

Figure 12. Coarse particle number concentrations (left) and VOC concentrations (right) generated during the three different cleaning scenarios tested in the human chamber exposure study. The averages for all subjects are shown as the bold lines and the shaded areas represent the standard deviation. After the 30-minute cleaning session, the chamber was express ventilated.

More specific characterizations of the particle phase were also conducted for the seven different cleaning sprays included in our study. Initially, both the SMPS and APS were used to measure the primary particles generated from the sprays; however, very low concentrations were measured with the SMPS and in combination with the low time resolution (of minutes instead of seconds), the use of the SMPS was withdrawn. From the APS measurements, the mass median aerodynamic diameters (MMD_{ae}) were found to be in the range 1.9-3.7 µm for the different sprays, even though the major part of the aerosol (<99.9%) was in the gas phase. By using the DMS with high time resolution (seconds), we showed that the cleaning sprays were able to form new particles in the presence of ozone. Additionally, we also showed that there was an up to a 7 times difference in this capacity between the differently tested products (**Paper I**). This formation is most probably a result of reactions between generated VOCs and the ozone, since VOCs have been shown to be a precursor for new particle formation (Weschler and Shields 1999). The measurements conducted during the human chamber exposure study also confirmed the strong generation of VOCs (**Paper II**).

Health assessment

To relate the different aerosol measurements to potential health effects, particle respiratory deposition fractions were initially calculated using the MPPD model for the size distributions found primarily with the APS, but also with the DMS (**Paper I**). Standard (default) parameters, for a human during rest, were used with a breathing frequency of 12 min^{-1}, and nasal breathing (blue curves in **Figure 13**). For the primary and newly formed particles, this revealed pulmonary particle deposition fractions of 13-17% and 26-29%, respectively, and head airway deposition fractions of 36-63% and 9-13%, respectively.

Calculating the particle deposition fractions for a human during heavy physical work would probably be more realistic for the actual occupational situation (also indicated by the increased pulse readings registered during the human chamber exposure study). A breathing frequency of 24 min^{-1}, and oral breathing were used for these calculations (orange curves in **Figure 13**). They revealed pulmonary deposition fractions of 15-21% and 28-30%, and head airway deposition fractions of only 4-18% and 2-3% for the primary and newly formed particles, respectively. This clearly shows that the particle deposition in the head airways is relatively low during physical work, while the particle deposition in the alveoli is relatively high. Even though only a minor part of the total spray aerosol consists of particles, these particles can potentially have large local effects depending on where they deposit (Balashazy et al. 2003), especially considering exposures over a whole working life.

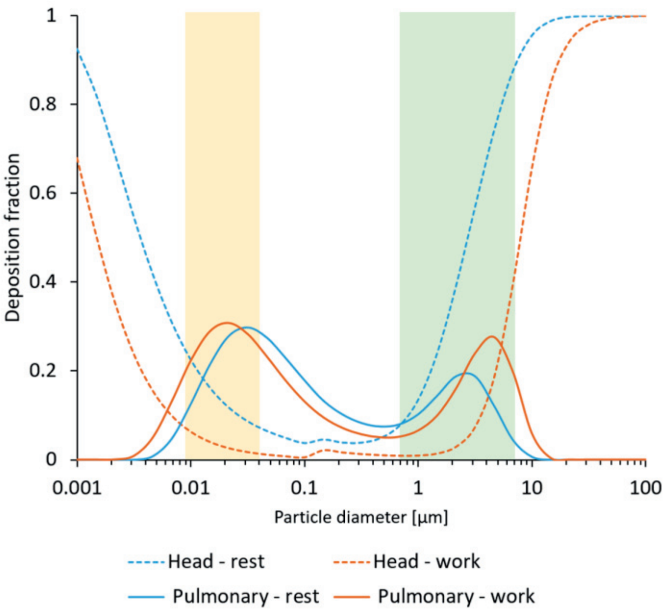

Figure 13. Head and pulmonary respiratory deposition fractions from the MPPD model (Netherlands National Institute for Public Health and the Environment (RIVM) 2002) for a human at rest with a breathing frequency of 12 min^{-1} and nasal breathing (blue curves) and for a human during physical work with a breathing frequency of 24 min^{-1} and oral breathing (orange curves). The whole size distribution for the primary particles formed when spraying (light green box), and the new particles formed from ozone reactions (light yellow box) are marked in the figure.

In the human chamber exposure study (**Paper II**), the specific health effects that arise during different types of cleaning work were further investigated. It was shown that the nose was the part of the respiratory system most affected by spray use. An increase in nasal symptoms and a decrease in the PNIF values, indicating increased nasal obstruction, were found during the spray/cloth exposure. **Figure 14** shows the average differences (for all subjects) in the PNIF values at each measurement time point (presented in **Figure 3**) compared to the first measurement in the morning. As can be seen, increases in the PNIF values were registered throughout the day during the water/cloth exposure, which corresponds to the normal trend of decreasing nasal obstruction (increased PNIF values) in the absence of chemical exposure over the course of the day (Enberg and Ownby 1991). With the chemical exposures (foam/cloth and spray/cloth), this increase is suppressed.

