Human health risks of exposure to carbon nanotubes

Keeping pace with innovation

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Human health risks of exposure to carbon nanotubes - Keeping pace with innovation

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Keeping pace with innovation

Humane gezondheidsrisico's van blootstelling aan koolstofnanobuisjes

Gelijke tred houden met innovatie

(met een samenvatting in het Nederlands)

Proefschrift

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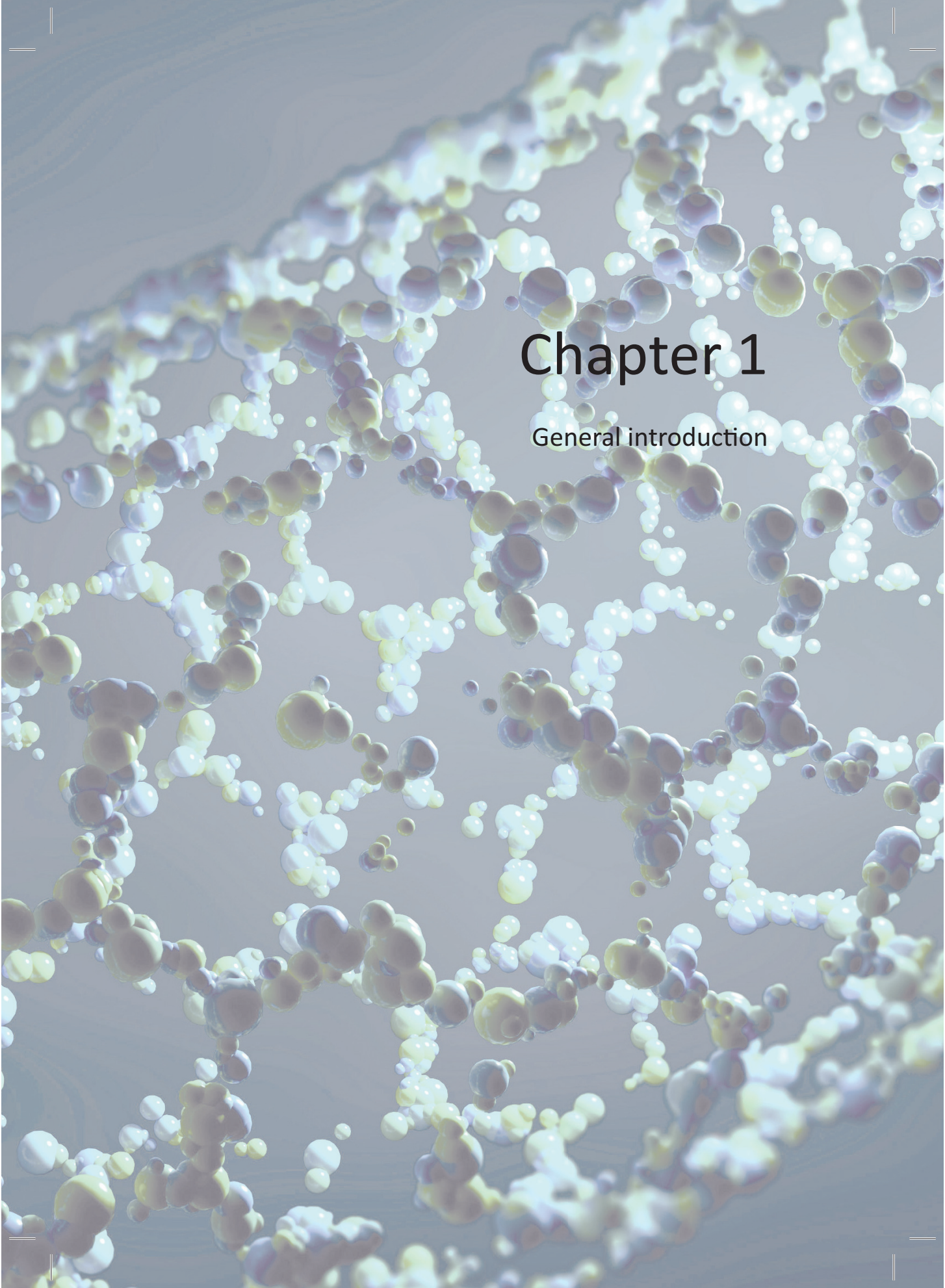
Table of contents



Chapter 1

General introduction

In 1952 the Russian scientists Radushkevich and Lukyanovich described carbon nanotubes (CNTs) and provided visual evidence for this finding using electron microscopy.[1] Due to the Cold War, the fact that Russian was used to describe the material, and that access to Russian peer-reviewed publications in the west was limited, it was not until 1991 that CNTs attracted global attention.[2] Nowadays, CNTs are one of the most promising relatively "new" materials resulting from nanotechnology. However, a growing body of literature on in vitro and in vivo experimental studies has highlighted a potential hazard of exposure to different types of CNTs.

Stronger than steel, as light as air and more flexible than rubber

Carbon nanotubes are graphene sheets which are composed of carbon; every carbon atom has three covalent bonds to other carbon atoms. Rolling up a graphene sheet (single-walled carbon nanotubes; SWCNT) or multiple graphene sheets (double-walled or multi-walled carbon nanotubes; DWCNT, MWCNT) visually illustrates the man-made CNT formulations (Figure 1). Double-walled CNTs have one outer and one inner wall; whereas MWCNTs can have more than 100 walls. Nowadays, CNTs are produced in different shapes, sizes, chemical compositions and other physical and chemical characteristics in order to optimize the required beneficial properties. The diameter of CNTs can be as thin as a few nanometers, while the length of CNTs can be up to hundreds of microns. Furthermore, depending on the production process of CNTs, the material can range from very flexible tubes to much more rigid forms. According to the EU and the WHO, CNTs are classified as a nanomaterial and as a fiber. [3, 4]

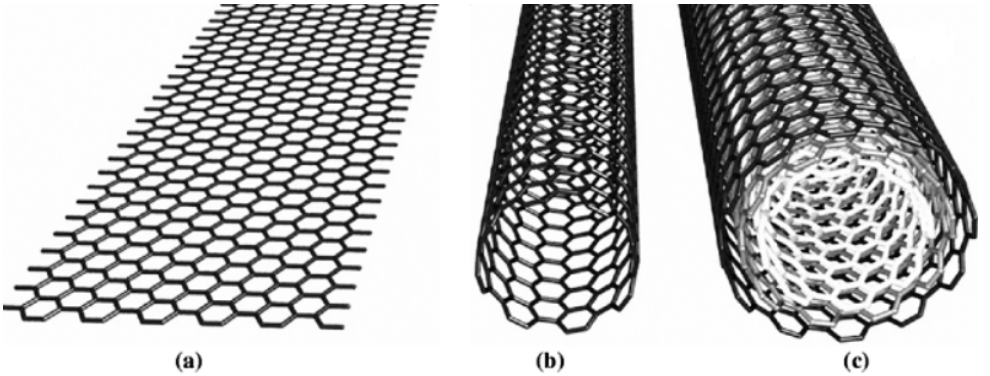


Figure 1: Graphene sheets within a) Graphene, b) SWCNT and c) MWCNT. Source Kreupl et al. 2004 [5]

The discovery of CNTs changed our beliefs about the world's strongest natural and manmade substances, which were previously believed to be diamond and steel, respectively. In addition, other beneficial properties of other forms of CNTs include its high electrical conductivity, flexibility, low weight, and thermal conductivity. With production capacity scale-ups driving prices down, CNTs and especially the relatively cheap MWCNTs are being

increasingly applied in commercial products. Products with CNTs include (flexible) electronics, batteries, bicycle components, sports gear, solar cells, thermoplastics, polymer composites, coatings and inks, human tissue repair, drug delivery, and medical diagnostics.[6] In addition, due to the decreasing market prices, the material is becoming a beneficial alternative to the more conventional particulate carbon black filler with about 1 million tons produced every year.[7] In 2013 the CNT market for electronics was estimated by IDtechEx to have reached 50 million dollars.[8] This market is expected to grow to over 2.8 billion dollars by 2023.

Hazards of CNTs

The expected increase in the CNT market, with the growing production and application of CNTs, is expected to result in the increased exposure of workers, consumers, and environments. While innovation of CNTs continues, hazard evaluations are trailing behind. Results from acute and sub chronical animal studies have showed that some types of CNTs can induce genetic lesions, oxidative stress, acute or persistent pulmonary inflammation, pulmonary fibrosis, cardiovascular effects, and with some types of CNTs, cancers such as mesothelioma.[6, 9] These results, especially mesothelioma, which is comparable to those health effects caused by asbestos, have caused much debate about the safe use of CNTs.[6]

In 2014, the international agency for research on cancer (IARC) classified a specific rigid type of multi-walled carbon nanotubes (MWCNT-7) as possibly carcinogenic to humans (group 2B), while other forms of CNTs – including other forms of MWCNTs – were assessed as unclassifiable (group 3).[10] These classifications of different CNTs were formed on an evaluation of the mechanistic evidence based on six key events in cancer pathways and key data gaps in assessing the potential carcinogenicity of CNTs in humans.[10] Evidence was found in peer-reviewed literature based on animal studies that CNTs are inhalable and deposit in the lungs.[11, 12] Long-term retention of CNTs has not been measured in animals, but reduced clearance has been reported in rodents for some forms of CNTs.[11, 13] Specific forms of MWCNTs in pleural lung tissue have been observed in animal studies associated with exposure via inhalation, but uncertainty is moderate to high regarding the used doses.[13, 14] Furthermore, acute exposure to CNTs is associated with (reversible) lung inflammation often considered a marker for carcinogenic risks, while long-term exposure to CNTs induces non-reversible inflammation associated with granuloma formation, fibrosis, and subpleural thickening.[11, 15, 16] Only a very limited number of studies have evaluated cell proliferation, hyperplasia, and cell signaling associated with CNT exposure.[17, 18] Nevertheless, lung epithelial cell proliferation was observed in rats exposed to a form of MWCNTs by subchronic inhalation.[19] In addition, inhalation of MWCNT-7 was associated with alveolar hyperplastic lesions in mice.[17] Altered gene expression and activation of cell cycle signaling pathways was found in an in vitro study that examined exposure to SWCNTs.[20] Genotoxic effects were observed in animal studies induced by SWCNTs and MWCNTs. Both SWCNTs and MWCNTs are believed to be genotoxic to the lungs and pleura.[21] However, in vivo studies have not been

conducted, and more research is needed to evaluate the possible correlations to cancer-related responses.[10] Lastly, while some cancer studies are available, they are mainly focused on the highly studied MWCNT-7.[17] For the development of lung cancer or mesothelioma, dose-response relations are not available.[10] The diversity in forms of CNTs and the data gaps mentioned above for most of these CNTs cause much uncertainty concerning the assessment of potential carcinogenicity. Epidemiological studies and chronic animal studies are needed to evaluate potential health effects including carcinogenicity caused by different types of CNTs.[10]

Epidemiology studies on worker exposure to CNTs

A cornerstone of responsible development is worker safety and health: Workers are normally the first individuals to be exposed to new materials, and they provide the initial evidence for potential health effects. In addition, occupational exposure is generally higher than exposure in non-working conditions via the environment or consumer goods. While chronic animal studies for CNTs are ongoing and results will become available in the upcoming years, epidemiological research focused on health endpoints in exposed workers is currently a challenge. The development of health effects as a result of CNT exposure may only become visible after a long time. The relatively recent introduction of CNTs means that the worker may have had a relatively short experience with CNTs, and thus exposure might have been too limited, in terms of duration and level, to have led to chronic health effects. In addition, large exposed populations are needed for the assessment of potential subtle health effects of CNT exposure. Consequently, recent epidemiologic studies have focused on the detection of potential markers of early health effects on populations exposed to CNT.[22-26] These studies are generally limited in size, but due to the fact that one looks at a continuous endpoint, the statistical power of such studies might still be reasonable.

After the tragedy of September 11, 2001 in New York, several rescue workers and debris removal personnel were diagnosed with respiratory lesions. In the lung biopsies performed for the most severe cases, CNTs were found. These CNTs were formed due to the high combustion temperatures, similar to how current CNTs are artificially produced.[27, 28] In addition to these cases, a few small-scale studies reported the association between CNT exposure and (early) health effects in occupationally exposed humans.[23-25] Lee et al. (2015) have studied nine manufacturing workers and four office workers at a MWCNT manufacturing facility and found significant increases in biomarkers of oxidative stress.[25] In a cross-sectional study with 10 exposed workers, significantly elevated inflammatory biomarkers (IL-1b, IL-4, IL-10, and TNF-α) were associated with MWCNT exposure.[23] Shvedova et al. (2016) evaluated changes in non-coding RNA and observed several changes in the mRNA and non-coding RNA in eight MWCNT exposed workers, which are associated with pulmonary, cardiovascular, and carcinogenic outcomes.[24] More larger-scale cross-sectional studies focused on early health

effects potentially associated with CNTs are needed to evaluate the risks for exposed workers and to develop evidence-based occupational health guidance.

Occupational exposure to CNTs

Due to the potential hazard of CNTs, there is a need to measure exposure; it is important for epidemiological research and for more insight into exposure determinants and activities. Early exposure studies demonstrated the exposure potential during several occupational activities including during the transfer, weighing, and mixing of the CNT powders, and in experimental settings during the cutting and drilling of products containing CNTs.[29-36] Mass exposure in the personal breathing zone (PBZ) of the workers varied substantially among these different activities from 0.008 mg/m^3 to 2.4 mg/m^3 during the weighing and the dry cutting activities, respectively.[35, 36] In addition, during weighing and sonification of CNTs, particle number concentrations (between 10 – 1,000 nm) ranged between 1,500 and 2,500 particles/cm.[3, 31, 32]

The majority of these early exposure assessments were conducted in R&D facilities; a growing CNT market is expected to result in more large-scale manufacturing and handling of CNTs in the near future.[37] In addition, most workers are currently exposed to CNTs during the production and processing of these materials, while later stages of the product life cycle (use, disposal and recycling) will result in more and higher exposed workers with application of CNTs. Consequently, the number of exposed workers is expected to increase with more workers potentially at risk across the life cycle of CNT-products. It is important to obtain more insight into exposure determinants and the activities of CNT exposure across this product life cycle in order to better protect and guarantee workers' safety.

Exposure measurements for CNTs

There is no consensus regarding the optimal sampling methodology of assessing exposure to CNTs.[38] Current challenges to assessment include the difficulty in distinguishing between background exposure and CNT exposure.[25] Moreover, the recommended exposure levels for CNTs are relatively low, while the detection limits of several methods are relative high.[25] In addition, there is the complicated nature of some of the analytical methods.[25] Early exposure studies used either direct-reading instruments (DRIs) and/or filter-based gravimetrical methods for the quantification of CNT exposure. Quantification of CNT exposure with DRIs is difficult, since these instruments are only valid for spherical particles while traditional gravimetrical methods are not sensitive and selective enough.[39-41] Recently, a more refined method for the exposure assessment of CNT was published based on elemental carbon (EC) as a proxy for CNT exposure.[42] Only a few recent studies used the more refined EC analytical method for workers in R&D facilities.[39, 43] In addition to the EC analytical method, two alternative selective analytical methods are available for the detection of CNT exposure

(electron microscopy/energy dispersive X-ray spectroscopy [EM/EDX) and inductive coupled plasma-mass spectrometry [ICP-MS)], all of which have their own advantages and disadvantages. Table 1 describes the measurement methods available for the assessment of CNT exposure using several key characteristics.

Table 1: Available measurement methds for CNT exposure and their key characteristics

Measurement method	Selective for CNT	Measurement metric	Time[1]	Particle size[2]
Direct-reading instruments (DRIs)	No	Particles	Time resolved	Both size integrated and size resolved
Gravimetrical methods	No	Mass	Time integrated	Size integrated
Carbon analyses	Yes	Mass	Time integrated	Size integrated
Electron microscopy/energy dispersive X-ray spectroscopy (EM/EDX)	Yes	Particles	Time integrated	Size resolved
Inductive coupled plasma-mass spectrometry (ICP-MS)	Yes	Mass	Time integrated	Size integrated

[1] Time resolved: Continuous real-time results; Time integrated: Aggregated result over sampling time.

[2] Size resolved: Specific information about the size of the exposure; Size integrated: Aggregated result for measurement size range.

There is a need to optimize the current and more refined methods of CNT exposure and to develop a comprehensive measurement approach in order to study both the type and the quantity of exposure. In addition, the developed measurement approach needs to be used at (large-scale) CNT manufacturing and/or handling facilities to evaluate workers' exposure and to ensure responsible development.

Risk evaluation of CNTs

Uncertainty in the risk assessment of exposed individuals leads to industry's unwillingness to invest in beneficial nanotechnologies; this inhibits the full potential of CNTs for society.[44] The scientific knowledge of (many) different forms of CNTs is too limited to derive specific health-based exposure limits. In the Netherlands and other European countries, due to the absence of health-based occupational exposure limits (OELs) or derived no-effect levels (DNELs) for CNTs, warning levels are set based on the precautionary principle.[45,46] The precautionary principle is often conservative and based on the worst case scenario, aiming to manage poorly quantified risks. Gaining an overview of accurate exposure levels at the workplace and more insight into potential early health effects associated with CNT exposure would be the first step towards a more evidence-based risk assessment for CNT exposure.[47]

Thesis aims and outline

Based on the information presented above the primary objectives of this thesis are:

1. To develop of a comprehensive approach to assess both the nature and level of (MW)CNT exposure based on the evaluation and optimization of different available methods of measurement.
2. To use the developed measurement approach discussed above in order to obtain insight into exposure determinants and activities of MWCNT exposure during the (large-scale) production and handling of MWCNTs and during abrasive activities in the later stages of the life cycle of products containing MWCNTs.
3. To evaluate the association between occupational exposure to MWCNTs and early cardiovascular effects in a cross-sectional epidemiologic study.

In **Chapter 2** of this thesis, a systematic review of current knowledge is provided on the emission potential of nano-objects and their agglomerates, and aggregates (NOAA) including CNTs, across various product life cycle stages. Key gaps in the data are identified, and future research recommendations are suggested.

Chapter 3 describes the evaluation and optimization of different methods for the exposure assessment of MWCNTs at the workplace, resulting in a comprehensive approach to study both the nature and level of exposure. The three analytical methods considered are EC analysis (as a proxy for CNT mass concentrations), (S)EM with EDX and ICP-MS.

Chapter 4a evaluates an exposure assessment during the synthesis and handling of MWCNTs in a commercial production facility in which the developed measurement method (as discussed in **Chapter 3**) was used. Personal shift-based exposure levels are assessed and specific activities contributing to MWCNT exposure are identified. **Chapter 4b** discusses an experiment that evaluates the potential exposure of workers during abrasive activities (sawing, drilling) with MWCNT and products that contain OPs; this experiment tested variable realistic workplace determinants (energy level of the abrasive activity, ventilation in the room).

Chapter 5 describes the outcome of a cross-sectional epidemiologic study among workers from a company producing MWCNTs commercially and a matched unexposed population (this is the same population as described in **Chapter 4a** and **Appendix 1**). Cardiovascular markers were measured in the participants' blood, and possible associations with personal exposure to MWCNTs were assessed.

Chapter 6 summarizes the results and analyzes them from a broader perspective. The state-of-the-art knowledge of CNTs is discussed regarding 1) the exposure assessment, 2) the evidence of associated potential health effects in humans, 3) risk assessment, and 4) risk management. Furthermore, future perspectives and considerations are evaluated; these are

focused on 1) read across and grouping, 2) safe innovation, 3) risk governance, and 4) exposure registration and epidemiological research.

Appendix 1 describes a cross-sectional biomarker study (based on the same study population as discussed in **Chapter 4a** and **Chapter 5**) that evaluated and observed indications of an association between exposure to MWCNTs and immunological effects and lung health.

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Chapter 2

Understanding workers' exposure: Systematic review and data-analysis of emission potential for NOAA

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Chapter 2

Abstract

Exposure assessment for nano-objects, and their aggregates and agglomerates (NOAA) has evolved from explorative research towards more comprehensive exposure assessment, providing data to further develop currently used conservative control banding (CB) tools for risk assessment. This study aims to provide an overview of current knowledge on emission potential of NOAA across the occupational life cycle stages by a systematic review and subsequently use the results in a data analysis.

Relevant parameters that influence emission were collected from peer-reviewed literature with a focus on the four source domains (SD) in the source-receptor conceptual framework for NOAA. To make the reviewed exposure data comparable, we applied an approach to normalize for workplace circumstances and measurement location, resulting in comparable "surrogate" emission levels. Finally, descriptive statistics were performed.

During the synthesis of nanoparticles (SD1), mechanical reduction and gas phase synthesis resulted in the highest emission compared to wet chemistry and chemical vapor condensation. For the handling and transfer of bulk manufactured nanomaterial powders (SD2) the emission could be differentiated for five activity classes: 1) harvesting; 2) dumping; 3); mixing; 4) cleaning of a reactor and 5) transferring. Additionally, SD2 was subdivided by the handled amount with cleaning further subdivided by energy level. Harvesting and dumping resulted in the highest emissions. Regarding processes with liquids (SD3b), it was possible to distinguish emissions for spraying (propellant gas, (high) pressure and pump), sonication and brushing/rolling. The highest emissions observed in SD3b were for propellant gas spraying and pressure spraying. The highest emissions for the handling of nano-articles (SD4) were found to nano-sized particles (including NOAA) for grinding.

This study provides a valuable overview of emission assessments performed in the workplace during the occupational handling of NOAA. Analyses were made per source domain to derive emission levels which can be used for models to quantitatively predict the exposure.

Introduction

Inhalable nano-objects, and their aggregates and agglomerates (NOAA) [1], can exhibit toxicological properties which might be different from those of conventional materials of the same chemical composition. Hazard assessments of NOAA are difficult due to their variability in structure and size, but a lack of data on (workplace) exposure to NOAA makes a comprehensive risk assessment is even more difficult.

Exposure models are crucial in (regulatory) risk assessment as it would be expensive, impracticable and time consuming to carry out case-by-case studies with individual exposure measurements for each chemical under every circumstance. For the emerging risks of

nanoparticles, various control banding (CB) tools were developed as a pragmatic approach to manage the risks in a context of large uncertainty by controlling exposure.[2] The conceptual framework for the assessment of inhalation exposure to NOAA [3] was used as a basis for many CB tools. For example, Stoffenmanager Nano [4], describes a stepwise transfer of NOAA from the source (emission) via the various transmission compartments to the receptor (exposure). It is assumed that the potential for emission is determined by both the substance emission potential (e.g. dustiness, weight fraction, viscosity) and the activity emission potential (e.g. level of energy transfer related to activities, scale and product-to-air interface). We define the emission as the transfer process of liberated nanomaterial to the workplace air, usually expressed as a flow (particles per unit time or area). The framework assumes that local control measures during transmission and personal protective equipment at the receptor reduce exposure at similar levels of effectiveness for NOAA compared to conventional particles.[3-6]

In the past decade, numerous risk assessments have been published regarding workplace NOAA exposure assessment. However, Kuhlbusch et al.[7], Clark et al.[8], Losert et al.[9] and Virji et al.[10] reviewed workplace exposure assessments and concluded there is an urgent need for a more systematic and standardized measurement approach to be able to compare results for modelling purposes. In parallel, the market for nanomaterials is expanding with potentially more workers exposed.[11] With several recent initiatives to make exposure assessment results more comparable, systematic data analyses of field and experimental studies are rapidly needed to derive more insight into quantitative exposure assessment and exposure modeling.[12] Such analysis would improve precision of exposure estimates and could settle controversies from apparently contradicting results. Brouwer (2012) evaluated available control banding models for NOAA and concluded that the models are either based on the emission potential or on (personal) exposure estimates generated by the underlying source-receptor model.[2] The evaluated control banding tools use no measurement data, for several variables the multipliers for bulk materials are used and numerous data gaps are filled based on expert elicitation. Present NOAA-specific qualitative risk prioritization or banding tools (e.g. ANSES, Guidance, CB Nanotool and Stoffenmanager, Nano NanoSafer respectively) or conventional exposure tools which need refinements for NOAA (e.g. Advanced REACH tool (ART), ConsExpo) could be made applicable for NOAA and turned into tools for predicting quantitative exposure of NOAA.[4-6, 13-16]

The primary objective of this study was to provide a systematic review on emission potential of NOAA across various occupational life cycle stages, transform the reviewed data into comparable measures and provide descriptive statistics of available data. Future investigations can build on this study for further modeling purposes.

Method

Scope and boundaries

Schneider and colleges [3] identified four source domains (SD) in the NOAA source-receptor conceptual framework. Based on this description and relevant adjustments for SD3 of this concept, the source domains are: synthesis of nanomaterials (SD1), handling/transfer of bulk nanopowders (SD2), handling of powder intermediates/ready-to-use products (SD3a), handling of liquid intermediates/ready-to-use products (SD3b), and handling of nano-enabled products (SD4).

Within each SD, the parameters that influence emission are believed to be different (or differently scaled) and therefore the starting point of this project was a review of measurement data per SD. This review consisted of evaluating exposure assessment studies for relevant parameters available through peer-reviewed papers and (public) reports. The parameters that were addressed during the analysis and extracted (if available) from the included peer-reviewed articles were: 1) type and amount of nanomaterial handled, 2) moisture content, dustiness, weight fraction and/or viscosity of nanomaterial, 3) primary particle size of the nano-objects, 4) measured scenario description of the performed activity, 5) circumstances of the measurement with information about modifying factors of the emission, 6) measurement location (distinguishing emission from 'near-field (NF)' sources in the breathing zone (< 1 m) of the worker and emissions from 'far-field (FF)' sources (the remaining space of the work area), 7) the measurement devices used and their detection range and 8) Workplace air or breathing zone concentrations due to nanomaterial related activity (corrected for background concentration by subtraction).

Due to lack of knowledge about the toxicological mechanism of NOAA, the most appropriate metric to assess exposure to NOAA is still under debate, i.e. particles size or number-, surface area-, mass concentration. However, most of the reviewed data contained information about the particle number concentration and it was decided for practical reasons to use this metric in the current study.

The peer-reviewed articles were selected by a systematic literature search, which included searches via Medline for recently published peer-reviewed papers, with the search terms (and combinations) nanomaterial(s), NOAA, ENM, workplace exposure, occupational exposure and exposure. Furthermore, recent reviews [7, 9, 10, 12] were used for the identification of relevant literature. For this review, we have taken into account exposure related NOAA measurements at workplaces, where nanomaterials or nanomaterials incorporated products are produced, processed or used. These include industrial facilities, R&D facilities as well as down scale users. Identified data (published between 2000 and 2015) was reviewed by two experts in the field of exposure assessment. All reviewed data is referenced in the supplementary information (S1).

Data processing

The reported exposure in the identified peer-reviewed literature were 'normalized' to make the reviewed data comparable. We used a 'backwards calculation' procedure, correcting for different workplace circumstances and measurement locations by using the relevant variables included in the Stoffenmanager Nano 1.0 algorithm (adapted from Marquart et al.[17]). Thereby we obtained comparable, normalized emission values for each activity in all SDs, without distinguishing between different types of nanomaterials. Substance emission potential research focusing on dustiness concluded that not the type of nanomaterial determines the emission but physical and chemical factors are important (e.g binding strength, energy, coating of the particles).[18, 19] As research is ongoing and we believe there is currently no method available that could correct for substance emission potential, we decided to focus on variables relevant for activity emission potential. Data was normalized for the effect of localized controls, transmission factors i.e. , dilution/dispersion (based on the room volume and the ventilation type) and separation or reduction of the immission at the receptor by respiratory protective equipment. The correction for dilution/dispersion was preformed based on estimations of the position of the receptor in relation to the source (NF or FF).[3, 4, 17] Final comparison of the normalized data took place on the total multiplier level of 1. In more detail, we corrected the exposure to the situation of no control measures at the source, a room volume > 1000 m^3 with no general ventilation and a worker not working in a cabin during the measurement. Supplementary information (S2) provides examples of application of this procedure. Tables S2-1-S2-3 shows in detail the different variables we used for correction with corresponding multipliers.

Descriptive statistics

Descriptive statistics (N, mean, median, 25%, 75%,min, max) were derived for the various SD, regarding the relevant activities and other independent variables influencing the emission potential. All statistical analyses were performed using the R statistical software STATS package (R Development Core Team, 2011).

Results

The concentrations presented in this result section represent the normalized concentrations after data processing.

Synthesis (Source domain 1)

NOAA synthesis processes mostly take place under well controlled circumstances with workers often not in close proximity of the source (industrial scale), although R&D facilities have reported small scale open synthesis processes. The ISO guidelines for control banding approaches identified four relevant synthesis groups, namely 1) Gas phase synthesis (including flame pyrolysis, laser ablation, electro-spraying); 2) Mechanical reduction or

attrition (grinding and cutting); 3) Chemical vapor condensation and 4) Wet chemistry (into or within a solution).[20] Laser ablation and sintering were excluded as the aim of these methods was not the synthesis of manufactured nanoparticles.

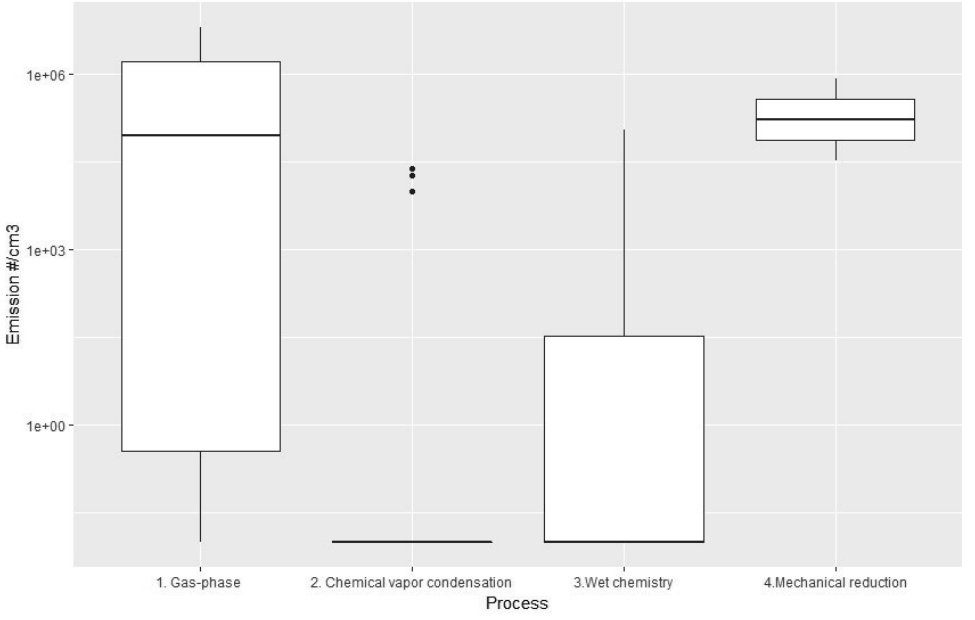

Figure 1: Normalized emission caused by four groups of synthesis processes (source domain 1). Boxplots displays 25%, 50% and 75%.

For gas-phase synthesis, NF emissions were found between 100,000 and 1,000,000 #/cm^3.[21-25] In general, the number concentration upon emission is very high, but particle agglomeration rapidly reduces the number concentration, although not the mass concentration.[24] Gas-phase processes have been described as the only type of nanoparticle synthesis methods which can lead to inhalation exposure of primary nanoparticles as a result of reactor leaks [26], and with particle size distributions of the data showing emission with a size range between 20-200 nm.[21-25]

Mechanical reduction is a top-down process where nanoparticles are produced from larger (micro) particles by cutting or grinding. Limited data showed a relatively high emission compare to other synthesis processes, up to 830,000 #/cm^3 with most NOAA between 10 – 100 nm.[27]

Nanoparticles created with chemical vapor condensation are manufactured on a substrate, which makes emission into the workplace air unlikely.[26] Data showed high particle number concentrations which are believed to be process generated particles.[28] The required harvesting and reactor cleaning for this production process is part of activities related to SD2.

Synthesis with wet chemistry methods is not regularly measured but emission is believed to be low because historical industrial hygiene experience has shown that exposures tend to be lower for wet processes with dust compared to dry processes.[10] Two studies indicated no elevated concentration of particles during wet chemistry synthesis of nanoparticles.[23, 29] One study reported increased particle numbers during wet chemistry synthesis of NOAA but measured a very high background level of nanosized particles during the closed reactor period.[30]

Emissions observed during synthesis of nanoparticles (N median, min, max (# / cm^3)) were relatively high for gas-phase (N=38) 8,9E4 (0 – 6,1E6) and mechanical reduction (N=3) 1,7E5 (3,3E4– 8,3E5), compared to chemical vapor condensation (N=16) 0 (0 – 2,4E4) and wet chemistry (N=3) 0 (0 – 1,1E5) (Figure 1). The boxplots display 25%, 50% and 75% percentiles.

Handling pure nanopowder (Source domain 2)

Source domain 2 includes the handling and transfer of ~100% pure manufactured nanomaterial powders. Data from 24 peer-reviewed publications and additional internal projects resulted in 131 emission scenarios. Information about relevant activity emission potential variables including handled amount of NOAA (< 1 g, 1 – 1000 g, > 1 kg) and the subjectively assigned energy transfer (low, medium, high) during the activity was recorded. Five activity categories were distinguished, namely cleaning (of the reactor/workplace), dumping, harvesting, mixing and transferring (i.e. handling of heavily contaminated objects, vacuum transfer and scooping of powder).

Dumping activities were often performed with large amounts (> 1 kg) whereas the other activities were most frequently performed with < 1 kg of nanomaterial. Overall, emission levels (N, median, min, max (# / cm^3)) observed during handling > 1 kg and 1 g – 1 kg of nanomaterial were relatively high, (N=27) 1,5E4 (0 – 6,0E6) and (N=50) 1,1E4 (0– 4,2E6) respectively, compared to handling < 1 g ((N=54) 5,9E2 (0 – 6,7E5)). Furthermore, emission levels observed during the activities dumping and harvesting were relatively high ((N=23) 1,9E5 (0 – 6E6)), ((N=18) 7,9E3 (0 – 4,2E6)) respectively, compared to transferring ((N=43) 1,7E3 (0– 5,1E5)), cleaning ((N=38) 6,9E2 (0 – 6,7E6)) and mixing ((N=9) 0 (0 – 2E4)). Alternatively, the results are presented per activity category and per handled amount of nanomaterial in Figure 2. Overall, the handled amount seem to be a relevant variable for most activities, but some odd trends can be observed as the data in some categories is very limited.

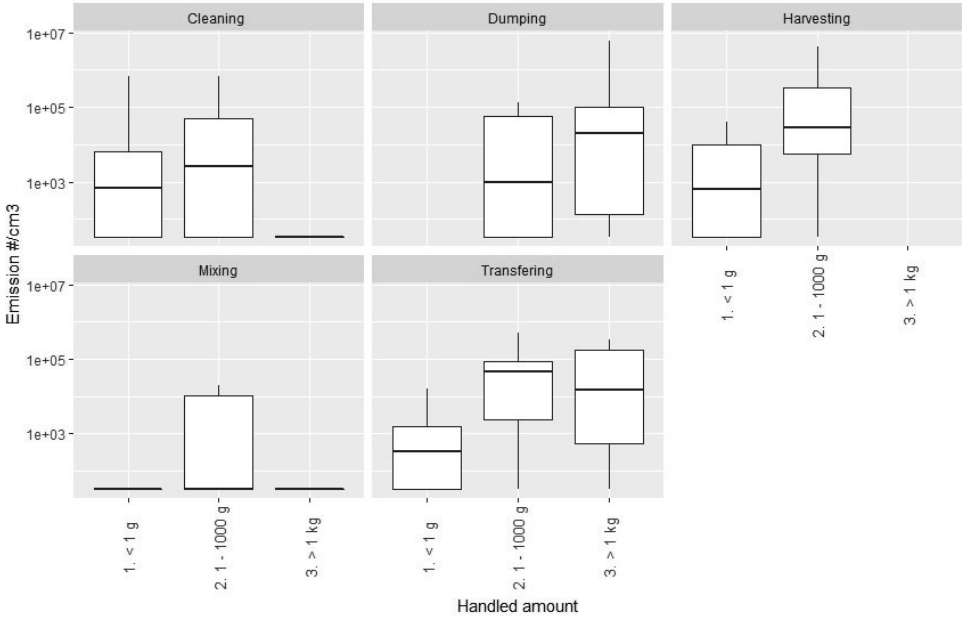

Figure 2: Normalized emission from five groups of activities per handled amount (source domain 2). Boxplots displays 25%, 50% and 75%.

A third potential determinant effecting the emission of nanopowder is the level of energy transferred in the process. However, the energy level of the used process is believed to be directly related to the activity. Energy analyses were only possible for cleaning as it was not possible to distinguish enough between the different energy transfer levels within other activity classes. Energy classes included low energy transfer (cleaning reactor with a wet duster or by scraping), medium energy transfer (cleaning reactor with a brush, a dry duster or a sourcing pad), and high energy transfer (cleaning reactor by sanding, vacuum cleaner or compressed air). An overview regarding the effect of energy transfer for cleaning activities with different amounts of nanomaterial handled is shown in Figure 3. Disregarding the amount handled, emission levels (N median, min, max (# / cm^3)) observed during cleaning with a high energy transfer were relatively high ((N=9) 7,7E3 (0 – 6,7E5)) compared to a medium energy transfer ((N=23) 5,3E2 (0– 5,2E5)) and low energy transfer ((N=6) 0 (0 – 0)).

One study concluded that the individual patterns of the particle size distribution (<500 nm) during the handling of pure nanopowder showed a limited change compared to the background trends.[31] The geometric mean diameter (GMD) of the particle size distributions (N=29) varied between 20–114 nm (background) and 21–84 nm (SD2 activity, NF). Despite this fact, several studies showed an increase of particle number concentrations between 20 – 500 nm, with mean particle size above 100 nm, with the exception for activities with Multi-walled carbon nanotubes (MWCNTs), which mostly show large clusters of > 500 nm.[28, 32-36]

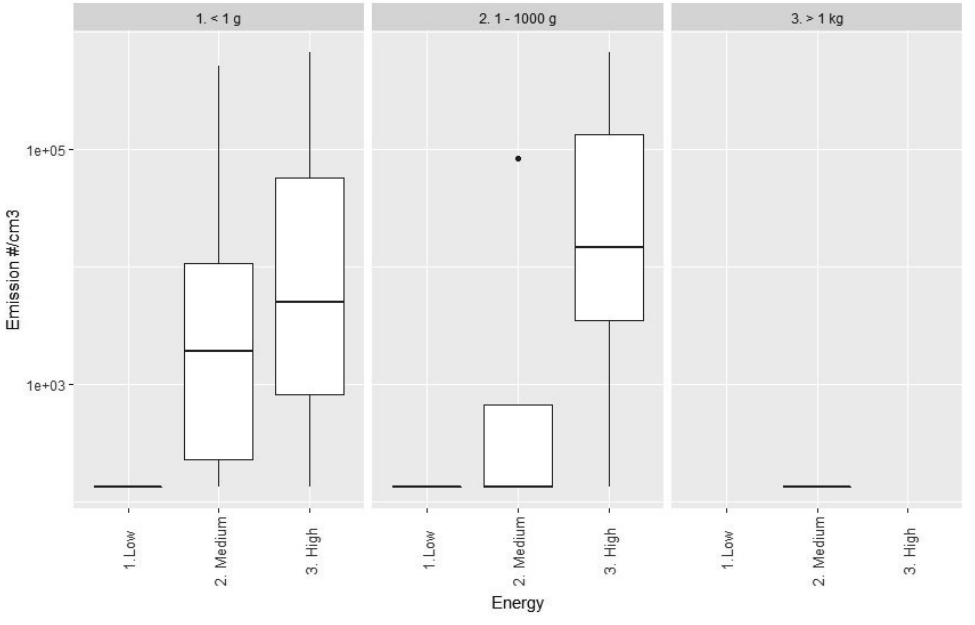

Figure 3: Normalized emission from cleaning per handled amount per energy level (source domain 2). Boxplots displays 25%, 50% and 75%.

The principal factor determining the emission of aerosols from handling or processing powdered materials is the dustiness of the material.[37] Dustiness of powder or bulk material is a relative measure, thus the results of a dustiness test will always depend on the applied method (e.g. rotating drum, continues drop) and ambient conditions. Nevertheless, dustiness is considered to be an important determinant for the emission potential of substances in predictive (nano- and conventional) exposure models.[3, 38] The inconsistency of product rankings from different dustiness sampling methods provides evidence that the level of energy used in the process might be an emission determinant.[39, 40] In general, the various dustiness methods can be ranked according to their dispersion energy and assumed to mimic low- to high energy activities. Thereby, certain dustiness measurement methods could be representative for certain activities as the energy levels are comparable. However, at this moment further research is needed as the relation between dustiness measurement methods and activities within the SD is lacking.

Emission of NOAA observed during handling of pure nanomaterials is relatively high for dumping and harvesting activities, for activities with high quantities of nanomaterials and for cleaning methods with a high energy transfer. However, correct statistical testing of this data using a mixed model was not possible as no normal distribution (after transformation) of the data was found. The non-normal distribution was most likely caused by the high number of measurements with no increase compared to background and the large variation within exposure situations.

Handling powder intermediates/ready-to-use products (Source domain 3a)

Six peer-reviewed publications were identified related to the dispersion of powder intermediates or application of ready-to-use nanoproduct powders.[41-46] Emission levels were identified up to 2,0E5 #/cm^3, but the direct reading instruments were not able to distinguish between NOAA and other substances in the product. Due to similar handling techniques, it was concluded that emission potentials as identified for SD2 are equally applicable for activities related to SD3a. The difference lies in the fact that in SD2 emission is of 100% NOAA, whereas in SD3 emission is of particles composed of only a fraction of NOAA.

In the absence of evidence, we assume that the NOAA are uniformly distributed over the emission and the emitted particles. Three publications were identified that reported the weight fraction of nanoparticles in powders, varying between 3 – 15% w/w, mostly nanoparticles in another powder.[42, 44-46] No indications were provided regarding the relation between weight fraction and the emission. Consequently, emission estimates using conventional models adapted for NOAA with particle number concentrations as output, should not correct the emission for the fraction of NOAA but should collate and process information about the percentage of nanomaterial in the product as this is of high interest for the risk assessment.

Handling liquid intermediates/ready-to-use products (Source domain 3b)

During handling and application of liquid intermediate or ready-to-use nanoproducts, workers are potentially exposed to airborne droplets containing NOAA. Peer reviewed papers report emission of airborne droplets containing NOAA during spraying (N=22), sonication (N=10) and brushing/rolling (N=3) (Figure 4).

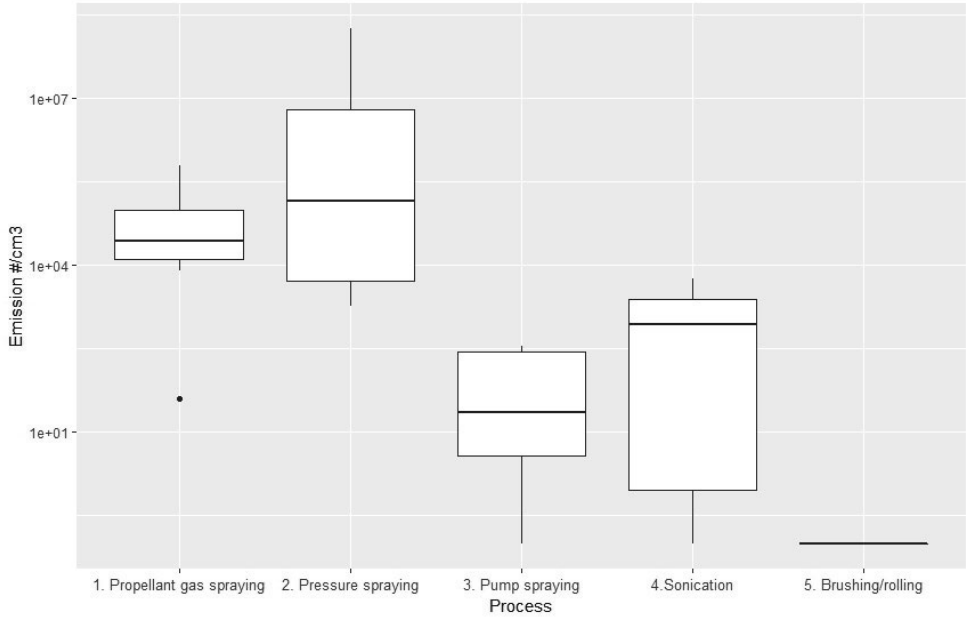

Figure 4: Normalized emission from five groups of during handling of liquid intermediate nanoproducts or application of liquid ready-to-use nanoproducts (source domain 3b). Boxplots displays 25%, 50% and 75%.