Through a linear mixed model analysis (comparing the spray/cloth to the water/cloth exposure), a significant increase in self-assessed nasal symptoms and a significant decrease in the PNIF values (-10.9 L/min) was determined. This corresponds to an almost 10% decrease from the PNIF values measured in the morning. For the foam/cloth exposure (compared with the water/cloth exposure), a -4.7 L/min (non-significant) difference was found, thus indicating a dose-response relationship between the air concentrations and the effects.

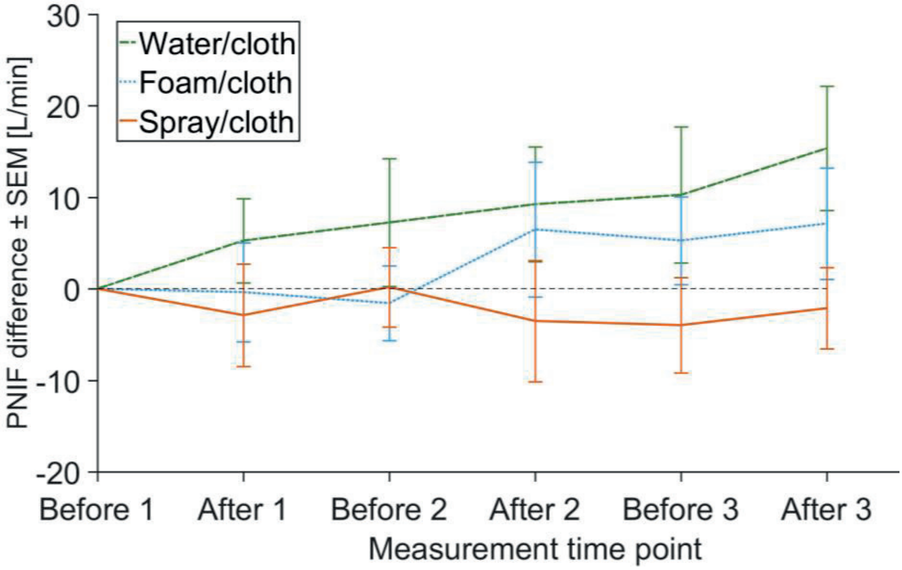

Figure 14. PNIF differences measured during the three different cleaning scenarios tested in the human chamber exposure study. The averages for all subjects are shown as the bold lines and the error bars represent the standard error of mean (SEM).

When analyzing cleaning workers and the comparative group of non-cleaning workers separately, only a slight difference was found in the PNIF values after the spray/cloth exposure, where the cleaning workers displayed a somewhat larger effect. However, for the self-assessed symptoms, a larger difference was seen between the two groups, where more effects were found in the group of non-cleaning workers. This result may indicate that the cleaning workers have grown accustomed to the cleaning spray exposure through their work and do not experience symptoms in the same way as non-cleaning workers, even though the objective PNIF measurements confirm such an effect.

Measurements of the ergonomic load were also recorded during the human chamber exposure study in addition to the aerosol characterizations and the medical assessments (**Paper II**). As anticipated, both tested methods (spray/cloth and water/cloth) yielded high loads, in accordance with previous studies (Unge et al. 2007). A somewhat higher muscular load on the right forearm muscles was found during the use of the spray/cloth method compared to that of the water/cloth method. This was probably due to the repeated handgrip motion when distributing the cleaning liquid with spray. In contrast, the arm elevation was lower during the use of the spray/cloth method compared to that of the water/cloth method. Thus, from an ergonomic point of view, no significant difference in the risk of developing musculoskeletal disorders seems to be associated with the different cleaning methods tested.

Evaluation of the nanotechnology occupational environment

Contextual information gathering

The nanotechnology companies included in this thesis research were handling different ENMs and were performing different work tasks. One company was manufacturing and handling metal (GaAs and silicon (Si)) nanowires (**Paper III**, Study 1 before and Study 2 after upscaling the production); one company was handling TiO_2 nanofibers (**Paper IV**, Study B) and two companies were handling graphene nanomaterials (**Paper IV**, Studies A and B). A few other nanomaterials were also handled at two of the companies including gold (Au) NPs (**Paper III**, Study 2), and TiO_2 NPs (**Paper IV**, Study A). The work processes performed included production of ENM, manual cleaning of different production equipment parts (**Paper III**), different ENM handling processes (**Papers III and IV**), and the use and testing of products containing ENM (**Paper IV**).