Twelve peer reviewed papers have studied emission of NOAA during spraying of NOAA containing liquid ready-to-use products. Four studies focused on spray activities in occupational setting, [27, 31, 42, 43] five on spray activities with consumer sprays in a small experimental room [47-51] and three peer-reviewed publications were excluded from further analyses as the spray generating mechanism was unknown [52, 53] or the measured exposure was not relevant for workplace exposure.[54] In total, 22 spray measurements were included, using various spray mechanisms, with emission levels (N median, min, max (# / cm^3)) observed for propellant gas ((N=7) 2,8E4 #/cm^3 (41 – 6,2E5)), (high) pressure ((N=7) 1,4E5 #/cm^3 (1,8E3 – 1,78E8)), and pump ((N=8) 2,4E1 #/cm^3 (0 – 3,5E2)). These results show that workers are potentially exposed to high levels of NOAA during spraying of liquid NOAA containing nanoproducts (and intermediates).

Emission of NOAA containing airborne droplets during sonication of a liquid nanodispersion was measured in total 10 times by four studies.[27, 31, 33, 55] These studies showed, with the exception of Bekker et al., 2015, that NOAA can become airborne during sonication processes with airborne droplets median (min – max) concentration 894 #/cm^3 (0 – 5,8E3) and the airborne droplets range in size from 10 nm - 1µm. The manual application of liquid ready-to-use products, i.e. brushing/rolling of a nanocoating, was concluded to not contribute to airborne emission of NOAA.[31]

The number concentration and size distribution of the airborne droplets is determined by the substance emission potential (e.g. viscosity of the liquid) and activity emission potential (e.g. combination of speed/energy, nozzle size, etc).[38] Liquids with a low viscosity are more prone to become airborne than highly viscous solutions and, under the same application conditions, emission of NOAA dispersed in liquids with a low viscosity will be higher than in a more viscous liquid.[56] Alternatively, spraying with a high pressure (e.g. propellant gas- or pressure spray) emits smaller and more airborne droplets than spraying with a pump spray.[48] Spray nozzle type and spacing also have a significant effect on the size of the emitted airborne droplets. The residence time of small airborne droplets is longer than that of big airborne droplets (sedimentation), and even long enough for the solvent to evaporate. Consequently, exposure to dry NOAA in the air is more likely during spraying with high pressure and nozzles with small spacing. Another factor influencing the measured concentration of airborne droplets is the spray direction and the position of the spray product with regard to the measurement equipment.

The presence of NOAA in the liquid product did not significantly influence the number concentration of airborne droplets generated or size distribution.[9, 47] Therefore, the above mentioned parameters (velocity, pressure, nozzle type and spray mechanism) have the same effect on NOAA containing liquid products compared to the conventional liquid products and already developed conventional exposure models (e.g. ART, ConsExpo, SprayExpo) can be used to estimate airborne droplet emission during handling of liquid intermediate nanoproducts or application of liquid ready-to-use nanoproducts.

However, the dispersion of the NOAA in the airborne droplets is a key element in determining the actual emission to NOAA. The applicability of conventional models for modelling NOAA emission and exposure needs to be further explored as these models are mass-based and use the weight fraction to correct for a specific substance. In the absence of evidence, we assume that the NOAA are uniformly distributed over the liquid used during the activity and that the NOAA will consequently be uniformly distributed over the emitted airborne droplets. As hypothesized by others, [9, 47] we believe the weight fraction only influences the percentage of NOAA in the airborne droplets and not the number of airborne droplets. Similar to SD3a, emission estimates using conventional models adapted for NOAA with particle number concentrations as an output should not correct the emission for the fraction of NOAA but should collate and process information about the percentage of nanomaterial in the product.

Machining of nano-enabled products (Source domain 4)

Twenty-five peer-reviewed publications were identified regarding handling of nanoparticles or fracturing and abrasion of nanoparticles (Figure 5) with embedded nanoparticles, using various abrasive activities, with overall emission levels including NOAA (N median, min, max (# / cm^3)) observed for abrasion ((N=13) 4,0E3 #/cm^3 (1,7E2 – 1,4E4)), cutting including sawing

and cleaving ((N=5) 2,9E3 #/cm^3 (5,7E2 – 2,9E5)), drilling ((N=6) 7,2E4 #/cm^3 (4,5E3 – 1,8E5)), grinding ((N=8) 3,8E3 #/cm^3 (0 – 1,7E6)), sanding ((N=24) 4,3E3 #/cm^3 (0– 2,5E8)) and weathering ((N=4) <4,3E0 #/cm^3 (<4,3E0 – <4,3E0)).

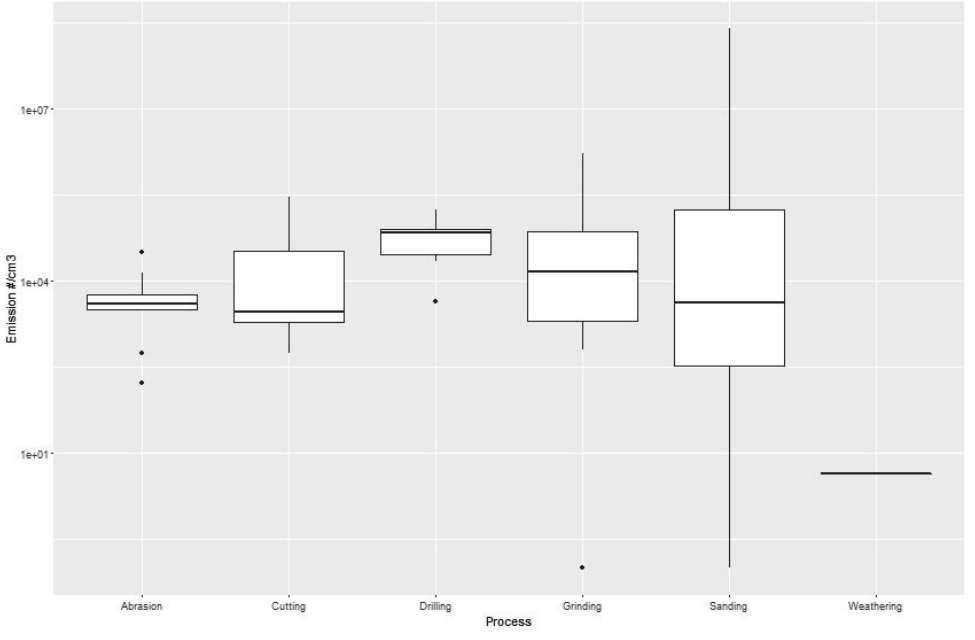

Figure 5: Normalized emission to nanoparticles from six groups of machining of nano-enabled products (source domain 4). Boxplots displays 25%, 50% and 75%.

Most studies performed experiments to investigate the release potential of NOAA from a matrix polymer. Workplace measurements were scarce and provided NF data related to total emission instead of NOAA emission. Quantitativily, almost no differences were observed in particle emissions of abrasive activities with NOAA-embedded composites and composites without NOAA.[57, 58] If measurements identified any individual reported NOAA emission levels, the overall emission during these measurements is dominated by either process generated particles (emitted by for example an electric motor used to perform the abrasive activity) or matrix material (with embedded NOAA).[43, 46] Qualitatively, free agglomerates of carbon nanotubes (CNT) and carbon nanofibers (CNF) containing composites have been identified in the air have been identified as a consequence of abrasion and machining. Detached, non-embedded CNT and CNF containing composites have been measured with a total emission (including NOAA) of 1,4E4 #/cm^3.[59] Furthermore, two peer-reviewed articles observed nano-sized particles (including NOAA) > 1,0E6 #/cm^3, during the polishing and grinding of composite material (with nanomaterial fraction up to 80%) related to dental care.[60, 61] Additionally, others found no free NOAA in airborne samples collected during abrasive activities and conducted with good dispersed NOAA in a matrix (total emissions including NOAA 4,2E2

#/cm^3 and 4,0E3 #/cm^3 respectively), except for tests performed with bad dispersed CNTs in a matrix, which resulted in some free NOAA.[62, 63]

Safe Work Australia reviewed available peer-reviewed literature and concluded that emissions from composites scenarios were largest for high energy activities like grinding, cutting and sanding.[57] Furthermore, emissions from sanding are influenced by the sanding speed and the roughness of the sandpaper.[62] In brief, substance emission variables like the hardness of the material, the position of NOAA on/in the matrix, the weight fraction NOAA and the dispersion of NOAA in the matrix determine the potential amount of emmision of NOAA caused by abrasive activities. As no NOAA specific differences were observed in the absolute number counts (emission), studies and models assessing the emission due to abrasive activities of conventional materials could be valuable. Future modelling would benefit from research focused on the applicability of conventional models for NOAA-embedded articles.

In the absence of evidence, we assume that NOAA are uniformly distributed over the matrix used during the activity and that the NOAA will consequently be uniformly distributed over the emitted aerosols. As hypothesized by others,[57,58] we believe the weight fraction only influences the percentage of NOAA in the aerosol and not the number of emitted aerosols. Similar to SD3b, modeling using conventional models adapted for NOAA with particle number concentrations as an output should not correct the emission for the fraction of NOAA but should collate and process information about the percentage and the position of nanomaterial in the product as these are of highest interest.

Discussion

In this study, we provided an overview of current knowledge regarding emission for NOAA across various occupational life cycle stages, transformed the reviewed data into comparable measures and provided a brief analysis of said data. During the synthesis of nanoparticles (SD1) several process types can be ranked according the highest emissions to NOAA: 1) mechanical reduction, 2) gas phase synthesis, 3) wet chemistry and 4) chemical vapor condensation. For the handling and transfer of bulk manufactured nanomaterial powders (SD2) the emission could be differentiated for five activity classes 1) harvesting; 2) dumping; 3); mixing; 4) cleaning of a reactor and 5) transferring, subdivided by the handled amount and for cleaning further subdivided by energy level. Based on the available data, harvesting and dumping results in the highest emission. During the handling of solid (SD3a) / liquid (SD3b) intermediate nano-products, including solid intermediates, the emission consists of NOAA and solid/liquid particles with or without NOAA. For processes with liquids, it was possible to distinguish emissions for spraying (propellant gas, (high) pressure and pump), sonication and brushing/rolling. The highest emissions were observed for propellant gas spraying and pressure spraying. Lastly, for the handling of nano-articles (SD4) emission can occur as matrix

material, matrix material with NOAA embedded and incidentally free NOAA, with highest emissions to nano-sized particles (including NOAA) for grinding.

Although the exposure assessment for NOAA has progressed, exposure modelling for NOAA is still in its infancy. Current exposure models are precautious, which results in limited resolution in the outcomes due to their relatively simple and conservative nature. To further develop quantitative exposure models, a number of variables need to be calibrated. In this study, descriptive statistics were used to analyze different variables but unfortunately the results of additional statistical techniques had no added value due to non-normal distributions of data. Nevertheless, we believe meta analyses offer more possibilities with data collected following the same measurement approaches and focusing on the same determinants of emission and exposure. Future studies should focus on the validation of emission results provided by the present study, aiming for further underpinning of variables with measurement data and consequently resulting into more accurate and precise quantitative exposure models.

The exposure and hazard potential, together the risk, cannot be evaluated absolutely independently.[7] Future models should aim for a more integrated approach, with exposure focusing more on the changes in appearance/ transformation that NOAA undergo from source to worker, from the breathing zone to the lungs and subsequent hazard assessment focusing on the deposited particles. Preferably, an exposure model would estimate particle sizes and the number of particles in order to create a more quantitative hazard model with variables for deposition of particles in the lung. The present study provides general information about the mean size of NOAA per SD, which can be a starting point for risk and hazard modeling purposes. In addition, process generated nanoparticles are important and contribute to aggregate lung deposited dose of nanoparticles and should not be disregarded from the perspective of health impact.

It is important to note that the descriptive statistics derived in this study should be interpreted with caution due to the necessary assumptions made during data transformation. Heterogeneous methods and scenarios are limiting the data analysis needed for calibration and further development of exposure modelling. As a corrective step, recent initiatives to harmonize measurement strategies resulted in reliable formal methodologies for conducting consistent exposure measurements for NOAA. Still, there is a large need for these formal methodologies to result in more consistent collected data in near future.[64, 65] Additionally, this study showed large variability within the emission situations caused by numerous reasons including the inability of the current activity classes to distinguish differences in how these activities are performed, differences between and within measurement instruments, the different measurement approaches and within and between worker variability. For example, normalized emission levels during (high) pressure spraying ranged between 2,0E3 and 1,8E8 #/cm^3 and wet chemistry synthesis between 0 and 1,0E5 #/cm^3. Thus, there is a need to

increase the number of available exposure measurement data to underpin an evidence based exposure model. There is also a need for repeated measurements to provide more insight in between- and within- worker variability.[31] Furthermore, the standardization of the data is based on relations for modifying factors only tested for bulk substances and additional assumptions. The correction for FF measurements is arbitrary for NOAA and is still under debate among scientists. The direct reading instruments used to monitor exposure to NOAA offer the advantage of providing real time information during a short period of handling the material. However, distinguishing between NOAA and other nanoscale particles (process generated, naturally occurring) is difficult.[10] A well designed measurement approach enables correction for naturally occurring nanoparticles, but quantitative elimination is not easily achievable for process generated nanoparticles which is believed to be a realistic error for SD1, SD3 and SD4. Also, as there was no clear relation available between intrinsic substance properties (e.g. adhesive binding forces, particle density, particle morphology) and the release, the applied approach assumed no effect of the type of substance on the emission which introduced variability in the results of this study. Clearly emission may differ by intrinsic properties, especially for situations such as carbon nanotubes (high adhesive binding force, non-spherical morphology). Lastly, as for every meta-analysis, publication bias could be another source of variability that influenced the validity of the assessment. As a consequence of this large variability, the results of this study contribute to range finding and are a next step into more quantitative exposure models. We believe the results can be used for modelling purposes, by still applying a precautionary approach, by using the maximum reported value for less than 9 measurements and by using the 75 percentile for scenarios with 9 or more measurements.[66]

In conclusion, this study provides a valuable overview of emission assessments performed in the field of occupational handling of NOAA. Analyses were made to derive emission levels which can be used for quantitative exposure models, per SD and describing the vast majority of current and near future occupational emission situations for NOAA.

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Supporting information S1

Table SI1-1: Exposure levels measured during synthesis methods (source domain 1)

Information source	Nanoproduct (primary particle diameter nm)	Circumstances	Individual reported exposure levels (particles / cm^3)	Correction factor (corrected for)	Normalized emission (particles / cm^3)
Gas-phase synthesis method					
[1]	CeO_2 (20-40)	Synthesis and coating was performed in a small unventilated area.	160,000	0.1 (small unventilated area)	16,000
[1]	CeO_2 (20-40)	Synthesis was performed in furnace placed in enclosure. Measurements were performed in the surrounding (far field).	9,200	30 (enclosure, partial segregation FF, 100-1,000 ventilation)	276,000
[2]	Fe_xO_y (-)	Synthesis (20 mg/min) was performed inside closed fume cupboard.	40,000,000	0.1 (small unventilated area)	4,000,000
[2]	Fe_xO_y (-)	Synthesis (20 mg/min) was performed inside closed fume cupboard.	30,000,000	0.1 (small unventilated area)	3,000,000
[2]	TiO_2 (-)	Synthesis (40 mg/min) was performed inside closed fume cupboard.	10,000	11.11 (Small ventilated room, Fumehood)	111,111
[2]	TiO_2 (-)	Synthesis was performed inside fume cupboard (1 side open).	25,000	11.11 (Small ventilated room, Fumehood)	277,778
[3]	BiPO4 (40)	Production of nanoparticles in a lab (511 m3 (2-10 ACH)) using an open flame spray reactor placed in a fume hood.	1,000	33.33 (NF, Medium sized ventilated room, fumehood)	33,333

[3]	BiPO4 (40)	Production of nanoparticles in a lab (511 m3 (2-10 ACH)) using an open flame spray reactor placed in a fume hood.	500	111.11 (FF, Medium sized ventilated room, Fumehood)	55,556
[3]	BiPO4 (40)	Production of nanoparticles in a lab (511 m3 (2-10 ACH)) using an open flame spray reactor placed in a fume hood.	80,000	33.33 (NF, Medium sized ventilated room, fumehood)	2,666,667
[3]	BiPO4 (40)	Production of nanoparticles in a lab (511 m3 (2-10 ACH)) using an open flame spray reactor placed in a fume hood.	55,000	111.11 (FF, Medium sized ventilated room, Fumehood)	6,111,111
[3]	NaCl (60-80)	Production of nanoparticles in a lab (511 m3 (2-10 ACH)) using an open flame spray reactor placed in a fume hood.	6,000	33.33 (NF, Medium sized ventilated room, fumehood)	200,000
[3]	Bi2O3 (-)	Production of nanoparticles in a lab (511 m3 (2-10 ACH)) using an open flame spray reactor placed in a fume hood.	0	33.33 (NF, Medium sized ventilated room, fumehood)	0
[3]	Bi2O3 (-)	Production of nanoparticles in a lab (511 m3 (2-10 ACH)) using an open flame spray reactor placed in a fume hood.	45,000	33.33 (NF, Medium sized ventilated room, fumehood)	1,500,000
[3]	CaSo4 (20-50)	Production of nanoparticles in a lab (511 m3 (2-10 ACH)) using an open flame spray reactor placed in a fume hood.	2,000	33.33 (NF, Medium sized ventilated room, fumehood)	66,667

[3]	CaSo4 (20-50)	Production of nanoparticles in a lab (511 m3 (2-10 ACH)) using an open flame spray reactor placed in a fume hood.	2,000	33.33 (NF, Medium sized ventilated room, fumehood)	66,667
[3]	CaSo4 (20-50)	Production of nanoparticles in a lab (511 m3 (2-10 ACH)) using an open flame spray reactor placed in a fume hood.	40,000	33.33 (NF, Medium sized ventilated room, fumehood)	1,333,333
[3]	TiO2 (-)	Production of nanoparticles in a lab (259 m3 (2-10 ACH)) using an open flame spray reactor placed in a fume hood.	0	33.33 (NF, Medium sized ventilated room, fumehood)	0
[3]	SiO2 (-)	Production of nanoparticles in a lab (259 m3 (2-10 ACH)) using an open flame spray reactor placed in a fume hood.	0	33.33 (NF, Medium sized ventilated room, fumehood)	0
[3]	SiO2 (-)	Production of nanoparticles in a lab (259 m3 (2-10 ACH)) using an open flame spray reactor placed in a fume hood.	0	111.11 (FF, Medium sized ventilated room, Fumehood)	0
[3]	Cu/ZnO (-)	Production of nanoparticles in a lab (259 m3 (2-10 ACH)) using an open flame spray reactor placed in a fume hood.	1,000	33.33 (NF, Medium sized ventilated room, fumehood)	33,333
[3]	Cu/SiO2 (-)	Production of nanoparticles in a lab (259 m3 (2-10 ACH)) using an open flame spray reactor placed in a fume hood.	1,500	33.33 (NF, Medium sized ventilated room, fumehood)	50,000

[3]	Cu/SiO2 (-)	Production of nanoparticles in a lab (259 m3 (2-10 ACH)) using an open flame spray reactor placed in a fume hood.	0	111.11 (FF, Medium sized ventilated room, Fumehood)	0
[3]	Cu/ZrO2 (-)	Production of nanoparticles in a lab (259 m3 (2-10 ACH)) using an open flame spray reactor placed in a fume hood.	1,000	33.33 (NF, Medium sized ventilated room, fumehood)	33,333
[3]	Cu/ZrO2 (-)	Production of nanoparticles in a lab (259 m3 (2-10 ACH)) using an open flame spray reactor placed in a fume hood.	0	111.11 (FF, Medium sized ventilated room, Fumehood)	0
[3]	WO3 (-)	Production of nanoparticles in a lab (259 m3 (2-10 ACH)) using an open flame spray reactor placed in a fume hood.	16,523	33.33 (NF, Medium sized ventilated room, fumehood)	550,767
[3]	WO3 (-)	Production of nanoparticles in a lab (259 m3 (2-10 ACH)) using an open flame spray reactor placed in a fume hood.	16,091	111.11 (FF, Medium sized ventilated room, Fumehood)	1,787,889
[3]	Ta2O5/SiO2 (10)	Production of nanoparticles in a lab (550 m3 (10 ACH)) using an open flame spray reactor placed in a specially designed ventilated enclosure.	50,000	33.33 (NF, Medium sized ventilated room, fumehood)	1,666,667
[3]	Ta2O5/SiO2 (10)	Production of nanoparticles in a lab (550 m3 (10 ACH)) using an open flame spray reactor placed in a specially designed ventilated enclosure.	25,000	111.11 (FF, Medium sized ventilated room, Fumehood)	2,777,778

[3]	Pt/Ba/Al2O3 (-)	Production of nanoparticles in a lab (130 m3 (2-10 ACH)) using an open flame spray reactor placed in a fume hood.	15,000	33.33 (NF, Medium sized ventilated room, fumehood)	500,000
[3]	Pt/Ba/Al2O3 (-)	Production of nanoparticles in a lab (130 m3 (2-10 ACH)) using an open flame spray reactor placed in a fume hood.	15,000	111.11 (FF, Medium sized ventilated room, Fumehood)	1,666,667
[4]	CaCo3 (50)	Production of nanoparticles in a 300 m3 lab with multiple ventilation sinks using a flame spray pyrolysis setup placed in a ventilated fume hood.	5,000	111.11 (FF, Medium sized ventilated room, Fumehood)	555,556
[4]	CaCo3 (50)	Production of nanoparticles in a 300 m3 lab with suction pump failure a flame spray pyrolysis setup placed in a ventilated fume hood.	50,000	33.33 (FF, Medium sized unventilated room, fumehood)	1,666,667
[4]	CaCo3 (50)	Production of nanoparticles in a 300 m3 lab without ventilation using a flame spray pyrolysis setup placed in a not working fume hood.	3,000,000	1 (FF, Medium sized unventilated room)	3,000,000
[5]	Ag (20-30)	Enclosed production of nanoparticles. Measurements taken outside the collector.	6,675	3.33 (FF, Medium sized ventilated room, containment of the source)	22,250
[6]	SiC (30)	Synthesis took place in a 160 m3 lab with a ventilation rate of 36 ACH.	0	1 (NF, Medium sized ventilated room)	0
[7]	Si (60)	Synthesis took place a closed process with negative pressure in a factory with mechanical ventilation.	0	1 (NF, Medium sized ventilated room)	0
[8]	Ag (<100)	NF assumed in small room, closed reactor, general ventilation	0	1 (NF, Medium sized ventilated room)	0

Chemical vapor deposition					
9	CNTs (-)	SWCNT were generated using the super growth method. Sampling was performed both in and outside the protected enclosures (glovebox or fumehoods). The work rooms were ventilated with hepa filters resulting in low background particle numbers.	0	1 (NF, Medium sized ventilated room)	0
9	CNTs (-)	SWCNT were generated using the super growth method. Sampling was performed both in and outside the protected enclosures (glovebox or fumehoods). The work rooms were ventilated with hepa filters resulting in low background particle numbers.	0	1 (NF, Medium sized ventilated room)	0
9	CNTs (-)	SWCNT were generated using the super growth method. Sampling was performed both in and outside the protected enclosures (glovebox or fumehoods). The work rooms were ventilated with hepa filters resulting in low background particle numbers.	0	1 (NF, Medium sized ventilated room)	0
9	CNTs (-)	SWCNT were generated using a continuous process. The reactor was almost fully enclosed and ventilated. The work rooms were ventilated with hepa filters resulting in low background particle numbers.	0	1 (NF, Medium sized ventilated room)	0

[9]	CNTs (-)	SWCNT were generated using a continuous process. The reactor was almost fully enclosed and ventilated. The work rooms were ventilated with hepa filters resulting in low background particle numbers.	0	1 (NF, Medium sized ventilated room)	0
[9]	CNTs (-)	SWCNT were generated using a continuous process. The reactor was almost fully enclosed and ventilated. The work rooms were ventilated with hepa filters resulting in low background particle numbers.	0	1 (NF, Medium sized ventilated room)	0
[10]	SWCNT (-)	SWCNT production takes place in a reactor placed in a fumehood under normal conditions.	0	33.33 (NF, Medium sized ventilated room, fumehood)	0
[10]	SWCNT (-)	SWCNT production takes place in a reactor placed in a fumehood under normal conditions.	0	33.33 (NF, Medium sized ventilated room, fumehood)	0
[11]	MWCNT (15)	Workplace C manufactured MWCNTs 5 times/day using the CVD method.	0	1 (NF, Medium sized ventilated room)	0
[12]	MWCNT, DWCNT (15)	In workplace A, the production process for MWCNTs was enclosed and process gases exhausted out of the building.	7,200	3.33 (FF, Medium sized ventilated room)	24,000
[12]	MWCNT, DWCNT (15)	In workplace A, the production process for MWCNTs was enclosed and process gases exhausted out of the building.	0	3.33 (FF, Medium sized ventilated room)	0
[12]	MWCNT, DWCNT (15)	In workplace B the entire production process was being under vacuum.	5,400	3.33 (FF, Medium sized ventilated room)	18,000
[12]	MWCNT, DWCNT (15)	In workplace B the entire production process was being under vacuum.	0	3.33 (FF, Medium sized ventilated room)	0

[12]	MWCNT, DWCNT (15)	In workplace C, the production of MWCNT took place in a reactor under vacuum.	3,000	3.33 (FF, Medium sized ventilated room)	10,000
[12]	MWCNT, DWCNT (15)	In workplace C, the production of MWCNT took place in a reactor under vacuum.	0	3.33 (FF, Medium sized ventilated room)	0
[13]	CNT (8)	Atmospheric pressure CVD using a sealed horizontal quart tube furnace.	0	1 (NF, Medium sized ventilated room)	0
Wet chemistry					
[5]	Ag (-)	Wet chemistry.	0	1 (NF, Medium sized ventilated room)	0
[14]	Al (-)	Electrolyse was used to manufacture aluminium nanoparticles.	0	1 (NF, Medium sized ventilated room)	0
[15]	Ag (-)	Wet chemistry took place in opened reactor.	1,100,000	0.1 (NF, Small sized ventilated room)	110,000
Attrition					
[14]	Al (50-60)	The semi cake from the drying oven is grinded to produce smaller particles (57 nm).	99,984	0.33 (NF, Medium sized unventilated room)	33,328
[15]	Ag (-)	The grinding process was performed in a closed system which is regular opened.	2,500,000	0.33 (NF, Medium sized unventilated room)	833,333
[16]	CNF (<100)	Wet sawing took place in a negative pressure room with lab hood.	5,000	33.33 (FF, small sized ventilated room, fumehood and wetting)	166,667

Table SI1-2: Exposure levels measured during handling and transfer of bulk manufactured nanomaterials (source domain 2)

Information source	Nanoproduct (primary particle diameter nm)	Circumstances	Individual reported exposure levels	Correction factor (corrected for)	Normalized emission (particles / cm^3)
Cleaning					
[8]	CNM -CNT (-)	Cleaning reactor with a dry duster (<1g).	850	1 (NF, Medium sized ventilated room)	850
[8]	SiO (-)	Cleaning reactor with a dry duster (<1g).	527	1 (NF, Medium sized ventilated room)	527
[8]	SiO (-)	Cleaning reactor with a dry duster (<1g).	1,208	1 (NF, Medium sized ventilated room)	1,208
[8]	Zinc Oxide (10)	Cleaning sieve (>1 kg).	0	1 (NF, Medium sized ventilated room)	0
[8]	Graphene (20)	Cleaning reactor with vacuum cleaner (1 - 100 g).	233	33.33 (NF, Medium sized ventilated room, fumehood)	7,767
[12]	CNM - SWCNT (1,1)	Cleaning reactor with vacuum cleaner (1 - 100 g).	800	3.33 (NF, Medium sized ventilated room, LEV)	2,667
[12]	CNM - SWCNT (1,1)	Cleaning reactor with vacuum cleaner (1 - 100 g).	8,500	3.33 (NF, Medium sized ventilated room, LEV)	28,333
[16]	Ag (20-50)	Cleaning reactor with a brush (< 1 g).	0	3.33 (NF, Medium sized ventilated room, LEV)	0
[16]	Co (20-50)	Cleaning reactor with a brush (< 1 g).	1,900	3.33 (NF, Medium sized ventilated room, LEV)	6,333
[16]	Manganese (20-50)	Cleaning reactor with a brush (< 1 g).	100	3.33 (NF, Medium sized ventilated room, LEV)	333
[16]	Ag (20-50)	Cleaning reactor with a brush (< 1 g).	6,100	1 (NF, Medium sized ventilated room)	6,100
[16]	Co (20-50)	Cleaning reactor with a brush (< 1 g).	12,900	1 (NF, Medium sized ventilated room)	12,900

[16]	Manganese (20-50)	Cleaning reactor with a brush (< 1 g).	16,900	1 (NF, Medium sized ventilated room)	16,900
[16]	Al (50-80)	Cleaning reactor with a brush (< 1 g).	6,700	33.33 (NF, walk in booth)	223,333
[16]	Al (50-80)	Cleaning reactor with a brush (< 1 g).	10,450	33.33 (NF, walk in booth)	348,333
[16]	Al (50-80)	Cleaning reactor with a brush (< 1 g).	15,580	33.33(NF, walk in booth)	519,333
[9]	CNM -CNT (-)	Cleaning reactor with by scraping (< 1 g).	0	1 (NF, Medium sized ventilated room)	0
[9]	CNM -CNT (-)	Cleaning with compressed air (<1 g).	5,000	1 (NF, Medium sized ventilated room)	5,000
[6]	SiC (30)	Cleaning with compressed air (1 - 100 g).	0	10 (NF, enclosure)	0
[6]	SiC (30)	Cleaning with compressed air (1 - 100 g).	226,000	1 (NF, Medium sized ventilated room)	226,000
[7]	Si (60)	Cleaning reactor with a wet duster (<1g).	3,000	1 (NF, Medium sized ventilated room)	3,000
[17]	Silica (-)	Cleaning reactor by sanding (1 - 100 g).	20,000	33.33 (NF, cleanroom)	666,667
[17]	Ti, Cr, Au, Pt (-)	Cleaning reactor by sanding (1 - 100 g).	20,000	33.33 (NF, cleanroom)	666,667
[17]	Si (-)	Cleaning reactor with a dry duster (<1g).	0	1 (NF, Medium sized ventilated room)	0
[17]	Co (5)	Cleaning reactor with a dry duster (<1g).	4,000	1 (NF, Medium sized ventilated room)	4,000
[17]	Ga	Cleaning reactor with a dry duster (1 - 100 g).	0	33.33 (NF, cleanroom)	0
[17]	Pt (-)	Cleaning reactor with a wet duster (<1g).	0	1 (NF, Medium sized ventilated room)	0
[17]	Si (-)	Cleaning reactor with a wet duster (<1g).	0	1 (NF, Medium sized ventilated room)	0
[17]	SnCl4 and NH4F (-)	Cleaning reactor with a wet duster (<1g).	200	1 (NF, Medium sized ventilated room)	200
[17]	Ge, Pt, Co, and Cu (-)	Cleaning reactor with a wet duster (1 - 100 g).	0	33.33 (NF, Medium sized ventilated room)	0

[17]	Ti, Cr, Au, Pt (-)	Cleaning reactor with a wet duster (1 - 100 g).	0	33.33 (NF, Medium sized ventilated room)	0
[17]	Ag (-)	Cleaning reactor with by scraping (< 1 g).	0	33.33 (NF, Medium sized ventilated room)	0
[17]	Al (-)	Cleaning reactor with by scraping (< 1 g).	0	33.33 (NF, Medium sized ventilated room)	0
[17]	Au (-)	Cleaning reactor with by scraping (< 1 g).	0	33.33 (NF, Medium sized ventilated room)	0
[17]	Zn (-)	Cleaning reactor with by scraping (< 1 g).	0	1 (NF, Medium sized ventilated room)	0
[17]	Ga (-)	Cleaning reactor with by scraping (1 - 100 g).	0	33.33 (NF, Medium sized ventilated room)	0
[17]	As (-)	Cleaning reactor with sourcing pad(1 - 100 g).	2,500	33.33 (NF, Medium sized ventilated room)	83,333
[17]	Si (-)	Cleaning with compressed air (<1 g).	0	1 (NF, Medium sized ventilated room)	0
Dumping					
[8]	TiO2 (-)	Dumping powder (> 1kg)	12,638	3.33 (NF, LEV)	42,127
[8]	Graphene (20)	Dumping powder (> 1kg)	560	33.33 (NF, fumehood)	18,667
[8]	TiO2 (50)	Dumping powder (> 1kg)	0	3,.33 (NF, LEV)	0
[8]	Silica (-)	Dumping powder (> 1kg)	51,980	1 (NF)	51,980
[8]	Silica (-)	Dumping powder (> 1kg)	9,092	1 (NF)	9,092
[8]	Zinc Oxide (10)	Dumping powder mechanical (> 1kg)	0	3.33 (NF, LEV)	0
[8]	Zinc Oxide (10)	Bagging (> 1kg)	0	33.33 (NF, containment, LEV)	0
[8]	Zinc Oxide (10)	Dumping powder manually (> 1kg)	6,551	3.33 (NF, LEV)	21,837
[18]	CNM – CNF (-)	Dumping powder (> 1kg)	800,000	1 (NF)	800,000
[18]	CNM –CNF (-)	Dumping powder (> 1kg)	800,000	1 (NF)	800,000
[19]	TiO2 (-)	Dumping powder (> 1kg)	11,500	1 (NF)	11,500
[16]	TiO2 (40)	Dumping powder (> 1kg)	3,500	3.33 (NF, LEV)	11,667

[20]	CNM – MWCNT (9.5)	Dumping powder (> 1kg)	0	10 (NF, Enclosure)	0
[21]	CaCO3 (94)	Dumping powder (> 1kg)	3,800	33.33 (NF, Enclosure, LEV)	126,667
[21]	Carbon black (32)	Dumping powder (> 1kg)	180,000	33.33 (NF, Enclosure, LEV)	6,000,000
[21]	Silica (15)	Dumping powder (> 1kg)	19,000	10 (NF, Enclosure)	190,000
[22]	Al (27-56)	Dumping powder (1 - 100 g)	0	33.33 (NF, Enclosure, LEV)	0
[22]	Nanoclay (-)	Dumping powder (1 - 100 g)	0	33.33 (NF, Enclosure, LEV)	0
[22]	Nanoclay (-)	Dumping powder (1 - 100 g)	100	10 (NF, Enclosure)	1,000
[22]	Al (27-56)	Dumping powder (1 - 100 g)	17,280	3.33 (NF, LEV)	57,600
[22]	Nanoclay (-)	Dumping powder (1 - 100 g)	130,980	1 (NF)	130,980
[23]	Carbon black (10 – 100)	Dumping powder (> 1kg)	22,240	1 (NF)	22,240
[7]	Si (60)	Dumping powder (> 1kg)	0	10 (NF, Enclosure)	0
Harvesting					
[8]	CNT (-)	Harvesting (< 1 g)	300	33.33 (NF, fumehood)	10,000
[13]	CNM –CNT (8)	Harvesting (1 - 100 g)	0	1 (NF)	0
[12]	CNM – MWCNT (15)	Harvesting (1 - 100 g)	0	1,000 (NF, glove box)	0
[12]	CNM – MWCNT (15)	Harvesting (1 - 100 g)	4,200	1,000 (NF, glove box)	4,200,000
[12]	CNM – SWCNT (1.1)	Harvesting (1 - 100 g)	100	1,000 (NF, glove box)	100,000
[12]	CNM – SWCNT (1.1)	Harvesting (1 - 100 g)	4,200	1,000 (NF, glove box)	4,200,000
[12]	CNM – MWCNT (15)	Harvesting (1 - 100 g)	28,900	1 (NF)	28,900
[12]	CNM – MWCNT (15)	Harvesting (1 - 100 g)	5,800	1 (NF)	5,800
[12]	CNM – DWCNTs (15)	Harvesting (1 - 100 g)	28,900	1 (NF)	28,900

[5]	TiO2 (-)	Harvesting (1 - 100 g)	10,000	33.33 (NF, fumehood)	333,333
[20]	CNM – MWCNT (9.5)	Harvesting (< 1 g)	644	1 (NF)	644
[9]	CNM –CNT (-)	Harvesting (< 1 g)	0	1.000 (NF, glove box)	0
[9]	CNM –CNT (-)	Harvesting (< 1 g)	4,400	1 (NF)	4,400
[9]	CNM –CNT (-)	Harvesting (< 1 g)	0	1 (NF)	0
[24]	CNM (-)	Harvesting (< 1 g)	1,167	33.33 (NF, fumehood)	38,900
[24]	CNM (-)	Harvesting (< 1 g)	0	33.33 (NF, fumehood)	0
[24]	CNM (-)	Harvesting (< 1 g)	0	33.33 (NF, fumehood)	0
[24]	CNM (-)	Harvesting (< 1 g)	41,717	1 (NF)	41,717
Mixing					
[12]	CNM - MWCNT (12)	Mixing powder in a liquid (100 - 1000 g)	0	1 (NF)	0
[25]	CNM – Fullerenes (-)	Mixing powder in a liquid (> 1 kg)	0	3.33 (NF, LEV)	0
[26]	CNM –CNF (-)	Mixing powder in a liquid (< 1 g)	0	3.33 (NF, LEV)	0
[27]	ZnO (30)	Mixing powder in a powder (1 - 100 g)	8,462	1 (NF)	8,462
[22]	Al (27-56)	Mixing powder in a liquid (1 - 100 g)	0	33.33 (NF, Enclosure, LEV)	0
[22]	Nanoclay (-)	Mixing powder in a liquid (1 - 100 g)	0	33.33 (NF, Enclosure, LEV)	0
[22]	Nanoclay (-)	Mixing powder in a liquid (1 - 100 g)	0	10 (NF, full enclosure)	0
[22]	Al (27-56)	Mixing powder in a liquid (1 - 100 g)	6,060	3.33 (NF, LEV)	20,200
[22]	Nanoclay (-)	Mixing powder in a liquid (1 - 100 g)	12,390	1 (NF)	12,390
Transferring					
[8]	Carbon black (30)	Handling heavy contaminated objects (> 1kg)	37,673	3.33 (NF, LEV)	125,577
[8]	Zinc oxide (30)	Handling heavy contaminated objects (> 1kg)	0	3.33 (NF, LEV)	0
[8]	Silica (16)	Handling heavy contaminated objects (> 1kg)	4,426	3.33 (NF, LEV)	14,753

8	Carbon black (30)	Handling heavy contaminated objects (> 1kg)	76,133	3.33 (NF, LEV)	253,777
8	Carbon black (-)	Handling heavy contaminated objects (> 1kg)	8,420	1 (NF)	8,420
8	CNM –CNT (-)	Scooping powder (< 1g)	477	33.33 (NF, fumehood)	15,900
8	Silica (70)	Scooping powder (< 1g)	0	1 (NF)	0
8	CaCO3 (40-50)	Scooping powder (< 1g)	0	1 (NF)	0
8	Silica (70)	Scooping powder (< 1g)	0	1 (NF)	0
8	Al2O3 (13)	Vaccuum transfer (> 1 kg)	99,583	3.33 (NF, LEV)	331,943
13	CNM –CNT (8)	Scooping powder (< 1g)	0	1 (NF)	0
28	CNM –CNT (10-50)	Scooping powder (< 1g)	166	1 (NF)	166
12	CNM – MWCNT (2,5)	Scooping powder (1 - 100 g)	510	1000 (NF, glove box)	510,000
12	CNM – CNF (140)	Scooping powder (100 - 1000 g)	5	10 (NF, enclosure)	50
29	TiO2 (anatase) (15)	Scooping powder (1 - 100 g)	194,000	1 (NF)	194,000
25	CNM – Fullerenes (-)	Vaccuum transfer (> 1 kg)	0	1 (NF)	0
30	Carbon black (15)	Scooping powder (1 - 100 g)	0	1 (NF)	0
30	CNM - MWCNT (Functionalized) (20-30)	Scooping powder (1 - 100 g)	676	1 (NF)	676
30	CNM - MWCNT (Raw) (10-20)	Scooping powder (1 - 100 g)	1,576	1 (NF)	1,576
30	CNM – Fullerenes (-)	Scooping powder (1 - 100 g)	1,476	1 (NF)	1,476
26	CNM –CNF (-)	Scooping powder (< 1g)	0	3.33 (NF, LEV)	0
16	CNM - MWCNT (Raw) (20)	Scooping powder (< 1g)	1,480	1 (NF)	1,480
16	CNM - MWCNT (Raw) (20)	Scooping powder (< 1g)	1,580	1 (NF)	1,580

[16]	CNM -MWCNT (functionalized) (20)	Scooping powder (< 1g)	680	1 (NF)	680
[16]	TiO2, CuO, Ag (6-40, 60, 15)	Scooping powder (< 1g)	1,020	1 (NF)	1,020
[16]	TiO2, CuO, Ag (6-40, 60, 15)	Scooping powder (< 1g)	1,680	1 (NF)	1,680
[31]	CNM –CNF (-)	Scooping powder (< 1g)	0	33.33 (NF, fumehood)	0
[31]	CNM –CNF (-)	Scooping powder (< 1g)	1,697	1 (NF)	1,697
[31]	CNM –CNF (-)	Scooping powder (< 1g)	1,476	1 (NF)	1,476
[27]	ITO - Indium tin oxide (40-50)	Scooping powder (1 - 100 g)	9,198	1 (NF)	9,198
[27]	ITO - Indium tin oxide (40-50)	Scooping powder (1 - 100 g)	10,050	1 (NF)	10,050
[9]	CNM –CNT (-)	Scooping powder (< 1g)	0	1000 (NF, glovebox)	0
[9]	CNM –CNT (-)	Scooping powder (< 1g)	2,000	1 (NF)	2,000
[9]	CNM –CNT (-)	Scooping powder (< 1g)	0	1 (NF)	0
[32]	Al (27-56)	Scooping powder (1 - 100 g)	1,400	33.33 (NF, fumehood)	46,667
[33]	Al (27-56)	Scooping powder (1 - 100 g)	2,752	33.33 (NF, fumehood)	91,733
[33]	Al (27-56)	Scooping powder (1 - 100 g)	3,093	33.33 (NF, fumehood)	103,100
[33]	Al (27-56)	Scooping powder (1 - 100 g)	1,575	33.33 (NF, fumehood)	52,500
[33]	Al (27-56)	Scooping powder (1 - 100 g)	2,447	33.33 (NF, fumehood)	81,567
[33]	Al (27-56)	Scooping powder (1 - 100 g)	3,124	33.33 (NF, fumehood)	104,133
[33]	Al (27-56)	Scooping powder (1 - 100 g)	1,698	33.33 (NF, fumehood)	56,600
[33]	Al (27-56)	Scooping powder (1 - 100 g)	12,014	3.33 (NF, LEV)	40,047
[33]	Al (27-56)	Scooping powder (1 - 100 g)	13,260	3.33 (NF, LEV)	44,200

Table SI1-3: Exposure levels measured during handling powder intermediates / ready-to-use products (source domain 3a)

Information source	Nanoproduct (primary particle diameter nm)	Weight percentage ENP in nanoproduct	Circumstances	Individual reported exposure levels
[34]	MWCNT (-)	Unknown	Mixing (1 – 100 g), CNT research facility, blending to formulate composites,	194 tubes
[34]	MWCNT (-)	Unknown	Mixing (1 – 100 g), CNT research facility, blending to formulate composites, With the process inside an encapsulation,	0.018 tubes
[35]	CNT (-)	15	Mixing (1 – 100 g), mixing of 15 w% CNT with a melt polymer in a clean room	0
[36]	Silica (-)	Unknown	Mixing (> 1kg), Mixing of 25 kg NanoCrete,	178,745
[36]	Silica (-)	Unknown	Mixing (> 1kg), Mixing of 25 kg NanoCrete with bad weather conditions,	5,139
[35]	CNT (-)	3	Scooping (1 – 100 g), weighing operation under a with and without ventilated hood 3,	0
[8]	CeO2 (20)	Unknown	Melt blending (1 – 100 g), melt blending (extrusion with polymer)	804
[8]	Graphene (20)	Unknown	Melt blending (1 – 100 g), melt blending (extrusion with polymer)	558
[8]	Graphene (20)	Unknown	Melt blending (1 – 100 g), melt blending (extrusion with polymer)	0
[33]	Al (27-56)	7	Dumping (100 – 1000 g), feeding into a twin screw extruder, Each trial used 2.3 kg of polymer pellets and 0.16 kg of nanoalumina particles,	280,000
[37]	Metals including iron, zinc, titania, chromium, nickel and manganese	2- 8 wt%	Commercial photocopy centre ID 1, weekly GM	13,633
[37]	Metals including iron, zinc, titania, chromium, nickel and manganese	2- 8 wt%	Commercial photocopy centre ID 2, weekly GM	12,966

[37]	Metals including iron, zinc, titania, chromium, nickel and manganese	2- 8 wt%	Commercial photocopy centre ID 3, weekly GM	9,486
[37]	Metals including iron, zinc, titania, chromium, nickel and manganese	2- 8 wt%	Commercial photocopy centre ID 4, weekly GM	11,435
[37]	Metals including iron, zinc, titania, chromium, nickel and manganese	2- 8 wt%	Commercial photocopy centre ID 5, weekly GM	6,278
[37]	Metals including iron, zinc, titania, chromium, nickel and manganese	2- 8 wt%	Commercial photocopy centre ID 6, weekly GM	3,670
[37]	Metals including iron, zinc, titania, chromium, nickel and manganese	2- 8 wt%	Commercial photocopy centre ID 7, weekly GM	4,376
[37]	Metals including iron, zinc, titania, chromium, nickel and manganese	2- 8 wt%	Commercial photocopy centre ID 8, weekly GM	33,743

Table SI1-4: Exposure levels measured during handling liquid intermediates/products (source domain 3b)

Information source	Nanoproduct (primary particle diameter nm)	Amount ENP in nanoproduct	Circumstances	Individual reported exposure levels
Propellant gas				
[38]	SiO_2 (-)	n.a.	Consumer sprays in a small experimental room	28,000
[38]	TiO_2 (-)	n.a.	Consumer sprays in a small experimental room	130,000
[39]	Ag (-)	1040 mg/L	Consumer sprays in a small experimental room	8,000
[40]	Ag (-)	6.8 mg/kg	Consumer sprays in a small experimental room	620,000
[40]	ZnO (-)	584 mg/kg	Consumer sprays in a small experimental room	76,000
[41]	n,a, (-)	n.a.	Consumer sprays in a small experimental room	21,000
[41]	n,a, (-)	n.a.	Consumer sprays in a small experimental room	41
(High) pressure				
[8]	SiO2 (50)	n.a.	Occupational setting	144,324
[8]	SiO2 (20–50)	n.a.	Occupational setting	796,296
[8]	SiO2 (-)	n.a.	Surface protection coating, activity took place outside,	1,841
[8]	SiO2 (50–100)	n.a.	Surface protection coating, activity took place outside,	2,258
[36]	TiO_2 (-)	n.a.	Occupational setting, activity took place outside,	12,219
[29]	TiO_2 (-)	2%	Spray in a small experimental room	51,900,000
[29]	TiO_2 (-)	2%	Spray in a small experimental room	178,000,000
Pump				
[39]	Ag (-)	1040 mg/L	Consumer sprays in a small experimental room	0
[40]	Ag (-)	9.1 mg/kg	Consumer sprays in a small experimental room	0
[41]	Silane (-)	n.a.	Consumer sprays in a small experimental room, Measurement range 6-523 nm	350
[41]	Silane (-)	n.a.	Consumer sprays in a small experimental room, Measurement range 0.5-19.8 µm	16

[41]	TiO_2 (-)	n.a.	Consumer sprays in a small experimental room, Measurement range 6-523 nm	280
[41]	TiO_2 (-)	n.a.	Consumer sprays in a small experimental room, Measurement range 0.5-19.8 µm	32
[41]	TiO_2 (-)	n.a.	Consumer sprays in a small experimental room, Measurement range 6-523 nm	300
[41]	TiO_2 (-)	n.a.	Consumer sprays in a small experimental room, Measurement range 0.5-19.8 µm	13
Sonication				
[42]	Fullerenes (-)	100 mg/L	Sonication deionized water	2,176
[42]	Raw MWCNT (-)	100 mg/L	Sonication water with natural organic matter	2,776
[42]	Functionalized MWCNT (-)	100 mg/L	Sonication water with natural organic matter	726
[42]	Carbon black (-)	100 mg/L	Sonication in deionized water	1,057
[11]	CNT (-)	n.a.	Occupational setting	5,840
[16]	Raw MWCNT (-)	n.a.	Occupational setting	2,500
[16]	Functionalized MWCNT (-)	n.a.	Occupational setting	730
[8]	CNT (-)	n.a.	Occupational setting, academic and research	0
[8]	CeO2 (50–105)	n.a.	Occupational setting, academic and research	0
[8]	Ag (75)	n.a.	Occupational setting, academic and research	0
Brushing/rolling				
[8]	SiO2 (-)	n.a.	Occupational setting, brushing and rolling: Surface protection/ coating	0
[8]	SiO2 (-)	n.a.	Occupational setting, brushing and rolling: Surface protection/ coating	0
[8]	SiO2 (-)	n.a.	Occupational setting, brushing and rolling: Surface protection/ coating	0

Table SI1-5: Exposure levels measured during handling of nano articles and fracturing and abrasion of nanoparticles-embedded end products (source domain 4),

Information source	Nanoproduct (primary particle diameter nm)	Circumstances	Amount ENP in nanoproduct	Overall reported emission levels nanoparticles (including NOAA)	Individual reported NOAA emission levels
[8]	CNT (-)	Cleaving of wafers containing CNT	-	566	-
[8]	Unknown (-)	Sanding nanospecific coating	-	3,729	-
[8]	CNT (-)	Sanding stainless steel	-	428	
[8]	Silica (-)	Sawing of nano-coated synthetic material	-	2,895	
[43]	CNT (-)	Dry cutting – CNT – alumina	2 wt%	33,180	0
[43]	CNT (-)	Dry cutting – CNT - carbon	0,03 wt%	289,180	0
[44]	CNT (-)	Dry drilling - CNT – alumina – High speed	2 wt%	175,800	-
[44]	CNT (-)	Dry drilling - CNT – alumina – Low speed	2 wt%	80,800	-
[44]	CNT (-)	Wet drilling - CNT – alumina – High speed	2 wt%	4,500	-
[44]	CNT (-)	Dry drilling - CNT – carbon – High speed	0.03 wt%	79,800	-
[36]	Silica (-)	Drilling – outdoor up-wind Nanocrete	-	21,940	-
[36]	Silica (-)	Drilling – outdoor down-wind Nanocrete	-	63,376	-
[28]	CNT (-)	Sanding (Manual) - epoxy samples (integrated)	2 wt%	3,889	
[35]	CNT (-)	grinding - polymer nanocomposites	-	3,000	-
[45]	Zinc oxide (-)	sanding - polyurethane coating	-	250,000,000	70,000,000
[45]	Zinc oxide (-)	sanding - architectural coating	-	2,100,000	400,000
[45]	Fe2O3 (-)	sanding - architectural coating	-	2,100,000	400,000

[46]	TiO2 (-)	abrasion (wet/dry) - coatings different surfaces (glass, PVC)	-		170
[47]	CNT (-)	Abrasion PA6	4 wt%	4,000	3,975 -
[47]	CNT (-)	Abrasion polycarbonate	4 wt%	4,000	3,930
[47]	SiO2 (-)	Abrasion paint with and without nano-SiO2	0.8 wt%	3,000	2,000
[14]	Al (-)	grinding	100 wt%	29,791	29,791
[48]	CNT (-)	Weathering - composites	-		In order of mg/m2/year
[49]	CNT (-)	sanding - epoxy resin test sticks, medium sanding speed and medium sandpaper roughness	4 wt%	455	94
[49]	CNT (-)	sanding - epoxy resin test sticks, medium sanding speed and medium sandpaper roughness	3 wt%	340	0
[49]	CNT (-)	sanding - epoxy resin test sticks, medium sanding speed and medium sandpaper roughness	2 wt%	332	0
[49]	CNT (-)	sanding - epoxy resin test sticks, medium sanding speed and medium sandpaper roughness	1 wt%	200	0
[49]	CNT (-)	sanding - epoxy resin test sticks, fast sanding speed and medium sandpaper roughness	2 wt%	3,140	-
[49]	CNT (-)	sanding - epoxy resin test sticks, slow sanding speed and sandpaper roughness	2 wt%	92	-
[49]	CNT (-)	sanding - epoxy resin test sticks, medium sanding speed and coarse sandpaper roughness	2 wt%	4,670	-
[49]	CNT (-)	sanding - epoxy resin test sticks, medium sanding speed and fine sandpaper roughness	2 wt%	214	-

[50]	W2730X (< 100)	sanding - surface coating	10 wt%	416,000	336,000
[50]	CB (95)	sanding - surface coating	2.5 wt%	118,000	16,000
[50]	SiO2 (7)	sanding - surface coating	10 wt%	145,000	-
[50]	CaCO3 (-)	sanding - surface coating	-	329,000	-
[50]	SiO2 (50)	sanding - surface coating	5 wt%	374,000	0
[51]	TiO2 (17)	sanding - surface coating	-	10,300	0
[51]	carbon black (95)	sanding - surface coating	-	23,800	5,700
[31]	CNF (-)	Wet saw cutting	-	1,934	-
[31]	CNF (-)	Surface grinding	-	491,599	-
[31]	CNF (-)	Belt sanding	-	0	-
[31]	CNF (-)	Hand sanding	-	0	-
[52]	Nanoclay (-)	shredded for recycling - nanocomposites	5 wt%	32,483	0
[53]	Silica (-)	Abrasion	-	3,200	600
[54]	CNT (-)	abrasion - epoxy based nanocomposite	-	14,000	0
[55]	CNT (-)	weaving of MWCNT-coated yarn	-	5,000	0
[56]	Zinc oxide (-)	wear – polyurethane coating - Fiberboard plate	-	< 4.29	0
[56]	Zinc oxide (-)	wear – polyurethane coating - Steelpanel		< 4.29	0
[56]	Zinc oxide (-)	wear – UV curableclearcoat - Fiberboard plate		< 4.29	0
[56]	Zinc oxide (-)	wear – White pigmented architectural coating – Fiber cement plate		< 4.29	0
[57]	SiO2	Abriasion - polyamide	4 wt%	4,700	1,000
[57]	CNT	Abrasion - polyoxymethylene	2 wt%	5,800	300
[57]	CNT	Abrasion - Cement	2 wt%	3,900	0

[57]	**Calcium silicate hydrates (-)**	**Abrasion - Cement**	**2 wt%**	**6,700**	**0**
[58]	CNT (-)	Sanding - thermoplastic polyurethane	-	25,000	0
[58]	CNT (-)	Abrasion - thermoplastic polyurethane	-	550	-
[58]	CNT (-)	Wearing - thermoplastic polyurethane	-	-	-
[59]	Glass fibers (-), Carbon Fibers (-)	Grinding – sanding grit AG36	35 wt%, 65 wt%	6,900	-
[59]	Glass fibers (-), Carbon Fibers (-)	Grinding – sanding grit AG60	35 wt%, 65 wt%	39,000	-
[59]	Glass fibers (-), Carbon Fibers (-)	Grinding – sanding grit ROS40	35 wt%, 65 wt%	0	-
[59]	Glass fibers (-), Carbon Fibers (-)	Grinding – sanding grit ROS120	35 wt%, 65 wt%	640	-
[59]	Glass fibers (-)	Grinding	70 wt%	1,700,000	-

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Supporting information S2

Introduction Stoffenmanager Nano 1.0

Stoffenmanager Nano (version 1.0) is a precautionary risk-banding tool to prioritize health risks occurring as a result of exposure to manufactured nano objects (MNOs) for a broad range of worker scenarios.[1, 2] In order to prioritize the health risks, the Stoffenmanager Nano combines the available hazard information of a substance with a qualitative estimate of potential for inhalation exposure.

The inhalation exposure algorithm of Stoffenmanager Nano is based on Schneider et al. (2011) [3] and applies a source-receptor approach (immission, transmission and emission). The model describes a stepwise transfer of a NOAA from the source via the various transmission compartments to the receptor.

The emission model includes activity emission potential and substance emission potential as the variables. Transmission is also considered and this includes the dispersion/dilution (near-field/far-field), surface deposition (indirectly), re-suspension (surface contamination), localized controls in the modeling. Immission by the worker is addressed, e.g. whether a protective mask is used or a personal enclosure.

The relative exposure score underlying the exposure bands within Stoffenmanager Nano are derived by multiplication of relative multipliers (on a logarithmic scale) for the various model inputs, based on the available information.

The normalization procedure

In Table SI2-1, SI2-2 and SI2-3 the different variables we used for correction are included with corresponding multipliers.

Table SI2-1: Multipliers for localized controls (published in Van Duuren-Stuurman et al. 2012, table 11)

Category	Examples	Multiplier
No control measures at the source		1
Use of a product that limits the emission	Wetting a powder, spraying of water	0.3
Local exhaust ventilation	Removal of air at the source of the emission; the dangerous substances are captured by an air stream leading them into a hood and duct system	0.3
Containment of the source	The source is fully contained; however, no local exhaust ventilation is used within the containment	0.3
Containment of the source with local exhaust ventilation	Containment of the source in combination with local exhaust ventilation, e.g. a fume cupboard	0.03
Glove boxes/bags	Any form of permanent encapsulation or encasing of the source (which are not opened during the given activity)with a well-designed local exhaust ventilation system	0.001

Table SI2-2: Multipliers for ventilation type and room size, near-field and far-field (published in Van Duuren-Stuurman et al. 2012, table 12)

Room size (volume)	No general ventilation (0.3–1 ACH)	Mechanical and/or natural ventilation (3 ACH)	Spraying booth (>10 ACH)
A			
Volume < 100 m^3	10	3	0.1
Volume 100 -1000 m^3	3	1	0.3
Volume > 1000 m^3	1	1	1
Work performed outside	-	1	-
B			
Volume < 100 m^3	10	3	-
Volume 100 -1000 m^3	1	0.3	-
Volume > 1000 m^3	0.3	0.1	-
Work performed outside	-	0.1	-

ACH = air changes per hour

Table SI2-3: Multipliers for separation (published in Van Duuren-Stuurman et al. 2012, table 13)

Category	Examples	Multiplier
The worker does not work in a cabin		1
The worker works in a cabin without specific ventilation system	A cabin of a tractor or truck, a cabin not equipped with filters, overpressure system, etc., or behind a screen	0.1
The worker works in a separated (control) room with independent clean air supply	The workplace of the worker is in a (control) room that is equipped with an air supply system independent of the air in the room where the source is	0.03

Example 1: Peer reviewed literature reported about a concentration of 4000 particles/cm^3 during the weighing of TiO_2, using local exhaust ventilation in a large room (>1000m^3) with the worker near field to the source. Corrected exposure we used in the analyses is 4000 particles/cm^3 x (1/0.3) = 13333 particles/cm^3. 0.3 is the multiplier for local exhaust ventilation. The other variables already correspond to the multiplier level of 1.

Example 2: Peer reviewed literature reported about a concentration of 10000 particles/cm^3 during the dumping of ZnO, using no control measured at the source, with the activity performed in a very small room < 100 m^3 with no general ventilation with the worker in a cabin without specific ventilation system. Corrected exposure we used in the analyses is 10000 particles/cm^3 x (1/10) x (1/0.1) = 10000 particles/cm^3. 10is the multiplier for a small room and no general ventilation. 0.1 is the multiplier for working in a cabin without specific ventilation. The local exhaust ventilation variable already correspond to the multiplier level of 1.

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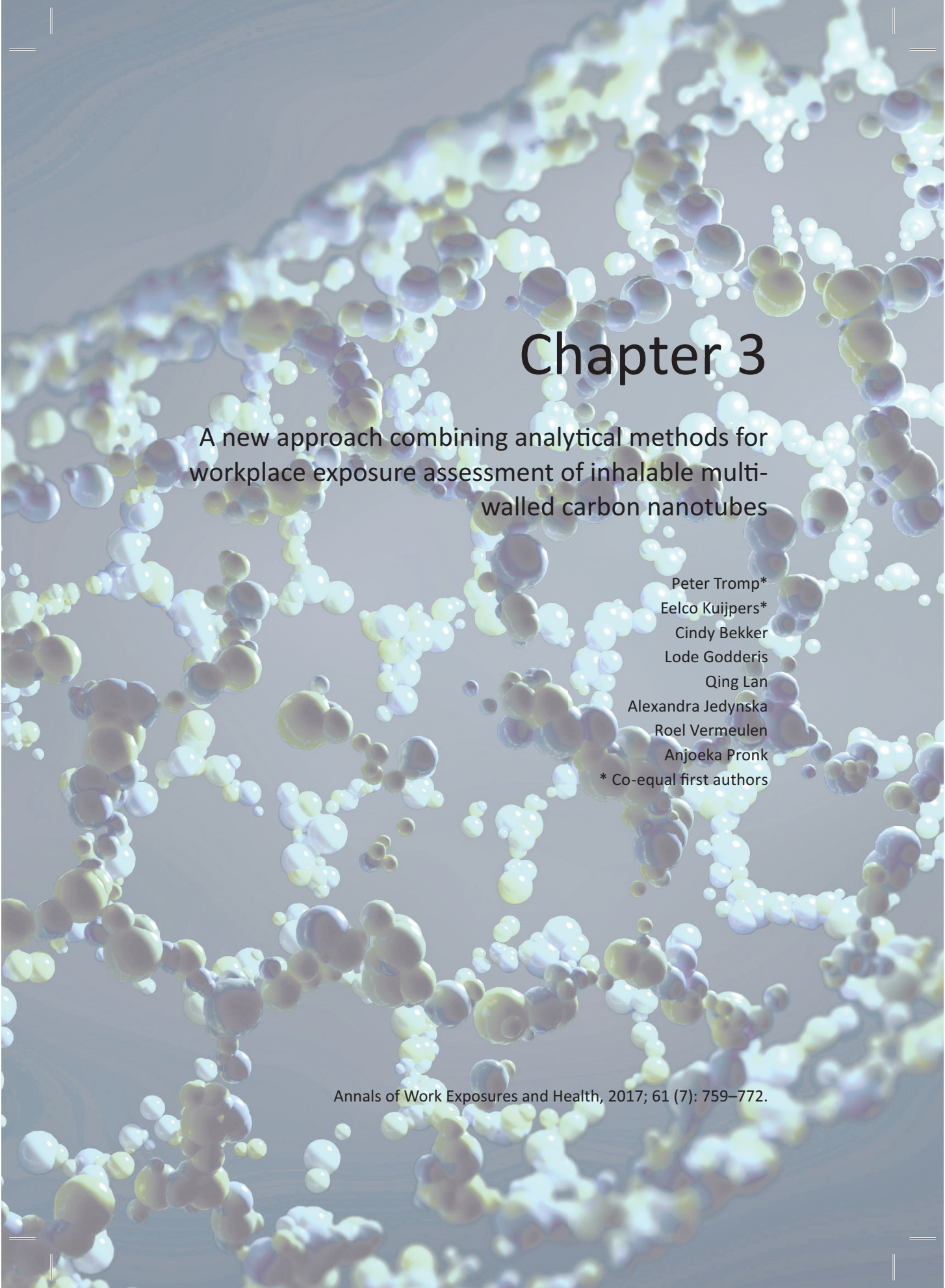

Chapter 3

A new approach combining analytical methods for workplace exposure assessment of inhalable multi-walled carbon nanotubes

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Abstract

To date there is no consensus about the most appropriate analytical method for measuring carbon nanotubes (CNTs), hampering the assessment and limiting the comparison of data. The goal of this study is to develop an approach for the assessment of the level and nature of inhalable multi-wall CNTs (MWCNTs) in an actual workplace setting by optimizing and evaluating existing analytical methods.

In a company commercially producing MWCNTs, personal breathing zone samples were collected for the inhalable size fraction with IOM samplers; which were analyzed with carbon analysis, inductively coupled plasma mass spectrometry (ICP-MS) and scanning electron microscopy / energy dispersive X-ray spectroscopy (SEM/EDX). Analytical methods were optimized for carbon analysis and SEM/EDX. More specifically, methods were applied and evaluated for background correction using carbon analyses and SEM/EDX, CNT structure count with SEM/EDX and subsequent mass conversion based on both carbon analyses and SEM/EDX.

A moderate to high concordance correlation coefficient (R_c) between carbon analyses and SEM/EDX was observed (R_c=0.81, 95% CI 0.59-0.92) with an absolute mean difference of 59 µg/m^3. A low R_C between carbon analyses and ICP-MS (R_C=0.41, 95% CI 0.07-0.67) with an absolute mean difference of 570 µg/m^3 was observed. The large absolute difference between EC and metals is due to the presence of non-embedded inhalable catalyst particles, as a result of which MWCNT concentrations were overestimated. Combining carbon analysis and SEM/EDX is the most suitable for quantitative exposure assessment of MWCNTs in an actual workplace situation.

Introduction

Carbon nanotubes (CNTs) are nanoscale cylinders of carbon (essentially consisting of 'rolled' sheets of graphene) with very large aspect ratios.[1, 2] The production of CNTs has increased greatly in the last decade due to the development of a wide range of CNT-based applications in a multitude of products, like batteries and fuel cells, packaging material, electronics and pharmaceutical composites.[3, 4] There is a growing body of toxicological research indicating a potential health risk of CNTs.[5-11] Especially certain types of multi-walled carbon nanotubes (MWCNTs) may have the potential for pulmonary toxicity due to their morphological similarity to asbestos.[8, 12-14] Recently, a working group of the International Agency for Research on Cancer (IARC) concluded that one of the rigid MWCNT, MWCNT-7 is possibly carcinogenic for humans (group 2B).[15] As a result, there is a need to assess (occupational) exposure to MWCNTs in order to monitor and minimize exposure levels.

In 2012, Brouwer et al. concluded there was no consensus about the most appropriate measuring method and exposure metric (e.g. number counts, mass, surface area) to investigate occupational exposure to CNTs.[16] Direct-reading instruments (DRIs) and/or filter-based sampling methods have been used.[1, 16-27] Quantification of CNTs with DRIs is complicated since these instruments are calibrated for spherical particles. Accordingly, several studies have confirmed that DRIs are not suitable to assess exposure to CNTs.[2, 21, 22, 25] For exposure to CNTs, a filter based sampling technique is considered to be the most suitable method.[16, 22] A few potentially more selective (filter based) analytical methods for detection and quantification of CNTs have been used in a workplace environment, based on physical and chemical properties of CNTs, all of which have their own advantages and disadvantages. The three commonly used methods are 1) (scanning) electron microscopy / energy dispersive X-ray spectroscopy (SEM/EDX), 2) carbon analysis and 3) inductive coupled plasma-mass spectrometry (ICP-MS).[20] SEM is a technique for physico-chemical qualitative characterization of CNTs, however objective criteria are lacking for quantitative counting CNTs.[16] In addition, due to heterogeneity in size, shape and composition of CNTs quantification remains difficult and time consuming.[23] Carbon analysis is commonly used for CNTs and is a practical quantification technique based on the elemental carbon content of CNTs but it is not straightforward to discriminate between CNTs, carbonaceous background or other process generated particles with this method.[24] Finally, ICP-MS, is used as a technique to detect embedded metals as proxies for CNTs. CNTs are commonly synthesized by a catalytic process causing low levels of residual catalyst metals embedded in the carbon structure of the tubes.[19]

The goal of this study is to develop an approach for the assessment of the level and nature of inhalable MWCNTs, combining different methods. We therefore optimized and evaluated the three analytical methods SEM/EDX, carbon analysis and ICP-MS for the quantification of inhalable MWCNTs in an actual workplace exposure situation.

Material and Methods

Field survey

This study is part of a study on occupational exposure and potential health effects at a commercial industrial MWCNT production facility. A detailed description of this facility, the activities performed and MWCNT product is given by Kuijpers et al.[28] Exposure measurements were performed during two periods in 2013 at the production facility. 4-8 hour shift-based personal breathing zone (PBZ) samples were collected inside the production area (three and four days in respectively May without any synthesis activities and November during a period of full-scale synthesis), the R&D area (two days in May) and offices (two days in November). In brief, results show comparable personal MWCNTs exposure during both phases in the production area due to relatively high contamination of the workplace. In the R&D facility exposure was lower, mainly due to handling of lower quantities of MWCNTs. In addition,

stationary samples were collected outdoors (5 meters from the facility) for background comparison.

Samples were collected in parallel for scanning electron microscopy / energy dispersive X-ray spectroscopy (SEM/EDX, N=10) and for carbon analysis and ICP-MS analysis on the same filter (N=10), using IOM inhalable dust samplers (SKC Inc., USA). SEM/EDX analysis sampling was performed with nickel coated track-etched polycarbonate filters (0.4µm 25mm, Nuclepore). Due to high air resistance of this filter the flow rate was set at 0.7 L/min (normally operating at 2 L/min). Sampling for the carbon analysis and ICP-MS analysis sampling was performed with pre-heated (2h at 800 °C) quartz fiber filters (QMA 25mm, Whatmann) at a flow rate of 2 L/min.

Analytical methods

SEM/EDX

Automated particle analysis provides many advantages over manual analysis including speed, thoroughness and reliability, but due to the heterogeneity in morphology of MWCNT structures (and agglomerates), their direct identification is complicated. Besides CNTs, particulate matter on these filters consisted of inorganic particulate matter (Fig 1B,C: white dots), organic carbonaceous matter (Figure 1B) and soot (Figure 1A). Automated detection of inorganic particulate matter, organic carbonaceous matter and of total particulate matter was feasible. Automatic detection of soot was not possible. Soot structures were manually counted based on their unique morphological properties: fractal chain-like aggregates of spherical primary particles between 10-50nm (Figure 1A), which makes them easy to distinguish from other types of particles, including MWCNT aggregates (Figure 1C,D,E).

An indirect approach was developed to quantify the MWCNT concentration, by both using automated and manual particle analyses according the following equation:

MWCNT concentration = Total (automated) - inorganic (automated) - organic carbonaceous matter (automated) - soot (manual)

A Tescan MIRA-LMH FEG-SEM microscope was used at an accelerating voltage of 15kV, working distance 10mm, spot size 5nm. The EDX spectrometer was a Bruker AXS spectrometer with a Quantax 800 workstation and a XFlash 4010 detector. The SEM/EDX was equipped with Scandium SIS software package (Olympus Soft Imaging Solutions GmbH, Germany) for automated particle analysis. With this system the polycarbonate filter area was automatically inspected on a field-by-field basis. In 100 randomly selected fields of view, covering the complete filter surface, particles were recognized manually (soot) or by using a pre-selected grayscale video threshold (detection threshold level) to discriminate between a particle and filter background. Particle analysis was performed using the secondary electron

mode (SE-mode) for detection of total particles and soot and backscattered electron mode (BE-mode) for detection of organic carbonaceous particulate matter (Figure 1B: black particle) and inorganic particles (Figure 1B,C: white dots). In the BE-mode, inorganic particles and organic carbonaceous particles were distinguished from MWCNTs (Figure 1C: grey particle), based on the greyscale of the particles.

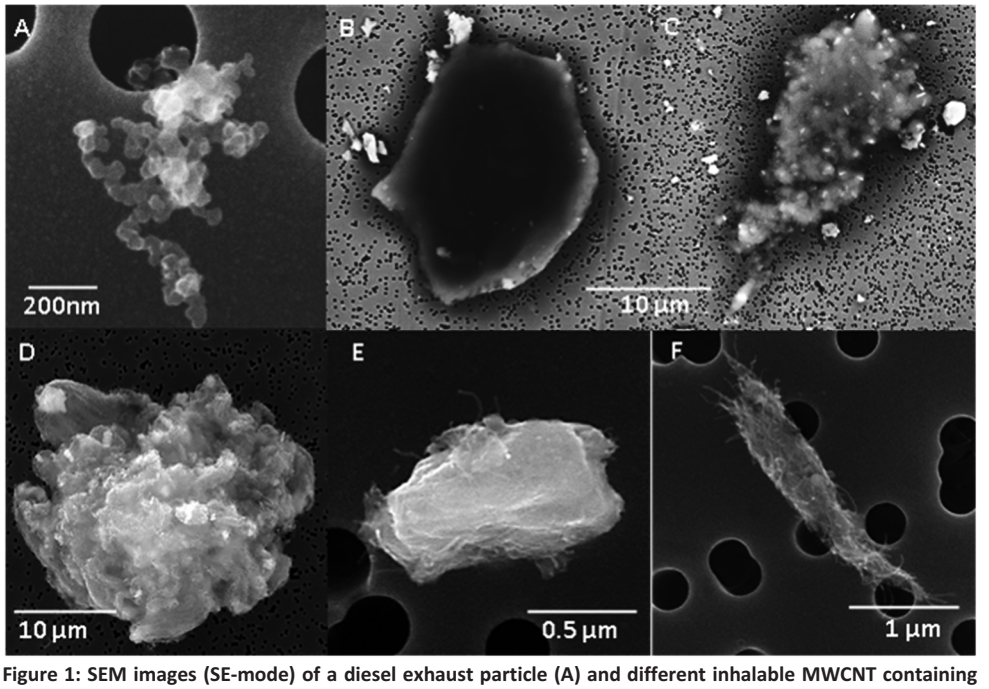


Figure 1: SEM images (SE-mode) of a diesel exhaust particle (A) and different inhalable MWCNT containing structures collected in the production facility: MWCNT hairball (D), MWCNT composite particles (E) and MWCNT bundle (F) and SEM images (BE-mode) of an organic carbonaceous particle (B: black flake), large CNT structure (C: grey irregular shape) and inorganic particles (B, C: white dots).

In addition, to derive mass concentrations and mass size distributions of the different types of particles, for each particle (or cluster of particles) the projected area equivalent diameter (d_{pa}) was measured. Magnifications were chosen so that their measurable size ranges overlap slightly and cover the particle sizes of interest. To analyse diameters between 25 nm and 100 µm, four magnifications were selected, namely 200X, 1.000X, 5.000X and 25.000X. These magnifications cover in total 18 size bins: 25-40, 40-65, 65-100, 100-160, 160-250, 250-400, 400-650, 650-1000nm and 1.0-1.6, 1.6-2.5, 2.5-4.0, 4.0-6.5, 6.5-10, 10-16, 16-25, 25-40, 40-65 and 65-100µm. Soot structures were manually counted at magnifications 5.000X and 25.000X covering the size bins between 25nm and 2.5µm. Per size bin a minimum of 50 particles were detected with in total approximately 5000 particles per filter (including MWCNTs, soot, inorganic and carbonaceous particles). The numerical concentration per particle type and size bin was calculated in accordance with ISO14966.[29] Per size bin the mean

diameter and the standard deviation (95% confidence interval) was calculated on the basis of the Poisson distribution.

Based on the numerical concentration ($N_{1\text{-}18}$) of particles per size bin, mass per size bin (M_p) and total mass concentrations were calculated for all particle types, using the particle density (ρ_p), (calculated) particle size ($d_{pa1\text{-}18}$) and volumetric shape factor (S_v),[30, 31] by applying the following equation:

$$\text{Total mass concentration}_{SEM} = \sum_{i=1}^{18} M_p \ \ with\ Mp = \left(\left(\frac{\pi}{6}\right) \times p_p \times \left(\frac{d_{pa\ 1-18}}{S_v}\right)^3 \right) \times N_{1-18}$$

For carbon-based particles, like soot, MWCNTs and other carbonaceous particulate matter, an average ρ_p of 1.5 g/cm^3 was used. The density was based on literature; for soot and other carbonaceous particles [32-35] and for MWCNTs. [36-38] For inorganic non-carbonaceous articles, an average ρ_p of 3.0 g/cm^3 was calculated based on the chemical composition of the particles, known from EDX-analysis. By introducing the S_v, the particle size of non-spherical particles can be expressed in the three-dimensional equivalent-volume diameter (d_{ev}) instead of the two dimensional d_{pa}.[30] Since little is published about volumetric shape factors of specific (inhalable) particle types, we used an average S_v of 1.5 for all particle types based on published data; for inorganic particles, [30-32] for soot [33-35] and for MWCNTs.[36, 39]

Carbon analysis

The analysis of elemental carbon (EC) and organic carbon (OC) was based on the thermal optical method varying in treatment temperature and atmosphere composition (with Helium and Oxygen) resulting in three OC stages and three EC stages as described in the American Standard Method NIOSH 5040,[40] A modified IMPROVE protocol (specific for MWCNTs) was used for the temperature and atmospheric gas settings.[24] According to this protocol the sum of EC_2 and EC_3 was used as a good quantitative estimate of MWCNTs. Because soot was also present in EC_2, the sum concentration of EC_2 and EC_3 was corrected for background soot levels.

EC/OC method

For the carbon analyses 1 cm^2 from each quartz filter was analysed for elemental (EC) and organic (OC) carbon, using a thermal/optical carbon monitor (Sunset Laboratory Inc., USA). All OC was removed from the filter in the temperature range of 120-550°C in a non-oxidizing carrier gas (Helium). EC was removed in the temperature range of 550-920°C at a mixture of helium and 2% oxygen (2% O_2/He). The resulting CO_2 was converted to methane and detected by flame ionisation detection (FID). Correction for pyrolysis of OC was carried out by measurement of light transmission. EC was categorized into EC_1 (550°C), EC_2 (650°C) and EC_3 (920°C).[24]

The oxidizing temperature of CNTs depends greatly on the type, size, agglomeration state, diameter of the fibers and embedded metal particles it was necessary to validate the heating conditions for the target MWCNTs of interest.[20] Beside the CNT characteristics, the temperature also depends on the filter load of other particulate matter (PM). This is acknowledged in literature [41-43] but the effect has never been quantified. In this study the influence of PM on the oxidation temperature of MWCNTs was determined with pre-loaded filters, using the standard addition technique (for results see Supporting Information 1). The filters were pre-sampled at an urban road site in the vicinity of the MWCNT facility and spiked with known amounts of MWCNTs. The addition of urban dust particulate matter decreases the oxidation temperature of MWCNTs, resulting in a shift to EC_2, but not EC_1. Based on these results the sum of EC_2 and EC_3 were selected as a good quantitative estimate of the MWCNT concentration.

During laboratory validation performance characteristics of the analytical method was determined in accordance with ISO 5725 (for results see Supporting Information 1).[44] For MWCNTs the limit of detection (LOD) was 0.3-0.5 μg/cm^2 corresponding to an inhalable MWCNT concentration of 1.5 μg/m^3 (8hr sampling period with flow rate 2 L/min), the reproducibility was between 7.4% (2.5 μg/cm^2) and 10.6% (25 μg/cm^2) and the recovery was between 85 and 106%.

Background soot correction

Indoor soot detection with carbon analyses based on samples that do not contain MWCNTs was not possible as there was a constant process of synthesis and/or handling of MWCNTs. Normally, a direct (daily) background correction for soot can be applied based on outdoor soot concentrations, assuming a stable proportion of soot outdoors entering the production facility. Depending on the air circulation inside the facility (e.g. ventilation, recirculation) the concentration soot indoors is usually 80% (±15%) of the concentration soot outdoors.[45] However, it is likely that soot is generated inside the production facility, for example from combustion sources of the reactor unit, resulting in incorrect MWCNT concentrations when using a proportion of the outdoor concentrations.

Therefore, a different method was developed to investigate potential indoor soot sources and to estimate the indoor soot concentrations. Concurrent to the sampling inside the production facility using similar measurement equipment, outdoor background samples were collected in parallel on each measurement day/shift combination. These were analyzed for soot by SEM/EDX (manual counting) and carbon analyses (EC_2+EC_3) to validate the mass conversion (μg/m^3) from soot number concentrations (#/m^3) and establish the correlation between SEM/EDX and carbon analysis. Subsequently this correlation was used to calculate the indoor EC background concentrations based on SEM/EDX analyses.

In order to calculate the mass concentration of inhalable MWCNTs corrected for background soot, we used the following equation, which includes the individual exposures (parallel PBZ samples) of EC_2 + EC_3 and soot (SEM/EDX soot):

$$Total\ mass\ concentration_{Corrected} = EC_2 + EC_3 - Soot$$

ICP-MS analysis

During the production process of MWCNTs, transition metals like molybdenum, nickel, cobalt, yttrium and iron are typically used for catalytic growth of the carbon structures. Consequently, residual metal catalyst particles frequently persist within the carbon structure of MWCNTs after manufacturing and generally account for several percent of the particle mass.[19] At this production facility ICP-MS analysis showed low percentages of residual transition metals in bulk MWCNT samples. Although this could be promising, not all metals can be used as selective markers (proxies) for the presence of inhalable MWCNTs because of high background concentrations (e.g. Fe is also presence in natural and anthropogenic sources.[21, 46] Information about the catalyst metals used in the analysis is not reported to protect companies' intellectual properties and is therefore further referred to as metal proxy.

After the carbon analyses, residual parts of the quartz filters were digested with aqua regia (mixture of concentrated hydrochloric acid and nitric acid 3:1) in a Microwave Digestion System (CEM Corporation, USA) and analyzed with high resolution ICP-MS. The ICP-MS used was the Element XR High Resolution Inductively Coupled Plasma Mass Spectrometer (Thermo, Bremen, Germany). All data acquisitions were carried out in high resolution mode, to avoid the influence of spectral interferences on the results. The quantification was carried out by external five-point-calibration. The stock solutions were diluted to relevant concentration levels. In general, metal impurities of quartz filters were low and for the metal proxy ≤ 0.002 µg / filter. Indoor concentrations of the metal proxy (0.02 – 1.0 µg / filter) were corrected for background metal proxy levels outdoors (0.003 – 0.007 µg / filter). Sensitivity of the filter sampling technique in combination with analysis with HR-ICP-MS was approximately 2.5 ng/m^3 for an 8 hour sampling period with a flow rate of 2.0 L/min.

Statistical analysis

Performance characteristics of the three analytical methods were determined in accordance with ISO5725.[44] Accuracy and precision of SEM/EDX (for MWCNTs and EC background) and ICP-MS (for MWCNTs) were determined with the concordance correlation coefficient (R_C) and the arithmetic mean (AM) ratio in comparison with carbon analysis. The concordance correlation coefficient is a modified version of the Pearson correlation coefficient, not only taking into account the linear covariation between two methods but also the degree of correspondence between these methods.[47]

Reproducibility was determined with the corresponding standard deviation (SD) of the AM ratios. In addition, the uncertainty of the counting method with SEM/EDX and the subsequent mass conversion was determined, based on a 95% confidence interval and the estimated uncertainty in the chosen values of S_v and ρ_p for soot and MWCNTs, expressed as the coefficient of variation (CV). The overall uncertainty of the method was determined by summing all CVs according to standard error propagation procedures.[44]

Results and discussion

MWCNTs with SEM/EDX

Qualitative characterization with SEM/EDX showed a variety of MWCNT structures, heterogeneous in size, shape, morphology and agglomeration state, including hairballs (highly entangled agglomerate/aggregate networks), composite particles (agglomerates of fiber structures and inorganic particles) and bundles (Fig.1C,D,E). Overall particle size of these inhalable structures varied between approximately 0.25 and 100 µm and no single MWCNTs were detected.

Besides MWCNTs also soot structures, inorganic particles and organic particulate matter were present. Inorganic particles consisted of metal oxides, calcium carbonate, transition metals, silicates (soil dust) and salts (sea salt). Inorganic particles were present as single particles, agglomerates with MWCNTs or embedded in MWCNT agglomerates. A small percentage of organic particulate matter consisted of biological particles (plant fragments, textile fibers) but the majority of these particles were production related carbonaceous structures with large particle sizes (5-200 µm).

Quantitative particle number concentrations (based on semi-automatic counting and subtraction) were dominated by soot structures, including DEP (96.5%) with only small proportions of MWCNTs (1.8%), inorganic particles (1.6%) and organic particulate matter (0.1%) (Fig.2A). Whereas, due to the size of the particles the majority of the calculated particle mass concentration comes from MWCNTs (56.2%) and organic particulate matter (39.7%), while the contribution of inorganic particles (3.8%) and soot (0.4%) is only minor (Fig.2B).

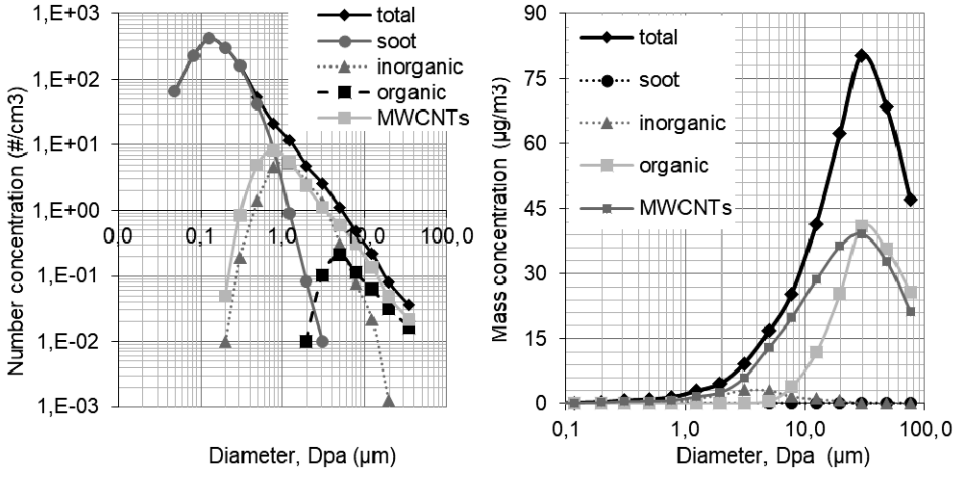


Figure 2: Particle number size distribution (#/cm^3) (A) and particle mass size distribution (µg/m^3) (B) of total particles, soot structures, inorganic particles, organic carbonaceous particles and MWCNTs determined with SEM/EDX.

To date, there is no standardized electron microscopy–based method for counting CNTs.[1, 16, 48] Besides counting procedures, also instrument settings of the electron microscope, like magnification(s), are not specified. Ogura et al.[49] used two magnifications of 1.000X and 10.000X, while Hedmer et al.[1] reported an image field of 9000 µm^2, corresponding to a single magnification of about 2.000X. Mattenklott and Thomas [23] suggests that because of the heterogeneity of MWCNTs, from small fibrous structures to large agglomerates, at least three magnifications should be applied. This study is consistent with the latter study and because the inhalable fraction was investigated rather than only the respirable fraction, four different magnifications were used (200X, 1.000X, 5.000X and 25.000X).

Manual counting of CNTs is difficult due to the many shapes and forms in which CNTs can occur: fibers/bundles, agglomerates/aggregates, hairballs, composite particles. Especially in a production facility, with large quantities of unpurified CNTs, a high percentage of CNTs are mixed agglomerates with inorganic particles from the reactor. Because, it is difficult to recognize CNTs in these agglomerates and composite particles (Figure 1E,F), there is a risk of underestimating CNT number concentrations using a conventional manual counting technique. In this study we used the semi-automatic subtraction technique. This is the first study using an indirect approach to quantify MWCNT concentration by using SEM/EDX. This method has the advantage over direct counting techniques that it is less time consuming and it is expected to be more unbiased. Secondly, the method prevents underestimation of agglomerates/composite particles, which are difficult to identify directly. The result of the subtraction technique should be considered as a conservative (maximum) MWCNT concentration in accordance with the precautionary principle. The method only has an added

value in case of relatively high MWCNT number concentrations and a clear distinction between other particulate matter (especially organic carbonaceous particles), which makes automated particle analysis feasible. In general, the method is not limited by the particle size of particulate matter including CNTs, however if CNTs appear as fibrous structures, automated particle analysis would not be possible due to the lack of contrast between single fibers and filter background.

To be able to compare between manual counting and semi-automatic counting and subtraction, for one sample both counting techniques were used. A clear difference in number concentration was observed between both techniques, which was statistically significant (95% confidence interval based on a Poisson probability distribution for SEM/EDX). With the subtraction technique the concentration was 6.0 (4.2-8.7) structures/cm^3 and with manual counting the concentration was 1.4 (0.7-3.1) structures/cm^3. After conversion to a mass concentration the difference was less, but still significant: 26.1 (18.3-37.8) μg/m^3 vs 12.2 (6.0-23.2) μg/m^3 for semi-automatic counting and subtraction and manual counting respectively. This difference is due to relatively larger estimated sizes for similar particles with manual counting compared to the subtraction technique and consequently end in different size bins. For comparison, the MWCNT concentration based on carbon analysis for this sample was 19 μg/m^3.

For estimating the quantitative mass concentrations based on SEM/EDX, the particle density is needed. The particle density of MWCNTs can vary over a very wide range depending not only on the number of walls, inner diameter or outer diameter of the tubes [38], but also on variables including fractal dimensions, agglomeration state and porosity of the CNT structures. For instance, MWCNT hairballs have typical particle densities between 0.12-0.17 g/cm^3 [23] while compact composite MWCNT material have densities similar to the skeletal density, reported to be 2.1 g/cm^3. Lehman et al.[50] and Kim et al.[37] have reported a mean density of 1.74±0.16 g/cm^3 for two different samples of MWCNTs (outer diameters of 15nm and 22nm), while Laurent et al.[38] reported mean values between 1.1-1.9 g/cm^3 for different types of CNTs. Based on this published data, taken into account the widely varying densities of MWCNTs, the CV in the chosen value of ρ_p (1.5 g/cm^3) on the MWCNT mass concentration would be approximately 25%.