Aerosol characterization

Since exposure to fiber-shaped nanomaterials is of special concern, the most important results from the workplace measurements were that all three fibrous nanomaterials that were handled (GaAs nanowires, Si nanowires and TiO_2 nanofibers) could be detected in the emission zone and identified through SEM analysis (**Figure 15**). Two of them (GaAs nanowires and TiO_2 nanofibers) could also be detected in the personal breathing zone, verifying possible worker exposure. Emissions of GaAs nanowires was further confirmed with Ga and As metal quantification by ICP-MS, and emissions of TiO_2 nanofibers was confirmed with Ti quantification by PIXE.

With the on-line emission zone measurements, CPC and APS, together with the work tasks registered in the logbook, the specific processes generating the particle emissions could be identified (**Figure 16**). Manually cleaning the inside of the production reactor by inserting a cleaning tube and pulling it up and down caused the GaAs nanowire exposure; removing the para-film covering the glass bowl after sonicating substrates with Si nanowires caused the Si nanowire emissions; and weighing the TiO_2 nanofiber powder gave rise to the TiO_2 nanofiber exposure (**Paper III and IV**). As can be seen in **Figure 16**, only using an on-line instrument that measures in the nanometer range (e.g., a CPC) is not always sufficient to evaluate the occupational environment. Previous workplace studies (Debia et al. 2016) have shown that nanofibers are mostly emitted as agglomerates, and an instrument that measures in the micrometer range (e.g., an APS) is a good complement to a CPC. This was also confirmed in our studies.

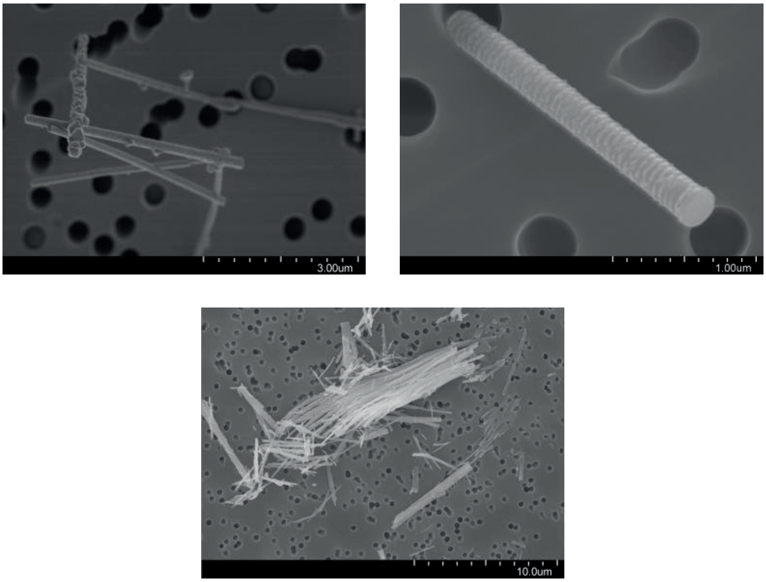

Figure 15. SEM images of GaAs nanowires (**Paper III**, Study 1, top left), Si nanowires (**Paper III**, Study 2, top right), and TiO_2 nanofibers (**Paper IV**, Study B, bottom) found in the emission zone.

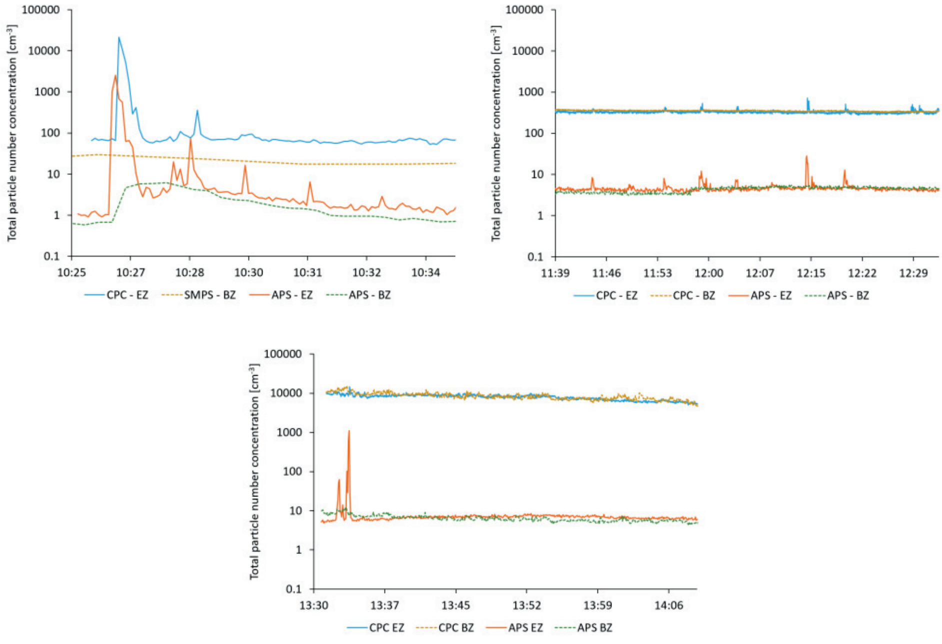

Figure 16. APS and CPC measurements in the emission zone (EZ) and the background zone (BZ) during manual cleaning of the inside of the GaAs nanowire production reactor (**Paper III**, Study 1, top left), sonication of substrates with Si nanowires (**Paper III**, Study 2, top right), and weighing and mixing the TiO_2 nanofiber powder (**Paper IV**, Study B, bottom).