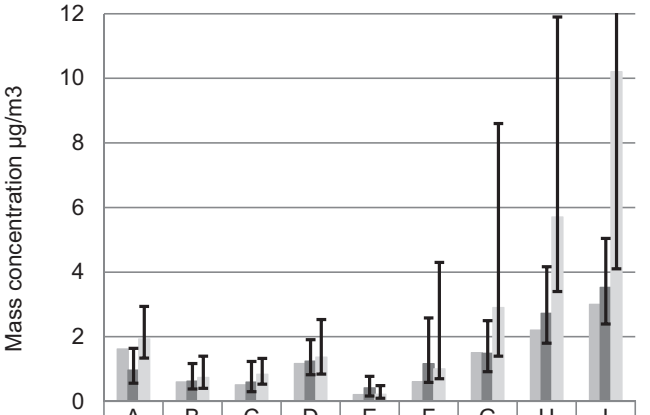
For MWCNTs there is no information about shape factors. Ku et al.[36] assumed a mean dynamic shape factor (χ) of 1.59 based on the fractal dimension and effective density of aerosolized carbon nanofibers (CNFs). From model simulations by Sturm[39] an χ of 1.54 is calculated for MWCNT structures with an aspect ratio of 10. However, the χ is not necessarily the same as the S_v. The dynamic shape factor does not exclusively depend upon particle geometry, like the volumetric shape factor, but is also influenced by the orientation of a particle relative to the direction of gas flow. Because of lacking data it is difficult to define a mean S_v and the uncertainty in this value. However, Ott et al.[31] suggested that the uncertainty in mass

concentration estimates may be eliminated by deriving S_v by microscopy. In this study >90% of the MWCNTs are present as hairballs, agglomerates and composite particles with small aspect ratios and not as fibrous structures. Based on the shapes of MWCNT structures in this study and known shape factors of other comparable shaped particles [30, 51], it is assumed that the S_v is in the range of 1.3-1.7. So, the uncertainty in MWCNT mass concentration due to the uncertainty of S_v is approximately 15%. The counting method of MWCNT structures with SEM/EDX results (similar as for soot) in an estimated CV (95% confidence interval) of 30%. The overall uncertainty in MWCNT mass concentration determined with SEM/EDX is estimated at approximately 60%. This overall CV is not dependent on the size of CNTs and heterogeneity in both density and shape of the particles are taken into account. However, if CNTs are also present as fibrous structures and single fibers the CV would be larger, due to the differences in shape factors.

For SEM/EDX analysis, sampling with IOM samplers was performed with a lower flow-rate (0.7 L/min) than prescribed due to practical reasons (lower resistance). Deviations from the specified flow-rate of 2.0 L/min can lead to a difference in inhalable size fraction. However, a clear advantage of the IOM sampler is that changes in the flow-rate do not result in significant errors in the sampling efficiency as size fractioning is achieved though the design of the sampler's head.[52] This is supported by a study of Zhou and Cheng[53], where the IOM sampler was found to behave similar when sampling at a higher flow-rate (10.6 L/min), except for particles >80 µm, for which a 20% lower sampling efficiency was measured. In addition, Vincent[54] and Sansone and Bernard[55] state that a lower flow rate has only an effect on larger particles, which are less efficiently captured, but for smaller particles in the size range of 2–20 µm no differences were observed. The expected effect of this flow-rate deviation is low for MWCNT mass concentrations calculated with SEM/EDX as the observed MWCNTs in this study showed particle sizes between 0.25 and 100 µm with a mode diameter between 650 and 1000 nm.

MWCNTs with carbon analyses: Background correction using SEM/EDX

Per day/shift per area of the two field campaigns in May 2013 and November 2013 soot concentrations were measured with SEM/EDX and carbon analyses outdoor and with SEM/EDX indoor (see Figure 3 for results and Table 1a for AM results). In both field campaigns the soot concentration inside the facility was higher than outside the facility, suggesting that soot is generated inside the production facility. In addition, there is a difference in soot concentration inside the production area comparing both field campaigns. The mean soot concentration in November was 5 times higher than in May, indicating that a major source of EC inside the facility is the MWCNT synthesis itself which was only performed in November.



	A	B	C	D	E	F	G	H	I
EC outside	1,61	0,60	0,51	1,16	0,21	0,60	1,50	2,20	3,00
SEM outside	0,96	0,62	0,59	1,24	0,41	1,16	1,48	2,72	3,53
SEM inside	1,93	0,74	0,84	1,36	0,22	1,00	2,90	5,70	10,20

Figure 3: EC outside concentration (µg/m^3) determined with carbon analysis (EC_2+EC_3) and SEM/EDX inside and outside (soot). Static air samples were collected outside the production facility in the field campaign of May 2013 (samples A-C in the production area, D-E in the R&D area without production of MWCNTs) and November 2013 (samples F-I, in the production area with production of MWCNTs). SEM inside samples were collected in the breathing zone of the workers. For SEM/EDX also the standard deviation (95% confidence interval based on a Poisson probability distribution) is shown.

For soot concentrations outside the facility there was a high concordance correlation coefficient between carbon analysis and SEM/EDX (R_C=0.92), with a non-significant difference ratio (p>0.05) of 124%±45%.

As an internal source was identified and the agreement between both methods was high, it was decided to use the personally measured daily indoor soot concentrations detected with SEM/EDX for the background correction of personal exposure to MWCNTs. The mass concentration of inhalable MWCNTs, corrected for background, was calculated as EC_2+EC_3 – SEM/EDX soot $_{\text{daily indoor concentration}}$. As background concentrations may differ for different locations, in composition and vary over time, it is recommended to identify possible internal sources of soot and consequently develop a method for the correct detection of the background.

The mass conversion from SEM/EDX soot structure counts depends on the S_v and ρ_p, which are average estimates based on published data.[32-35] However, the density and shape of soot is not uniform and depends on numerous factors, so S_v and ρ_p can deviate from published values. For instance, ρ_p depends on the organic carbon content[33] and hygroscopic growth[35]

and S_v depends on particle size and fractal dimensions. Based on published data the estimated uncertainty in the chosen value of S_v (1.5) and ρ_p (1.5 g/cm^3) was ±15%. By summing these CVs according to standard error propagation procedures[44] the uncertainty in mass concentration is approximately 45%. Additionally, the counting method of soot structures resulted in a CV (95% confidence interval) of approximately 30%. Therefore, in this study the overall uncertainty of the SEM/EDX method to determine soot mass concentrations is approximately 55%.

If indoor EC sources are present the SEM/EDX method is a better alternative for background correction than conventional methods using carbon analysis, especially when the CNT process itself is a source of EC. As can be seen from the high correlation (r=0.93) between carbon analysis and SEM/EDX it's an accurate method, despite the large uncertainty (CV). Because the majority of soot-structures are in the respirable size (Fig. 2), this SEM/EDX method is particularly of interest for measurements of respirable CNTs. There are no extra limitations of the method for the respirable fraction with respect to the inhalable size fraction. For higher accuracy even an actual personal background can be established by parallel PBZ sampling for SEM/EDX and carbon analysis. More information about the derivation of the respirable fraction is available in supporting information 2.

Comparison quantitative results MWCNTs

Quantitative MWCNT results of the side-by-side PBZ samples per day are presented in Figure 4 and AM results are presented in Table 1b, comparing carbon analyses with the other three methods.

Table 1: Comparison soot (a) and MWCNT (b) determined with the different analytical methods: carbon analysis (EC_2+EC_3), SEM/EDX and ICP-MS (metal proxy) and locations. Arrhythmic mean (AM) and ratios taking carbon analysis as the reference.

Analytical method	Parameter	Location (reactor) [1)]	AM ± SD concentration (µg/m^3)	Ratio ± SD (%) [2)]	Concordance correlation Coefficient (95% CI)[2]
a: Soot					
Carbon analysis	*EC_2 + EC_3*	*Outside*	*1.27 ± 0.91*		
SEM/EDX	Soot	Outside	1.41 ± 0.99	124 ± 45%	0.92 (0.71 – 0.98)
SEM/EDX	Soot	Facility (reactor off)	1.01 ± 0.65	126 ± 23%	0.93 (0.66 – 0.99)
SEM/EDX	Soot	Facility (reactor on)	4.95 ± 4.00	240 ± 77%	0.26 (0 – 0.58)

***b:* MWCNTs**					
Carbon analysis	*EC_2+EC_3*	Facility	*215 ± 355*		
ICP-MS	Metal proxy	Facility	784 ± 876	749 ± 687%	0.41 (0.07 – 0.67)
SEM/EDX	CNT mass	Facility	156 ± 161	87 ± 38%	0.81 (0.59 – 0.92)
SEM/EDX	CNT numbers	Facility	12.7 ± 15.9 #/cm3	28 ± 43%	0.001 (0 – 0.04)

[1] Reactor on/off: with/without production of MWCNTs; [2] Ratio in AM concentration between carbon analysis (CA), ICP-MS and SEM/EDX, calculated from the 9 individual measurements as follows: (SEM1/CA1 + SEM2/CA2 +… SEM9/CA9)/9.

Number concentrations by SEM/EDX

There was no correlation between MWCNT number concentration determined with SEM/EDX and mass concentration determined with carbon analysis (R^2= -0.01), but the correlation increased if only a larger fraction of the SEM/EDX number concentrations were used in the analyses. This result was expected as there is a large variation in size and shape of the MWCNTs. Dahm et al.[22] and Hedmer et al.[1] observed the same lack of correlation between EC mass concentration and CNT structure count. Both studies used a direct counting method by manual counting all CNT containing particles regardless length, width or size. In contrast, Dahm et al.[42] found a significant correlation for inhalable samples but with considerable data scatter explained due to measurement uncertainty. Furthermore, three other studies [18, 26, 27] were using a standard method for asbestos fiber counting in accordance with ISO14966 (only fibers/structures with length >5µm, width < 3µm and length:width ratio >3:1), but did not consider the correlation. Hedmer et al.[1] reported that 79% of the collected airborne CNTs did not fulfil the ISO fiber dimensions. In this study the estimated percentage is even higher; more than 90% of the MWCNT containing particles have no typical fiber dimensions but consist of agglomerates with aspect ratios <3. If only fibrous structures would be counted in this study, the MWCNT concentration would be less than 5 structures/cm^3.

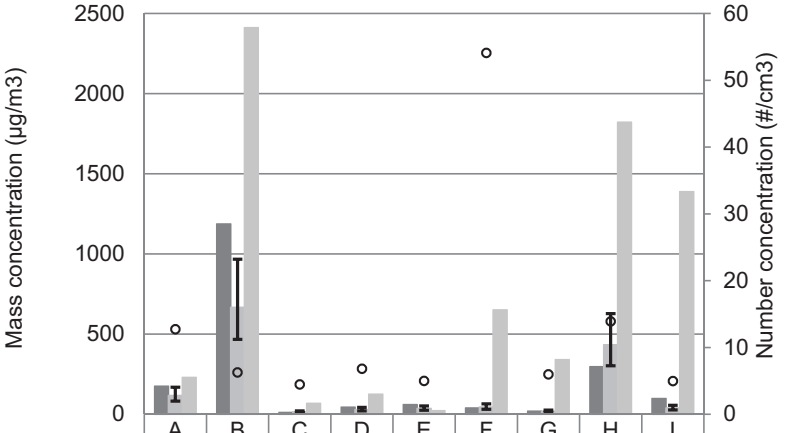
MWCNT mass concentrations by SEM/EDX

There was a high correlation between carbon analysis (EC_2 + EC_3 corrected for soot) and mass concentrations derived with SEM/EDX (R_C=0.81, absolute mean difference = 59 µg/m^3) (Table 1b). The ratio between the MWCNT concentration determined with SEM/EDX and carbon analysis was 87±38% but the difference in concentration was not significant (p>0.05). So, it is believed that SEM/EDX slightly underestimated as compared to carbon analyses, probably caused by the higher uncertainty of this method and the absence of a measurement standard. In comparison, exposure levels of MWCNTs observed during the field survey in the production area were (median (95% CI)) 35 µg/m^3 (20–88).[28] No other studies were identified using an approach to estimate the mass MWCNT concentration based on SEM/EDX.

MWCNT mass concentrations by ICP-MS

A moderate correlation was found between carbon analysis and ICP-MS (R_C=0.41, absolute mean difference = 570 µg/m^3). The MWCNT concentration based on catalyst metals as a proxy

was 7 times higher than determined with carbon analysis (Table 1b). Especially with MWCNT production (reactor on) metal concentrations were high (Figure 4) and would greatly overestimate the inhalable MWCNT concentrations if it would be used as a quantitative marker.



	A	B	C	D	E	F	G	H	I
EC2+EC3	175	1187	11	44	60	40	19	297	98
SEM mass	117	667	15	30	36	45	18	433	39
Metal proxy	229	2411	68	125	23	651	341	1822	1388
SEM numbers	12,7	6,3	4,5	6,8	5,0	54,1	6,0	13,9	5,0

Figure 4: Mass concentration MWCNTs (µg/m^3) determined with carbon analysis (EC2+EC3), SEM/EDX (both number and mass concentration) and mass concentration ICP-MS (metal proxy). Personal air samples were collected at the production facility in the field campaign of May 2013 (samples A-C in the production area, D-E in the R&D area, without production of MWCNTs) and November 2013 (samples F-I in the production area, with production of MWCNTs). For SEM/EDX mass also the standard deviation (95% confidence interval based on a Poisson distribution) is shown providing information about the uncertainty in extrapolation of SEM/EDX numbers into SEM/EDX mass.

To get a better understanding of the correlation between inhalable MWCNTs and catalyst metals, a random selection (N=21) of residual quartz filters, already reported in the occupational exposure article [28] were analyzed with ICP-MS. The correlation of the ratio between ICP-MS and carbon analyses (metal/EC_2+EC_3) and mass concentration (µg/m^3) detected with carbon analyses (EC_2+EC_3) is presented in Figure 5. In the production area there was a clear difference in the ratio metal/EC_2+EC_3 with production (2.2%) and without production of MWCNTs (0.4%).This is an explanation for the moderate identified correlation between carbon analysis and ICP-MS, as there is always MWCNT exposure including the metals but the reactor is only active during certain periods. In both cases the ratio metal/EC_2+EC_3 tended to go down as the MWCNT concentration was higher. This can be

explained by the fact that higher concentrations of MWCNT include relatively more MWCNT hairballs (Figure 1A) than MWCNT composite particles (Figure 1B). In inhalable MWCNT hairballs low levels of catalyst metals were embedded in the carbon structure of the tubes. MWCNT composite particles consist of a metal oxide, used as a carrier material for catalysts, with high concentrations of residual metal catalyst. These particles can be released from the reactor during production of MWCNTs. Also pure metal particles can be released during production of the catalyst material itself. In contrast to the production area, in the R&D area the ratio metal/EC_2+EC_3 was much lower (0.1%) and was not dependent on the concentration detected with carbon analyses. This can be explained by the fact that in the R&D area lower levels of inhalable MWCNTs were measured.[28]

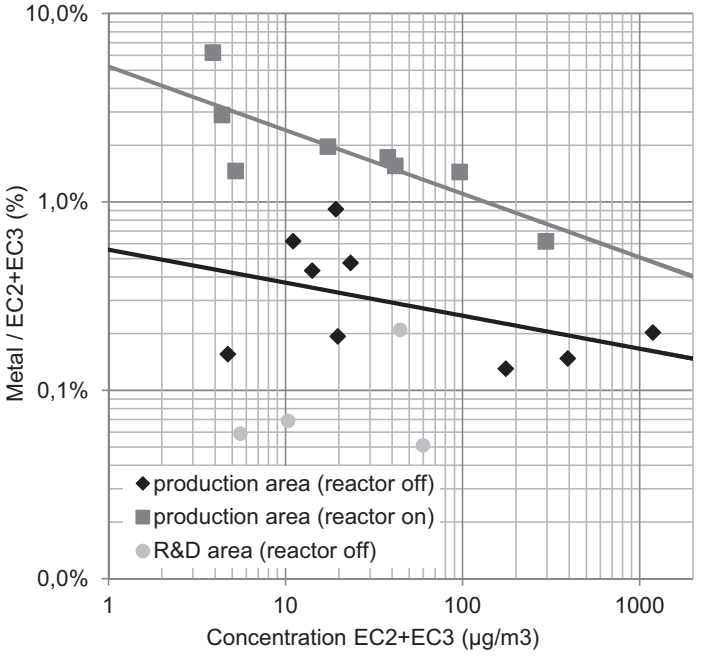


Figure 5. Correlation of the ratio between ICP-MS and carbon analyses (metal/EC2+EC3) and mass concentration EC2+EC3 (µg/m3) detected with carbon analyses in the production area with and without primary production (reactor on/off) and the R&D area. Additional samples were a random selection (N=21) of residual quartz filters, already reported in the occupational exposure article.

Previous studies also demonstrated that the release of non-embedded metal catalyst particles causes high and not well defined background concentrations resulting in inconsistent ratios between the tracer and the (single-walled) CNT.[46] These and our results indicate that metal catalysts are not reliable quantitative markers (proxies) for inhalable CNTs in the production facility itself. Other difficulties that have been identified are the variability of catalyst elements from batch-to-batch production, occurrence of catalyst metals in the local ambient environment and low concentrations of the catalyst impurities in the CNT

product.[21, 46] This can result in a poor accuracy and sensitivity of the quantification method and will disqualify the technique for this purpose in many situations. In downstream processes with already purified MWCNTs ratios between MWCNTs and metal catalysts tend to be more consistent, which enables ICP-MS as a possible quantification technique of exposure to MWCNTs. Due to low environmental background concentrations this applies especially to transition metals like nickel, molybdenum, cobalt and yttrium.[19]

Conclusion

The aim of this study was to develop an approach for the assessment of the level and nature of inhalable MWCNTs. We therefore applied the SEM/EDX method for background EC correction and mass conversion of CNT structure counting results using a semi-automated subtraction technique. Additionally we optimized the existing method for carbon analysis,[2, 43] by adjusting the heating conditions based on the 'non-CNT' PM load of the filter. Both adapted methods, and ICP-MS as a third technique, were then evaluated for quantification of inhalable MWCNTs in an actual workplace situation. Two of these techniques are based on the detection of proxies for MWCNT exposure, namely carbon (EC_2+EC_3) and metal catalysts.

ICP-MS seem to be the least appropriate as both accuracy and sensitivity were relatively low, making metal catalysts not reliable as quantitative markers (proxies) for inhalable CNTs in this large scale production facility. However, if a metal is toxic, and if there is exposure risk, monitoring may be warranted.[21]

SEM/EDX is an accepted technique for structure counting. However, the resulting number concentration is dependent on the counting procedure and electron microscope setting. This is especially the case for non-purified MWCNTs because of the heterogeneity of the structures and the presence of fiber composite particles which are difficult to identify. As different counting techniques have been used in previous studies and this study with different results, standardization of an electron microscopy–based method for counting (MW)CNTs seems crucial to be able to incorporate it in occupational exposure studies. Numerous studies used TEM for microscopic structure count [22, 43, 56]; in this study high resolution SEM is used. SEM offers a simpler analytical method than TEM and has the advantage to identify CNT structures based on morphology (SE-mode) as well as density/atom number (BE-mode). Moreover, although in this study a high correlation and calculated ratio (R_C=0.81, 87%) between SEM/EDX and carbon analysis was demonstrated (see Table 1b), given the relatively high uncertainty of ρ_p and S_v, (especially when fibrous structures are present), SEM/EDX should not be considered as a precise and accurate quantification technique for MWCNT mass concentration. However, the advantage of mass conversion is the distinction in separate mass size fractions of the total inhalable MWCNT concentration, In addition, the semi-automated subtraction technique provides a conservative (maximum) MWCNT concentration in

accordance with the precautionary principle, but has only an added value with relatively high, non-fibrous MWCNT concentrations.

Carbon analysis can be considered as the most appropriate method to quantify MWCNT concentrations. With carbon analysis a correct background subtraction is crucial, especially with lower inhalable concentrations near the recommend exposure limit (REL) of 1 μg/m^3 [2], as under- or overestimation of the estimated levels may occur. Even in the absence of obvious indoor background sources, caution should be taken when applying a background correction based on outside measurements, since the production process itself may be a source. Alternatively and in the absence of possibilities for correct background measurements, it can be decided applying the precautionary principle, not to correct for background. In this study, inhalable MWCNT exposure concentrations would have been overestimated with a median (min, max) of 1.4 μg/m^3 (0.2 – 10 μg/m^3); compared to the relatively high total MWCNT concentration this is approximately 5%. Although in this case relevancy is disputable, for respirable MWCNT concentrations the difference is substantial: approximately 37%. This is due to the lower concentrations, but also because the majority of background EC (soot) in the respirable size range. In addition, for respirable MWCNTs, the difference in SEM/EDX background correction compared to outside EC background correction is approximately 20% higher. Therefore, the background correction using SEM/EDX with indoor collected samples is particularly of interest for measurements of respirable CNTs, despite the larger uncertainty (CV) of the method. The derived particle density (1.5 g/cm^3) and volumetric shape factor (1.5) for mass conversion of soot number concentrations can be used in other studies as well, as this study showed a high concordance correlation coefficient between carbon analysis and SEM/EDX (R_C=0.92). In conclusion, the newly developed SEM/EDX method for background correction results in more accurate MWCNT mass concentrations in workplaces with internal sources of soot and other carbonaceous particulate matter.

Because the relationship between adverse health effects and physico-chemical properties of the exposed CNTs is not well understood, it's more appropriate to determine multiple metrics rather than a single metric [2, 16, 20] which include also oxidative damage.[57] The possibility to complement mass estimates and mass size distributions with MWCNT structure counts and additional physico-chemical analysis, makes SEM/EDX a powerful analytical technique to characterize occupational exposure. Based on our results we selected SEM/EDX and carbon analyses for the quantification of inhalable MWCNTs in an actual workplace exposure situation.

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Supporting information S1

Laboratory validation thermal-optical carbon analysis

Method

In this study for the determination of elemental carbon (EC) with thermal-optical carbon analysis the heating conditions are adopted from the IMPROVE protocol.[1, 2] Because the oxidizing temperature of CNTs depends greatly on the type and diameter of the fibers it was necessary to validate the heating conditions for the target MWCNTs of interest.[3] During laboratory validation the limit of detection (LOD), recovery (%) and reproducibility of the analytical method was determined in accordance with ISO 5725.[4] To determine possible positive interference from other EC sources several types of other carbonaceous materials were tested: carbon black, carbon powder, graphene, SWCNT, DEP and urban dust. The information on combustion temperature of these materials were used to optimize the analytical method in order to discriminate MWCNTs from background carbon.

MWCNT solutions were prepared by adding a known CNT mass to a known volume of MQ water that contained 1% w/w Triton-X-100 (Sigma-Aldrich). The CNTs were suspended by sonicating the mixture overnight in a low power (70 W) Branson ultrasonicator.[5] Aliquots of this suspension are applied to 800 °C pre-heated blanc quartz fiber filters and quartz fiber filters that were preloaded with urban dust particulate matter (PM) by air sampling in the vicinity of motorways. In addition, continuous drop tests with MWCNT were performed in accordance with EN 15051 [6] to generate equally loaded quartz fiber filters. These tests, normally performed to determine the dustiness of nanomaterials, were applied to generate more realistic samples (with heterogeneity in both size and shape of MWCNTs) in comparison with the suspension method. Sampling was performed with blanc quartz fiber filters as well as preloaded PM filters. Concentrations of MWCNT and PM varied between 1 – 50 μg/cm^2 filter.

Results

The LOD, reproducibility and recovery of the method were determined with 6-8 spiked quartz fiber filters with a MWCNT suspension at levels of 2.5 - 25 μg/cm^2, based on EC2 + EC3 (Table SI1-1). In addition the LOD of the method was determined for total EC (TEC) with quartz fiber filters that were preloaded with PM at levels of 3.5 μg/cm^2. For all parameters samples are analyzed on different days. The resulting LOD (3x SD) was 0.3 μg/cm^2 for PM and 0.5 μg/cm^2 for MWCNT, corresponding to an inhalable MWCNT concentration of 1.5 μg/m^3 (8hr sampling time with flow rate 2L/min). Because the suspension method could result in unequally loaded filters, performance characteristics of the method for MWCNTs are probably underestimated. The recovery was corrected for the carbon purity, quantified by EDX analysis. To test the interference of other carbonaceous particles on the method performance, parameters were also determined with PM pre-loaded filters (10 μg/cm^2). Concentration of MWCNTs were calculated by subtracting the TEC concentration of PM. This TEC concentration was

determined in a separate additional analysis of a filter that was not spiked with the MWCNT suspension. To investigate systematic errors of the above TEC "background" subtraction technique,the MWCNT concentrations were compared to MWCNT concentrations determined on clean (non pre-loaded) filters. Systematic errors could not be demonstrated; the average difference in the concentration MWCNT between clean and pre-loaded filters, expressed as the CV, is 14%. Additionally air samples were generated in a continuous drop test, wherein the concentration of MWCNTs was measured gravimetrically.

Table SI1-1. Performance characteristics thermal-optical carbon analysis MWCNT

Sample	Level ($\mu g/cm^2$)	n	LOD ($\mu g/cm^2$)	Reproducibility RSD (%)	Recovery (%, mean ± SD)
PM	2.5	8	0.3		
MWCNT suspension,	2.5	6	0.5	7.4	86 ± 14
blanc filter	25	6	-	10.6	
MWCNT suspension,	2.5	6	0.8 [1]	10.0 [1)]	85 ± 10 [1]
PM pre-loaded filter	10 (PM)				
MWCNT air sampling	50	3	-	-	106 ± 30
continuous drop test					

[1] Determined with TEC background subtraction derived from an additional analysis of a PM loaded filter that was not spiked with the MWCNT (MWCNT = filter PM+MWCNT – filter PM)

To decrease the positive interference from other carbonaceous materials during thermal-optical carbon analysis of MWCNTs, several instrument conditions were applied and tested: ratio O2/He (2-10%), the EC2 temperature (650-700°C) and the EC2 duration (360-1500sec). With none of the settings it was possible to discriminate MWCNTs from other types of particles containing elemental carbon, like carbon black, carbon powder, DEP and urban dust particulate matter (PM) (Table SI1-2).

Because the oxidation temperature is depending on size, shape and agglomeration state of the carbon nanotubes,[1] both suspension samples (with a uniform distribution of bundles and single tubes) and air samples (with a high percentage of big agglomerates) were used. As can be seen in Table SI3 an increasing size and agglomeration state of MWCNTs results in a small shift to EC3. Also external factors, like the presence of other carbonaceous particulate matter and transition metals can influence the oxidation temperature of MWCNTs.[1, 2] Therefore, the distribution in EC categories of MWCNTs was also determined with PM pre-loaded filters, using the standard addition technique. The addition of urban dust particulate matter decreases the oxidation temperature of MWCNTs, resulting in a big shift towards EC2.

Given this catalytic effect on the oxidation temperature together with the positive interferences of carbonaceous particulate matter, the ratio of EC3 to EC2 can vary depending

on the sampling location and conditions. Consequently, the ratio EC3/EC2 cannot be applied as an accurate indication of the presence of MWCNTs, as suggested in the study of Ono-Ogasawara et al.[2] However, based on the validation results the sum of EC2 and EC3 gives a good quantitative estimate of the MWCNT concentration (recovery 85 – 106%) provided that a correction is made for the amount of other carbonaceous particulate matter.

Table SI1-2. Validation heating conditions EC2 thermal-optical carbon analysis.

Product / substance	**% O2/He protocol**	**EC2 protocol**		**n**	**Distribution EC categories (%)**		
		Temp (°C)	time (sec)		EC1	EC2	EC3
Acetylene CB	2	650	360	3	<0.3	<0.3	100 (±0)
CB granulate				3	<0.3	3 (±0.5)	97 (±0.5)
Graphene platelets				3	<0.3	7 (±5)	93 (±5)
Carbon powder				3	7 (±2)	34 (±5)	59 (±5)
SWCNT				3	15 (±1)	61 (±2)	24 (±4)
DEP	2	650	360	3	2 (±1)	33 (±10)	65 (±10)
Urban dust				3	66 (±5)	29 (±6)	4 (±1)
MWCNT				3	0.5 (±0.3)	15 (±5)	85 (±5)
MWCNT (+urban dust)				3	< 0.3	80 (±5)	20 (±5)
DEP	2	650	1500	2	3 (±1)	95 (±5)	2 (±1)
Urban dust				2	69 (±5)	30 (±5)	1 (±0.5)
MWCNT				2	0.5 (±0.5)	68 (±1)	31 (±1)
DEP	2	700	360	3	3 (±1)	88 (±2)	9 (±3)
Urban dust				3	64 (±4)	35 (±4)	1 (±0.5)
MWCNT				7	0.5 (±0.5)	47 (±8)	53 (±8)
MWCNT (+urban dust)				7	2 (±2)	98 (±5)	< 0.3
DEP	10	650	360	3	3 (±1)	44 (±10)	53 (±10)
Urban Dust				3	68 (±1)	29 (±1)	3 (±1)
MWCNT				3	2 (±1)	23 (±5)	74 (±5)
MWCNT (+urban dust)				3	2 (±1)	97 (±5)	1 (±1)
DEP	10	700	360	3	3 (±1)	82 (±3)	16 (±3)
Urban dust				4	85 (±2)	15 (±2)	0.5 (±0.5)
MWCNT				3	1.5 (±0.5)	98 (±1)	0.5 (±0.5)
MWCNT (+urban dust)				3	< 0.3	99 (±1)	1 (±1)

N = number of analysis, ( ) = standard deviation, DEP = diesel exhaust particles (sampled from soot filter of a city bus), Urban Dust = particulate matter from urban background location, MWCNT(+urban dust)= carbon nanotubes on a filter already sampled with urban dust.

Table SI1-3. Distribution EC categories for different products containing elemental carbon (mean value ± SD)

Product/ substance	Sample type	Concentration	Distribution EC categories (%) [2]		
		µg/cm^2	EC1	EC2	EC3
Carbon black	suspension	5 – 40	<0.3	1.5 (±0.5)	98 (±2)
Carbon powder	suspension	5 – 20	7 (±2)	34 (±5)	59 (±5)
DEP [3]	suspension	2 – 35	2 (±1)	33 (±10)	65 (±10)
PM [4]	air sampling	3.5 - 10	66 (±5)	29 (±6)	4 (±1)
MWCNT	suspension	2.5	0.5 (±0.3)	15 (±5)	85 (±5)
	air sampling	10 – 35	0.5 (±0.3)	13 (±5)	87 (±5)
MWCNT (+PM) [1]	suspension	2.5 (PM 3.5)	< 0.3	85 (±5)	15 (±5)
	air sampling	20 (PM 10)	< 0.3	80 (±5)	20 (±5)

[1] carbon nanotubes on a filter already sampled with particulate matter (urban dust), [2] settings carbon-monitor: 2% O_2/He, EC2 650 °C (360sec), [3] diesel exhaust particles sampled from a soot filter of a city bus, [4] urban dust particulate matter sampled on the roadside with moderate traffic.

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Supporting information S2

Derivation of the respirable fraction

Respirable size fractions are derived from cumulative mass graphs (Figure SI2-1A). The correlation of the respirable fraction (D_{50} < 4µm) determined with SEM (D_{50} = 2 x D_{100}), and the inhalable MWCNT mass concentration, determined with carbon analysis ($EC_2 + EC_3$), are presented in Figure SI1B. There is a clear difference in respirable fraction with production (15.6%) and without production of MWCNTs (5.4%). During production smaller MWCNT structures are released in the air. Also, the respirable fraction tends to go down as the MWCNT mass concentration is higher, especially during production of MWCNTs. This can be explained by the fact that higher mass concentrations of MWCNT were observed in PBZ samples associated with activities with MWCNT bulkmaterial (e.g. big bag changing). These high exposures near direct sources include relatively more large CNT agglomerates which, relatively speaking, decreases the respirable mass fraction.

The respirable fraction was derived on the basis of particle size distributions determined with SEM. However, particle sizes were based on the volume equivalent diameter (d_{ve}) instead of the aerodynamic equivalent diameter (d_a), on the basis of which respirable cyclones and impactors operate. For low density particles with ρ_p <1.0 g/cm^3 and non-spherical (irregular) particles of standard density it is known that d_{ve} is bigger than d_a.[1] Thus, it is likely that for MWCNTs the derived respirable mass concentration with SEM underestimates the aerodynamic respirable fraction according to CEN definitions.[2] Comparing SEM measurements with DLPI and APS mass size distributions (see Supporting Information) a d_{ve}/d_a conversion factor of 1.2-1.5 was estimated for the MWCNT aerodynamic respirable fraction from SEM measurements. This implies that the underestimation of the aerodynamic respirable concentration by using SEM measurements is about 20 – 50%.

In addition, the variation in respirable fraction (%) makes it difficult to derive a generic conversion factor for the calculation of the respirable fraction from the inhalable mass concentration. Therefore, it is necessary to derive the respirable fraction for individual activities including external factors (e.g. reactor operation).

Comparing different techniques for deriving the respirable size fraction

To get a better understanding of the particle size distribution of MWCNTs and to evaluate the difference between d_{ve} and d_a, additional PBZ samples and areas samples (AS) were collected, using a Sioutas personal cascade impactor (SCI, SKC Inc., USA) and a Dekati Low Pressure Impactor (DLPI, Dekati Ltd., Finland). The SCI operates at a flow rate of 9 L/min and consists of 5 stages: 4 stages with 50% cut-off diameters of 2.5, 1.0, 0.5 and 0.25 µm, and a back-up filter as the final stage for particles smaller than 0.25 µm. Above the upper stage an additional collection stage was added with 50% cut-point of 6.6 µm in agreement with Ono-Ogasawara et. al (2009)[3]. The DLPI Dekati low pressure impactor consists of 13 stages to generate 14 different size fractions of particulate matter. Flow rate was set at 30 L/min. The 50% cut-off

diameters for the 13 stages were 0.028, 0.055, 0.093, 0.16, 0.26, 0.38, 0.61, 0.95, 1.6, 2.4, 4.0, 6.5 and 9,9 μm. In both impactors quartz fiber filters (QMA, Whatmann) were used as collection substrates for EC analysis; the same filters were used for additional ICP-MS and SEM analysis. Also an Aerodynamic Particle Sizer (APS, TSI, Model 3321) was used for measurement of particles with an aerodynamic diameter between 0.523 and 19.81 μm. With APS particle sizes were binned into 52 size channels.

Mass size distributions of total particles, inorganic particles and MWCNTs were determined. For this purpose, collection substrates of the impactor were subsequently analyzed with SEM-EDX for inorganic particle matter and carbon analysis for MWCNTs.

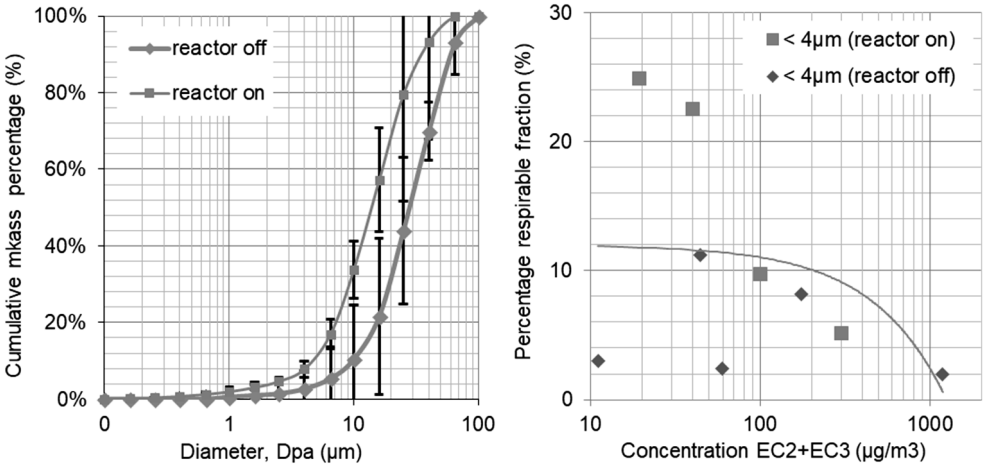


Figure SI2-1. Average cumulative mass size distributions with and without production (reactor on/off) including standard deviations on the basis of 95% confidence interval (A) and the correlation of the respirable size fraction (%) with the mass concentration $EC_2 + EC_3$ (μg/m³) for individual analysis (B).

In Figure SI2-1 cumulative mass size distributions of MWCNTs, inorganic particles and total particles are presented, determined on one location inside the production facility with simultaneous DLPI, SEM and APS measurements. For inorganic particles the DLPI and SEM cumulative mass size distributions are quite similar, which means that for inorganic particles d_{ve} approximates d_a. However, for a true comparison also the difference in cut-off diameters have to taken into account. DLPI is based on D_{50} cut-off diameters unlike SEM which is based on D_{100} cut-off diameters. After conversion of D_{100} into D_{50}, d_{ve} is actually slightly smaller than d_a. This can be explained by the fact that inorganic particles have a higher density than the unit density, on which the DLPI is calibrated.

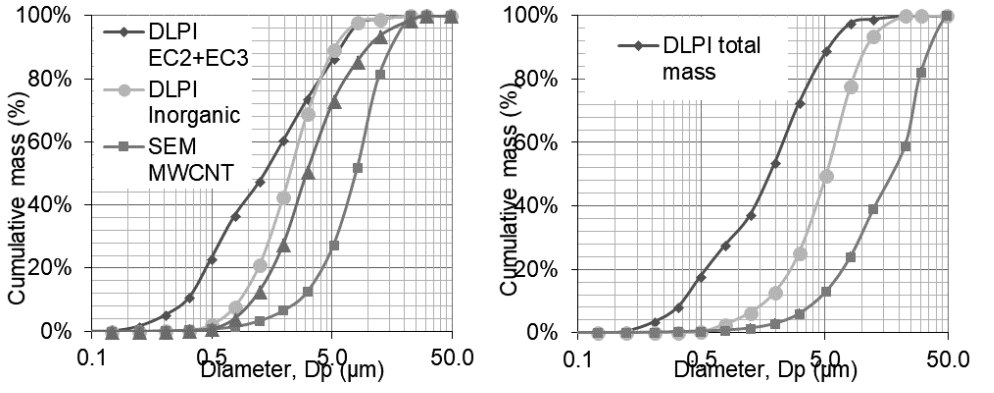
For MWCNTs and total particles (sum of MWCNTs, DEP, inorganic and organic carbonaceous particles) the DLPI, SEM and APS cumulative mass size distributions are quite different. With SEM particle diameters for MWCNTs and total particles are much bigger, which means that

d_{ve} is bigger than d_a. Even, after conversion of D_{100} into D_{50} for SEM, d_{ve} remains bigger than d_a. The difference between d_{ve} and d_a is demonstrated by the detection of large micrometer-sized MWCNT structures in the smaller submicron stages of the low pressure cascade impactor (Figure SI2-3).

The respirable fraction for MWCNTs based on SEM (D_{50}, d_{ve}) is approximately 35% and based on DLPI (d_a) the respirable fraction is 80%, implying an underestimation of the respirable concentration with more than a factor 2 when using SEM measurements. There are several explanations for this big difference. A large fraction of the particles, especially MWCNTs, deviate from the ideal spherical particles with unit density. In general CNTs are far from spherical and a part of the MWCNTs consist of porous structures with low densities. As a result, for MWCNTs d_{ve} is bigger than d_a. In addition, large agglomerated porous particles like MWCNTs (e.g. hairballs), may disperse in the DLPI, because of the shear force.[4] Also particle bounce leads to a distorted measurement of the size distribution. The distortion is attributed to particles bouncing off of the dry impaction surfaces and being collected on subsequent stages.[5] Both effects are resulting in an overestimation of the smaller particle size fractions and respirable particle concentration. Using greased collection plates is an option to reduce particle bounce, however this is not desirable due to the high carbon content of grease and the subsequent disturbance in the carbon analysis.

By comparing DLPI and APS particle size distributions in Figure SI2-2 it is shown that APS measurements results in a much bigger d_a than DLPI measurements. This suggests that de-aggregation and particle bounce are relevant factors to be taken into account when evaluating particle sizes of MWCNTs with DLPI. A real comparison cannot be made because APS doesn't detect particles smaller than 0.5 µm. However, the mass of particles <0.5 µm contributes only to a small extent to the total respirable mass. Taken into account both DLPI and APS results trying to rule out particle bounce and de-aggregation, the d_{ve} / d_a conversion factor to calculate the MWCNT aerodynamic respirable fraction from SEM measurements would be approximately 1.2-1.5. This implies that the underestimation of the aerodynamic respirable concentration by using SEM measurements is about 20 – 50%.

Figure SI2-2. Cumulative mass size distributions (%) of MWCNTs, inorganic particles and total particles, determined with low pressure impactor (DLPI) in combination with carbon analysis ($EC_2 + EC_3$), IOM sampler in combination with SEM particle count and APS.



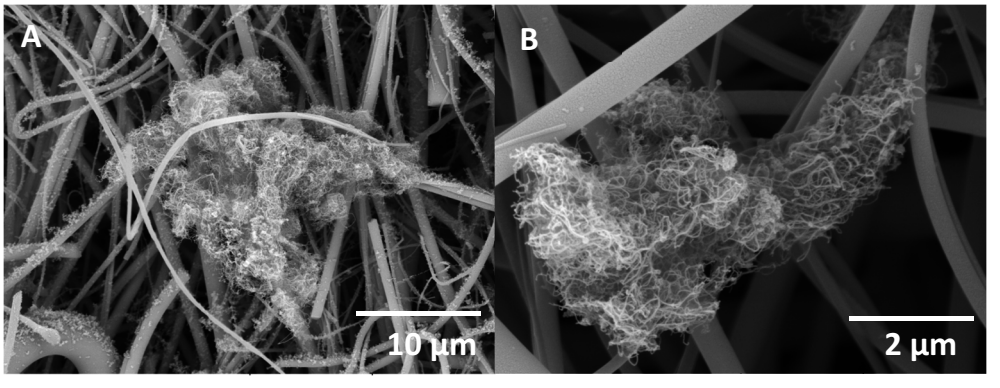


Figure SI2-3. MWCNT structures found in stage 3 (D_{50} = 90nm) of the low pressure cascade impactor.

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Chapter 4a

Occupational exposure to multi-walled carbon nanotubes during commercial production synthesis and handling

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Abstract

The worldwide production of carbon nanotubes (CNTs) has increased substantially in the last decade, leading to occupational exposures. There is a paucity of exposure data of workers involved in the commercial production of CNTs. The goals of this study were to assess personal exposure to multi-walled carbon nanotubes (MWCNTs) during the synthesis and handling of MWCNTs in a commercial production facility and to link these exposure levels to specific activities. Personal full-shift filter-based samples were collected, during commercial production and handling of MWCNTs, R&D activities, and office work. The concentrations of MWCNT were evaluated on the basis of EC concentrations. Associations were studied between observed MWCNT exposure levels and location and activities. SEM analyses showed MWCNTs, present as agglomerates ranging between 200 nm – 100 µm. Exposure levels of MWCNTs observed in the production area during the full scale synthesis of MWCNTs (N=23):) were comparable to levels observed during further handling of MWCNTs (N=19): (GM (95% LCL-95% UCL)) 41 µg/m^3 (20 - 88) vs 43 µg/m^3 (22 - 86)), respectively. In the R&D area (N=11) and the office (N=5) exposure levels of MWCNTs were significantly ($p<0.05$) lower: 5 µg/m^3 (2 – 11) and 7 µg/m^3 (2 – 28), respectively. Bagging, maintenance of the reactor and powder conditioning were associated with higher exposure levels in the production area, whereas increased exposure levels in the R&D area were related to handling of MWCNTs powder.

Introduction

Carbon nanotubes (CNTs) have the ability to improve the thermal, electrical, and mechanical properties of materials and are used, like other manufactured nano-objects (MNOs), to develop new products with improved characteristics. The market for CNTs is increasingly expanding, with applications in e.g. electronics, batteries, textile, concrete, sport equipment, solar cells, coatings, inks and pharmaceutical/biomedical devices.[1]

Alongside the enormous potential of CNTs, concerns have been raised about possible human health risks. The understanding of the specific hazard potential of CNTs is complicated by its variability in structure and size. Animal studies have demonstrated that certain types of CNTs can cause cancer of the pleura. Additionally, inhalation of some CNTs have been shown to induce acute or persistent pulmonary inflammation, granuloma formation, fibrosis, and bronchiolar or bronchioloalveolar hyperplasia in rodents.[2-12] While in vitro studies of cultured human lung or mesothelial cells have shown that CNTs, induce genetic lesions such as DNA strand breaks, oxidised DNA bases, mutations, micronucleus formation, and chromosomal aberrations.

Due to the potential hazard of CNTs and increasing use, insight in exposure levels and exposure conditions of workers potentially exposed to CNTs is needed. A number of studies demonstrated the potential of occupational exposure to CNTs during activities with CNTs.[13-31] The assessment is complicated as it is difficult to find a sensitive and selective analytical method for CNTs. In addition, so far, most exposure studies have been conducted in small

research and development (R&D) facilities, assessing the exposure during the synthesis and/or handling of a limited amount of CNTs (< 1 kg). However, with the growing market for CNTs, an increase in larger-scale industrial manufacturing of CNTs can be expected.[32] To date, only a few studies assessed frequently occupational exposure during synthesis and/or handling of larger quantities (> 1 kg) of CNTs.[22, 23, 27] Of these three studies only Takaya et al.[27] used a more refined mass-based method with elemental carbon (EC) as a proxy for CNTs exposure. However, none of the studies measured in the personal breathing zone (PBZ) frequently, which is the optimal strategy to assess a workers' personal exposure.

The primary objective of this study was to assess and characterize shift-based personal inhalable exposure to multi-walled carbon nanotubes (MWCNTs) during low volume (R&D) and commercial synthesis and subsequent handling of MWCNTs using a technique based on elemental carbon (EC) (as a proxy for CNT mass), and Scanning Electron Microscopy (SEM). A secondary objective was to link the personal exposure measurements to performed activities. The personal exposure measurements will form a basis for exposure assessment in a cross-sectional study of early effect markers among the workers of this facility.