The lack of exposure data during the down-stream use of graphene was addressed in **Paper IV**. SEM analyses showed that airborne graphene could be detected and identified in the emission zone both in Studies A and B. The EC concentration (assumed to originate from graphene) could further be quantified with thermal-optical analysis. In Study B, the on-line emission zone measurements, conducted with the APS and portable aethalometer, together with the work tasks registered in the logbook made it possible to identify "weighing and mixing the dry graphene powder" as the specific process that was generating the particle emissions. Thermal-optical analysis of the personal breathing zone filter also confirmed an EC exposure, likely of graphene origin. Using the thermal-optical analysis to measure EC, or the portable aethalometer to measure BC are less labor intensive and faster methods than the SEM for the identification and quantification of carbon containing nanomaterials, provided that no other sources of carbon are present in the workplace (such as diesel machinery or combustion processes). A previous study (J. H. Lee et al. 2016) has demonstrated that it is possible to use a combination of thermal-optical analysis and portable aethalometer measurements for graphene quantification at a graphene nanoplatelets manufacturer. We have confirmed that it is possible to also use these methods at down-stream handling facilities.

The use of four measurement zones for the workplace studies may seem excessive, especially including measurements in the supply air. However, during our study at the company in **Paper IV**, Study A, the inclusion of this zone was crucial. **Figure 17** shows the CPC and DMS measurements from the emission zone (CPC in the background zone as well) together with the P-Trak measurements in the supply air. It displays a sudden and rapid increase in the particle concentration in the supply air, which affects the particle concentration in the whole room. Had the supply air measurements not been included, we could have incorrectly interpreted this increase as a large nanoparticle emission event.

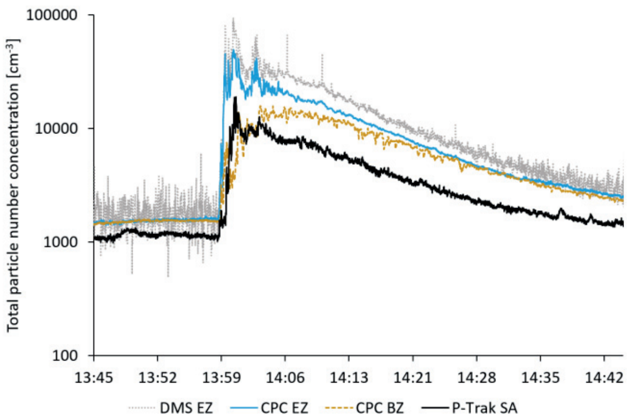

Figure 17. P-Trak, DMS and CPC measurements during the workplace measurements in **Paper IV**, Study A, from the emission zone (EZ), background zone (BZ) and the supply air (SA).

Surface contaminations were detected at all workplaces where surface sampling was conducted. However, no widespread nanomaterial contamination was found. In **Paper III**, surface contamination consisting of GaAs nanowires was only found on the floor inside the enclosure of the nanowire production reactor (both during Studies 1 and 2). In **Paper IV**, Study B, surface contamination of both TiO_2 nanofibers and graphene were found at the sink where the washing of equipment took place after preparation of coatings containing ENMs. A few other surfaces, related to the handling and testing of the products containing ENMs, were also found to be contaminated with either TiO_2 nanofibers or graphene, but no contamination in adjoining rooms could be detected. This indicates a limited risk for secondary inhalation exposure through particle resuspension.

Toxicity assessment

A toxicological study using ZnO NPs as a model ENM was conducted (**Paper V**) to relate the different exposure measurements carried out at the nanotechnology companies to the potential health effects in the workers. The two traditionally submerged exposure systems, SUB(growth) and SUB(iso), were compared with the more respiratory realistic ALI exposure system, NACIVT, which allows for NP exposure directly from the aerosolized phase. To achieve a high societal relevance of the study, the occupational exposure limit value for coarse ZnO in Sweden (Swedish Work Environment Authority 2018), and the British Standards Institution (BSI) suggested exposure limit value for ZnO NPs (BSI 2007) were considered as benchmark values for the doses used in our study. Assuming a particle deposition efficiency of 40% and a 1 h exposure, the corresponding deposited mass doses in the NACIVT system were 9.1 $\mu g/cm^2$ calculated from the Swedish limit value of 5 mg/m^3, and 0.6 $\mu g/cm^2$ calculated from the BSI limit value of 0.33 mg/m^3 (0.066 x workplace exposure limit of coarse material). **Table 3** shows the doses used in the three exposure systems, referred to as low, medium and high. The exact same doses were not achieved due to experimental challenges, but similar, comparable doses were still obtained. The low dose resembled the BSI suggested value, the medium dose was about the doubled BSI suggested value, and the high dose was about half of the Swedish limit value.