Methods

Facility and products description

We conducted this study at a company commercially producing MWCNTs. The facility consisted of two areas: a production area with attached the main office of the company and a R&D area not connected to the production or office area.

In the production area, chemical vapor deposition (CVD) is used to produce MWCNTs in a large reactor. During the continuous synthesis period, > 100 kg MWCNTs is produced per day (further referred to as synthesis period). Besides the synthesis of MWCNTs, handling activities with MWCNTs are performed year round, including packaging and integrating MWCNT powder in coatings, dispersions and plastics (during a period without synthesis further referred to as handling period). Both the synthesis process and the further handling of MWCNTs take place in a large open two-leveled area.

In the office workers perform administrative deskwork. The office is connected to the production area at the first floor via a dressing room. Although, no activities are performed with MWCNTs in the office, exposure measurements were taken to study potential secondary exposure coming from the production area, with a total estimated volume between 1,000 – 10,000 m^3

In the R&D area not connected to the production or office area, workers are responsible for research and development, quality control and technical support. Low quantities (< 500 g) of MWCNTs are handled per activity.

Sampling strategy

The sampling strategy focused on determining personal exposure to MWCNTs based on a specific EC method, characterizing MWCNT exposure by SEM and energy dispersive x-ray spectroscopy (EDX) and assessing the performed activities by observations and questionnaires. Workers in the production area (both during the synthesis and handling period), R&D area and office were included in the study.

Exposure measurements were performed during 3 days in May 2013 without any synthesis activities (handling period) and 4 days in November 2013 during a period of full scale synthesis of MWCNTs in the reactor (synthesis period). During the handling period, exposure measurements were performed in the production area and in the R&D area (2 days) while during the synthesis period measurements were performed in the production area and in the office (2 days). In the production area and the R&D area, every available worker (present between 6:00h and 22:00h) was assessed resulting in repeated measurements for individuals (with a maximum of 6), while in the office a random selection of the workers was measured based on availability.

Because pilot (static) measurements indicated levels of respirable size fraction below the limit of detection (data not shown), shift-based (4-8 hour) PBZ samples were collected for the inhalable size fraction. PBZ samples for analyses with SEM-EDX were collected from two randomly selected workers per measured day/shift with nickel coated track-etched polycarbonate filters (25mm, pore size 0.4µm, Nuclepore) in an IOM sampler connected to a personal pump (flow rate 0.7 L/min). Simultaneously, PBZ samples for EC analyses were collected from every available worker, on 25-mm diameter quartz fiber filters (Whatman, Kent, UK) in an IOM sampler, connected to a personal pump (flow rate 2 L/min), resulting in two double-equipped workers per day/shift.

From each measured worker information was obtained on the performed activities for the shift-based measurement by questionnaires completed by the workers at the end of the shift, personal observations of the fieldworkers and a daily interview with the production manager.

SEM-EDX analyses

SEM-EDX was used for physico-chemical characterization and determination of the particle size distribution of MWCNTs and a semi-quantitative estimate of the soot concentration (sources: ambient air and internal engines). The filters were screened at magnifications between 200x – 50,000x suitable for the detection of agglomerates of MWCNTs as well as individual MWCNTs. Qualitative data is obtained about the type, size and shape of sampled particles, the degree of agglomeration or aggregation and elemental composition.

All particles between 25 nm and 100 µm were counted with automated particle analysis software (Olympus Soft Imaging Solutions GmbH, Germany) and were distributed in 18 size bins: 25-40, 40-65, 65-100, 100-160, 160-250, 250-400, 400-650, 650-1,000nm and 1.0-1.6, 1.6-2.5, 2.5-4.0, 4.0-6.5, 6.5-10, 10-16, 16-25, 25-40, 40-65 and 65-100um. Using both the

secondary electron image and backscattered electron image of the microscope, MWCNTs could be distinguished from organic carbonaceous particles, soot and inorganic particles. Soot structures are identified based on the typical morphological characteristics: fractal chain-like aggregates of spherical primary particles. A detailed description of the quantification method with SEM-EDX is described by Tromp et al.[33]

Elemental carbon analyses

The analysis of elemental carbon (EC) is based on the thermal optical method as described in the American Standard Method NIOSH 5040. In agreement with Ono-Ogasawara & Myojo [34] a modified IMPROVE protocol was used for the temperature and atmospheric gas settings. In the present study, a thermal optical carbon monitor (Sunset Laboratory Inc., USA) was used. From each quartz filter 1 cm^2 is punched for carbon analysis. EC is removed in the temperature range of 550-920 °C at a mixture of helium and 2% oxygen (2% O2/He). The resulting CO2 is then converted to methane and detected by flame ionisation detection (FID). EC is categorized into EC1 (550 °C), EC2 (650 °C) and EC3 (920 °C). The LOD for a punched filter is 0.5 µg/cm^2 (based on reproducibility) corresponding to an airborne MWCNT concentration of 1.5 µg/m^3.

Calculation of inhalable and respirable CNT exposure levels

Ono-Ogasawara & Myojo [34, 35] described that MWCNTs are usually observed as EC3, which can be used to approximate MWCNT exposure. However, in this study MWCNTs are found in the EC2 fraction also, due to their small diameter and due to altered oxidation temperatures as a result of high concentrations of catalyzing metals and the presence of other elemental carbon particles (soot).

Because soot is also present in EC2 the mass soot concentration per day/shift per location was subtracted from the total sum of EC2 and EC3, to obtain the MWCNT mass concentration. The mass soot concentration was calculated using a mass equation $((\pi/6) \cdot \rho_p \cdot (d_{pa} / S_v)^3$, with ρ_p (particle density), d_{pa} (particle size) and S_v (shape factor). SEM analyses provided soot structure counts and the d_{pa}. A particle density of 1.5/g/cm^3 and a volume shape factor of 1.5 were used.[33] A detailed description of the adapted EC-based method for the assessment of MWCNTs mass and comparison with other approaches is described by Tromp et al.[33]

Statistical analyses

To link the personal inhalable mass concentrations of MWCNTs to the performed activities, statistical analyses were performed. Inhalable mass concentration data showed a right skewed distribution and were log-transformed prior to statistical analysis.

A linear mixed-effects model fit by restricted maximum likelihood (REML) was used to assess associations between inhalable MWCNTs mass concentrations and area (production area, R&D area, office) and period (synthesis period and handling period), taking into account repeated measurements on the same worker.[36]

In addition, linear mixed-effects models fit by REML were used to study associations between inhalable MWCNT mass concentrations (shift based) and performed activities for the production area (combining the synthesis and handling period) and the R&D area separately. For multivariate model building, backward stepwise model building based on the Akaike information criterion (AIC) was used to arrive at models with an optimal balance between goodness of fit and model complexity.

For the production area 42 measurements were available during which 15 activities were encountered. Activities were selected for inclusion in the multivariate model building based on univariate analyses with linear mixed-effects models fit by REML ($p<0.1$)) and the prevalence of the activity ($N>2$). Pearson correlation coefficients were calculated to evaluate the correlation between all activities.

For the R&D area 11 measurements were available and six activities were encountered. Due to this limited number of samples, activities were grouped into activities with direct contact to MWCNTs powder (n=3) and activities without direct contact to MWCNTs powder (n=3) (e.g. handling MWCNTs in a liquids or a matrix).

Geometric means (GMs) for the various areas and measurement periods were derived from the model estimates. All statistical analyses were performed using the statistical software R, with package NLME (R Development Core Team, 2011).

Results

Description of activities

A description of the performed activities with the used technical exposure control measures per area and per measurement period is given in Table 1. In the production area activities specifically related to the synthesis of MWCNTs only took place during the synthesis period and included the control of the reactor, catalyst production, big bag replacement and powder conditioning. Activities related to packaging and formulation of MWCNT enabled products took place during both the synthesis and handling period and included the bagging of powder MWCNTs and the incorporation of MWCNTs in coatings, dispersions and plastics. In addition, irregular activities performed during both periods included the maintenance of machines and cleaning with a vacuum cleaner (equipped with HEPA filters) of the area. Exposure control measures were used mainly during activities with MWCNTs powder (local exhaust ventilation, fume hood). General exhaust ventilation was active and doors/windows were more often opened during the synthesis period compared to the handling period, to cool down the production area.

Regularly performed activities in the R&D area were comparable with the production area but on a smaller scale and involved the use of a small-scale reactor, an extruder for the production of MWCNTs, composites and application of different analyzing techniques.

Exposure control measures were used including local exhausted ventilation, fume hoods and a closed reactor.

Table 1: Overview relevant activities per area and per measurement period. Information is included about the process and the available control measures.

Related activities	Description
Production area – synthesis	
Bagging	Dumping MWCNTs from big bags into smaller bags. The dumping is performed in a cabin with a semi-closed local ventilated bagging system.
Big bag replacement	The produced MWCNTs are collected (close system) in big bags. During the big bag replacement an employee manually changes the big bag and the closed system is temporary opened.
Catalyst production	The catalyst required for production is produced in the production area.
Cleaning	Cleaning the production area with a HEPA filtered vacuum cleaner.
Controlling operators	The production manager is regularly in contact with other operators.
Controlling reactor	The synthesis of MWCNTs is monitored.
Powder conditioning	Powder conditioning is a high energy process performed in a closed system but the system is manually opened for changing bags.
Handling research grades	Prepare samples for research and development and quality control. Activities are performed in a fume hood.
Maintenance	Maintenance of machines including the reactor.
Production coatings	MWCNTs applied in a coating. No control measures were observed.
Production granules	MWCNTs applied in granules. Process locally ventilated and partly enclosed.
Production area – handling	
Bagging	See above.
Cleaning	See above.
Control operators	See above
Handling research grades	See above.
Maintenance	See above.
Production coatings	See above.
Production granules	See above.
Stock management	Transferring bags of MWCNTs and preparing for distribution.
R&D area	
Manufacturing MWCNTs	Production of MWCNT at pilot scale, used to test new structures.
Micro compounding	Introduction of low volume powder in the micro compounder resulting after processing in a solid structure.
Production composites	Production and/or handling of composites with CNT.
Production granules	See above. In addition, volumes are lower compared to the production of granules in the production area.
Rheology / liquid handling	Testing with water-based liquids and applied MWCNTs.
Weighing of MWCNTs	Handling of low quantities of powder MWCNT for balancing purposes.
Office	
Deskwork	Activities behind the desk.

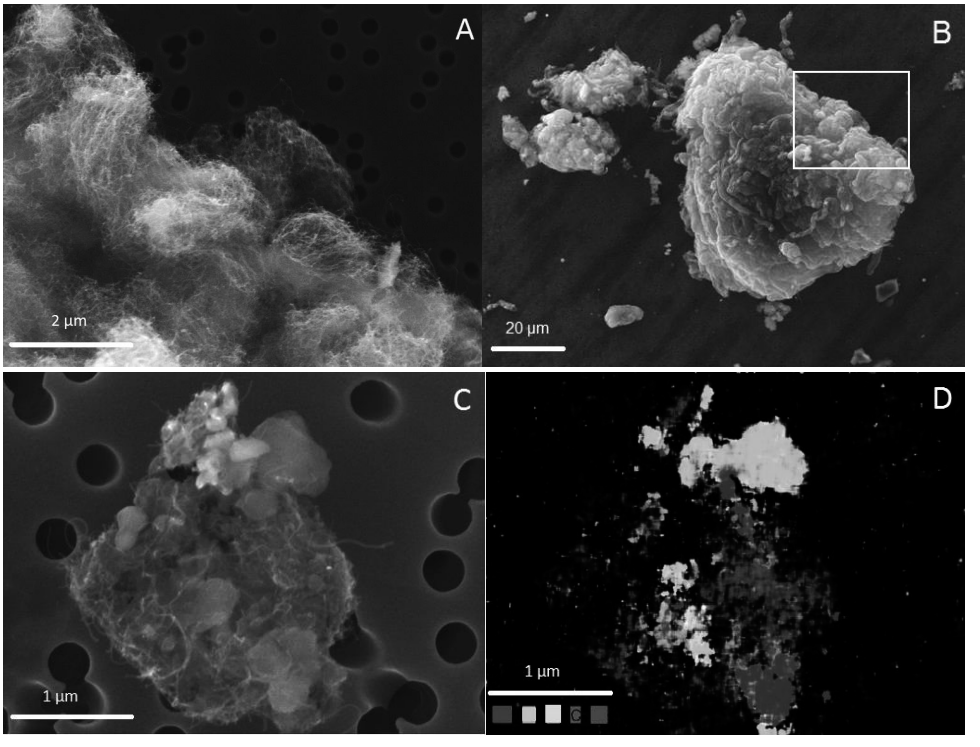

Figure 1: SEM images of collected PBZ samples, with A) Detailed image of MWCNTs, part of a agglomerate B) Different large MWCNT agglomerate C) Detailed image of agglomerate with MWCNTs, D) SEM mapping identifying attached particles at the agglomerate (as presented in C) with carbon in blue and other metals in red, pink, yellow and green.

Characterization and particle size distribution

In total, we collected 30 nickel coated nucleopore filters of which 10 filters were analyzed.Seven were taken in the production area, two in the R&D area and one in the office. SEM-EDX analyses demonstrated agglomerated MWCNTs on all of these filters. (Examples are shown in Figure 1A and Figure 1B). The filters contained large agglomerates with tangled and bundled MWCNTs structures with other particles consisting of other (catalyst) metals attached to them (Figure 1C and Figure 1D). No individual MWCNTs (diameter < 10 nm, length > 1µm) were observed. Soot concentrations (N=10) collected per day/shift and per location were found ranging from 0.2 µg/m^3 to 10.2 µg/m^3 with a GM of 1.25 µg/m^3 which were subtracted from the total sum of EC2 and EC3, to obtain the MWCNT mass concentration.

Figure 2a shows the mean particle size distributions obtained by SEM analysis for the production area (synthesis and handling period) and the R&D area, based on the percentage of particles of the total particle number concentration by size bin. In general, the particle size of the MWCNT agglomerates ranged from 200 nm – 100 µm, indicating a modal distribution with a mode diameter between 650 – 1,000 nm. More smaller particles seem to be present

during the synthesis period in the production area and in the R&D area, compared to the handling period in the production area. Figure 2b shows the mean particle size distributions based on the mass percentage by size bin. Using this representation also, the mode seemed to be smaller during the synthesis period (mode 10-20 µm) than the handling period (mode 20-50 µm). However, due to a high variation within the results, no clear conclusions can be drawn.

Particle size distributions for the office could not be obtained because the overall inhalable mass concentrations were too low to derive robust distributions. Nevertheless, visual inspection showed agglomerates of MWCNTs, mainly ranging between 1 and 10 µm.

Mass concentrations

Overall, 58 quartz fiber filters were collected for EC analysis. Figure 3 shows the GM of the inhalable MWCNT mass concentrations (including 95% lower confidence limit (LCL) and 95% upper confidence limit (UCL)) for the different areas and measurement periods. Inhalable mass concentrations were significantly higher in the production area than in the R&D area or the office ($p < 0.05$). Inhalable mass concentrations in the production area during the handling period were comparable to concentrations obtained during the synthesis period. Furthermore, no significant difference was found between inhalable mass concentrations in the R&D area and the office. In the supplementary information (in the online edition) a detailed overview of the obtained results is included per collected sample.

Determinants for inhalable mass concentrations MWCNTs

Table 2a shows the multivariate mixed effect model for the production area during synthesis, packaging and integrating MWCNT powder into products. Several activities significantly contributing to an elevation of the inhalable concentration were identified, all of which were performed in both the synthesis and the handling period. These activities are bagging, maintenance of the reactor and powder conditioning. Pearson correlation coefficients between all individual activities showed negligible or weak linear correlations ($r < 0.3$), with the exception for powder conditioning and big bag replacement ($r = 0.72$). The model explained 45 % of the total variance for the production area. The relatively high intercept indicates a high MWCNT background concentration, most likely caused by contamination of the production area.

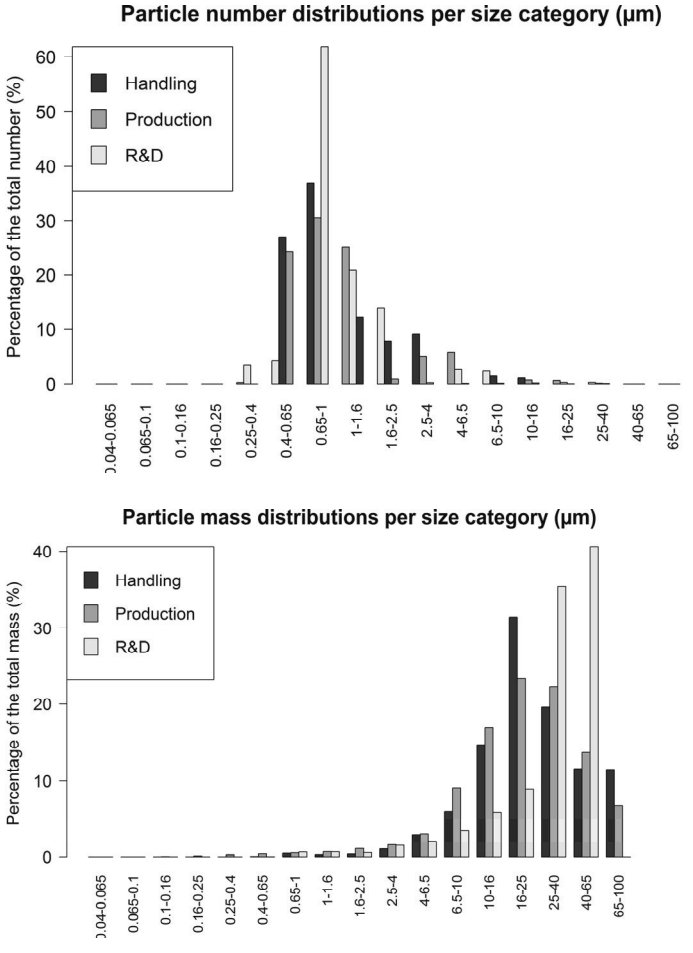

Figure 2: Particle size distributions analyzed semi quantitatively with SEM collected in the production area (production period and handling period) and in the R&D area. Particles were distributed in 15 size bins, a) results based per size bin on percentages of the total particles in the upper panel and b) with results per size bin based on the percentage of the total mass in the lower panel.

Table 2b shows the multivariate mixed effect model for the R&D area. The inhalable mass concentrations were significantly higher for measurements in which workers had direct contact to MWCNTs powder (weighing of MWCNTs, Micro compounding, manufacturing MWCNTs), compared to the group without direct contact to bulk MWCNTs powder (production composites and granules, rheology / liquid handling). The model for the R&D area explained 55 % of the total variance. The relatively low intercept suggests no additional sources of MWCNTs in the R&D area.

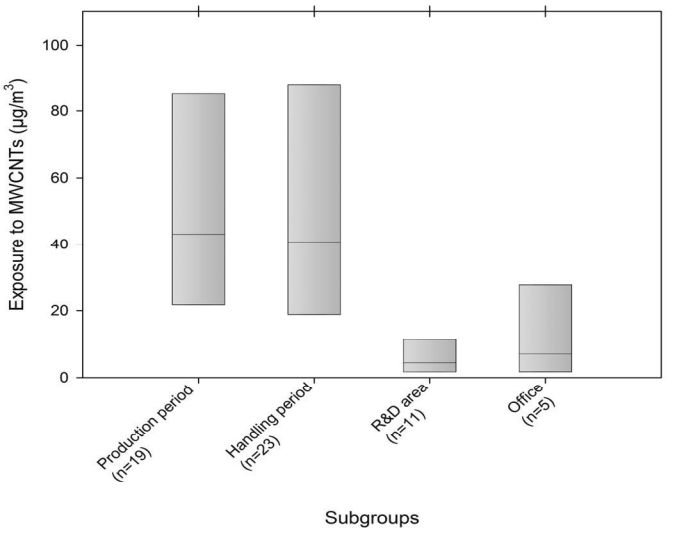

Figure 3: Estimated geometric mean inhalable personal inhalable MWCNT concentrations (TWA) at the production area (synthesis and handling period), R&D area and in the office. Bar ends represent the LCL 95%, and UCL 95%.

Table 2: Estimates of model variables in mixed effects models with a) the production area during the synthesis of MWCNTs and the handling of MWCNTs and b) the R&D area. The measured worker is included as a random effect.

A: Production area	**N[1]**	**N subjects[2]**	**β[3]**	**P-value**
Intercept[4]			2.90	0.0000
Bagging	4	2	2.81	0.0000
Maintenance	9	5	1.31	0.0030
Powder conditioning	9	8	1.46	0.0012
Var_b_w [5]			3.45×10^{-5} [6]	
Var_w_w [6]			1.06	
Total explained variability by model			45 %	

B: R&D area	**N[1]**	**N subjects[2]**	**β[3]**	**P-value**
Intercept[4]			-0.24	0.7109
Contact with MWCNTs	8	6	2.79	0.0117
Var_b_w [5]			1.73×10^{-10} [7]	
Var_w_w [6]			1.55	
Total explained variability by model			54.8%	

[1] # measurements, [2] # persons measured, [3] Fixed effect vector, [4] the intercept gives the exposure to MWCNTs, not performing tasks with MWCNT powder (e.g. handling liquids and dispersions), [5] variance component between workers, [6] variance component within workers, [7] variance between workers cannot be accurately estimated.

Discussion

Worldwide, the amount of produced MWCNTs and number of industrial applications are increasingly expanding, requiring more knowledge about the potential occupational exposure levels and related health effects. This study demonstrated significantly higher exposure levels during synthesis and subsequent handling of commercially produced MWCNTs (see Figure 3) in a production area compared to similar activities performed with lower volumes of MWCNTs in a R&D area of the same company. In the production area, exposure levels were comparable during a period of full-scale synthesis, packaging and integrating MWCNT powder into products (synthesis period) and a period of only packaging and integrating MWCNT powder into products (handling period). Bagging, maintenance of the reactor and powder conditioning of MWCNTs were associated with increased exposure levels in the production area. A high model intercept for the production area compared to the R&D area, suggested high background MWCNTs exposure as a result of contamination in the production area. In the R&D area handling MWCNTs powder (weighing of MWCNTs or manufacturing MWCNTs) was associated with significant increased exposure levels. MWCNTs were mostly present as large agglomerates ranging between 200 nm – 10 µm, with majority between 650 and 1000 nm for both the production area and the R&D area.

Comparison across studies assessing occupational exposure to (MW)CNTs is complicated by variability in exposure assessment methods used. The current study demonstrated the absence of single MWCNTs and the presence of predominantly respirable agglomerates of MWCNTs (mainly between 500 nm and 10 µm) with attached metals in the workplace air. These qualitative results are consistent with results from three other studies, which also used SEM or Transmission electron microscopy (TEM) to assess the type of exposure caused by activities with rigid and flexible MWCNTs, including synthesis, sonification in deionized water, transferring, harvesting, weighing and mixing.[19, 25, 26]

In addition to our study, four other studies [25, 26, 29, 31] were identified that applied a refined mass-based method based on EC as a proxy for CNTs exposure as described by Ono-Ogasawara & Myojo.[34] Dahm et al.[25], Methner et al.[26], Hedmer et al.[29] and Dahm et al.[31] assessed mainly activities with low volumes and found personal inhalable EC concentrations between 0.68 – 7.86 µg/m^3, 33 - 38 µg/m^3, 0.08 – 7.4 µg/m^3 and 0.01 – 79.57 µg/m^3, respectively which are consistent with our findings in the R&D area (0.17 – 59.50 µg/m^3). Furthermore, Dahm et al.[25] indicated that in most cases, the aerosols sampled were most likely within the respirable size fraction. This conclusion is consistent with our findings, but mass-based particle size distributions showed the enormous contribution of large MWCNTs agglomerates to the inhalable MWCNT mass concentrations.

Two other studies assessing exposures during more comparable synthesis and/or (subsequent) handling of high volumes of (MW)CNTs have been identified. However, these studies have used non-selective proxies for CNTs. Lee et al.[22] assessed exposure during the

production of MWCNTs in three industrial plants and obtained PBZ concentrations ranging between 21.2 µg/m^3 and 285.9 µg/m^3, by using a gravimetric method. However, no SEM or TEM analyses were performed to characterize the particles and to confirm CNT structures. The gravimetric results are most likely an overestimate of CNT exposure due to interferences from other (background) particle sources. In the present study the EC1 fraction, which is not included in the more specific measure for CNTs, was substantial (EC1 range 2.6 – 484.1 µg/m^3). Wang & Pui [23] measured exposure in an industrial production area for CNT-imbedded nano composites. The particle number concentration was between 90,000 –100,000 #/cm^3, but it was believed that volatile polymer fumes were a major particle source.

Recently, NIOSH proposed that exposures to respirable EC mass-based MWCNTs should be kept below a recommended exposure limit (REL) of 1 µg/m^3 as an 8-hour time weighted average (TWA).[37] The NIOSH REL is not a health-based exposure limit but is based on the current analytical limit of quantification with sampling and analytic methods. To get an indication of the respirable mass concentrations the measured inhalable mass concentrations were converted into corresponding respirable mass concentrations using the respirable convention as a percentage of the inhalable convention and the percentages MWCNTs per size category derived from the semi-quantitative SEM analyses according to CEN EN 481.[38] Respirable fractions ranging between 2 and 10% were obtained, resulting in converted respirable mass concentrations in the production area of 0.87 – 4.45 µg/m^3 (0.08 - 29.97) and 0.76 – 2.71 µg/m^3 (0.02 – 75.34) for the synthesis period and handling period, respectively and 0.07 – 0.30 µg/m^3 (0 – 3.84) for the R&D area. The calculated respirable mass concentrations for this study exceeded the REL frequently (67%, based on 4.95% respirable fraction) in the production area during both the synthesis period and handling period and occasionally (27%, based on 4% respirable fraction) in the R&D area. Because of the low MWCNT concentration in the office, no robust particle size distributions were obtained which made it impossible to calculate the respirable mass concentrations. The calculated respirable mass concentrations should be interpreted with caution, as the calculated respiratory levels may vary considerable as a result of the used methods and assumptions. First, PBZ samples for analyses with SEM-EDX were collected with flow rates of 0.7 L/min while the IOM sampler is usually operated at a flow rate of 2.0 L/min. Originally, these samples were only intended to be used for qualitative characterization of the MWCNTs and for practical reasons a lower flow rate was used. According to James H. Vincent (1989) [39] and Sansone & Bernard (1976) [40] a lower flow rate has a large effect on larger particles, which are less efficiently captured but for smaller particles in the size range of 2 to 20 µm no differences were observed. Therefore, the respirable fractions as presented with a lower flow rate (0.7 L/min) are likely to be comparable to the recommended flow rate of 2 L/min. Next, uncertainty in deriving the respirable size fraction with SEM analysis is introduced by the counting technique. Currently, no standard protocol has been developed, with counting rules, or semi-automatic routines for SEM/TEM analysis including the use of standard reference materials for sizing and characterizing particles.[41] The uncertainty in the derived respirable mass concentration due

to analyzing only a small fraction of the filter estimated based on the Poisson distribution is 20%. Furthermore, SEM volume equivalent diameters are derived in this study, while uniform respirable convention percentages are based on the aerodynamic diameters, resulting in an uncertainty of approximately 40%. Lastly, the respirable fractions were determined once per area and per measurement day and subsequently used for the conversion of every inhalable mass concentration introducing additional uncertainty.

This is one of the first studies that has evaluated determinants of MWCNT exposure. The identified high exposure activities bagging, maintenance of the reactor and powder conditioning in the production area, are consistent with the findings of previous studies that assessed the emission potential of activities with (powdered) nanomaterials (including CNTs) and performed task-based exposure assessments.[20, 42] It should be noted that the relatively low number of measurements, the numerous activities and the relatively high surface contamination suggested by the high model intercept may have hampered the identification of all activities that resulted in high exposure levels. In addition, since workers perform several activities over a day, often for a relatively short time period, the obtained shift-based results are a complex combination of activities. Nevertheless, the relatively low Pearson correlation coefficients suggest an independent character of the identified activities with increased exposure levels.

No clear differences were found in exposure levels and particles size distributions between both measurement periods in the production area. As the synthesis process of MWCNTs was an automatic process, activities related to the synthesis process were mostly performed in a control room, monitoring the reaction process. Individual measurements of workers only present in the control room, revealed relatively low inhalable exposure levels of MWCNTs (range 7.83 $\mu g/m^3$ – 32.62 $\mu g/m^3$, n=4). Therefore, the synthesis of MWCNTs itself does not appear to be a major source of exposure in this study.

Surprisingly, MWCNTs were detected in the office. This may be explained by a connection via two doors between the production area and the office that were regularly open, especially during the cleaning of the office, or by clothing contamination from several workers who work both in the production area and the office. Visual inspection during the fieldwork identified powder on most objects, floors and walls, suggesting that MWCNTs easily dispersed through the area.

In conclusion, this study demonstrated significantly higher MWCNT exposure during synthesis and subsequent handling of high volumes of MWCNTs compared to R&D activities. To our knowledge, this is one of the first study describing personal occupational exposure related to commercial activities, by using elemental carbon as a proxy for MWCNTs. We identified several activities that are associated with significantly increased exposure, which will give focus to interventions aimed at reducing exposure levels by the company.

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Supporting information

Table SI-1: Detailed results personal measurements production area during handling period.

Date	Personal ID	OC µg/m³	EC1 µg/m³	EC2 µg/m³	EC3 µg/m³	Inhalable CNT+BC (EC2+EC3) µg/m³	Background µg/m³	Inhalable CNT µg/m³
28-05-13	1	353.8	87.7	126.6	48.8	175.37	1.90	173.47
	2	65.3	19.2	16.0	2.3	18.23	1.90	16.33
	3	1805.7	484.1	432.5	20.7	453.21	1.90	451.31
	4	45.0	11.9	13.2	6.0	19.24	1.90	17.34
	5	50.5	8.2	5.3	1.3	6.60	1.90	4.70
	6	42.9	10.1	25.2	2.7	27.95	1.90	26.05
	7	118.1	66.0	35.3	3.0	38.33	1.90	36.43
29-05-13	1	113.1	34.3	410.9	776.3	1187.22	0.70	1186.52
	2	77.3	25.4	30.2	3.6	33.80	0.70	33.10
	5	50.5	13.1	16.7	2.6	19.29	0.70	18.59
	4	22.2	2.6	1.8	0.3	2.13	0.70	1.43
	6	48.7	13.4	20.8	2.5	23.28	0.70	22.58
	7	82.9	28.8	19.0	2.5	21.44	0.70	20.74
30-05-13	1	78.9	27.4	277.5	114.3	391.87	0.80	391.07
	2	76.7	36.5	187.9	68.7	256.61	0.80	255.81
	5	112.7	52.1	239.6	64.7	304.32	0.80	303.52
	4	92.8	27.7	12.5	1.1	13.62	0.80	12.82
	6	56.3	16.6	9.8	1.2	11.03	0.80	10.23
	7	57.7	20.7	37.1	11.2	48.25	0.80	47.45

Table SI-2: Detailed results personal measurements production area during synthesis period.

Date	Personal ID	OC µg/m³	EC1 µg/m³	EC2 µg/m³	EC3 µg/m³	Inhalable CNT+BC* µg/m³	Background µg/m³	Inhalable CNT µg/m³
07-10-13	3	72.7	12.9	79.8	27.0	106.9	5	101.9
26-11-13	6	66.4	14.5	35.0	4.6	39.62	1	38.62
	3	24.9	4.1	3.7	1.4	5.11	1	4.11
	8	97.9	19.5	35.5	12.4	47.90	1	46.90
27-11-13	4	57.8	13.2	12.6	2.3	14.84	2.9	11.94
	1	32.3	5.9	13.9	5.3	19.21	2.9	16.31
	5	33.3	8.9	13.2	4.8	17.98	2.9	15.08
	7	95.7	26.4	25.2	37.2	62.37	2.9	59.47
	9	58.3	12.1	12.5	5.5	17.95	2.9	15.05
	10	50.8	9.9	16.5	8.0	24.47	2.9	21.57
	2	202.1	59.4	189.2	71.3	260.47	2.9	257.57
28-11-13	4	71.1	23.1	79.8	31.1	110.81	5.7	105.11
	1	122.9	39.8	234.2	62.9	297.04	5.7	291.34
	5	40.8	9.0	14.7	6.4	21.10	5.7	15.40
	10	60.9	12.1	12.2	1.3	13.53	5.7	7.83
	2	103.1	33.5	47.6	32.9	80.52	5.7	74.82
	9	87.7	24.7	32.7	5.6	38.32	5.7	32.62
	7	145.8	36.4	51.9	27.3	79.20	5.7	73.50
	11	38.4	8.8	13.4	12.8	26.15	5.7	20.45
	8	110.0	30.4	173.3	57.6	230.88	5.7	225.18
29-11-13	5	72.2	24.4	74.5	23.6	98.08	10.2	87.88
	4	129.6	39.0	53.8	14.2	67.96	10.2	57.76
	3	548.2	76.9	237.9	15.5	253.38	10.2	243.18

Table SI-3: Detailed results personal measurements R&D area.

Date	Personal ID	OC µg/m³	EC1 µg/m³	EC2 µg/m³	EC3 µg/m³	Inhalable CNT+BC* µg/m³	Background µg/m³	Inhalable CNT µg/m³
29-05-13	12	94.6	10.6	37.0	7.4	44.44	1.4	43.04
	13	17.0	2.3	1.3	0.3	1.57	1.4	0.17
	14	53.1	14.7	4.7	0.8	5.57	1.4	4.17
	15	326.2	84.2	26.4	3.1	29.49	1.4	28.09
	16	33.0	5.4	3.6	1.1	4.64	1.4	3.24
	17	29.9	4.4	1.6	0.3	1.84	1.4	0.44
30-05-13	16	90.8	32.3	49.7	10.0	59.70	0.2	59.50
	12	56.6	10.8	7.6	2.7	10.33	0.2	10.13
	18	56.4	13.4	4.7	0.8	5.51	0.2	5.31
	17	32.1	3.6	1.2	0.3	1.43	0.2	1.23
	19	66.9	12.5	4.1	0.6	4.76	0.2	4.56

Table SI-4: Detailed results personal measurements offices.

Date	Personal ID	OC µg/m³	EC1 µg/m³	EC2 µg/m³	EC3 µg/m³	Inhalable CNT+BC* µg/m³	Background µg/m³	Inhalable CNT µg/m³
27-11-13	20	38.7	4.6	3.2	6.7	9.94	1.2	8.74
	21	36.0	5.9	4.5	7.2	11.71	1.2	10.51
	22	52.1	7.7	7.6	3.4	10.94	1.2	9.74
28-11-13	23	49.6	9.0	3.3	2.3	5.56	1.2	4.36
	22	56.2	7.3	4.4	1.5	5.87	1.2	4.67

Chapter 4b

Relative differences in concentration levels during sawing and drilling of car bumpers containing MWCNT and organic pigment

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Abstract

Improved knowledge on the exposure characteristics, including release of nanomaterials, is especially needed in the later stages of nano-enabled products' life-cycles to perform better occupational risk assessments. The objective of this study was to assess the concentrations during sawing and drilling in car bumpers containing multi-walled carbon nanotubes (MWCNT) and nano-sized organic pigment (OP) under variable realistic workplace situations related to the ventilation in the room and machine settings.

Twelve different experiments were performed in triplicate (N=36) using tools powered by induction engines that do not generate particles and allows interference-free particle measurements. A DiscMini was used to measure particle number concentrations, while particle size-distributions were measured using APS (TSI), SMPS (TSI) and ELPI (+) (Dekati). In addition, inhalable particles were sampled using IOM samplers on filters for SEM/EDX analyses. Data was analysed using Autoregressive Integrated Moving Average (ARIMA) models in the statistical software R to estimate the effects of individual exposure determinants.

In sawing experiments partly melted carbon-rich particles (100 nm – 20 micrometer) were identified with SEM/EDX, while drilling experiments revealed no activity related particles. In addition, no pristine engineered nanoparticles (MWCNTs and OP) were observed to be liberated from the matrix. Statistical analyses showed significant effects of a higher sawing speed, a reduction in air concentration due to mechanical ventilation and less exposure during sawing of car bumpers containing MWCNTs compared to bumpers containing OP.

The experiments in this study give an indication of the effects of different abrasive activities (sawing, drilling), machine settings (sawing speed, drill size), mechanical ventilation and material characteristics on the NOAA concentration levels and consequently on the potential worker exposure.

Introduction

Nanotechnology is a fast-growing and rapidly advancing technology, impacting global industry and society with numerous new manufactured nanomaterials and products containing these nanomaterials.[1] Nanotechnology is moving from small research and development (R&D) scale to larger industrial scale,[2] but the physical and chemical properties of MNOs raise health-related concerns.[3]

The potential exposure of workers to manufactured nano objects (MNO), their agglomerates and aggregates (NOAA) has received considerable attention in recent years,[4, 5] as occupational exposure levels are normally higher than consumer exposure levels and their safety is a cornerstone of responsible innovation. According to Schneider et al.[6] occupational activities with MNO cover the whole-life cycle of a nanotechnology-based product, which can be divided into four stages, namely: 1) synthesis of MNO, 2) handling and transfer of MNO, 3)

application of products containing MNO and 4) fracturing and abrasion of products which include MNO. In contrast to workers involved in the first stages of the product life cycle, the awareness of end-users about the presence of MNOs in their products is relatively low.[7] Increasing awareness requires more knowledge about scenarios with quantitative release of NOAA.[8]

Previous studies on the exposure potential of NOAA during the final stage (4) fracturing and abrasion predominantly focused on sanding activities on products containing multi-walled carbon nanotubes (MWCNT) and silica.[8-16] Only a limited number of (simulated) workplace studies focused on other occupational activities regularly performed, like sawing [11, 15 17, 18] and solid core drilling activities.[19, 20] Concentration levels of nanoparticles (including NOAA) were up to 1,6E6 #/cm^3 and 2,0E5 #/cm^3 for sawing and drilling, respectively. In general, interpretation of the results from abrasion and fracturing studies is challenging as measurements have shown that particles emitted by electrical tools themselves were repeatedly reported as a major source of nano-size particles.[4 7, 9, 12, 21, 22] More research is needed for realistic risk assessments in the later stages of the life-cycle of MNO for activities other than sanding, not influenced by particles emitted by electrical tools themselves.

The objective of this study was to assess the potential exposure (morphology, chemical composition, size and quantity) of NOAAs and other (formulated) nanosized particles during automated sawing and drilling. For this purpose, real-time aerosol concentrations were measured both in the vicinity and far from the activity in a controlled environment. In total, 12 simulated workplace experiments were completed to investigate various determinates of exposure, namely: type of MNO (car bumpers containing nanosized MWCNT or nanosized organic pigment (OP)), activity (automated sawing or drilling), instrument settings (speed for sawing, drill size for drilling) and mechanical ventilation (on / off). Furthermore, each of the experiment was repeated three times to assess the variability in concentration levels.

Methods

Studied materials

The test materials included two car bumpers: a red high density polyethylene (HDPE) polymer matrix containing 10 weight percent (wt. %) Organic Pigment (OP) Red 254 and a black polyurethane (PU) matrix containing 0.09 wt. % MWCNT.[23] The OP Red 254 particles have a diameter of 26 nm and a BET surface of 94 m^2/g. MWCNTs have on average a diameter of 9.5 nm, a length of 1.5 µm and a BET surface of 250 – 300 m^2/g. Both types of car bumpers have a comparable tensile stress which is the capacity of the car bumpers to withstand loads tending to elongate (ISO 527-2). Prior to the experiments, the car bumpers were cut over the length in several parts to create equally sized objects of ca. 50 cm x 10 cm x 1 cm. The nanomaterials and car bumpers were tested as one of the selected life-cycle test materials in the FP7 “Sustainable Nanotechnologies” (SUN) project (www.sun-fp7.eu).

Experimental set-up and environmental conditions

The experiments were performed in an experimental room of 19.5 m^3 (Length 3.90m × Width 2.10m × Height 2.38 m) which was previously described.[9, 24] Environmental conditions in the room were controlled during the experiments with temperatures and relative humidity ranging from 18 to 22 °C and 35 to 42%, respectively. Two different settings of the mechanical ventilation and the effect on concentration levels were evaluated with the experiments: 0 air change per hour (ACH) and 3.5 ACH as determined using an automatic air volume flow meter (TSI, Airflow Instruments ProHood Capture Hood PH731). The test room was flushed between two experiments using the maximum capacity of the mechanical ventilation (~20ACH) to allow a maximum background concentration prior to every experiment of < 200 #/cm^3. In addition, during this period between the experiments, the room was cleaned with a wet duster and a professional vacuum cleaner with a HEPA filter.

Previous release experiments reported significant particle emissions from electric motors in the machines used for abrasive activities,[7, 9, 12, 21, 22] which was explained by carbon brushes sliding over copper commutator contacts.[25, 26] As these particles influence the results of the release experiments, the present study used a bandsaw table and a drill table both with an induction motor. These induction motors were selected, as no particles are emitted by the motor, which was confirmed in the experimental room prior to the experiments. A bandsaw table was used for the sawing experiments (Metabo, BAS 318, EAN 4007430304940) with two different sawing speeds (410 and 880 m/min). The drill table used for the drilling experiments (Dedra DED7708, EAN 5902628770806) was operated (2700 rpm) using two different titanium drill bits (4mm and 8 mm).

The total measurement duration of every experiment was 13 minutes and consisted of 5 minutes of background measurements before the activity (phase 1), followed by 3 minutes of the activity (phase 2) and 5 minutes after the activity (phase 3). The fieldworker that performed the tests was in the experimental room during the entire experiment, using respirator protective equipment (P3 filter). To standardize the experiments, the numbers and duration of drill holes and the sawed length was equal in every experiment. An overview of the performed experiments and the studied variables is shown in Table 1. We performed four different experiments (all performed in triplicate) using the drill table, varying the type of car bumper (MWCNT or OP) and drill size (4 and 8mm). Eight different experiments (in triplicate) were completed using the band-saw table, varying the nanomaterial (MWCNT or OP) in the car bumper, sawing speed (410 and 880 m/min) and mechanical ventilation (0 and 3.5 ACH).

Table 1: Summary determinates and variables

Determinants	Variables
Car bumper	Red HDPE matrix with 10 wt. % OP
	Black PU matrix with 0.09 wt. % MWCNT
Abrasive machines	Bandsaw table
	Drill table
Machine settings	Speed for sawing (410 and 880 m/min)
	Drill size for drilling (4 mm and 8 mm)
Mechanical ventilation	0 ACH
	3.5 ACH (only for sawing)

HDPE: high density polyethylene, OP: organic pigment, PU: polyurethane, MWCNT: multi-walled carbon nanotubes, ACH: air change per hour.

Instrumentation

All measurement instruments were placed outside the room and attached with antistatic sampling tubes or with the tube type as provided by the instrument (<50 cm) to minimize the effect of the instruments on the experimental results. The inlets of the instruments were placed as close as possible to the source (near field; NF, ~ 20 cm) or in the opposite corner of the room (~4m, far field; FF). All instrument types were used for measurements at both the NF and FF measurement locations. For a schematic overview of the experimental setup and the position of the instruments see the supplementary information (Figure S1).

Inhalable dust particles were collected on 25-mm nickel-coated nucleopore filters (pore size 0.4 µm) for morphology, chemical composition and size using an IOM sampler and a volume flow of 2 L/min provided by Buck Basic-5MH pumps. Filters were loaded only during phase 2 and 3 and triplicate experiments were combined on one filter resulting in a total sampling duration of 24 minutes (48 L) per filter. Only a selection of the filters collected in the NF were analyzed by scanning electron microscope (SEM, model MIRA-LMH, Tescan) and in situ chemical analysis by energy-dispersive X-ray spectrometry (EDX spectrometer with XFlash 4010 detector; Bruker), as similar qualitative results may be expected in the FF.

In order to quantify the concentration levels, particle number concentrations were assessed in the range of 10 to 300 nm using the DiSCmini (Matter Aerosol, Switzerland). In addition, the particle size distributions were measured using a scanning mobility particle sizer (SMPS, model 3081 and 3786, TSI Inc), an aerodynamic particle sizer (APS model 3321, TSI Inc.) and an electrical low pressure impactor (ELPI+ (NF) and ELPI (FF), Model 9721 Dekati). The measured size range and response time for the SMPS, APS, ELPI and ELPI (+) were 11.3–514 nm (1 minute), 0.5–20 µm (1 second), 0.007-10 µm (1 second) and 0.006-10 µm (1 second), respectively. Due to technical issues no data was collected for four experiments with the ELPI + and for one experiment with the SMPS. Results for particle size distributions were normalized to dN/dlogDp to compare the distributions taken of the same aerosol using different instruments with different resolutions (mobility or aerodynamic diameter).

Statistical analyses

The methodology to analyse the particle number concentrations collected with the DiSCmini and the particle size distributions sampled with the SMPS, APS and ELPI (+) was previously described by [24]. In brief, a two-stage modelling strategy was applied: In stage 1 an autoregressive integrated moving average (ARIMA) models was used for individual experiments; In stage 2 the individual results of stage 1 were combined to evaluate and quantify the effect of the different determinants and variables.