Table 3. Deposited mass doses used in the three exposure systems in the toxicological study.

Exposure system	Low dose ($\mu g/cm^2$)	Medium dose ($\mu g/cm^2$)	High dose ($\mu g/cm^2$)
NACIVT	0.2	1.0	3.0
SUB(iso)	0.6	1.1	4.2
SUB(growth)	0.5	0.9	4.5

The deposited mass dose size distributions for these three doses in the NACIVT system are shown in **Figure 18** (more details concerning the dose calculations can be found in **Paper V**). The count median diameter (CMD) was 29 nm for the low and medium doses and 43 nm for the high dose. The SEM analysis further confirmed this difference in size distribution between the high dose (containing many large aggregates and few single particles) and the low and medium doses (instead containing fewer aggregates, which were smaller than for the high dose, and many single particles).

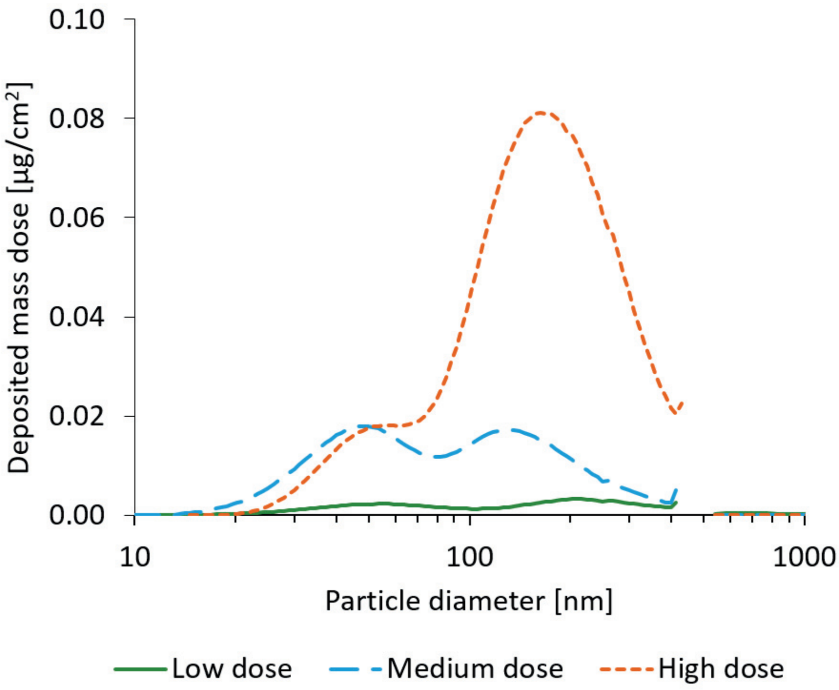

Figure 18. Deposited mass dose size distributions for the three different doses used in the NACIVT exposure system.

Comparing the toxicological responses after ZnO NP exposure in the three different exposure systems revealed that a significant increase from unexposed control in the cytokine levels of IL-8 and MCP-1, indicating proinflammatory responses, were only seen in our medium dose and only in the NACIVT system (**Figure 19**). Further analyses also showed significantly different responses between the NACIVT and the SUB(growth) system for both the IL-8 and MCP-1 release and between the NACIVT and the SUB(iso) system for MCP-1 release (**Paper V**). These clear differences demonstrate a higher sensitivity in the NACIVT system, with a response to lower doses of nanoparticle exposure compared to the submerged systems. This encourages the use of more physiologically realistic exposure systems.

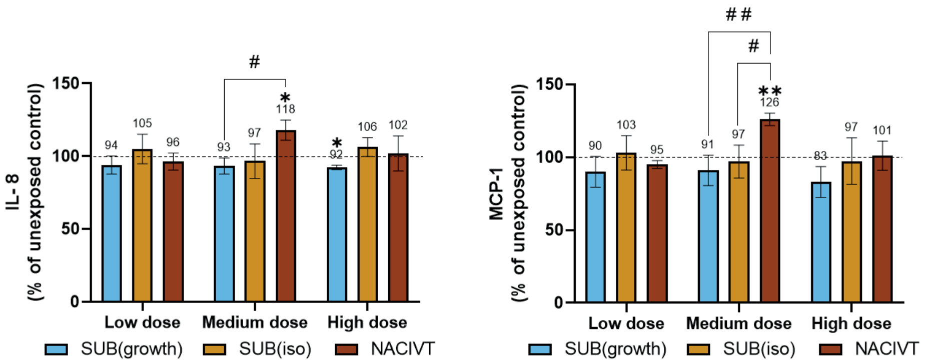

Figure 19. Comparison of toxicological responses after ZnO NP exposures in the three exposure systems 24 h after exposure. IL-8 release (left) and MCP-1 release (right) measured in basal medium and normalized to unexposed controls. The symbol (*) indicates a significant difference from unexposed control levels at p<0.05 and (**) at p<0.01. The symbol (#) indicates a significant difference between exposure systems at p<0.05 and (# #) at p<0.01. The average values are displayed in the graph and the error bars represent the standard deviation.