In stage 1 ARIMA models were used to take into account the pattern of autocorrelation in the sequential measurements collected with real-time instruments. In a stepwise approach which was previously described by Klein Entink et al.[27,28], the data was forced for stationarity as assumed by an ARIMA model. A second-order moving-average (MA) model was applied (100 iterations) which had the best fit with the measurement data. In addition, the dataset included for every data point a binary (0,1) variable, to indicate the data related to the background and the activity. As a result, model estimates were derived for both the average background and the average activity number concentration and size distribution. Next, activity-effect estimates (β) and standard error (SE) were used in stage 2 of the analysis.

In stage 2 it was assumed that the uncertainty in the stage 1 results is characterized by a lognormal model with the estimated mean (β) and standard error (SE). To account for this uncertainty, a Monte Carlo simulation was performed randomly selecting values (n=1000) from the distribution. The selected values were used in a multiple linear regression model with the evaluated determinants as independent variables. Regression coefficients (β) and standard error (SE) were pooled per experiment. Finally, β results were exponentiated (by squaring) to obtain geometric mean ratios (GMR). All statistical analyses were performed in R studio version 3.1.2 (R Core Team 2014).

Results

Characterization of released particles

In total, 24 nickel-coated nucleopore filters (12 different experiments, both NF and FF) were collected of which only 6 filters were analyzed by analytical SEM focusing first on the filters with the highest particle loading. The filters selected to qualify the release were all collected in the NF of the experiments, with three filters for the red car bumper containing OP and with three filters for the black car bumper containing MWCNTs. The three filters for both the red and black car bumper varied in the abrasive activities performed during the experiments (N=2x2 for sawing, N=2x1 for drilling). The filters for the sawing experiments varied in the bandsaw table setting (speed 410 and 880 m/min), while for drilling only the filters for the 8 mm drill bit were analyzed. In general, no MWCNTs and OP particles were observed to be liberated from the matrix. Furthermore, for the drilling experiments no activity related particles from the test materials were observed. But, for the sawing experiments activity related carbon-rich particles were observed as spherical conglomerates with sizes between

100 nm and 20 micrometer, which included either MWCNT or OP. These results can be extrapolated to the filters collected during other experiments which were not analyses, as the morphology, chemical composition and size of emitted particles are expected to be comparable. The smooth surface of the observed particles can be explained by heat production of the bandsaw table which (partly) melted the abrasively treated objects. Figure 1 shows four examples of the observed activity-related particles for the sawing experiments. Fragments of the OP containing HDPE matrix are shown in Figures 1A and B, while MWCNTs containing PU matrix fragments are shown in Figures 1C and D.

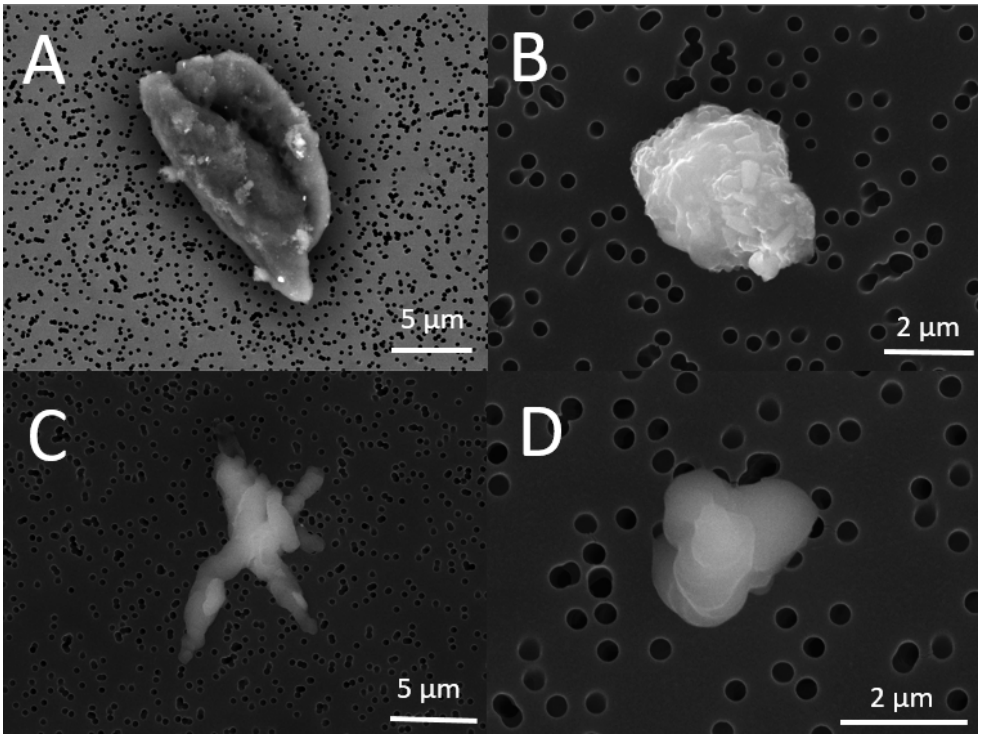

Figure 1: Four examples of activity related released matrix particles for sawing, with a smooth surface morphology, (partly) melted due to the bandsaw table. In 1A and 1B organic pigment (OP) containing high density polyethylene (HDPE) matrix fragments, in 1C and 1D MWCNT containing polyurethane (PU) matrix fragments.

Activity-effect estimates

During the drilling experiments, relatively low particle number concentrations were measured (DiSCmini, < 800 #/cm^3) as compared to background concentrations (100 - 200 #/cm^3), while no (visual) differences were observed in particle size distributions (10 nm - 20 µm; SMPS, APS, ELPI(+)). For the sawing experiments relatively high particle number concentrations were found (DiSCmini, up to 1.2E6 #/cm^3) as compared to comparable background levels (100 - 200 #/cm^3). Visual differences in particle size distributions were

observed (based on comparing the individual graphs) in the SMPS, APS and ELPI (+) data with more detected particles between 10 - ~200 nm compared to background concentrations.

Individual results of the experiments (geometric mean (GM) and geometric standard deviation (GSD) and the results of the statistical analyses of the individual experiments (stage 1) are available in the supplementary information (Table S1-4). The regression models (second stage) for drilling activities showed no significant relation between number concentrations, particle size distributions and the studied determinants (type of car bumper, machine settings) and corresponding variables (red HDPE matrix with 10 wt. % OP vs. black PU matrix with 0.09 wt. % MWCNT, drill size 4 mm vs. 8 mm) in the near field (Table 2a) and the far field (Table 2b). Significant differences in particle number concentrations and particle size distributions were found for the sawing experiments in the near field (Table 3a) and the far field (Table 3b). Regarding the number concentrations a higher sawing speed (880 m/min vs. 410) resulted in significantly higher concentrations with GMRs of 59 ($p = <0.01$) and 22 ($p = <0.01$) in NF and FF, respectively. In the FF a significantly higher concentration was found for the car bumpers containing OP compared to the ones containing MWCNTs (GMR = 0.45, $P = 0.02$), but results were not statistically significant in the NF although the direction of the effect was similar (GMR = 0.92, $p=0.81$). General room ventilation at 3.5 ACH reduced the particle number concentration in air when the ventilation was active, but results were only borderline statistically significant (GMR 0.53, $p = 0.06$). Regarding the particle size distributions, the ELPI + showed smaller particles for higher sawing speed in the NF (GMR = 0.92, $P = 0.01$), while in the FF larger particles were found for MWCNT containing car bumpers (GMR = 1.09, $P < 0.01$) and higher sawing speed (GMR = 1.08, $P < 0.01$), and smaller particles with ventilation (GMR = 0.86, $P < 0.01$).

Table 2: Activity-effect estimates for drilling experiments with in A: near field (NF) and in B: far field (FF) results. In bold markers determinants ($p < 0.05$) associations with air concentrations.

A: NF instruments	Determinant	GMR	SE	p-value
Number concentration	Material: MWCNTs vs. OP	1.02	1.41	0.94
DiSCmini: 10 – 300 nm	Drilling size: 4mm vs. 8 mm	0.75	1.41	0.40
Particle size	Material: MWCNTs vs. OP	1.14	3.61	0.92
SMPS, 11.3 – 514 nm	Drilling size: 4mm vs. 8 mm	1.03	3.53	0.98
Particle size	Material: MWCNTs vs. OP	1.06	1.09	0.49
APS, 500 nm – 20 µm	Drilling size: 4mm vs. 8 mm	0.98	1.09	0.85
Particle size	Material: MWCNTs vs. OP	1.03	1.03	0.25
ELPI +, 6 nm – 10 µm	Drilling size: 4mm vs. 8 mm	1.02	1.03	0.43

B: FF instruments	Determinant	GMR	SE	p-value
Number concentration	Material: MWCNTs vs. OP	0.86	1.15	0.28
	Drilling size: 4mm vs. 8 mm	1.08	1.15	0.57
DiSCmini: 10 – 300 nm				
Particle size	Material: MWCNTs vs. OP	0.86	4.15	0.92
	Drilling size: 4mm vs. 8 mm	1.01	4.18	0.99
SMPS, 11.3 – 514 nm				
Particle size	Material: MWCNTs vs. OP	0.95	1.10	0.59
	Drilling size: 4mm vs. 8 mm	1.04	1.10	0.71
APS, 500 nm – 20 µm				
Particle size	Material: MWCNTs vs. OP	0.93	1.04	0.09
	Drilling size: 4mm vs. 8 mm	0.99	1.04	0.87
ELPI, 7 nm – 10 µm				

Table 3: Activity-effect estimates for sawing experiments with in A: near field (NF) and in B: far field (FF) results. In bold markers determinants (p < 0.05) associations with air concentrations.

A: Instrument	Determinant	GMR	SE	p-value
Number concentration	Material: MWCNTs vs. OP	0.92	1.41	0.81
	Sawing speed: 410 vs. 880 m/min	**58.73**	**1.41**	**<0.01**
DiSCmini: 10 – 300 nm	Ventilation: 0 ACH vs. 3.5 ACH	0.53	1.41	0.06
Particle size	Material: MWCNTs vs. OP	0.86	2.18	0.85
	Sawing speed: 410 vs. 880 m/min	0.77	2.12	0.73
SMPS, 11.3 – 514 nm	Ventilation: 0 ACH vs. 3.5 ACH	0.96	2.13	0.96
Particle size	Material: MWCNTs vs. OP	0.98	1.04	0.64
	Sawing speed: 410 vs. 880 m/min	0.95	1.04	0.23
APS, 500 nm – 20 µm	Ventilation: 0 ACH vs. 3.5 ACH	1.01	1.04	0.72
Particle size	Material: MWCNTs vs. OP	0.91	1.05	0.06
	Sawing speed: 410 vs. 880 m/min	**1.15**	**1.05**	**0.01**
ELPI +, 6 nm – 10 µm	Ventilation: 0 ACH vs. 3.5 ACH	0.96	1.05	0.45

B: Instrument	Determinant	GMR	SE	p-value
Number concentration	**Material: MWCNTs vs. OP**	**0.45**	**1.42**	**0.02**
	Sawing speed: 410 vs. 880 m/min	**22.07**	**1.43**	**<0.01**
DiSCmini: 10 – 300 nm	Ventilation: 0 ACH vs. 3.5 ACH	0.74	1.42	0.39
Particle size	Material: MWCNTs vs. OP	1.06	2.16	0.94
	Sawing speed: 410 vs. 880 m/min	0.85	2.16	0.84
SMPS, 11.3 – 514 nm	Ventilation: 0 ACH vs. 3.5 ACH	1.15	2.18	0.86
Particle size	Material: MWCNTs vs. OP	0.96	1.04	0.28
	Sawing speed: 410 vs. 880 m/min	0.94	1.04	0.11
APS, 500 nm – 20 µm	Ventilation: 0 ACH vs. 3.5 ACH	1.02	1.04	0.57
Particle size	**Material: MWCNTs vs. OP**	**0.92**	**1.02**	**<0.01**
	Sawing speed: 410 vs. 880 m/min	**1.08**	**1.02**	**<0.01**
ELPI, 7 nm – 10 µm	**Ventilation: 0 ACH vs. 3.5 ACH**	**0.86**	**1.02**	**<0.01**

Discussion

This study provides a comprehensive overview of simulated occupational sawing and drilling in car bumpers containing either OP or MWCNTs, testing the effect of different variables on the concentration levels measured in the NF and FF, which included machine settings and mechanical ventilation. The controlled experimental environment, no disturbing particle emissions from the sawing and drilling machines, a consistently used method (in triplicate) of data collection and the detailed statistical analyses, allowed to study the contribution of potential determinants of the release of MNO both in the NF and FF. SEM/EDX analyses of filters collected during sawing revealed (partly melted) carbon-rich particles as spherical conglomerates (100 nm – 20 micrometer), while for drilling no activity related particles were observed. A higher sawing speed significantly contributed to more particles released, both in NF and FF. Ventilation reduced the particle number concentration and shifted the particle size distribution to the right when the ventilation was active, but results were only significant for the particle size distribution in the FF. The car bumpers containing MWCNTs showed fewer released particles for the sawing experiments, but only significant in the FF.

In two other comparable drilling experiments, different composites were investigated, which included nanosized silica, nanoclay, microsized polyamide and polypropylene composites.[20, 29] In both studies a hand held Makita angle drill was situated outside of the experimental testing room. Although the authors found large differences in dust generation between the different tested composites, the results were not conclusive on the effect of nanoparticles on the tensile stress of the composites and the type(s) of released particles explaining the differences in generated particles. Bello et al.[19] studied exposures to nanoparticles and nanofibers during drilling of two types of hybrid composites containing aluminum or carbon fibers and CNT. This study revealed airborne clusters of CNTs and ultrafine (< 5 nm) aerosols due to thermal degradation of the composite material. Both higher input energy and the type of the composite were identified as an important exposure modifying factor, with CNT-composites generally having a tendency to release less particles. The authors recommended additional work for a better understanding of the contributing particles on the total particles released. In contrast, in the present study we showed no significant release of airborne particles during drilling activities. Although a subtle effect on particle release due to drilling activities cannot be excluded, no concentrations up to 2E5 #/cm^3 were found, as observed by the previous studies.[19 20, 29] These contradicting results can be explained by differences in drilling methods with the settings of the machines (e.g. drill size and drill speed) and the characteristics of composites which contained MNO (e.g. the thickness of the sample drilled, tensile stress).

Sawing activities at the workplace were measured in two different studies [17, 18] with exposure to NOAA of 2000 and 3000 #/cm^3, respectively. In two experimental studies sawing activities were simulated.[11,15] Bello [11] evaluated sawing using hybrid advanced composites which included CNTs, but did not observe CNTs or bundles of fibers. Ultrafine particles were

detected, which were correlated to the different matrix thicknesses tested. Gomez et al.[15] studied the particle release effect of sawing with epoxy and paint nanocomposites which included CNTs. The sawing tests were performed with a Skil Masters (4585) jig saw. The different number concentrations with ELPI varied between 1.2E6 and 1.6E6 #/cm^3 which the authors explained by different hardness of the testing materials and subsequent differences in the motor load of the saw. These experimental studies support our data, but the relatively low concentrations found in the actual workplace deviate from our findings. Where Methner et al.[17] and Bekker et al.[18] performed personal measurements, the experimental studies including ours used stationary measurements in the NF and FF. These deviations may be explained by differences in the sawed material, the sawing machine and the circumstances at the workplace which is normally not comparable to a testing room.

In the present research we showed the effect of potential exposure determinants. Energy level (sawing speed) had a significant effect on both the particle number concentration and the particle size distribution. Ding and Riediker[30] evaluated the stability of particles with increasing energy levels, using different types of nanomaterials. An increasing energy applied to the materials was related to higher number concentrations and smaller airborne particles (11– 1083 nm), which was in agreement with our findings. Although not always significantly different, mechanical ventilation showed lower concentrations when comparing 3.5 to 0 ACH. The effect of mechanical ventilation as an exposure control for chemical substances was studied and summarized in ECEL and varied between a 35 and 83% reduction.[31] The authors are not aware of specific information on the mitigation of exposure to nanomaterials using mechanical ventilation. In the present study the (non-significant) effect for sawing was 47% in the NF (GMR=0.53, P = 0.06) and 26% in the FF (GMR=0.74, P = 0.39). However, such values are strongly dependent on room size and the effective speed of mixing of air in the study area.

Studying the release of NOAA due to abrasive activities on MNO containing materials in relation to real workplace situations will continue to be challenging as a large variation exist in treated materials, processes and circumstances at the workplace, subsequently influencing potential exposure. Taking this into consideration, the strengths of this study are the use of tools with no particle emissions potentially disturbing the experiments, the relative large number of experiments and the thorough statistical analyses. In addition, the reproducibility and repeatability of the results were good comparing the individual results of the three experiments performed in triplicate (see Supplementary information S1-4). For release rate measurements it is preferred to have direct sampling of the emission from the source or homogeneously mixed air from the source. The actual release rates in the present study can only be backwards modelled and currently represent concentrations levels.[8] Although experiments studying the release rates are relevant for modeling the human and environmental risks, the derived effects of the different determinants in the present study are directly applicable to the actual workplace. Also, the effect of ventilation on nanoparticle concentrations in air cannot be tested with release measurements, but needs more

quantitative data as its effectiveness for NOAA is currently only limited tested.[32] The three instruments we used for the particle size distribution (SMPS, APS, ELPI(+)) varied in the measured size range, response time and working mechanism, which resulted in different outcomes. Ideally, these instruments show similar trends and it should be possible to compare results and be conclusive about the outcomes. However, no significant results were found for the APS as the measurement range is 0.5–20 µm, while mostly small particles were detected. In addition, the SMPS had a response time of one minute, meaning only a limited number of data were collected per experiment which resulted in relatively large SEs. Due to the advantages and disadvantages of each instrument, a large set of direct reading instruments is still recommended in future research. In addition, offline analyses are needed as none of the currently available direct reading instruments are specific and distinguishes between particle types (e.g. process and non-process related nanoparticles).

In conclusion, the experiments in this study give a first indication of the effect of the evaluated determinants on NOAA release during sawing and drilling in polymer car bumpers and consequently on the potential worker exposure. An increase in the energy level of the abrasive activity results in melted carbon-rich particles during sawing, but the added nanomaterials were not liberated from matrix. Mechanical ventilation was (somewhat) effective in the reduction of exposure, while no differences in release were found for car bumpers with different MNO of the same tensile stress. Although the present study was conducted in an experimental setting, the outcomes are translatable to similar processes in a non-experimental setting like the workplace or a consumer scenario. As the toxicological properties of the released particles are still unknown, future efforts are needed to properly protect workers, which should aim for realistic risk assessments not only focused on the pristine engineered particles.

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Supporting information

Figure SI-1: Schematic layout of experimental setup including position of monitors SMPS, APS, ELPI(+), DISCmini (DM) and sampler (IOM).

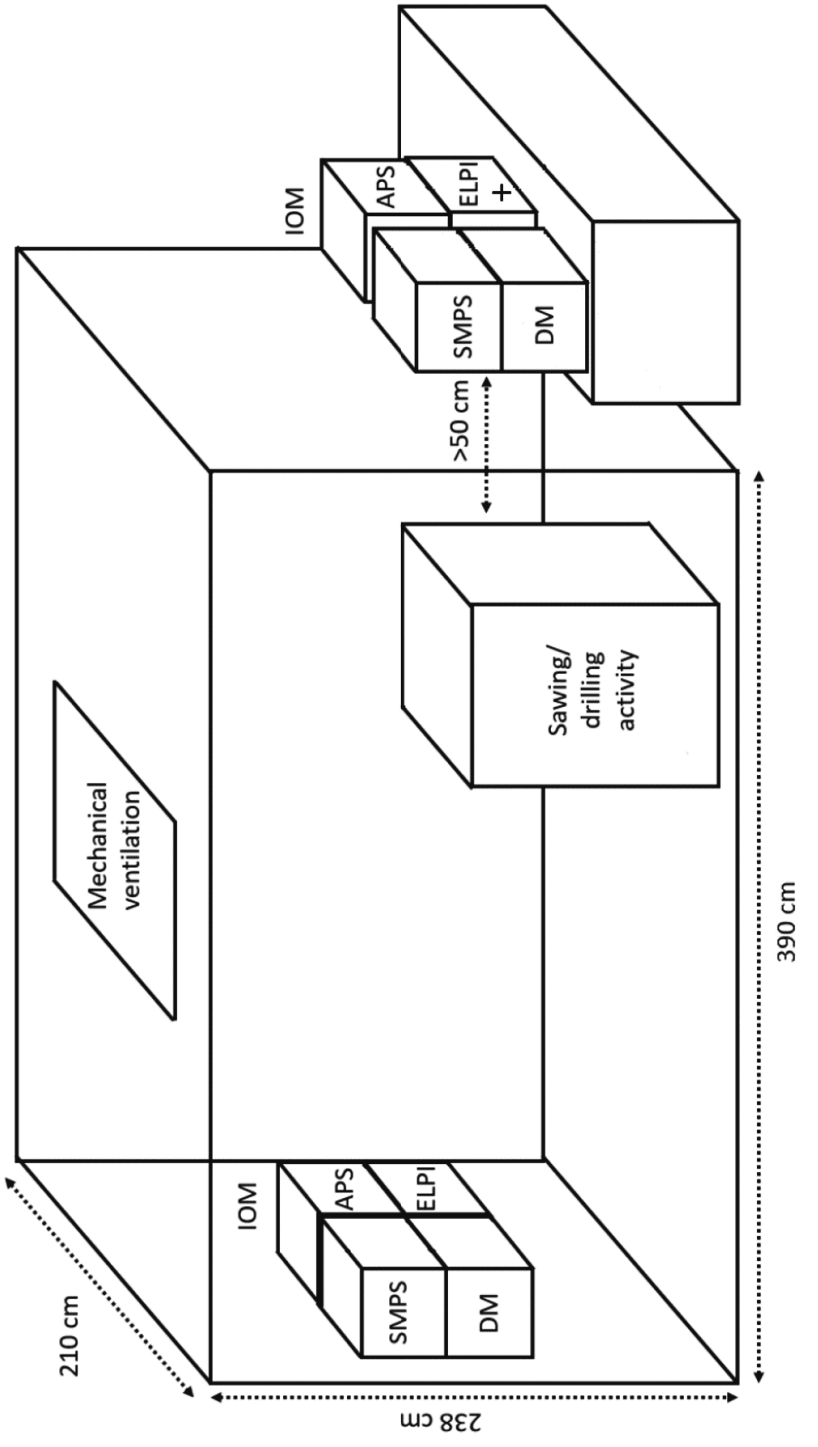

Table SI-1: Original results of the particle number concentration per experiment (GM and GSD) and results of the first stage analyses for DiSCmini estimated β and SE (log-transformed) of the activity-effect level (background corrected) and determinants.

	Activity-effect level NF				Activity-effect level FF				Exposure determinants			
Exp. No.	GM (#/cm^3)	GSD	Mean (β)	Standard error (SE)	GM (#/cm^3)	GSD	Mean (β)	Standard error (SE)	Activity	Car bumper	Settings machine	Mechanical ventilation
1	2.67	2.08	0.01	0.13	0	2.11	-0.39	0.05	Drilling	MWCNT	Drill size 4 mm	0 ACH
2	29.86	1.56	0.08	0.17	0	1.53	-0.33	0.16	Drilling	MWCNT	Drill size 4 mm	0 ACH
3	20.66	1.75	0.01	0.18	0	2.09	-0.32	0.10	Drilling	MWCNT	Drill size 4 mm	0 ACH
4	127.45	1.41	0.48	0.12	0	1.35	0.08	0.18	Drilling	MWCNT	Drill size 8mm	0 ACH
5	96.92	1.38	0.37	0.15	0	1.72	-0.12	0.15	Drilling	MWCNT	Drill size 8mm	0 ACH
6	65.52	1.78	0.33	0.04	0	2.27	-0.05	0.24	Drilling	MWCNT	Drill size 8mm	0 ACH
7	61.4	2.94	0.56	0.36	0	1.07	-0.02	0.04	Drilling	OP	Drill size 4 mm	0 ACH
8	87.71	1.9	0.78	0.22	0.17	1.14	-0.01	0.05	Drilling	OP	Drill size 4 mm	0 ACH
9	87.64	2.15	0.79	0.29	3.48	1.21	0.14	0.07	Drilling	OP	Drill size 4 mm	0 ACH
10	1.01	2.09	0.03	0.30	0.34	1.13	0.02	0.03	Drilling	OP	Drill size 8mm	0 ACH
11	66.12	1.98	0.34	0.23	0	1.08	-0.12	0.04	Drilling	OP	Drill size 8mm	0 ACH
12	0	1.71	-10.67	0.04	0	1.19	-0.27	0.01	Drilling	OP	Drill size 8mm	0 ACH
13	16978.69	2.48	32.11	0.26	2099.78	2.34	39.64	0.46	Sawing	MWCNT	Sawing speed 410m/min	3.5 ACH
14	20138.56	2.55	35.10	0.25	2487.85	1.56	41.39	0.39	Sawing	MWCNT	Sawing speed 410m/min	3.5 ACH
15	16551.69	1.7	35.41	0.05	2984.11	1.57	46.54	0.26	Sawing	MWCNT	Sawing speed 410m/min	3.5 ACH
16	11946.99	2.6	3.65	0.31	3169.64	2.12	31.29	0.38	Sawing	MWCNT	Sawing speed 880m/min	0 ACH
17	19107.07	1.4	42.49	0.14	3643.5	2.38	32.00	0.42	Sawing	MWCNT	Sawing speed 880m/min	0 ACH
18	17698.80	2.12	42.36	0.22	3736.35	1.85	33.57	0.33	Sawing	MWCNT	Sawing speed 880m/min	0 ACH

19	18223.09	2.72	3.67	0.40	5646.08	2.47	48.20	0.38	Sawing	MWCNT	Sawing speed 410m/min	0 ACH
20	28448.28	1.75	45.40	0.18	4394.86	2.59	49.93	0.28	Sawing	MWCNT	Sawing speed 410m/min	0 ACH
21	28062.63	1.35	45.31	0.12	3927.38	1.88	45.86	0.25	Sawing	MWCNT	Sawing speed 410m/min	0 ACH
22	30936.69	1.34	44.29	0.13	4076.39	2.1	42.49	0.54	Sawing	MWCNT	Sawing speed 880m/min	3.5 ACH
23	27353.53	2.38	41.73	0.29	5551.38	2.6	48.69	0.20	Sawing	MWCNT	Sawing speed 880m/min	3.5 ACH
24	27864.91	1.43	43.13	0.15	8437.64	1.65	55.11	0.18	Sawing	MWCNT	Sawing speed 880m/min	3.5 ACH
25	575611.7	1.78	79.40	0.49	109121.6	8.77	80.29	0.75	Sawing	OP	Sawing speed 410m/min	3.5 ACH
26	652493.9	2.02	80.85	0.50	203920.1	2.52	86.08	0.40	Sawing	OP	Sawing speed 410m/min	3.5 ACH
27	318540.5	5.11	69.00	0.72	144732.8	3.07	79.12	0.58	Sawing	OP	Sawing speed 410m/min	3.5 ACH
28	225283.5	2.88	68.58	0.41	35464.74	2.58	62.03	0.41	Sawing	OP	Sawing speed 880m/min	0 ACH
29	249832.9	2.06	71.42	0.16	54626.25	2.77	67.05	0.44	Sawing	OP	Sawing speed 880m/min	0 ACH
30	279657.5	1.64	72.88	0.20	53522.89	3	68.53	0.44	Sawing	OP	Sawing speed 880m/min	0 ACH
31	489647.7	1.57	81.45	0.25	91953.57	3.44	73.36	0.48	Sawing	OP	Sawing speed 410m/min	0 ACH
32	422143	3.74	78.49	0.48	75687.66	4.44	67.55	0.63	Sawing	OP	Sawing speed 410m/min	0 ACH
33	609705.8	1.35	83.68	0.24	105585.7	3.23	72.38	0.58	Sawing	OP	Sawing speed 410m/min	0 ACH

34	307012.6	1.9	94.84	0.23	97956.56	2.21	77.06	0.20	Sawing	OP	Sawing speed 880m/min	3.5 ACH
35	254985.8	1.88	92.25	0.25	63998.92	3.2	75.99	0.46	Sawing	OP	Sawing speed 880m/min	3.5 ACH
36	386476.6	2.31	96.13	0.29	103463.5	3.49	77.17	0.62	Sawing	OP	Sawing speed 880m/min	3.5 ACH

GM: Geometric mean, GSD: Geometric standard deviation, NF: near field, FF: far field, OP: organic pigment, MWCNT: multi-walled carbon nanotubes, ACH: air change per hour

Table SI-2: Original results per experiment (GM and GSD) of the particle size and results of the first stage analyses for SMPS estimated β and SE (log-transformed) of the activity-effect level (background corrected) and determinants.

	Activity-effect level NF				**Activity-effect level FF**				**Exposure determinants**			
Exp. No.	GM (nm)	GSD	Mean (β)	Standard error (SE)	GM (nm)	GSD	Mean (β)	Standard error (SE)	Activity	Car bumper	Settings machine	Mechanical ventilation
1	58.77	1.03	-0.13	1.79	63.75	1.08	0.19	1.17	Drilling	MWCNT	Drill size 4 mm	0 ACH
2	60.04	1.02	0.06	0.94	64.98	1.09	-0.09	1.47	Drilling	MWCNT	Drill size 4 mm	0 ACH
3	61.61	1	-0.43	1.59	59.77	1	-1.19	1.91	Drilling	MWCNT	Drill size 4 mm	0 ACH
4	56.22	1.08	-0.26	1.08	60.15	1.06	0.05	1.54	Drilling	MWCNT	Drill size 8mm	0 ACH
5	59.71	1.05	0	1.56	58.40	1.04	-0.07	1.45	Drilling	MWCNT	Drill size 8mm	0 ACH
6	56.81	1	-0.66	2.21	57.42	1	-0.62	2.2	Drilling	MWCNT	Drill size 8mm	0 ACH
7	55.48	1.01	0.11	1.31	56.10	1.02	-0.15	1.5	Drilling	OP	Drill size 4 mm	0 ACH
8	53.29	1.01	0.09	1.17	56.42	1.04	0.17	1.8	Drilling	OP	Drill size 4 mm	0 ACH
9	58.82	1	-0.73	1.61	52.88	1	-0.45	1.92	Drilling	OP	Drill size 4 mm	0 ACH
10	51.17	1.04	0	1.47	52.09	1.05	-0.14	1.11	Drilling	OP	Drill size 8mm	0 ACH
11	50.47	1.09	0.19	1.18	50.66	1	-0.3	1.75	Drilling	OP	Drill size 8mm	0 ACH
12	-	-	-	-	-	-	-	-	Drilling	OP	Drill size 8mm	0 ACH
13	38.33	1.72	-0.71	1.39	35.22	1.89	-0.58	1.36	Sawing	MWCNT	Sawing speed 410m/min	3.5 ACH
14	31.57	1.53	-0.46	1.28	31.65	1.58	-0.61	0.57	Sawing	MWCNT	Sawing speed 410m/min	3.5 ACH
15	34.91	1.47	-0.51	1.18	33.06	1.56	-0.44	0.91	Sawing	MWCNT	Sawing speed 410m/min	3.5 ACH

16	37.92	1.61	-0.37	1.1	31.95	1.58	-0.62	0.64	Sawing	MWCNT	Sawing speed 880m/min	0 ACH
17	34.68	1.64	-0.61	1.34	33.22	1.59	-0.51	1.23	Sawing	MWCNT	Sawing speed 880m/min	0 ACH
18	48.10	1	-0.9	2.08	48.50	1	-0.75	2	Sawing	MWCNT	Sawing speed 880m/min	0 ACH
19	32.72	1.76	-0.62	1.15	32.36	1.78	-0.63	1.34	Sawing	MWCNT	Sawing speed 410m/min	0 ACH
20	30.88	1.65	-0.73	0.89	30.38	1.55	-0.5	1.21	Sawing	MWCNT	Sawing speed 410m/min	0 ACH
21	24.44	1.14	-0.24	1.39	43.72	1	-0.82	1.82	Sawing	MWCNT	Sawing speed 410m/min	0 ACH
22	34.75	1.77	-0.48	1.45	31.72	1.76	-0.63	1.69	Sawing	MWCNT	Sawing speed 880m/min	3.5 ACH
23	30.22	1.64	-0.72	0.89	30.65	1.77	-0.61	1.46	Sawing	MWCNT	Sawing speed 880m/min	3.5 ACH
24	51.93	1	-0.8	1.55	31.13	1.77	-0.88	1.25	Sawing	MWCNT	Sawing speed 880m/min	3.5 ACH
25	28.45	1.91	-0.84	1.15	29.31	2.04	-0.86	1.38	Sawing	OP	Sawing speed 410m/min	3.5 ACH
26	22.69	1.38	-0.55	1.27	25.33	1.61	-0.84	0.94	Sawing	OP	Sawing speed 410m/min	3.5 ACH
27	22.25	1.06	-0.48	1.46	21.41	1	-0.96	1.72	Sawing	OP	Sawing speed 410m/min	3.5 ACH
28	34.03	2.39	-1.13	1.09	33.58	2.32	-0.75	1.68	Sawing	OP	Sawing speed 880m/min	0 ACH
29	29.84	2.10	-1.26	1.24	18.20	1.01	-0.35	1.01	Sawing	OP	Sawing speed 880m/min	0 ACH
30	24.06	1.50	-1	1.01	17.94	1	-0.67	1.67	Sawing	OP	Sawing speed 880m/min	0 ACH
31	58	1.16	-0.89	1.59	62.56	1.06	-0.53	1.46	Sawing	OP	Sawing speed 410m/min	0 ACH
32	25.97	1.53	-0.95	1.2	28.93	1.93	-0.67	1.02	Sawing	OP	Sawing speed 410m/min	0 ACH
33	21.55	1.29	-0.56	1.29	27.05	1.75	-1.05	1.05	Sawing	OP	Sawing speed 410m/min	0 ACH
34	32.58	2.31	-0.87	1.33	34.84	2.56	-1.18	0.9	Sawing	OP	Sawing speed 880m/min	3.5 ACH
35	27.84	1.87	-0.85	1.1	27.18	1.81	-0.67	1.13	Sawing	OP	Sawing speed 880m/min	3.5 ACH
36	24.83	1.60	-0.85	1.35	25.39	1.60	-0.93	1.15	Sawing	OP	Sawing speed 880m/min	3.5 ACH

GM: Geometric mean, GSD: Geometric standard deviation, NF: near field, FF: far field, OP: organic pigment, MWCNT: multi-walled carbon nanotubes, ACH: air change per hour.

Table SI-3: Original results per experiment (GM and GSD) of the particle size and results of the first stage analyses for APS estimated β and SE (log-transformed) of the activity-effect level (background corrected) and determinants.

	Activity-effect level NF				Activity-effect level FF				Exposure determinants			
Exp. No.	GM (μm)	GSD	Mean (β)	Standard error (SE)	GM (μm)	GSD	Mean (β)	Standard error (SE)	Activity	Car bumper	Settings machine	Mechanical ventilation
1	1.04	1.06	-0.03	0.11	1.28	1.07	-0.07	0.09	Drilling	MWCNT	Drill size 4 mm	0 ACH
2	0.96	1.04	-0.08	0.08	1.14	1.08	-0.17	0.09	Drilling	MWCNT	Drill size 4 mm	0 ACH
3	0.99	1.02	-0.05	0.09	1.10	1.06	-0.19	0.11	Drilling	MWCNT	Drill size 4 mm	0 ACH
4	1.02	1.07	-0.02	0.11	1.19	1.08	0.02	0.1	Drilling	MWCNT	Drill size 8mm	0 ACH
5	0.98	1.08	-0.03	0.12	1.02	1.05	-0.12	0.09	Drilling	MWCNT	Drill size 8mm	0 ACH
6	0.96	1.05	-0.04	0.09	1.00	1.09	-0.15	0.1	Drilling	MWCNT	Drill size 8mm	0 ACH
7	1.04	1.10	0.11	0.12	1.15	1.10	0.04	0.11	Drilling	OP	Drill size 4 mm	0 ACH
8	0.97	1.05	0.04	0.1	1.00	1.13	-0.12	0.1	Drilling	OP	Drill size 4 mm	0 ACH
9	0.90	1.07	-0.02	0.09	0.94	1.08	-0.13	0.14	Drilling	OP	Drill size 4 mm	0 ACH
10	1.04	1.07	0.02	0.11	1.14	1.09	-0.04	0.11	Drilling	OP	Drill size 8mm	0 ACH
11	0.86	1.08	-0.1	0.1	0.86	1.08	-0.11	0.14	Drilling	OP	Drill size 8mm	0 ACH
12	1.06	1.10	0.04	0.08	1.15	1.10	0	0.09	Drilling	OP	Drill size 8mm	0 ACH
13	1.06	1.03	-0.02	0.06	1.16	1.08	-0.12	0.06	Sawing	MWCNT	Sawing speed 410m/min	3.5 ACH
14	1.10	1.10	0.01	0.04	1.29	1.11	0.01	0.05	Sawing	MWCNT	Sawing speed 410m/min	3.5 ACH
15	1.04	1.05	-0.04	0.05	1.20	1.06	-0.07	0.04	Sawing	MWCNT	Sawing speed 410m/min	3.5 ACH
16	1.12	1.06	-0.13	0.06	1.35	1.09	-0.05	0.06	Sawing	MWCNT	Sawing speed 880m/min	0 ACH
17	1.08	1.05	-0.14	0.06	1.26	1.07	-0.11	0.06	Sawing	MWCNT	Sawing speed 880m/min	0 ACH
18	1.11	1.10	-0.12	0.05	1.32	1.11	-0.05	0.06	Sawing	MWCNT	Sawing speed 880m/min	0 ACH
19	1.10	1.04	0	0.05	1.26	1.07	0.01	0.05	Sawing	MWCNT	Sawing speed 410m/min	0 ACH
20	1.12	1.03	0.01	0.06	1.30	1.06	0.01	0.05	Sawing	MWCNT	Sawing speed 410m/min	0 ACH
21	1.12	1.04	0.02	0.05	1.28	1.05	0.01	0.05	Sawing	MWCNT	Sawing speed 410m/min	0 ACH
22	1.05	1.03	-0.03	0.06	1.16	1.07	-0.03	0.06	Sawing	MWCNT	Sawing speed 880m/min	3.5 ACH
23	1.16	1.06	0.07	0.06	1.33	1.08	0.07	0.06	Sawing	MWCNT	Sawing speed 880m/min	3.5 ACH

24	1.07	1.06	0	0.05	1.17	1.09	-0.05	0.06	Sawing	MWCNT	Sawing speed 880m/min	3.5 ACH
25	1.14	1.05	-0.03	0.06	1.27	1.04	-0.07	0.06	Sawing	OP	Sawing speed 410m/min	3.5 ACH
26	1.04	1.05	-0.15	0.05	1.15	1.05	-0.14	0.06	Sawing	OP	Sawing speed 410m/min	3.5 ACH
27	1.09	1.04	-0.1	0.05	1.27	1.09	-0.04	0.05	Sawing	OP	Sawing speed 410m/min	3.5 ACH
28	1.01	1.03	-0.05	0.05	1.07	1.05	-0.03	0.06	Sawing	OP	Sawing speed 880m/min	0 ACH
29	0.97	1.04	-0.12	0.07	1.11	1.08	-0.14	0.08	Sawing	OP	Sawing speed 880m/min	0 ACH
30	1.00	1.05	-0.05	0.06	1.12	1.07	-0.02	0.06	Sawing	OP	Sawing speed 880m/min	0 ACH
31	0.99	1.04	-0.09	0.05	1.14	1.07	-0.07	0.06	Sawing	OP	Sawing speed 410m/min	0 ACH
32	0.93	1.06	-0.15	0.06	1.16	1.12	-0.08	0.06	Sawing	OP	Sawing speed 410m/min	0 ACH
33	0.95	1.05	-0.1	0.05	1.06	1.09	-0.12	0.07	Sawing	OP	Sawing speed 410m/min	0 ACH
34	1.06	1.05	-0.02	0.06	1.21	1.08	-0.08	0.06	Sawing	OP	Sawing speed 880m/min	3.5 ACH
35	0.98	1.04	-0.1	0.05	1.10	1.06	-0.17	0.05	Sawing	OP	Sawing speed 880m/min	3.5 ACH
36	1.03	1.07	0.01	0.05	1.13	1.12	-0.17	0.07	Sawing	OP	Sawing speed 880m/min	3.5 ACH

GM: Geometric mean, GSD: Geometric standard deviation, NF: near field, FF: far field, OP: organic pigment, MWCNT: multi-walled carbon nanotubes, ACH: air change per hour

Table SI-4: Original results per experiment (GM and GSD) of the particle size and results of the first stage analyses for ELPI estimated β and SE (log-transformed) of the activity-effect level (background corrected) and determinants.

	Activity-effect level NF				**Activity-effect level FF**				**Exposure determinants**			
Exp. No.	GM (nm)	GSD	Mean (β)	Standard error (SE)	GM (nm)	GSD	Mean (β)	Standard error (SE)	Activity	Car bumper	Settings machine	Mechanical ventilation
1	10.93	1.02	0	0.01	46.22	1.31	-0.03	0.02	Drilling	MWCNT	Drill size 4 mm	0 ACH
2	10.82	1.01	0.01	0.01	50.66	1.41	0.05	0.02	Drilling	MWCNT	Drill size 4 mm	0 ACH
3	11	1.10	-0.01	0.01	46.01	1.33	-0.04	0.02	Drilling	MWCNT	Drill size 4 mm	0 ACH
4	12.12	1.16	0.05	0.01	43.96	1.22	-0.27	0.02	Drilling	MWCNT	Drill size 8mm	0 ACH
5	11.95	1.06	0.03	0.000001	55.91	1.22	-0.02	0.02	Drilling	MWCNT	Drill size 8mm	0 ACH
6	12.15	1.09	0.05	0.01	42.84	1.16	-0.29	0.01	Drilling	MWCNT	Drill size 8mm	0 ACH
7	13.14	1.08	0.02	0.01	54.87	1.09	-0.04	0.01	Drilling	OP	Drill size 4 mm	0 ACH

8	12.78	1.09	-0.01	0.01	54.67	1.09	-0.05	0.01	Drilling	OP	Drill size 4 mm	0 ACH
9	15.63	1.29	0.19	0.01	57.33	1.08	-1E-06	0.01	Drilling	OP	Drill size 4 mm	0 ACH
10	-	-	-	-	33.48	1.06	-0.11	0.01	Drilling	OP	Drill size 8mm	0 ACH
11	-	-	-	-	50.95	1.10	0.31	0.01	Drilling	OP	Drill size 8mm	0 ACH
12	-	-	-	-	44.96	1.09	0.19	0.01	Drilling	OP	Drill size 8mm	0 ACH
13	10.68	1.04	0.08	0.01	50.91	1.19	0.01	0.01	Sawing	MWCNT	Sawing speed 410m/min	3.5 ACH
14	27.22	3.10	1.02	0.07	59.64	1.19	0.17	0.01	Sawing	MWCNT	Sawing speed 410m/min	3.5 ACH
15	10.09	1.02	0.02	0.000001	51.04	1.23	0.02	0.01	Sawing	MWCNT	Sawing speed 410m/min	3.5 ACH
16	10.52	1.04	0.05	0.000001	58.16	1.29	0.62	0.01	Sawing	MWCNT	Sawing speed 880m/min	0 ACH
17	10.66	1.05	0.06	0.000001	64.48	1.33	0.73	0.01	Sawing	MWCNT	Sawing speed 880m/min	0 ACH
18	13.55	1.60	0.29	0.03	46.47	1.30	0.4	0.01	Sawing	MWCNT	Sawing speed 880m/min	0 ACH
19	12.84	1.07	0.21	0.01	51.63	1.16	0.14	0.01	Sawing	MWCNT	Sawing speed 410m/min	0 ACH
20	17.91	1.26	0.54	0.02	56.18	1.07	0.22	0.01	Sawing	MWCNT	Sawing speed 410m/min	0 ACH
21	17.30	1.17	0.51	0.01	58.06	1.05	0.25	0.01	Sawing	MWCNT	Sawing speed 410m/min	0 ACH
22	21.81	1.28	0.72	0.01	56.31	1.20	0.46	0.01	Sawing	MWCNT	Sawing speed 880m/min	3.5 ACH
23	19.59	1.40	0.62	0.02	77.41	1.28	0.77	0.01	Sawing	MWCNT	Sawing speed 880m/min	3.5 ACH
24	21.59	1.37	0.72	0.02	73.55	1.24	0.72	0.01	Sawing	MWCNT	Sawing speed 880m/min	3.5 ACH

25	17.50	1.41	0.46	0.02	48.18	1.25	0.11	0.02	Sawing	OP	Sawing speed 410m/min	3.5 ACH
26	-	-	-	-	41.37	1.10	-0.05	0.01	Sawing	OP	Sawing speed 410m/min	3.5 ACH
27	15.41	1.23	0.07	0.01	44.54	1.18	0.03	0.01	Sawing	OP	Sawing speed 410m/min	3.5 ACH
28	11.95	1.02	0.05	0.01	49.22	1.19	0.46	0.01	Sawing	OP	Sawing speed 880m/min	0 ACH
29	10.62	10.07	0.01	0.00001	48.70	1.17	0.45	0.01	Sawing	OP	Sawing speed 880m/min	0 ACH
30	10.22	1.04	0.06	0.00001	35.83	1.11	0.15	0.01	Sawing	OP	Sawing speed 880m/min	0 ACH
31	10.21	1.02	0.07	0.01	45.17	1.17	-0.05	0.01	Sawing	OP	Sawing speed 410m/min	0 ACH
32	10.39	1.08	0.08	0.01	45.48	1.22	-0.04	0.02	Sawing	OP	Sawing speed 410m/min	0 ACH
33	10.49	1.03	0.11	0.00001	47.80	1.17	0.01	0.01	Sawing	OP	Sawing speed 410m/min	0 ACH
34	11.12	1.05	0.08	0.01	59.46	1.13	0.61	0.01	Sawing	OP	Sawing speed 880m/min	3.5 ACH
35	10.74	1.06	0.16	0.01	57.67	1.20	0.58	0.01	Sawing	OP	Sawing speed 880m/min	3.5 ACH
36	11.74	1.19	0.08	0.01	49.01	1.18	0.41	0.01	Sawing	OP	Sawing speed 880m/min	3.5 ACH

GM: Geometric mean, GSD: Geometric standard deviation, NF: near field, FF: far field, OP: organic pigment, MWCNT: multi-walled carbon nanotubes, ACH: air change per hour

Chapter 5

Cardiovascular effects among workers exposed to multi-walled carbon nanotubes

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Abstract

The increase in production of Multi-Walled Carbon Nanotubes (MWCNTs) has led to growing concerns about health risks. In this study, we assessed the association between occupational exposure to MWCNTs and cardiovascular biomarkers.

A cross-sectional study was performed among twenty-two workers of a company commercially producing MWCNTs (subdivided into lab personnel with low or high exposure and operators), and a gender and age-matched unexposed population (n=42). Exposure to MWCNTs and twelve cardiovascular markers were measured in participants' blood (phase 1). In a sub-population of thirteen exposed workers and six unexposed workers these measures were repeated after five months (phase 2). We analyzed associations between MWCNT exposure and biomarkers of cardiovascular risk, adjusted for age, BMI, sex and smoking.

We observed an upward trend in the concentration of endothelial damage marker ICAM-1, with increasing exposure to MWCNTs in both phases. The operator category showed significantly elevated ICAM-1 geometric mean ratios (GMR) compared to the controls (phase 1: GMR=1.40, p=1.30E-3; phase 2: GMR=1.37, p=0.03). The trends were significant both across worker categories (phase 1: p=1.50E-3, phase 2: p=0.01) and across measured geometric mean (GM) MWCNT concentrations (phase 1: p=3.00E-3, phase 2: p=0.01). No consistent significant associations were found for the other cardiovascular markers.

The associations between MWCNT exposure and ICAM-1 indicate endothelial activation and an increased inflammatory state in workers with MWCNT exposure.

Introduction

The Multi-Wall Carbon Nanotube (MWCNT) industry is growing due to the thermal, electrical, and mechanical properties of the material.[1, 2] Current production amounts are unknown, but due to the decreasing market price, MWCNTs are becoming a good alternative for the more conventional particulate carbon black filler, which has a production of about a million tons per year.[3] Consequently, the production of MWCNT is moving from R&D scale to larger scale production facilities, with more workers potentially exposed.[4]

Concerns have been raised about health risks due to MWCNT exposure.[5] Evidence from in vitro and animal studies have shown that MWCNTs can induce genetic lesions, oxidative stress, acute or persistent pulmonary inflammation, pulmonary fibrosis, cardiovascular effects, and for the rigid types of MWCNTs mesothelioma-like effects.[2, 6] Results from in vitro and in vivo studies clearly indicate the need for human epidemiological evidence. However, only a few relatively small studies have reported on health effects associated with exposure to MWCNT in humans.[7-12]

These studies gave some indications of early human effects of occupational exposure to MWNCTs. However, interpretation of the results is hindered due to the limited

characterization of the MWCNT exposure in many of these studies and due to the small number of exposed individuals included (generally less than 10). Reported biological effects of MWCNT in exposed humans include oxidative stress markers [9], lung inflammation markers [7, 8, 11] (including monocyte cell counts), and gene expression changes including messenger-RNA and non-coding RNA.[10] No associations between MWCNT exposure and lung function [9, 11] monocyte cell counts [9], and pneumoproteins [11] were found, which partly contradicts with the reported biological effects. Two biomarker studies specifically looked at cardiovascular endpoints among workers handling engineered nanomaterials including MWCNTs, and reported inconsistent results.[13, 14]

We previously reported on personal exposure levels to MWNCTs [15] and the association with immunological effects and lung health [11] among workers in a MWCNT production facility. Based on the same study population we here report the association between occupational exposure to MWCNTs and early cardiovascular effects. We hypothesize that exposure to MWCNTs, comparable to what is found in epidemiological studies on traffic related particulate matter (PM) [16] and more specifically ultrafine particles (UFP) [17], can induce cardiovascular effects. Based on Brook et al.[16] and several related papers, [18-21] cardiovascular biomarkers associated with PM exposure, were selected including endothelial activation and damage markers, systemic inflammatory markers and thrombosis and coagulation markers.

Methods

In 2013 a cross-sectional biomarker study was conducted in a company commercially producing MWCNTs. The control population was recruited from four different companies. One group of controls was working at the MWCNT producing company but not involved in producing or handling of MWCNTs (Controls A). In addition, controls were recruited from three different industries in the vicinity of the MWCNT production facility with no history of MWCNT production, handling or use. These included chemical controllers in a chemical plant not involved in production of MWCNTs, retail workers in a large warehouse, and health service employees in a health center (Controls B-D). The study design has been described previously in more detail.[11] Here, we briefly describe the methodology and specify deviations relevant to the reported cardiovascular endpoints.

Study population

For the cardiovascular markers, we recruited twenty-two exposed individuals and forty-two controls in June 2013 (phase 1). In October 2013 (phase 2), a subset of the phase 1 study population (and two additional highly exposed participants) were included resulting in thirteen exposed individuals and six matched controls. The number of participants slightly deviate from the study population described by Vlaanderen et al.[11], with three more non-exposed controls for both phases in the present study, as only data on cardiovascular markers was available for these participants. The study was approved by the Commission for Medical Ethics of UZ Leuven (reference number S54607).

Exposure assessment

Both study phases were preceded (the week before) by repeated personal exposure measurements in the exposed population. The exposure study has been reported previously by Kuijpers et al.[15] Shift-based (4-8h) personal breathing zones were sampled for the inhalable size fraction. The collected filters were analysed by scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDX), and elemental carbon (EC) analyses, according to a modified IMPROVE protocol.[22] In addition, individual daily work activities were recorded. These were used together with the quantitative exposure results to stratify the exposed population into three groups with increasing levels of exposure. i.e. lab low exposure, lab high exposure, and operators. To evaluate any indication of exposure-response, exposure was assigned by two different approaches, namely by trends across ordinal exposure categories (1, 2, 3, respectively) and by measured inhalable geometric mean (GM) MWCNT concentrations with different exposure levels for the operators in phase 1 and phase 2 (1, 7, and 45 or 57 μg/m^3 EC, respectively). The matched controls were assigned 0 μg/m^3 EC MWCNT exposure.

Questionnaires

During phase 1 participants completed a general health and lifestyle questionnaire that was validated within the ELON study.[23] Information collected included demographic information, general health, weight, length, respiratory health, asthma and allergies, complaints of the circulatory system, lifestyle factors including smoking and alcohol consumption, radiation exposure history, family medical history and work history. In addition, participants completed a questionnaire before biological sampling in both study phases, to obtain information on smoking, alcohol consumption, health, and medication use in the 24 hours before biological sampling.

Cardiovascular markers

Based on a literature search that identified relevant reviews,[16,18-21] several cardiovascular biomarkers were selected:1) endothelial activation and damage markers: intercellular adhesion molecule-1 (ICAM‑1), vascular cell adhesion molecule-1 (VCAM‑1), and E-selectin; 2) systemic inflammatory markers: interleukin 1-β and 6 (IL1-β and IL-6), tumor necrosis factor alpha (TNF-α), and transforming growth factor beta (TGF-β), C-reactive protein (CRP); and 3) thrombosis and coagulation markers: von Willebrand factor (vWF), plasminogen activator inhibitor-1 (PAI-1), tissue polypeptide antigen (tPA), fibrinogen, and fibrin degradation product (D-Dimer).

Blood samples for cardiovascular biomarker analyses were collected in EDTA vacutainer tubes before midday from each study participant while seated, using standard phlebotomy of venipuncture of forearm veins during both phases.

Biomarkers were determined by ELISA kits following the instructions of the manufacturer (R&D Systems, Minneapolis, MN, USA). Results were quantified with SoftMaxPro 5.4.5 ELISA

analyses software (Molecular Devices, Sunnyvale, CA, USA). Briefly, for every biomarker a standard calibration curve was generated using a set of calibration standards, and an optimal dilution was defined in pilot experiments. Thereafter, the plasma samples of the participants were assessed (single analysis) relative to the calibration curve. In addition, as part of a Luminex multi-analyte analyses for inflammatory markers we measured the C-reactive protein (CRP).[11] CRP is a marker of acute phase inflammation and associated with cardiovascular disease.[24]

In addition to the cardiovascular biomarker analyses, lipid profiles were quantified using a Roche-Hitachi cobas 8000 c702 Chemistry Autoanalyzer, to be able to correct for potential confounding of the cardiovascular marker levels. Total cholesterol and high-density lipoprotein (HDL) cholesterol were determined using colorimetric analysis (cholesterol oxidase (CHOD) / phenol and aminophenazone (PAP) method). Triglyceride was analysed using colorimetric analysis with glycerol-3-phosphate oxidase (GPO) instead of CHOD. Low-density lipoprotein (LDL) cholesterol was determined using the Friedewald equation.[25]

Statistical analyses

A censored regression approach (Tobit model) was used to account for information below the limit of detection. This method was applied separately for phases 1 and 2, assuming a lognormal distribution for the cardiovascular markers. We adjusted the analyses for potential confounding parameters: age, body mass index (BMI), smoking, and gender. We conducted categorical analysis comparing the different groups of exposed workers (operators, lab high, lab low) to non-exposed workers (controls). Furthermore, trends across exposed and non-exposed workers were assessed, based on their ordinal exposure ranking (values 0-3) and based on the actual assigned geometric mean exposure for each category (0,1, 7 and 45 $\mu g/m^3$ EC for phase 1; 0,1, 7 and 57 $\mu g/m^3$ EC for phase 2). Kruskal-Wallis tests (test if measurements from different exposure groups originated from same distribution) and Wilcoxon tests (test for significant difference between controls and operators) were performed for the most robustly associated markers, using a statistical significance cut-off p-value of 0.05.

We conducted several sensitivity analyses for phase 1 only, as power was more limited in phase 2. Sensitivity analyses included correcting separately for alcohol use (continuous), doctor diagnosed cardiovascular (yes/no), chronic (yes/no), inflammatory (yes/no) and metabolic (yes/no) diseases, statin use (yes/no), educational level (high, middle, low), recent infections (self-reported by participant) (yes/no), white blood cell count (continuous), cholesterol levels (continuous), and previous exposure to chemicals (yes/no), nanoparticles (yes/no), or particulates (yes/no). In addition, we tested if the associations were sensitive to misclassification of exposure by excluding laboratory workers that potentially had previous exposure as operator (n=2) and by excluding the manager of the operators (n=1). Furthermore, a jack-knifing technique was used to sequentially eliminate all controls from location A to D, smokers and (the limited number of participating) females from the analyses. All statistical analyses were performed in R version 3.1.2 (R Core Team 2014).

Results

Study population

Seven operators, six lab workers with high exposure, nine lab workers with low exposure and forty-two controls were included in phase 1, while in phase 2 nine operators, one lab worker with high exposure, three lab workers with low exposure, and six controls were included. A description of the demographic characteristics of the study population is given in Table 1. Operators were exclusively male with slightly higher age compared to other participants. A relatively high percentage of participants, in particular in the control group and the low exposed worker group, was diagnosed with a chronic disease. The population exposed to MWCNTs had a higher previous exposure to other particulates, chemicals and various nanoparticles than the controls. Most participants had completed high school and/or university and no differences were observed between groups in smoking habits, BMI and alcohol use.

Table 1: Demographic characteristics of the study population. Numbers in parenthesis represent the individuals or percentages of individuals that participated in phase 2.

	Controls[a]	Lab low[a]	Lab high[a]	Operators[a]
Participants (n)	42	9	6	7
	(6)	(3)	(1)	(9)
Median age	31.8	32.2	30.1	36.2
	(37.1)	(32.1)	(28)	(33.4)
Women (%)	24	33	17	0
	(0)	(0)	(0)	(0)
Median BMI	24.3	25.7	25.4	25.3
	(26.1)	(24.9)	(32.4)	(25.7)
Current / Former smoker (%)[b]	26/17	22/33	17/33	29/0
	(17/0)	(0/17)	(0/100)	(33/17)
Mean alcohol use last 24hr (n units)	1.2	0.7	0.3	0.6
	(0)	(0.3)	(0)	(0)
Diagnosed chronic disease (%)[c]	71	78	50	29
	(83)	(33)	(0)	(22)
Diagnosed cardiovascular disease (%)[d]	10	0	0	0
	(17)	(0)	(0)	(0)
Diagnosed inflammatory disease (%)[d]	45	78	33	0
	(33)	(33)	(0)	(0)

Diagnosed metabolic disease (%)[d]	**17**	**0**	**17**	**0**
	(0)	(0)	(0)	(0)
Education level Higher / Middle / Lower (%)[e]	43/55/2	89/11/0	83/17/0	29/43/0[f]
	67/33/0	100/0/0	100/0/0	22/44/11[f]
Previous exposure to chemicals (%)	19	44	33	29
	(50)	(33)	(0)	(22)
Previous exposure to nanoparticles (%)	0	78	83	71
	(0)	(67)	(100)	(56)
Previous exposure to particles (%)	5	0	17	43
	(17)	(0)	(0)	(44)
Exposure ranking	0	1	2	3
	(0)	(1)	(2)	(3)
Assigned GM EC exposure (µg/m3)	0	1	7	45
	(0)	(1)	(7)	(57)

[a] Controls: individuals not exposed to MWCNT, Lab low: Laboratory employees performing tasks with relatively low exposure, Lab high: Laboratory employees performing tasks with relatively high exposure and Operators: Working in the production area with high exposure. [b] The question about smoking divided the participant in current smokers, former smokers and participants who never smoked. [c] Chronic diseases includes autoimmune, hepatitis, rheumatoid arthritis, myocardial infarction, stroke, cardiac dysrhythmia, hypertension, diabetes, allergic, asthma, bronchitis, pulmonary emphysema, pneumonia, renal failure, eczema, other chronic diseases and cancer. [d] Subset of chronic diseases (c) with: Metabolic diseases are diabetes; Cardiovascular diseases includes myocardial infarction, stroke, cardiac dysrhythmia and hypertension and; Inflammatory diseases includes autoimmune, hepatitis, rheumatoid arthritis, allergic, asthma, bronchitis, pulmonary emphysema and eczema. [e] Higher education level is university, middle education is high school and lower education is elementary school. [f] Two participants did not complete the question about education.

Cardiovascular biomarkers

We measured 12 cardiovascular markers in participants' blood in both phases of the study. PAI-1 and IL1-β were excluded from statistical analyses as the majority of the measured concentrations were below the limit of detection (>60%). The median Pearson correlation coefficient between cardiovascular markers in phase 1 and phase 2 for participants measured in both phases (N=17) was 0.50 ranging from 0.32 to 0.95 (tPA 0.74, ICAM-1 0.59, VCAM-1 0.78, TGF-β 0.33, IL-6 0.34, E-selectin 0.91, TNF α 0.32, Fibrinogen 0.32, vWF 0.40, D-Dimer 0.95).

Both in phase 1 and phase 2 we observed an upward trend in the concentration of ICAM-1 with increasing exposure to MWCNTs (table 2a, table 2b, figure 1). The operator category showed significantly elevated ICAM-1 geometric mean ratios (GMR) compared to the controls (phase 1: GMR=1.40, p=1.30E-3; phase 2: GMR=1.37, p=0.03). The lab category with low

exposure showed a significantly elevated ICAM-1 GMR compared to the controls for phase 1 only (GMR=1.22, p=0.04). No significant associations were found for the lab category with high exposure (phase 1: GMR=1.09, p=0.45; phase 2: GMR=1.02, p=0.96). The trends were significant both across ordinal exposure categories (phase 1: p=1.50E-3, phase 2: p=0.01) and across measured GM MWCNT concentrations (phase 1: p=3.00E-3, phase 2: p=0.01).

Table 2: Difference in cardiovascular marker concentrations between workers exposed to MWCNT and controls in (a) phase 1 and (b) phase 2. Geometric mean ratio (GMR)1 estimates and p-values from Tobit regression, corrected for age, BMI, sex, and smoking. In bold markers significantly (p < 0.05) associations with MWCNT exposure.

Marker	Lab low	Lab high	Operators	Trend ranking (P value)	Trend GM (P value)
(a) Phase 1					
tPA (ng/mL)	1.14 (p=0.38)	1.22 (p=0.25)	0.91 (p=0.57)	0.89	0.53
Fibrinogen (mg/mL)	1.32 (p=0.28)	0.82 (p=0.51)	0.75 (p=0.30)	0.32	0.23
ICAM1 (ng/mL)	**1.22 (p=0.04)**	1.09 (p=0.45)	**1.40 (p=1.30E-3)**	**1.50E-3**	**3.00E-3**
VCAM1 (ng/mL)	1.16 (p=0.06)	1.07 (p=0.42)	1.09 (p=0.32)	0.17	0.43
vWF (ng/mL)	1.07(p=0.83)	1.31 (p=0.45)	1.34 (p=0.37)	0.27	0.35
IL 6 (pg/mL)	1.78 (p=0.76)	1.61 (p=0.36)	1.11 (p=0.63)	0.53	0.45
TGF- β (pg/ml)	1.13 (p=0.50)	1.01 (p=0.97)	**0.61 (p=0.02)**	0.66	0.43
E-selectin (ng/ml)	1.01 (p=0.31)	1.33 (p=0.39)	0.86 (p=0.32)	0.39	0.60
TNF-α (pg/ml)	0.80 (p=0.34)	1.03 (p=0.85)	0.75 (p=0.31)	0.22	0.13
D-Dimer (ng/mL)	0.79 (p=0.42)	1.07 (p=0.85)	1.39 (p=0.29)	0.39	0.23
CRP (ng/mL)[2]	0.85 (p=0.53)	**3.10 (p=3.00E-4)**	0.71 (p=0.20)	0.47	0.47
(b) Phase 2					
tPA (ng/mL)	1.14 (P=0.52)	0.89 (P=0.78)	0.85 (P=0.33)	0.25	0.18
Fibrinogen (mg/mL)	0.74 (P=0.11)	0.67 (P=0.27)	0.96 (P=0.77)	0.94	0.55
ICAM1 (ng/mL)	1.07 (P=0.68)	1.02 (P=0.96)	**1.37 (P=0.03)**	**0.01**	**0.01**
VCAM1 (ng/mL)	0.89 (P=0.49)	0.83 (P=0.61)	1.00 (P=0.97)	0.83	0.63
vWF (ng/mL)	0.83 (P=0.49)	**6.91 (P=6.30E-3)**	0.96 (P=0.87)	0.71	0.50
IL 6 (pg/mL)	1.13 (P=0.76)	0.46 (P=0.36)	1.17 (P=0.63)	0.53	0.45
TGF- β (pg/ml)	0.91 (P=0.68)	**4.46 (P=7.90E-3)**	0.96 (P=0.81)	0.66	0.43

E-selectin (ng/ml)	1.54 (P=0.31)	2.09 (P=0.39)	1.38 (P=0.32)	0.39	0.60
TNF-α (pg/ml)	0.78 (P=0.34)	1.12 (P=0.85)	1.26 (P=0.31)	0.22	0.13
D-Dimer (ng/mL)	1.01 (P=0.99)	0.49 (P=0.65)	1.76 (P=0.35)	0.25	0.19
CRP(ng/mL)[2]	1.53 (P=0.72)	1.13 (P=0.95)	0.97 (P=0.97)	0.76	0.84

[1] GMR is calculated using GM exposed population / GM non-exposed population. [2] Previously reported by Vlaanderen et al.[11] BMI, body mass index; CRP, C reactive protein; GMR, geometric mean ratio; ICAM-1, intercellular adhesion molecule-1; IL-6, interleukin-6; MWCNT, multiwalled carbon nanotube; TGF-β, transforming growth factor beta; TNF-α, tumour necrosis factor alpha; tPA, tissue polypeptide antigen; VCAM-1, vascular cell adhesion molecule-1; vWF, von Willebrand factor.

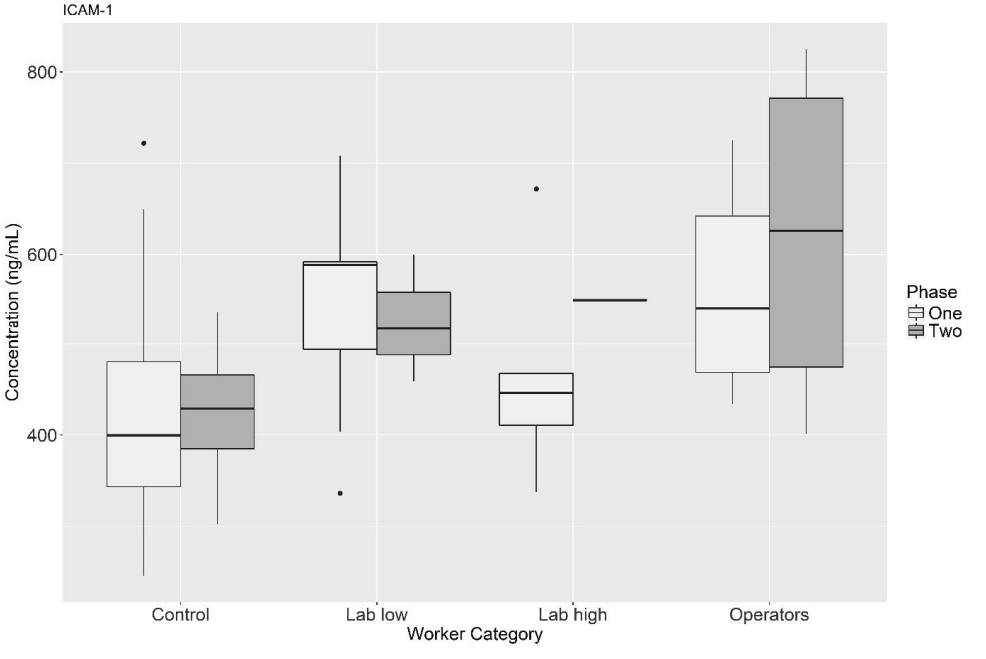

Figure 1: Boxplot showing the distribution of intercellular adhesion molecule-1 (ICAM-1) concentrations during phases 1 and 2 for every exposure category. Kruskal-Wallis rank-sum test P values were 4.60E-3 and 0.13, respectively for phases 1 and 2. Wilcoxon rank-sum test P values for operators versus controls were 6.36E-3 for phase 1 and 0.04 for phase 2.

In addition to the consistent significant finding for ICAM-1, other more incidental findings were observed. A significant decrease in TGF-β in the operators category was found compared to the control group during phase 1 (GMR=0.61, p=0.02) but not in phase 2. Furthermore, a significant increase was found for CRP in the lab category with high exposure compared to the control group during phase 1 (GMR=3.10, p=3.00E-4).[11] During phase 2, significant increases in cardiovascular markers compared to the control group were found for the lab

category with high exposure for TGF-β (GMR=4.46, p=7.90E-3) and for vWF (GMR=6.91, p=6.30E-3). No significant associations were identified for the other cardiovascular markers (tPA, Fibrinogen, VCAM-1, IL-6, E-selectin, TNF-α, and D-Dimer) with increasing exposure to MWCNTs.

Sensitivity analyses

Results for ICAM-1 were generally stable across sensitivity analyses (table 3), in which several potential confounders were separately included, participants with potentially misclassified exposure were excluded and the separate control groups, smokers and women were eliminated sequentially. Generally, in these sensitivity analyses, an increase in GMR was observed for laboratory workers with both low and high exposure and the operators, which was only significant among operators. In addition, trends across categories based on ranking and based on GM exposure were robust. Sensitivity analyses corrected for previous exposure to nanoparticles attenuated the results for ICAM-1 (Operators: GMR=1.16, p=0.49, trend ranking: p=0.68, trend GM: p=0.76). However, previous exposure was correlated to current exposure categories and therefore the two cannot be separated (Pearson R = 0.71). Loss of statistical significance ($p<0.05$) was also observed in analyses corrected for triglyceride (Operators: GMR=1.29, p=0.09, trend GM: p=0.12) and without controls from company B (Operators: GMR=1.33, p=0.079, trend GM: p=0.10) and D (Operators: GMR=1.32, p=0.09, trend ranking: p=0.09, trend GM: p=0.19), but GMRs remained essentially similar and p-values were generally around $p \sim 0.1$.

Table 3: Tobit regression estimates (GMR)[1] corrected for age, BMI, gender and smoking for ICAM-1 from a set of sensitivity analyses, assuming a lognormal distribution for the markers. In bold markers significantly ($p < 0.05$) associations with MWCNT exposure.

Sensitivity analysis	Lab low	Lab high	Operator	Trend ranking (P value)	Trend GM (P value)
Tobit regression. corrected for age, BMI, sex, and smoking (table 2a)	**1.22 (p=0.04)**	1.08 (p=0.45)	**1.40 (p=1.3E-3)**	**1.5E-3**	**3.0E-3**
Corrected for alcohol use	1.27 (p=0.09)	1.21 (p=0.16)	**1.38 (p=0.02)**	**6.4E-3**	**0.03**
Corrected for doctor diagnosed cardiovascular disease[2]	1.32 (p=0.06)	1.18 (p=0.20)	**1.37 (p=0.02)**	**0.01**	**0.04**
Corrected for doctor diagnosed chronic disease	1.32 (p=0.06)	1.21 (p=0.20)	**1.40 (p=0.02)**	**0.01**	**0.04**

Corrected for doctor diagnosed inflammatory disease	1.26 (p=0.06)	1.22 (p=0.14)	**1.40 (p=0.02)**	**7.1E-3**	**0.03**
Corrected for doctor diagnosed metabolic disease	**1.32 (p=0.04)**	1.18 (p=0.18)	**1.35 (p=0.02)**	**9.7E-3**	**0.04**
Corrected for educational level	1.34 (p=0.24)	1.32 (p=0.15)	1.38 (p=0.05)	**0.02**	**0.04**
Corrected for 'recent infection'	1.29 (p=0.06)	1.17 (p=0.21)	**1.35 (p=0.03)**	**0.01**	**0.04**
Corrected for white blood cell count	1.29 (p=0.07)	1.19 (p=0.25)	**1.35 (p=0.03)**	**0.01**	**0.04**
Corrected for previous exposure to chemicals	1.28 (p=0.07)	1.20 (p=0.17)	**1.35 (p=0.02)**	**9.0E-3**	**0.03**
Corrected for previous exposure to nanoparticles	1.18 (p=0.34)	1.06 (p=0.75)	1.16 (p=0.49)	0.68	0.76
Corrected for previous exposure to particulates	1.29 (p=0.07)	1.17 (p=0.22)	**1.35 (p=0.03)**	**0.01**	**0.04**
Corrected for total cholesterol	1.26 (p=0.09)	1.14 (p=0.33)	**1.38 (p=0.02)**	**0.01**	**0.02**
Corrected for HDL	1.31 (p=0.05)	1.16 (p=0.25)	**1.35 (p=0.02)**	**0.01**	**0.04**
Corrected for LDL	1.19 (p=0.21)	1.14 (p=0.30)	**1.42 (p=0.01)**	**5.0E-3**	**7.0E-3**
Corrected for triglyceride	1.30 (p=0.06)	1.16 (p=0.26)	1.29 (p=0.09)	**0.04**	0.12
Without laboratory workers that potentially had previous exposure as operator	1.29 (p=0.07)	1.24 (p=0.16)	**1.37 (p=0.03)**	**8.1E-3**	**0.04**
Without 'manager operators'	1.29 (p=0.07)	1.18 (p=0.21)	**1.38 (p=0.03)**	**0.01**	**0.04**
Without controls A[3]	1.29 (p=0.06)	1.17 (p=0.21)	**1.35 (p=0.03)**	**0.01**	**0.04**
Without controls B[3]	1.29 (p=0.11)	1.18 (p=0.26)	1.33 (p=0.07)	**0.04**	0.10
Without controls C[3]	1.32 (p=0.06)	1.20 (p=0.19)	**1.35 (p=0.04)**	**0.02**	0.06
Without controls D[3]	1.43 (p=0.06)	1.16 (p=0.30)	1.32 (p=0.09)	0.09	0.19

[1] GMR is calculated using GM exposed population / GM non-exposed population.[2] Corrected for statins provide same results (medicine which reduce CVD and mortality in high risk groups). [3] Controls were selected from different locations (A – D). Controls from location A were working in the office area of the MWCNT factory.

Discussion

This cross-sectional study indicates an effect of MWCNTs exposure on the endothelial damage marker ICAM-1. An upward trend in ICAM-1 was positively associated with MWCNT exposure in both phases of the study and was stable across sensitivity analyses.

Increased ICAM1 levels as a result of exposure to MWCNT, might suggest that high MWCNT exposure stimulates the attachment of leukocytes to the endothelial cell layer, which is an important step in trans endothelial migration of leukocytes and recruitment of these cells to sites of inflammation.[26] An upward trend of ICAM-1 has been observed in humans in relation to the development of atherosclerotic lesions, which is the underlying pathology of cardiovascular diseases including coronary heart disease.[18, 27-30] In the initiation of vascular inflammation, TNF-α plays a key role and is responsible for the expression of ICAM-1 and VCAM-1.[31, 32] In the present study, no effect of MWCNT exposure was found on circulating levels of TNF-α and VCAM-1. It is possible that MWCNTs only have a local effect on ICAM-inducing inflammatory cytokines and that both cytokines are not elevated in plasma which is in line with their role as local inflammatory mediators between cells of a tissue. Lack of an effect on plasma TNF-α levels may also be related to the short half-life of this cytokine and it is plausible that ICAM-1, which is a more stable inflammation marker down-stream of TNF-α, better reflects the integral effect of MWCNT exposure over longer periods of time. This is to some extend reflected in the correlation between these markers over time between phase 1 and 2 with Pearsons Rs of 0.32 and 0.59 for TNF-α and ICAM-1, respectively. We observed no increase in VCAM-1, which mediates the adhesion of lymphocytes, monocytes, eosinophils, and basophils to the vascular endothelium. This may be a consequence of differences in gene regulation or shedding from the endothelial cell layer under the conditions studied. Alexeeff et al. found a similar differential effect for these cell adhesion molecules, with an upward trend of ICAM-1 and no effect of VCAM-1 in subjects exposed to traffic-related air pollution.[18] The potential importance of ICAM-mediated vascular processes is supported by the previously reported increase in circulating monocytes (based on standard cell counting) in our population,[11] which in itself is only moderately correlated with ICAM-1 (Pearson R = 0.3). Monocytes can bind to ICAM-1 in order to migrate from the bloodstream into the vasculature or other tissues.

The effect of exposure to MWCNT on ICAM-1 observed in this study, is inconsistent with the null effect observed by Liao et al.[14] Liao and colleagues [14] performed a six-month longitudinal study among workers producing and handling several engineered nanomaterials (SiO_2 and TiO_2, Ag, CNT and other nanomaterials). The study group included 23 participants exposed to carbon nanotubes (CNT, type not specified). No significant effects on cardiovascular disease markers (including ICAM-1, VCAM-1 and IL-6) after 6-months of follow-up were reported for these participants. Significant upward trends for ICAM-1 and VCAM-1 were however observed among employees working with silicon dioxide (n=24) and titanium dioxide (n=3). In a study by Liou et al.[13], an increase in ICAM-1 (and not for VCAM-1) in workers handling nanomaterials including CNT, compared to unexposed controls was reported, consistent with the present study. In these workers, Liao et al. also reported increases in cardiovascular marker IL-6 (which was non-significantly elevated in our study), and related to this, increases in the IL-6 inducible acute phase protein fibrinogen.[13]

In addition to human studies, in vitro and animal studies provide indications of an upward trend of ICAM-1 in response to MWCNT exposure.[33-35] Cao et al.[35] studied vascular effects of two types of MWCNTs (flexible and comparable to the types in the present study) in mice and cultured cells. Results suggest that exposure to MWCNT is associated with the development of atherosclerosis, related to increased adherence of monocytes (due to increased levels of ICAM-1 and VCAM-1) onto the endothelium and an oxidative stress-mediated change of monocytes to foam cells. As such, the increase in ICAM-1 in the present study could both reflect an inflammatory response and an cardiovascular effect. In addition, Pacurari and colleagues [34] found that MWCNT exposure elevated the levels of monocyte chemoattractant protein-1 (MCP-1) and ICAM-1 at the cellular level. In the same population as the present study, Vlaanderen et al.[11] observed no association between MWCNT exposure and MCP-1 in phase 1, but found a downregulation with exposure for MPC-1 in phase 2 of the study.

In addition to endothelial activation and damage markers, we included systemic inflammatory markers in the study. A significant effect with MWCNT for the markers CRP and TGF-β was shown in phase 1, when comparing the non-exposed population with the lab category with high exposure and the operators category, respectively. However, these results did not replicate in phase 2 of this study. We are not aware of scientific literature relating MWCNT exposure to effects on CRP and TGF-β. Based on analogy in elemental characterization it may be hypothesized that exposure to MWCNTs results in comparable changes in cardiovascular markers as found for PM exposure from other sources. For instance, CRP was found to be significantly affected by ambient air pollution (including ultrafine particles).[24] A significant upward trend was reported in the literature for two other systemic inflammatory markers included in our study (IL-6 and TNF-α) in serum of 11 workers exposed to MWCNTs compared to 14 non-exposed controls.[8] In the present study we detected a non-significant upward trend for IL-6 and a non-significant downward trend for TNF-α. A short-term (1 hour) exposure study reported significant associations after 24 hours between diesel exhaust inhalation and IL-6 and TNF-α, though tPA and soluble ICAM-1 were not elevated.[24] However, comparing this short-term exposure study with the present work is difficult due to the focus on acute changes rather than on (sub)chronic changes in markers. Nevertheless, the non-significant upward trend in the present study and the significant findings in peer-reviewed literature [8] for IL-6 and TNF-α, indicate for a potential and subtle (small) effect on systemic inflammatory markers associated with MWCNT exposure.

No significant effects were observed for the third category of cardiovascular markers, the thrombosis and coagulation markers. The significant association between PM air pollution and increased risk of cardiovascular diseases was supported by reported associations between PM air pollution and the thrombosis and coagulation markers fibrinogen [36-38], vWF [39], and D-dimer [38] in experimental studies among humans and fibrinogen [19] and vWF [40] in epidemiological studies. It has been hypnotized in the present study that exposure to MWCNT has the potential to induce systemic responses resulting in an increased risk of cardiovascular

diseases comparable to PM air pollution. However, an alternative explanation could be a difference in human response to MWCNTs compare to PM air pollution, but a mechanistic rationale for this hypothesis is currently lacking. Also, the wide range of physicochemical properties among different MWCNTs could influence potential adverse health effects, resulting in different human responses within the global MWCNT exposed populations.[5]

Studying health effects of MWCNT exposure in humans is challenging and probably will continue to be challenging as occupationally exposed populations are fragmented, and small populations exposed to distinct types of MWCNTs are distributed between different industries, within individual industries and over time. In addition, the assessment and characterization of MWCNTs is challenging due to their small size, chemical composition, structure, low surface charge, and the rapid agglomeration in air to larger conglomerates. Taking this into consideration, the strengths of the present study include the quantitative and personal exposure assessment over time (2 phases),[15] the observed relatively high contrast in exposure, and the systematically collected information regarding potential confounding factors. Although, this is the largest study that has evaluated early cardiovascular effects due to MWCNT exposure, the size of the assessed population still results in limited statistical power for the assessment of subtle health effects. Large-scale collaborations between research groups and companies would be needed to overcome this. Collaborative study solutions need to be found in order to address the variation in exposure to different types of MWCNTs between companies and over time within companies.

In conclusion, in this cross-sectional epidemiologic study we observed an indication for endothelial activation associated with exposure to MWCNTs. Although the significant upward trend in ICAM-1 levels in both phases was found to be robust in sensitivity analyses, the limited statistical power hampers strong conclusions. However, published in-vitro and in-vivo studies support the hypothesis of an induction of ICAM-1 in response to MWCNT exposure providing important insight into the potential detrimental cardiovascular effects of MWCNT exposure.

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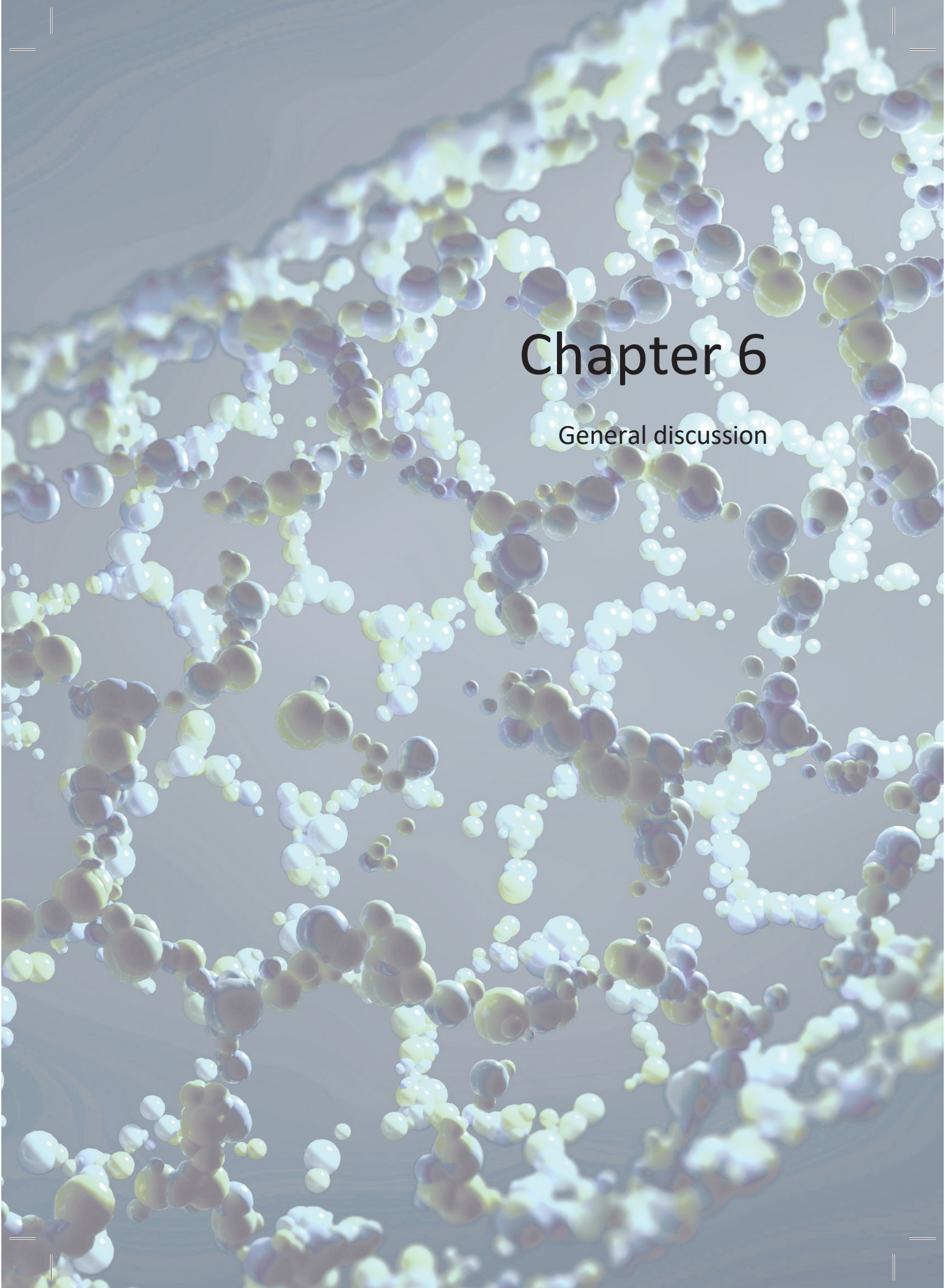

Chapter 6

General discussion

Main findings

Concerns have been raised about the potential health effects associated with carbon nanotube (CNT) exposure. In order to achieve full economic and societal benefits for industry and society, it is crucial to ensure the safety of the workers, consumers and environment by reducing uncertainty in risk assessment of CNTs. There are several gaps in research that limit accurate risk assessment; this is a result of difficulties in measuring CNT exposure, limited exposure data for the different stages of the CNT product life cycle, and incomplete data on the hazards of most different forms of CNTs – this includes (very) limited data on health effects in humans that are associated with exposure to the various types of CNTs. The aim of this thesis is 1) to develop a comprehensive measurement approach to assess (multi-walled (MW))CNT exposure at the workplace, 2) subsequently to use this approach and measure workers' exposure across the various life cycle stages of products containing MWCNTs, and 3) to evaluate the association between occupational exposure to MWCNTs and early cardiovascular health effects.

A systematic review and data-analysis of the standardized emission potential of nano-objects and their NOAA including CNTs was conducted in order to gain an overview of available data across the various product life cycle stages (**Chapter 2**). The results showed that NOAA emission substantially differs between activities and their operating procedures in the workplace and as a result of their intrinsic properties, such as the shape of NOAA. This study identified the need for future research to include more measurements during large-scale synthesis of nanomaterials and machining of nanomaterial-enabled products.

Quantification of CNT exposure is complicated as direct reading instruments (DRIs) are only calibrated for spherical particles; this leads to measurement bias, and more conventional filter-based sampling techniques are often not selective and sensitive enough for the detection of CNT exposure. After the evaluation and optimization of three more selective methods of measuring CNT exposure, it was concluded that more accurate CNT mass concentrations can be obtained (**Chapter 3**) by combining results from carbon analysis and scanning electron microscopy / energy dispersive X-ray spectroscopy (SEM/EDX) analysis with the developed method for background correction (using soot concentrations determined with SEM/EDX). Inductive coupled plasma-mass spectrometry (ICP-MS) was found to be unreliable as a quantitative marker for inhalable CNTs, because in production facilities metal catalysts (required for the production of CNTs) often cause high and variable background concentrations.

Applying this developed measurement approach in a production facility demonstrated significantly higher personal exposure levels to MWCNTs during synthesis and subsequent handling in the production area compared to similar activities with lower volumes of MWCNTs in a R&D area. In addition, bagging of MWCNT powder, maintenance of the MWCNT reactor,

and powder conditioning of MWCNTs were identified as activities which significantly contributed to personal exposure (**Chapter 4a**).

With the manufacturing of CNTs moving from R&D to larger production plants and the increasing applications of CNTs, workers and consumers are also potentially more exposed in the later stages of the product cycle. In an evaluation of the particle release during abrasive activities with products containing MWCNT under experimental conditions, significant effects on released MWCNT embedded particles were observed. Furthermore, while there was a significant increase in released particles during sawing experiments (and a further increase with a higher sawing speed), there was no increase during drilling experiments. In addition, measured concentrations were significantly reduced by mechanical ventilation (**Chapter 4b**). Finally, in a cross-sectional epidemiologic study among the work force in an MWCNT production facility (the same population as described in **Chapter 4a**), we observed an indication of endothelial activation based on increased ICAM-1 levels in participants' blood. This increased level is associated with exposure to MWCNTs in two measurement phases (**Chapter 5**). This finding was further supported by the observation of an increased inflammatory reaction in these workers (**Appendix 1**).

In this chapter, the overall findings are discussed with respect to the ongoing developments which contribute to the safe(r) use of CNTs.

Current status – anno 2018

In this section, the current status of CNTs is discussed regarding 1) exposure assessment, 2) evidence for potential adverse health effects, 3) risk evaluation, and 4) risk management.