Because the low and medium doses for the NACIVT exposures had similar size distributions with only different concentrations, a dose-response relationship can be shown for these doses (no significant response from the low dose exposure and a significant response from the medium dose exposure). The lack of further significant response from the high dose exposure could be a result of the different size distributions with a high presence of large aggregates, since the size of the ZnO NPs particles has been shown to influence the cellular responses with smaller particles inducing greater toxicological responses (Wang et al. 2018).

To evaluate if the doses used in our NACIVT exposures are relevant for occupational exposures, and thus if the cellular responses are relevant to consider, a rough estimate was conducted to calculate the number of workdays corresponding to the doses used. A human during moderate physical work with a breathing frequency of 18 min^{-1}, a tidal volume of 625 ml, a lung area of 70 m^2, and a particle deposition efficiency of 40% was assumed. All three of our doses corresponded to about one year of 8 h occupational exposure daily, making the study reasonably relevant, especially considering the large amount of toxicological studies that use unrealistically high doses, mainly in traditionally submerged cultures.

Research ethical considerations

Something that has been touched upon earlier in the thesis, but that deserves further discussion, are the ethical considerations one has to consider when conducting research in the area of occupational exposure and health effects.

One motivation for the Cleaning Spray Project was that it is about an occupation where not many studies have been conducted, especially not at the detailed level as we now have done. This is also a low-wage and female-dominated occupation, and most studies investigating occupational environments have been carried out from a male perspective (MYNAK 2020). Additionally, a previous study has shown that the lungs of women are more sensitive to chemical exposure than men's (Svanes et al. 2018), making it even more important to investigate this group. One problem, though, with studying this group was the recruitment of actual professional cleaning workers to participate in the project, both in the survey and in the human chamber exposure study. Some employers and some individual cleaning workers expressed the difficulty in scheduling participation, partly because of the struggle to find substitutes, which made it especially challenging to conduct the studies.

The human chamber exposure study was designed to generate exposure levels comparable to a normal working day for a hotel cleaning worker. Both the survey and the human chamber exposure study were approved by the Regional Ethical Review Board in Lund, Sweden (2015/389) and followed the principles of informed consent in the current revision of the Declaration of Helsinki (2013).

The reference group, including representatives from cleaning unions, trade organizations and product companies, was consulted on some of the design aspects of the project to reflect the most relevant questions. Their input was very important for the project as a whole, but could possibly be considered as a conflict of interest since several of the participants had their own interest and ideas of project outcomes. This could have compromised the scientific reliability of our research and violated good research practice. However, we were aware of these possible influences and discussed them in the research group, and we selected the input from the reference group carefully, for example what types of cleaning methods to include in the human chamber exposure study. Additionally, we did not let the reference group influence the data analysis or the interpretation and reporting of the results by either diminishing or enhancing them.

During the Nanosafety Project, measurements were conducted at actual companies active in the nanotechnology industry. This is an important aspect to consider when motivating the start of these types of workplace studies. The value of capturing the real-life situation is high, but as in all workplace studies, the risk of the company or the individual worker influencing the study, consciously or unconsciously, has to be considered. It could, for example, be difficult to determine if the worker is moving or working in the usual way when equipped with several measuring devices or when being observed by researchers. However, we have had good collaboration with the companies and have managed to capture as realistic processes as possible, while not letting the companies influence the data analysis or the interpretation and reporting of the results in any way.

When it comes to investigating the health effects caused by exposure to ENMs, a cell toxicity study was performed in the research presented in this thesis. A human chamber exposure study would, of course, have been interesting to carry out, but it would have been unethical! Since it is still widely unknown what type of effects exposure to ENMs can have, it would be irresponsible to expose humans to it. Furthermore, it would be difficult to determine the relevant exposure levels to be used in a human chamber exposure study because the exposure levels present in the nanotechnology industry are still not fully known. In addition, for many work tasks, PPEs are used which means that exposing workers to materials from which they are normally protected would not mirror reality and would be impossible to ethically motivate. An animal exposure study could also have been an option, but cell toxicity studies are the best choice when underlying toxicological mechanisms are to be studied and dose-response relationships are to be determined. This would also minimize the unwarranted use of animals.

Exposing the exposures…

Worker's exposures could be determined in both the occupations examined in this thesis research. It was shown that the use of cleaning sprays, and especially the use of spray bottles with adjustable nozzles, gave rise to the highest emission and exposure levels to both airborne particles and gas phase VOCs generated from the liquid cleaning chemicals. At the nanotechnology companies, the emission of and exposures to fibrous nanomaterials were found foremost during the manual cleaning of the ENM production reactor and handling of both dry ENM powder and ENM-containing liquid. Emissions of and exposures to a few other nanomaterials were also found.

… and what to do about them

Now that we know that these exposures occur, is there something we can do to reduce or completely eliminate them? According to the hierarchy of controls (**Figure 1**), the first step to protect the worker is to eliminate the chemical, material or method used. In the case of the cleaning occupation, removing the chemicals completely would eliminate the chemical exposure seen by the aerosol measurements, and reduce the respiratory effects, as exemplified by the PNIF measurements (**Paper II**). Cleaning with only microfiber cloths premoistened with water (our water/cloth method) is done in some areas today and if no chemicals can be justified, this would be the recommended practice. However, it must be pointed out that in some situations or locations, chemical use is necessary.

The second step is to substitute the chemical, material or method used with a suitable safer alternative. In the cleaning occupation, this means for instance to replace cleaning products containing irritants or disinfectants, with products without them (if possible). However, the results of our study showed that even milder products (i.e., products without bleach, chlorine and ammonia) give rise to health effects. Replacing the chemical application method would instead be an option. The study results showed that using a foaming nozzle instead of a spraying nozzle greatly decreases the aerosol exposure and the health effects (**Paper II**). Another option would be to develop and modify the design of the spraying nozzle to decrease the airborne mass fraction if sprays are the preferred application method (**Paper I**).

The third (use of engineering controls) and the fourth (use of administrative controls) steps may not be the main control measures in the cleaning occupation, but increasing the ventilation rate or open windows during cleaning, and providing education or instructions to new employees can help in the process of lowering the exposures. When it comes to the use of PPE (fifth step) (e.g., respiratory protection), one concern for the cleaning workers is that with their already physically high workload that results in increased breathing rates, the added use of PPE can make breathing more difficult and make them feel warm or stuffy. Another concern can be that they cannot use the PPE in areas where they meet or can be seen by other people residing in the facilities they are cleaning. The appearance of the cleaning workers handling something hazardous could raise concerns for the public's health and thoughts that they too should protect themselves. This could be dealt with, in part, by educating the public of the consequences of working with chemicals daily.

In contrast, in the nanotechnology industry, it can be difficult to eliminate or replace (first and second step) the nanomaterials handled since it is the materials' specific properties that are desired. However, a safe-by-design approach should always be considered as early on as possible in the development of new materials or products, and could in some cases be applied for instance by producing shorter nanofibers or replacing a compound that could decrease the hazard. The mitigation processes could otherwise typically start at the third step of the hierarchy of controls by using engineering controls. Applying these controls, such as enclosing the ENM production in a reactor or using fume hoods, was shown in our studies to significantly reduce the particle exposures, for example, during manual cleaning of the reactor parts for metal nanowire production (**Paper III**). Reducing the workers' exposure by using engineering controls has also been emphasized by Debia et al. (2016). By changing the way the work is performed (fourth step), we showed in our studies that exposures can be reduced, for example, by changing how NP containing powder is weighed and mixed with a liquid (**Paper IV**). However, since we also showed that ENMs can be detected in the personal breathing zone of the workers at all companies, the use of PPE (fifth step) is necessary until it can be ensured that no particle exposures are present or that it can be proven that the particles do not pose a hazard, which would also comply with the precautionary principle.

If workplace emission and exposure measurements cannot be performed, one approach can be to use control-banding tools to estimate worker exposure. These are techniques used to guide the assessment and management of workplace risks, and they are based on the type of emission and exposure measurements conducted in this thesis research. A few different tools have been developed with different perspectives in mind. Some (Stoffenmanager Nano, NanoSafer, ANSES CB nanotool) were developed for occupational risk assessment and management during production and down-stream use of ENM. Another one (IVAM Guidance) was developed for supporting employees and employers in discussing their workplace safety; one (CB Nanotool) was developed for protecting the nanotechnology researcher; and one (The Swiss Precautionary Matrix 3.0) was developed for risk identification and prioritization (Liguori et al. 2016). To help facilitate workplace risk assessments, a mobile app called *Safety Observer* (NRCWE 2019) has also been developed where you can easily document both safe and unsafe observations at a workplace and monitor the risk management implementations over time.

Conclusions

It can be challenging to conduct research on occupational exposures and to gain a comprehensive and holistic view of the entire occupational situation. Depending on the occupation, the working environment, the potential aerosol source and the adverse effects those aerosols may cause, the methodologies used have to be adapted for the specific situation. The objectives of this thesis were to characterize the aerosol emissions and exposures present in two different occupational settings, and to evaluate the potential health and toxicological effects they might cause the workers, with the overall aim to provide a basis for the implementation of improvements of the working environments.

To gain a comprehensive view of the occupational conditions, it was essential to collect contextual information regarding the real-life work situations prior to the aerosol characterizations and the evaluations of the effects regardless of which occupation was being studied. This is an important first step to be able to conduct realistic and societally relevant occupational evaluation studies.

Evaluating the working environment for the cleaning workers required a careful and comprehensive methodological approach. The contextual information provided a basis for the choice of relevant products and application methods. The extensive aerosol and health effect evaluations revealed possible ways to improve the occupational environment from multiple aspects.

Using the developed method for measuring the total airborne mass fraction revealed that the possible aerosol exposure varied between 2.7 and 32.2% for different sprays and choosing spray bottles without adjustable nozzles could lower the exposure. The aerosol measurements showed, furthermore, that switching from a spray to a foam nozzle resulted in a 7 times lower particle concentration and a 2.5 times lower VOC concentration. The PNIF measurements also confirmed this improvement, showing that the nose effects decreased by half when the chemical application method was changed.

Assessing the working environment in the nanotechnology industry also required a thoughtful methodological approach. The contextual information contained specifications about the materials used and the processes carried out at the workplaces. This information was then used for the detailed planning and conduction of workplace measurements, which revealed critical exposure events and included ways to mitigate them.

The release of fibrous nanomaterials, for example, was found during several processes, including during the handling of both dry ENM powder and ENM-containing liquid, but applying engineering controls such as fume hoods, significantly reduced the particle exposures. In addition, the toxicity testing with different methods revealed the advantages of using a more physiologically realistic exposure system such as the NACIVT system, to find responses at lower doses compared to what the traditionally submerged systems could.

When focusing on aerosol characterizations, it is important to consider what type of instruments and other measurement techniques that are to be used. On one hand, a simple off-line method can result in valuable information initially, before more advanced on-line instruments are used for more detailed characterizations (as with the cleaning evaluation). On the other hand, on-line instruments can be used to find the specific processes that can create an exposure situation, while off-line measurements can confirm the specific type of exposure (as with the evaluation in the nanotechnology industry). A combination of currently available techniques is key to get the full picture of the occupational exposure situation.

For health and toxicity effect assessments, it is equally important to choose relevant evaluation methods. Methods that are fast and easy to conduct can be used to get an initial idea of the effects caused by aerosol exposure, as with the PNIF measurement during the cleaning evaluation. More advanced and time-consuming methods can be necessary to achieve adequate comparisons between different aerosol exposure conditions, as with the cytokine analyses during the nanotoxicity evaluation. A combination of different medical assessments or evaluations of toxicological responses are important to fully identify and understand the effects initiated by aerosol exposure.

The knowledge generated through the research presented in this thesis can be used to promote safe working environments. Exposure data together with hazard/toxicity information can be used to provide improved risk assessments, and for modeling or control-banding tools. Comparisons and evaluations of measurement techniques can be used in recommendations to occupational hygienists, or for continued research in the area. The identification of processes causing exposure events can be used by companies to implement suitable mitigation systems. All this can be done so that healthier workplace conditions can be achieved!

Outlook and Future Work

It is becoming clear that a multidisciplinary approach is vital for continued research in the area of occupational exposures and their possible health effects. Understanding the societal and medical aspects of the specific occupation being studied when designing aerosol measurements and health or toxicity evaluations is important to be able to provide relevant new knowledge.

In the cleaning occupation, performing a long-term intervention study would be desirable where one widely used cleaning method, such as spray, is exchanged with another method, such as foam. The health effects should be evaluated before and after the exchange, while at the same time evaluating the cleaning efficiency of the methods. This will, though, be challenging with all factors that have to be considered when trying to study something in a complex and dynamic environment.

In the nanotechnology industry, further emission and exposure assessments are needed, for example during down-stream use of ENMs, but especially during the end-of-life treatment of ENMs, in order to provide a balanced knowledge base for the different parts that go into a risk assessment and a life cycle analysis. Repeated measurements at the same company should be prioritized to be able to assess the effects of relevant control measures. These studies should include the same work tasks and evaluating day-to-day, season-to-season and year-to-year differences and improvements. Development of personal aerosol instruments that can determine chemical content in real-time would also be most valuable for continued monitoring.

Toxicological studies involving nanoparticle exposures have been performed extensively in the past, but perhaps not always for relevant doses. Toxicological assessments in the future have to be performed for realistic doses and exposure situations not previously studied. The use of systems that resemble the bodily exposure situation should be prioritized and further developed. Applying co-cultures with different cell types in synergy, or exposure devices that mimic bodily movements are examples of more realistic systems to use.

It is important to continue to investigate these different aspects of occupational environments to ensure a sustainable and healthy work situation for everyone. And as Marie Skłodowska Curie stated:

> "Nothing in life is to be feared, it is only to be understood.
> Now is the time to understand more, so that we may fear less."

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