Exposure assessment for CNTs

In the beginning of the product life cycle stage, workers are potentially exposed to ~100% pure CNTs, whereas at a later stage, CNTs are mixed with other powders/liquids or are embedded in solid products; this affects the potential exposure during handling. The expected type(s) of exposure together with the study objective (e.g. compliance check, evaluation of exposure control measures) resulted in the use of different measurement strategies in reviewed exposure studies.[1] At the first CNT product life cycle stages (synthesis and handling of ~100% pure material), early CNT exposure assessment studies used either DRIs or gravimetrical methods.[2-4] Compared to spherical nanomaterials, exposure assessment of CNTs at these stages is more complex, and the available and utilized methods often lack selectivity and validity for CNT (fiber) exposure, especially in the presence of other carbon contaminants (e.g. soot).[5] At the later stages of the CNT product life cycle, during the handling of intermediates or ready-to-use products, often more spherical-released aerosols (which include CNTs) can be expected; this makes a measurement approach using DRIs more valid.[6] In addition, across the product life cycle (S)EM/EDX analyses are needed to confirm the

type(s) of CNT exposure. For asbestos (S)EM/EDX analyses are also used to quantify exposure, but analyses are time consuming and expensive. In the case of CNT exposure, a standardized electron microscopy–based method is not available due to the large variation in size and shape of the CNTs.[7] As a result, the use of (S)EM/EDX for quantification of CNT exposure is not recommended.

Chapter 4a of this thesis focuses on worker exposure during commercial production and handling of MWCNTs. A more selective methodology, taking shift-based time-integrated measurements with a combination of carbon analyses and SEM/EDX analyses was developed as discussed in **Chapter 3**. By using carbon analysis with elemental carbon (EC) as a proxy for MWCNT exposure, no real-time information about the exposure was collected. Consequently, a combination of observations during the fieldwork and statistical analyses were used to identify activities which contributed to personal exposure to MWCNT. Selective and valid DRIs for the exposure assessment of fibers (e.g. CNTs), could add a high time and space resolution enabling better activity-based assessments. In addition, real-time information collected with sensors applicable for fibers would provide the opportunity for real-time feedback to the workplace; this would provide real-time possibilities for workers and occupational hygienists to reduce exposure if needed. However, currently available DRIs are not sensitive and selective enough for the detection of CNT fibers.[5] Recently, Kim and colleagues (2016) reported promising results on the detection of CNT at workplaces with a real-time Aethalometer monitor, which is normally used for measurements of black carbon in the general environment.[8] Nevertheless, for the selective quantification of exposure to CNT, a solution is needed to correct for background sources of carbon (e.g. soot). In addition, the Aethalometer monitor measures the blackness of a sample, which is equivalent to the carbon content. Therefore, in order to detect robust CNT concentration differences, a relatively high CNT exposure or a long measurement period is required. As sensor development continues, more selective DRIs for CNT exposure are expected to become available, which could add to or possibly replace (parts of) the current measurement methodology as discussed in **Chapter 3**.

In **Chapter 4b** of this thesis, the potential of worker exposure is evaluated during experimental abrasive activities with (end) products that contain MWCNT. Different abrasive techniques and different determinants for release were evaluated; this provided knowledge about the exposure potential under different workplace conditions. Based on other peer-reviewed literature, the release of more spherical particles was expected during abrasive activities with products containing low quantities of embedded MWCNTs.[6, 9] Consequently, a commonly used combination of DRIs and offline SEM/EDX analyses were used for the assessment of these released spherical particles.[10] This hypothesis that spherical particles would be released under these conditions was confirmed, as measured emissions were predominantly (roughly spherical) matrix particles from the abrasively treated material containing MWCNTs, with no

single MWCNT fibers observed. In contrast to the methodology used for the exposure assessment during the first stages of the product life cycle (**Chapter 3**), carbon analysis was not used as the matrix materials could (significantly) influence the results. In addition, for a realistic risk assessment it is important to focus not (only) on the (~100% carbon-based) CNTs, but also on the exposure forms and composition (e.g. matrix material with protruding CNTs) as they are encountered in the workplace.[11, 12]

Evidence for potential adverse health effects associated with CNTs

In 2014, the international agency for research on cancer (IARC) evaluated the potential carcinogenicity of CNTs.[13] While one type of CNT (MWCNT-7, rigid structure, ~50 nm in diameter; ~4 µm in length) was classified as a possible carcinogen (IARC classification group 2B), the scientific evidence was not strong enough to draw firm conclusions about other CNTs (IARC classification group 3, not classifiable). In 2017 Kuempel et al. provided an extended review of the mechanistical evaluation of the potential carcinogenicity of CNTs, using the IARC Monograph 111 as the basis. [14] The most studied type of CNT in cancer studies on animals is MWCNT-7,[15] but it is unclear if the conclusions of these studies can be extended to other types of CNTs as chronic animal studies are lacking. Despite the heterogenicity of the types of CNTs, the inadequate systematic evaluations performed, and the limited number of chronic studies available, Kuempel et al. (2017) concluded that the mechanistic evidence in animals is relevant to humans. This evidence gathered from animal studies concerns several types of CNTs and includes the deposition and retention of inhalable CNTs in the lung, translocation from the lung, progression of inflammation, lung and pleural injury, fibrosis, and genotoxicity.[14] However, information about responses in humans, which points to potential underlying mechanisms, is missing; this is considered to be an important gap in the scholarship on evaluation.

Most chemicals evaluated by IARC are classified as possible carcinogens (group 2b, n=299) or unclassifiable (group 3, n=502), while 120 chemicals are classified as carcinogenic to humans (group 1).[16] A chemical is placed in group 1 when there is sufficient evidence of carcinogenicity involving direct (usually epidemiological) observations in humans. In addition, with insufficient evidence of carcinogenicity in humans, the evidence of carcinogenicity in experimental exposed animals together with knowledge that the chemical acts through a relevant mechanism of carcinogenicity in humans, can result also in a group 1 classification of a chemical.[17] Due to the substantial evidence requirements, chemicals in group 1 can be considered to represent cancers that might have been prevented, with better and earlier predictions of cancer hazards.[18] A group 1 classification is not expected in the (near) future for CNTs, as no informative studies can be conducted due to the diversity of types of CNTs, the low number of workers exposed to these CNTs, the relatively short duration of exposure, and long latency period of developing cancers. Consequently, human studies focused on early

health effects associated with exposure to MWCNT are important when examining the potential long-term risks of developing a disease.

Currently, only a few recent small-scale studies reported the association between different types of CNT exposure and early health effects in occupationally exposed humans.[19-24] Results from the cross-sectional study, as described in this thesis, focused on cardiovascular effects associated with MWCNT exposure (**Chapter 5**) and were part of a larger campaign which included a quantitative and personal exposure assessment over time (**Chapter 4a**). This study also included an evaluation of immunological effects and lung health (**Appendix 1**) and an evaluation of potential changes in DNA methylation. [24] The recent small-scale studies in humans and cross-sectional study described in this thesis focused on the detection of potential markers of early health effects and found indications associated with exposure to CNTs: oxidative stress, lung inflammation, increased monocyte cell counts, and gene expression changes (including messenger-RNA and non-coding RNA)[19-25] (**Chapter 5** and **Appendix 1**). Although results from these relatively small studies are suggestive, the evidence on early health effects is still limited. These studies found different (significantly) increased markers associated with CNT exposure, which can potentially be explained by limited statistical power in these studies, by findings that are possibly false positives, and by the use of different methods of measurement both for the exposure assessment and the assessment of biological markers.[26] As an example, Fatkhutdinova et al. (2016) found a significant association between exposure to MWCNT and upregulation of TNF-α, but the cross-sectional study described in this thesis did not replicate this finding; although the effect was in the same direction (**Appendix 1**).[20] In addition, in 2014 Wu et al. studied the effects of exposure to nanoparticles including non-specified types of CNTs on fractional exhaled nitric oxide (FENO) and found no depression in FENO, which was also observed in this thesis (**Appendix 1**).[19] Furthermore, the observed increase in the cardiovascular marker ICAM-1 and a null effect for IL-6 associated with MWCNT exposure, as described in **Chapter 5,** is inconsistent with the null effect for ICAM-1 observed by Liao et al. (2014) and the increase in IL-6 found by Liou et al. (2012).[27, 28] Despite these differences in reported outcomes, the results from these studies are worrisome and warrant follow-up in other populations that are exposed to MWCNT for more scientific evidence.

In the present cross-sectional study, a cardiovascular effect of MWCNTs was observed with increased ICAM-1 concentrations over time (effects were replicated in phase II of the study after five months), indicating endothelial activation (**Chapter 5**). The evaluation of immunological effects and lung health for the same population showed upward trends for immune markers C-C motif ligand 20, basic fibroblast growth factor and soluble IL-1 receptor II associated with MWCNT exposure (**Appendix 1**).[25] However, the upward trend in C-C motif ligand 20 observed in this study surprisingly contradicts the negative association found with cigarette smoke in an in vitro experiment.[29, 30] Effects related to MWCNT were observed on FENO and several complete blood cell count parameters (including monocytes) (**Appendix**

1).[25] A depression of FENO suggests that MWCNT exposure may have an inhibitory effect on nitric oxide (NO) synthase in the lungs, as previously reported in human responses to NO in cigarette smoke.[31] Ghosh et al. (2017) studied changes in DNA methylation, which is associated with carcinogenesis, in the same population as the present study. They observed significant effects on the DNA methylation markers DNMT1, ATM, SKI, and HDAC4 promoter CpGs, which are associated with MWCNT exposure.[24] For the type of MWCNT studied in **Chapter 5**, **Appendix 1** and by Ghosh et al. (2017), in vitro and animal studies showed adverse effects on biopersistent, interstitial retention, bronchiolalveolar hyperplasia, genotoxicity, fibrosis and persistent inflammation.[14 32-35] In addition to the IARC evaluation for Monograph 111 and the extended review by Kuempel et al. (2017), this cross-sectional study and previous studies in humans observed indications of cardiovascular effects, lung inflammation and epigenetic changes, which are now repeatedly reported in animals and humans.

Although it may be expected that the health effects of exposure to CNTs embedded in matrix material are less severe than those effects that are the result of exposure to pristine CNTs, the toxicological properties of embedded CNTs are still largely unknown. In a preliminary risk assessment of comparable forms of CNT exposure as presented in **Chapter 4b**, the human health risks to workers and consumers were low, as no free CNTs were released during abrasive activities.[36] In addition, the airborne particles were often too large to reach the alveoli, and no cytotoxicity potential was observed for these forms of exposure.[36] Furthermore, in a preliminary (acute) in vivo instillation experiment, no differences in the hazard potential were observed between nanocomposites (including CNTs) and composites without nanomaterials.[37] In contrast, Schlagenhauf et al. (2015) reported released matrix material with protruding CNTs. These CNTs may make direct contact with lungs cells and result in toxic impact comparable with that of pristine CNT fibers.[38] Much is still unknown about the health effects of the different forms of CNT exposure across the product life cycle; this stresses the need for hazard assessment to focus on actual workplace exposure instead of only on the pristine CNTs.

The selection of biomarkers (**Chapter 5** and **Appendix 1**),[24] the quantitative and personal exposure assessment over time across the different product life cycle stages (**Chapter 3**, **4a** and **4b**), and the systematically collected information concerning potential confounders included in this thesis provides a basis for future epidemical research on CNTs. However, this study also shows the need for studies on larger populations, as it has limited statistical power due to a relatively low number of participants, which prevents us from drawing firm conclusions. Although more results from epidemiological studies will become available, it is unlikely that current limitations (heterogenicity of CNTs, small study populations) will be (fully) addressed in the near future.

Risk evaluation of CNTs

Uncertainty about the health effects of different types of CNTs results in the use of the precautionary principle and consequently minimization of exposure in order to ensure the safety of workers at their workplaces. The limitations and gaps in data in current research on the health effects of CNTs require making assumptions in risk assessment; this results in (more) worst-case and conservative evaluations. In addition, as the number of and the variety in CNTs is still growing, especially when taking into account functionalization possibilities, it is undoable and unpractical to study every type of CNT and their corresponding health associations in exposed humans. Although a large amount of uncertainty exists in the hazard assessment of CNTs, several occupational exposure limits (OELs) and recommended exposure limit values (RELs) have been proposed and used by governmental institutes in different countries. Table 1a summarizes the proposed OELs (μg/m^3) from literature mainly based on subacute and sub-chronic animal studies; Table 1b presents different mass-based (μg/m^3) or number-based (#/cm^3) RELs from national governmental institutes.

Table 1: Overview of a) proposed occupational exposure limits (OELs) and b) recommended exposure limit values (RELs).

Material	Specification	Proposed OEL (μg/m^3)	REL[1]	Reference
a: Proposed exposure limits from scientific publications				
CNT	MWCNT (Shenzhen Nanotech Port Co. China)	0.67	-	Stone et al. (2009) [39]
CNT	MWCNT Nanocyl NC7000	2.5	-	Luizi (2009) [40]
CNT	MWCNT Baytubes®	50	-	Pauluhn (2010) [41]
CNT	MWCNT Baytubes®	2	-	Aschberger et al. (2011) [42]
CNT	MWCNT Nanocyl NC7000	1	-	Aschberger et al. (2011) [42]
CNT	2x SWCNT (AIST, CNI), 2x DWCNT (Toray Industries, Inc.), 3x MWCNT (Nikkiso Co., Mitsui & Co., Showa Denko K.K.)	30	-	Nakanishi et al. (2015) [43]
b: Exposure limits using a grouping approach proposed by governments				
Fibers	Fibrous nanomaterials	-	0.01 #/cm^3	UK (BSI, 2007) [44]
Fibers	-	-	0.01 #/cm^3	Netherlands (SER, 2012) [45]
CNT and CNF	-	-	<1 μg/m^3	USA (NIOSH, 2013) [5]
Carbon	Carbon fibers length >5 μm, diameter <3 μm, aspect ratio >3	-	2 #/cm^3	Belgium (ELSD, 2014) [46]
Fibers	Non-entangled fibrous NM	-	0.01 #/cm^3	Germany (BAUA, 2016) [47]

[1] Mass-based (μg/m^3) or number-based (#/cm^3) recommended exposure levels (REL). Based on assumptions on the density, diameter and length of CNTs, a conversion between both metrics is achievable.

The proposed OELs derived in scientific research all used subacute or sub-chronic rat studies, while chronic studies are desirable to derive an OEL while taking into account the most critical expected and potential health effects. The variation in the proposed OELs shows the diversity in methods to derive OELs and the types of CNTs that have associated effects in animal studies. Both for the extensively studied flexible MWCNT Baytubes® and MWCNT Nanocyl NC7000, two different OELs were proposed based on the same animal study and point of departure, but by applying different assessment factors.[32, 40-42] Stone et al. (2009) proposed the lowest OEL (0.67 μg/m^3) for a type of MWCNT based on a 14-day inhalation study on mice with a relatively high exposure level.[39] However, they also applied a relatively high extrapolation factor (150) to convert the short-term exposure to ling-term exposure for the general public. [39] In addition, Nakanishi et al. (2015) proposed a relatively high OEL (30 μg/m^3) for different CNTs based on a 28-day inhalation study and observed no health effects.[43] In contrast to Stone et al. (2009), a relatively low extrapolation factor (6) was used to convert the results to human (which was limited to 15 years), correcting only for interspecies difference (3) and the difference in the exposure period (2). In the present study, the calculated personal respirable MWCNT exposure ranged between 0.76 and 4.45 μg/m^3 in the production facility, which is in the same order of magnitude as most of the proposed OELs. As chronic studies are largely missing for the different types of CNTs, and different extrapolation factors are used to convert results from animal studies to humans for different populations and periods, these proposed OELs should be considered with caution when determining the risk assessment for humans. Taking into account the available evidence for potential adverse health effects associated with CNTs and the important knowledge gaps identified in this evaluation, there is a need for governmental institutions to propose the use of more generic and conservative limit values rather than the currently proposed OELs.

The proposed limit values by governmental institutions for different countries are largely based on health effects associated with asbestos. Interestingly, limit values for asbestos were recently lowered in the Netherlands from 0.01 fibers/cm^3 to 0.002 fibers/cm^3.[48] The limit value for CNTs in the Netherlands (0.01 fibers/cm^3) was recently evaluated based on available scientific knowledge and the lowered limit for asbestos to determine if an adjustment is needed.[49] The evaluation was inconclusive about the conservativeness of the current value used for CNTs and whether lowering this value is scientifically justifiable and necessary. The authors concluded that it is not clear whether CNTs cause carcinogens via the same mechanism as asbestos. It is also unclear if the potency of CNTs is comparable to asbestos and if a linear extrapolation of observed dose-response relationships to low doses as used for asbestos is valid for CNTs.[49] Although a scientific motivation for lowering the limit value for CNTs was not found, lowering this limit value to 0.002 fibers/cm^3 should be considered to guarantee conservativeness and to ensure worker safety.

In addition to the Dutch proposed limit value, the American National Institute for Occupational Safety and Health (NIOSH) in 2013 performed a detailed health-based analysis

using available literature and proposed a REL which deviates from the asbestos exposure limit value (<1 μg/m^3).[5] NIOSH concluded that there are many uncertainties in assessing the risks of CNTs and that carcinogenic health effects cannot be ruled out. Consequently, the proposed limit value is based on the analytical limit of quantification, and it is recommended that exposure to all CNTs should be restricted to <1 μg/m^3 respirable elemental carbon as an 8-hour time-weighted average (TWA). Furthermore, for unknown reasons the Belgian federal government ELSD (2014) deviates from the asbestos exposure limit value with their legally binding exposure value.[46] Unless health effects caused by asbestos (mesothelioma, lung cancer) can be excluded for a specific type of CNT, the use of exposure limit values either based on (lowered) limit values for asbestos or based on the analytical limit of quantification is recommended.

Ideally, for every type of CNT a health-based limit value will be derived to minimize uncertainty in risk assessment. More realistically in the near future, the limited number of performed and ongoing chronic animal studies with CNTs will be used together with other relevant outcomes of in vitro and in vivo studies. These results can be used for read-across and aim to group different types of CNTs based on their physical and chemical properties and derive more specific limit values. However, more research is needed for the development of scientifically correct read-across approaches, as it is largely unknown how physical and chemical properties of CNTs influence health effects in humans.

Risk management for CNTs

As health effects of CNTs are still largely unknown, regulatory agencies are focused on the minimization of worker exposure to CNTs. However, recent relatively large-scale studies indicated rather high personal exposure to CNTs at different workplaces (**Chapter 4a**).[50] Risk management measures provide solutions to reduce personal exposure by applying the hierarchy of controls .[51] The hierarchy of controls enumerates different methods in the following order, starting with the potentially most effective, protective, and preferred method:

1) Elimination (physically remove the CNTs);
2) Substitution (replacement of the specific form of CNTs);
3) Technical controls (isolation of workers from the CNTs);
4) Administrative controls (changes in workers' procedures);
5) Personal protective equipment (equipment placed on the worker).

Traditional workplaces that have workers handling conventional materials have applied the hierarchy of control. However, for nanomaterials including CNTs, the effectiveness of the five different methods is often unknown. A recent evaluation of the available information on the effectiveness of risk management measures of nanomaterials showed that technical,

administrative and personal controls used for conventional materials are also effective in reducing exposure to nanomaterials including CNTs.[52] However, drawing quantitative conclusions about the effectiveness is difficult due to the limited available data.[52] More specific in relation to CNTs, several peer-reviewed publications confirmed the effectiveness of reducing CNT exposure for several technical and personal controls.[53-58] Cena et al. have observed the good performance of a biosafety cabinet reducing CNT exposure and a relatively poor performance of a custom hood, which may have been explained by the lack of a front sash.[55] Vo et al. have demonstrated the effectiveness of several filtering facepiece respirators when working with CNTs, but they have also highlighted that the effectiveness of the protection depends mainly on the fit of the mask and the breathing volume of the worker.[56-58] In the United States, producers and users of CNTs use technical, administrative and personal protective methods, but the risk management methods of elimination and substitution received less attention in the workplace.[59] With ongoing research into the efficacy of exposure controls and the protection of workers from exposure to CNTs, a combination of risk management methods that aim to protect workers should be considered the best approach. The hierarchy of control needs to be applied, and the use of technical (e.g. local exhaust ventilation), administrative (e.g. restrictions on the time spent performing an activity with CNTs) and personal (e.g. protective equipment) controls should be considered simultaneously especially when elimination and substitution are not possible.

Future perspectives and considerations

The health effects associated with exposure to CNTs and asbestos are often compared in society. This is based on in vitro and in vivo studies which reported similarities between the biological responses to asbestos and the biological responses to some forms of CNTs, which are characterized as long thin biopersistent fibers.[60] However, information about the specific characteristics that account for the differences in the hazard potential of various types of CNTs is lacking. Nevertheless, it is unlikely that those health effects observed as a response to asbestos can be expected for all forms of CNT.[13] However, the combination of widespread use and uncontrolled exposure could presents risks when working with every toxic substance. Much can be learned from the asbestos case about CNTs and also about new fibers, which have not yet received much attention (e.g. nanocellulose, mineral fibers, ceramic fibers, nanosilver fibers).[61] Future perspectives and these potential discoveries are discussed in the next section which includes 1) read-across and grouping, 2) safe innovation, 3) risk governance, and 4) exposure registration and epidemiological research.

Read-across and grouping

Ideally, for every type of CNT human health-based information would be available to derive specific limit values. At present, but likely also in the near future, data gaps in important

knowledge will continue to be present in the risk assessment of CNTs. As only limited data is available, which will increase over subsequent years, optimal use of the existing data is needed. The correct use of read-across and grouping approaches can help achieve this.[62] Currently, Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) requires all companies manufacturing or placing CNTs on the European market to register if the quantities are greater than 1,000 kg/year. Recently, the European Commission (EC) held a consultancy meeting with stakeholders regarding a proposal for the use of read-across and grouping, in which they amended the REACH Annexes for 2020 to address nanoforms of substances. The EC proposes to allow grouping for nanoforms with similar properties that cause only limited variations in risk assessment. A justification needs to be provided if grouping and read-across is applied.

In peer-reviewed literature, several proposals exist for the grouping of nanomaterials including CNTs based on exposure, hazard and risk potential.[62-68] Drew and colleagues (2017) constructed a database with data from 25 different rodent studies and identified similar nanomaterials based on their potency in causing inflammation.[62] The various types of MWCNTs considered in this study had wide-ranging hazard potencies, which can be explained by their different characteristics. In general, more comprehensive data is needed for validation purposes and to extend proposed frameworks, but these approaches could help address the relevant physical and chemical properties for different nanomaterials including CNTs.[62, 64]

Specifically with regard to CNTs the relevant physical and chemical properties (influencing the hazard potential) that are believed to be important are the length and diameter of the tubes, structural defects, the rigidness of the fiber, metal impurities, post-synthesis treatments, and surface functionalization.[14, 69, 71] Rittinghausen et al. (2014) observed stronger carcinogenic effects in animals in longer (biopersistent) fibers when compared with shorter fibers, as alveolar macrophages are not able to take up the longer fibers.[70] Furthermore, Muller et al. (2008) studied inflammation and fibrogenicity in vivo and observed stronger associations with broken C-C bonding defects caused by imperfect CNT synthesis or post-synthesis treatment.[35] In addition, Sager et al. (2014) reported reduced bioactivity and pathogenicity for surface modified MWCNTs with an additional -COOH group.[72] Nonetheless, it is currently difficult to define the key parameters for the hazard potential and to describe their relative importance; this requires more research to overcome these knowledge gaps. However, pragmatic and flexible read-across and consequently grouping provides opportunities and is the next step towards more evidence-based risk assessment without industry having to shoulder an unreasonably large registration burden.

Safe innovation

The aim of safe innovation is to address safety issues of new innovations in a timely manner, preferably before the product enters the market.[73-75] Safe innovation requires interaction between stakeholders (regulators, innovators, scientific experts, risk assessors) and supports products that are safe(r) by design. Ideally, the safety of CNTs is already considered at the early stages of product development, when decisions are made regarding the type of CNT used in the product. As CNTs often have unique properties in products compared to conventional materials, the optimal balance between added value of CNTs and safety needs to be evaluated. While epidemiological data regarding the health effects of CNTs is largely lacking, data, preferably from in vitro studies (rather than animal studies), can be used to identify less hazardous forms of CNTs. Testing the redox potential at an early stage of product development could contribute to the identification and use of less hazardous functionalized forms of CNTs.[76] Moreover, with increased understanding about physical and chemical properties and their effect on the hazard potential, less hazardous forms of CNTs (with the required properties) can be identified and used. In addition, safe innovation is not only about the form of CNTs incorporated in the product. The material in which the CNTs are embedded is also relevant and should be included in the discussion about the hazardous potential of the product. Therefore, a realistic hazard assessment and safe innovation requires evaluation of the relevant forms of exposure at the workplace throughout the life cycle of the product.

Technical, administrative, and personal protective methods are already used during activities involving CNTs within the workplace. However, according to the hierarchy of controls safe innovation also focuses on elimination and substitution, which are believed to be more effective in minimizing the risks for workers potentially exposed to CNTs. Safe innovation is expected to result in more alternatives that will allow for the removal of hazardous types of CNTs and the substitution of less hazardous forms of CNTs. For example, Vlasova et al. 2016 demonstrated the effectiveness of reducing the bio-persistence of CNTs by using doping techniques.[77] Finally, successful safe innovation needs successful communication and for information to be shared between regulators and innovators. Policy makers have suggested that regulators and innovators share what they have discovered about substitution approaches to CNTs. The EC funded a European Centre (EC4SafeNano, Horizon 2020) to support this; it aims to promote safe innovation by connecting (national) nano safety centers and encouraging mutual cooperation.

Risk governance

Nanotechnological development proceeds quickly, and health and safety regulators have difficulty keeping pace with progress. Traditional approaches to health and safety regulation of nanotechnology are constantly under debate and require more research. Uncertainty about how to implement CNTs safely leads to an unwillingness to invest in beneficial nanotechnologies. In addition, although evidence-based regulations are missing specifically

for nanomaterials, employers are responsible for the safe development with nanomaterials including CNTs. Soft laws (regulatory agreements) in risk governance of nanomaterials are introduced to cope with uncertainty in current risk assessment.

Soft law approaches are based on non-binding requirements, and unlike in traditional hard law approaches, they are not directly enforceable by the government.[78] In the Netherlands, the Social and Economic Council developed the nano reference values (NRVs) for managing unknown and potential health risks associated with nanomaterials; the NRVs can be considered a soft regulatory agreement.[45] In 2011, the Dutch risk governance for nanotechnology was evaluated, with the evaluation focused on already operative soft regulatory agreements (NRVs and Stoffenmanager Nano).[79] Although both approaches were introduced just before the Dutch risk governance evaluation in 2011, it was concluded that soft regulatory approaches can contribute to responsible nanotechnological development. In order for the soft regulatory approaches to be successful, it is important that the agreements 1) are specific enough for regulators, 2) should lead to awareness among occupational hygienists and managers in companies with enough financial and professional resources, and 3) require continuous and adequate adaptation based on new insights into the field of occupational safety and nanomaterials.

After this evaluation in 2011, other soft law approaches became publicly available, which are comparable to the NRVs and Stoffenmanager Nano. Liguori et al. (2016) evaluated six risk categorization- and control banding tools including Stoffenmanager Nano.[80] It was concluded that both Stoffenmanager Nano and NanoSafer are more advanced for compliance purposes. The many soft law initiatives in Europe together with the often unknown conservativeness of these initiatives made the EC decide to fund research into the development of an EU risk governance council for nanomaterials. This independent council needs to be transparent, self-sustained and science-based, and provide sustainable solutions using both soft and hard law approaches to nano risk governance. It may be expected that the council will provide scientific and independent information about the conservativeness of individual soft law proposals. An EU risk governance council for nanomaterials further helps society adapt to the pace of innovation, fully and safely benefitting from the potential of CNTs.

Exposure registration and epidemiological research

The results of this thesis together with other epidemiological studies are not conclusive, and more data needs to be collected to study the health effects of CNTs in humans. The health effects in animal studies observed for some types of CNTs are worrisome. In 2012, the Dutch Health Council recommended introducing an exposure register (at the company level) for workers exposed to nanomaterials.[81] In 2016, Dutch employers (VNO/NCW) and employees (FNV/CNV) published the results of a pilot study which tested the feasibility of an exposure register for nanomaterials.[82] This pilot demonstrated that a register is practically feasible within the current Dutch risk regulation, but is not recommended with exposures below the

NRVs. Stakeholders who participated in this pilot concluded that exposure registration is always needed only for CNTs with probable asbestos-like health effects. Nevertheless, strategically large-scale exposure registration was not suggested. It was concluded that raising awareness of applying the precautionary principle by using effective control measures during the handling of nanomaterials is a preferable option to encourage safe workplaces.[82] Currently in 2018 no exposure register is required in the Netherlands for companies handling CNTs. The Netherlands together with several other European countries are waiting for a European registration system, while other countries have already introduced national registration systems (France 2013, Denmark 2014 and Belgium 2016). Waiting for a European consensus seems to be an understandable decision from an industry perspective, as several European countries with a national register system in place complain about high administrative costs and unfair competition with other European countries.

Data collected from an exposure register allows for retrospective analyses that can relate diseases to past exposure. The Dutch Health Council advised in 2012 to link results from an exposure register to already existing (passive) health surveillance systems (e.g. data on mortality and diseases of the general population).[81] Medical screening, health monitoring and medical surveillance were not recommended by the Council due to unclear health effects associated with nanomaterials and the absence of sensitive and specific tests.[81, 83] In addition, another important recommendation made by the Dutch Health Council was to focus epidemiological research on the early health effects associated with exposure to nanomaterials. The results of this thesis as well as other epidemiological studies have showed some promising biomarkers for the early detection of potential health effects. Although these markers are believed to be nonspecific for CNT exposure, results could be more meaningful with repeated measurements over time. On an individual level, these biomarkers can be used over time to monitor health status and to identify possible preclinical stages of an illness. On a group level, results can be used to study possible causal pathways of diseases associated with CNT exposure and individual susceptibility to these diseases.[84]

The growing number of workers exposed to CNTs and the still largely unknown health effects increases the imperative of introducing a mandatory European exposure registration for workers exposed to CNTs for retrospective research on mortal diseases. As current proposed limit values are not health based, registration is recommended with an exposure level above 10% of the limit value (which is commonly used in occupational hygiene). In addition, results from registration should be used to identify exposed workers, to work towards more proactive epidemiological research focused on early effects, and to overcome the limited power of statistics due to a relatively low number of participants, which prevents us from drawing strong conclusions. To study potentially subtle health effects, epidemiological research should be a worldwide collaboration that focuses first on CNTs that are commercially produced and/or handled in large manufacturing facilities. Epidemiological research needs to focus on the assessment of biomarkers that have already been tested to study trends over

time in relation to exposure and should include newly identified more specific markers for CNT exposure.

Conclusion

Recently CNTs have received global attention due to their unique properties when compared to more conventional materials. However, concerns have been raised about the exposure of individuals based on the health effects observed in animal studies. This thesis provides an overview of the current scholarship regarding the health effects of CNTs and contributes to 1) a more comprehensive method of exposure assessment for MWCNTs using both refined carbon analyses and SEM/EDX, 2) the insights into the personal occupational exposure levels and exposure determinants during production activities with high quantities of MWCNTs and abrasive activities with products that contain MWCNT, and 3) the knowledge of the association between occupational exposure to MWCNTs and early cardiovascular health effects.

Uncertainty about the potential health effects of CNTs limits innovation. Moreover, it is expensive for industry to ensure their workplaces are safe as the precautionary principle requires minimizing worker exposure. Future studies should focus on 1) read-across and grouping for an optimal use of available data, 2) safe innovation ensuring a safe product, which includes CNTs and maintains its benefits, 3) soft laws that adapt to the pace of innovation in nanotechnology, and 4) the registration of workers exposed to CNTs together with passive health surveillance and new epidemiological research.

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Appendix 1

A cross-sectional study of changes in markers of immunological effects and lung health due to exposure to multi-walled carbon nanotubes

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Appendix 1

Abstract

Multi-wall Carbon nanotubes (MWCNTs) are manufactured nanomaterials to which workers and the general population will be increasingly exposed in coming years. Little is known about potential human health effects of exposure to MWCNTs, but effects on the lung and the immune system have been reported in animal and mechanistic studies.

We conducted a cross-sectional study to assess the association between occupational exposure to MWCNTs and effects on lung health and the immune system.

We assessed 51 immune markers and three pneumoproteins in serum, complete blood cell counts (CBC), fractional exhaled nitric oxide (FENO), and lung function among 22 workers of a MWCNT producing facility and 39 age- and gender-matched, unexposed controls. Measurements were repeated four months later among 16 workers also included in the first phase of the study. Regression analyses were adjusted for potentially confounding parameters age, body mass index, smoking, and sex and we explored potential confounding by other factors in sensitivity analyses.

We observed significant upward trends for immune markers C-C motif ligand 20 (p=0.005), basic fibroblast growth factor (p=0.05), and soluble IL-1 receptor II (p=0.0004) with increasing exposure to MWCNT. These effects were replicated in the second phase of the study and were robust to sensitivity analyses. We also observed differences in FENO and several CBC parameters between exposed and non-exposed, but no difference in lung function or the pneumoproteins.

We observed indications of early effects of occupational exposure to MWCNTs on lung health and the immune system.

Introduction

Multi-Wall Carbon nanotubes (MWCNTs) are a type of manufactured nanomaterials that have many potential (industrial, medical) applications due to their unique physicochemical properties.[1] Though production is currently generally small scale, increased production of MWCNTs is expected in the coming years which will increase exposure to both producers (workers involved in manufacture and application) and users of MWCNTs.[2, 3]

There is considerable evidence from animal and in vitro studies that MWCNTs induce inflammation, oxidative stress, pulmonary fibrosis, mesothelioma-like effects, and cardiovascular effects.[3-5] Data in humans relating to biological perturbations involving the lungs and immune system due to exposure to MWCNTs is available from a handful of epidemiological studies conducted in occupationally exposed populations. Lee et al. (2015) reported an effect of occupational exposure to MWCNT on oxidative stress markers (hydrogen peroxide, malondialdehyde, 4-hydroxy-2-hexenal, and n-hexanal) in exhaled breath condensate in a small Korean population (n=18).[6] No effects on lung function or

hematology were reported. Wu et al. (2014) reported a null effect of occupational exposure to carbon nanotubes (type not further specified) on fractional exhaled nitric oxide (FENO; a marker of lung inflammation) based on a cross-sectional analysis of a Taiwanese population (n=57).[7] In further publications based on this population, but augmented with workers that were exposed to other engineered nanomaterials (n=364), associations were reported with worsening of allergic dermatitis, increased levels of small airway damage marker (Club Cell Secretory Protein 16; CC16) and lung function test parameters.[8, 9] Fatkhutdinova et al. conducted a small scale pilot study among 11 workers with more than 1 year exposure to MWCNT and 14 non-exposed controls.[10-12] The authors reported significant associations between exposure to MWCNT and levels of interleukins (IL)-1β, IL-4, IL-5, IL-6, IL-8, tumor necrosis factor α (TNF-α), and Krebs von den Lungen-6 (KL-6) measured in sputum and levels of IL-1β, IL-4, IL-10, and TNF- α measured in serum.[11] The same authors also reported genome-wide differential expression in messenger-RNA and non-coding RNA between workers exposed to MWCNT and non-exposed controls.[12]

Although suggestive, evidence from these studies is limited due to their size and the absence of quantitative exposure assessment and the heterogeneity of the study populations in terms of the engineered nano particles to which subjects were exposed.

We conducted a cross-sectional study among workers occupationally exposed to MWCNTs in a MWCNT production facility and non-exposed controls. We assessed the association between quantitative measures of MWCNTs [13] and a set of markers of lung health and early perturbations of the immune system; complete blood cell counts (CBC), fifty-one circulating inflammation markers,[14, 15] three pneumoproteins, lung function: forced expiration volume in 1 second and forced vital capacity (FEV1, FVC), and FENO. We were able to collect repeated measurements for a subset of markers.

Methods

Study population

We conducted a cross-sectional study among twenty-two workers of a MWCNT producing facility and thirty-nine age- and gender-matched, unexposed controls. Controls were selected from four different locations in the vicinity of the MWCNT producing facility among which three locations were at companies not involved in the production or use of MWCNTs (a consumer electronics store, a chemical plant, and an occupational health services company). The fourth location was a department at the MWCNT facility not involved in producing or handling of MWCNTs. The study was approved by the Commission for Medical Ethics of UZ Leuven (reference number S54607) and conducted in two phases. During phase 1 (June 2013) previously synthesized MWCNTs were bagged and incorporated into coatings, dispersions, and plastics. During the phase 2 (October 2013), in addition to these activities, MWCNTs were also actively synthesized. In phase 2, a subset of the study population (10 exposed individuals and 6 matched controls) were included.

Appendix 1

Assignment of MWCNT exposure

The collection of the exposure measurements that formed the basis of the MWCNT exposure assignment in this study has been described before.[13] Briefly, breathing zone measurement of inhalable particulate matter was taken from workers in different parts of the production and research & development process across seven days. MWCNT mass was estimated by determining elemental carbon (EC) levels in the collected particulate matter. The procedure is described in Tromp et al.[16] and is summarized in the Supplementary material. Based on exposure measurements and individual task patterns, workers at the MWCNT production and laboratory facility were divided into three exposure groups: operators, lab personnel with relatively high exposure (lab high), and lab personnel with relatively low exposure (lab low). Exposure to MWCNTs was assigned using an exposure score (i.e. 1,2,3) and by estimating the geometric mean (GM) MWCNT mass concentration for each exposure group using a mixed model.

Assessment of health outcomes

Questionnaires

All individuals participating in the study completed a questionnaire that was previously validated within the ELON study.[17] The questionnaire was used to acquire information on general demographic information, health history, respiratory health, asthma and allergies, complaints of the circulatory system, lifestyle factors including smoking, and alcohol consumption, radiation exposure history, family medical history and work history. This baseline questionnaire was distributed once per individual included in the study during phase 1. Study participants also completed a questionnaire that was used to acquire information on smoking, alcohol consumption, health, and medication use (among other factors) in the 24 hours before the biological samples were collected.

Complete blood cell counts, immune markers and pneumoproteins

Whole blood was collected in the morning hours (before midday) by standard phlebotomy of venipuncture of forearm veins in a sitting position. CBC were determined in fresh blood at the clinical laboratory of the University Hospitals Leuven, Gasthuisberg, Belgium. Circulating blood cytokines, interleukins, and chemokines were determined by Luminex (Austin, TX, USA) multi-analyte profiling kits according to a procedure described in Shiels et al.[15] Markers were selected to reflect several key components of inflammation, including acute-phase proteins, pro- and anti-inflammatory cytokines, chemokines, growth factors, and angiogenesis factors [15] and based on their performance and reproducibility in multiplexed assays.[14] To combine the information available from independent immune markers, we calculated two inflammation scores based on four independent markers (CRP, BCA-1/CXCL13, MDC/CCL22, and IL-RA; and CRP, SAA, CXCL9, and sTNFRII) that were reported to be significantly predictive of lung cancer risk.[15, 18] Risk scores were calculated by summing the z-scores of the independent markers. We assessed the impact of adding white blood cell count

measurements to the risk scores. Serum samples were also assayed for pneumoproteins CC16, SP-A and SP-D using standard ELISA kits from R&D Systems (Minneapolis, MN, USA) according to the instructions of the manufacturer and quantified using SoftMaxPro 5.4.5 ELISA analysis software (Molecular Devices, Sunnyvale, CA, USA). All markers in blood were analyzed in duplicate and the average concentration was used for further statistical analysis. To assess technical variability in the assessment of these markers we calculated the intraclass correlation coefficient (ICC) for each marker, using duplicate split samples (ICC_{dup}).

Lung function

Lung function was performed using the EasyOne electronic spirometer (ndd Medizintechnik, Zurich, Switzerland), which meets standards by the European Respiratory Society (ERS) and American Thoracic Society (ATS).[19] Tests were done in sitting positions, repeated until at least three technically correct maneuvers were obtained, and were validated by a certified lung function technician. The best value from the technically correct maneuvers was selected according to the maximum value method of the European Respiratory Society.[20] Individuals for which not at least two acceptable and reproducible tests were collected were excluded from the analysis. We measured FEV1 and FVC and calculated the percentage of predicted values for these measures using European Respiratory Society equations.[20]

Fractional exhaled nitric oxide (FENO)

FENO was measured in ppb using the NIOX MINO (Aerocrine, Solna, Sweden). Subjects inhaled filtered air through the monitor until reaching full lung capacity. Next the subject exhaled through the device at an approximate flow of 50 mL/s. FENO was measured with an electrochemical sensor.

Statistical analyses

To assess the volatility of markers measured in blood over time we calculated an ICC based on marker measurements from phase 1 and phase 2 (ICC_{rep}). Analyses of the association between markers measured in blood, FENO, and lung function parameters (FEV1, FVC, and FEV1/FVC) and exposure were conducted using multiple linear regression.

We used Tobit regression models to account for left censoring caused by values below the detection limit for all markers (including, for consistency, markers that were not left censored). We excluded markers from statistical analysis if the percentage of concentrations below the limit of detection was higher than 60%. We specified a lognormal distribution for all continuous outcome markers in the Tobit regression. We used simple multiple linear regression to model FEV1 and FVC on a linear scale. All analyses were adjusted for potentially confounding parameters age, body mass index, smoking, and sex. Analyses were conducted separately for phase 1 and 2.

We conducted categorical analysis comparing three categories of exposed workers (operators, lab high and lab low) to non-exposed controls. In addition, we conducted analyses

assessing the trend across exposure categories (assigning values of 0-3 to the exposure categories based on their exposure ranking), and conducted linear regression using the assigned actual exposure estimates for each category.[13] Analysis of the risk scores (both on a linear scale and after natural log-transformation) was conducted in phase 1 only, following the same strategy as for individual markers, but using linear regression.

We conducted a series of sensitivity analyses for phase 1 to assess the robustness of our noteworthy findings by additionally correcting univariably for information from the baseline questionnaire: alcohol use (reported by 51 subjects), doctor diagnosed cardiovascular disease (n=3), doctor diagnosed chronic disease (n=24), doctor diagnosed inflammatory disease (n=17), metabolic disease (n=2), educational level, previous exposure to chemicals (n=16), previous exposure to nanoparticles (n=17), previous exposure to particulates (n=6); the latter three categories were created based on reported jobs and tasks in the workers occupational history, for information from the questionnaire covering the 24 hours before blood collection: self-reported 'recent infection' (n=1), and for white blood cell count (as a marker of infection) , excluding laboratory workers that potentially had previous exposure as an operator (n=2), excluding the manager of the operators (n=1; initially categorized as operator), jack-knifing controls by location, and excluding smokers and females.

For our most robustly associated markers we conducted a Kruskal-Wallis test to assess whether measurements from operators, lab workers, and controls could have originated from the same distribution and a Wilcoxon test to assess whether measurements from operators and controls were significantly different from each other.

A *p*-value of 0.05 was used as cut-off value to declare statistical significance. Because we consider this an exploratory pilot study with limited statistical power, we do not report which of our findings survived a correction for multiple testing.[21] All statistical analyses were conducted in R version 3.03 (R Core Team 2014).

Results

We included 22 workers exposed to MWCNTs and 39 non-exposed workers (controls) (phase 1). A subset of 10 exposed workers and 6 controls was assessed a second time (phase 2). We provide further details on the study population in Table 1. In phase 1, we assigned GM MWCNT mass concentrations of 1, 7, and 45 EC $\mu g/m^3$ to exposure groups 'lab low', 'lab high', and 'operators', respectively. We measured higher exposure levels among operators during phase 2 as a result of the primary production process which was active in phase 2, resulting in an assigned GM MWCNT mass concentration of 57 $\mu g/m^3$. No individual MWCNTs (diameter <10 nm, length >1 µm) were observed in the collected inhalable particulate matter samples. In general, the particle size of the MWCNT agglomerates ranged from 200nm to 100 µm, indicating a modal distribution with a mode diameter between 650 and 1000nm.[13] Although the exposure groups were generally matched on gender and age, the operators

were on average slightly older than the rest of the study population and the only group that was exclusively male.

Table 1: Demographic characteristics of the study population. In between brackets characteristics of the subset of individuals that also participated in phase 2.

	Controls[b]	Lab low[c]	Lab high[d]	Operators[e]
Individuals (n)[a]	39 (6)	9 (2)	6 (1)	7 (7)
Median Age[a]	31.7 (37.1)	32.2 (32.2)	30.1 (28)	36.2 (36.2)
Percentage women[a]	18 (0)	33 (0)	17 (0)	0 (0)
Median BMI[a]	24.4 (26.1)	25.7 (31.9)	25.4 (32.4)	25.3 (25.2)
Percentage smokers[a]	37 (0)	56 (50)	50 (100)	29 (29)
Exposure ranking[a]	0 (0)	1 (1)	2 (2)	3 (3)
Assigned GM EC exposure (µg/m3) [a]	0 (0)	1 (1)	7 (7)	45 (57)

[a] Top row: phase 1, June 2013. Second row (in between brackets): phase 2, October 2013, [b] Individuals not exposed to MWCNT working in the MWCNT factory or in nearby companies, [c] Laboratory personnel performing tasks with relatively low exposure, [d] Laboratory personnel performing tasks with relatively high exposure, [e] Operators working with the reactor.

We measured 51 immunological markers in blood collected in phase 1 and 2 of the project. Five markers (IL-3, IL-33, thymic stromal lymphopoietin, thrombopoietin, and stem cell factor) were excluded from statistical analysis because the percentage of concentrations below the limit of detection was higher than 60%. Median ICC_{dup} of the immunological markers in phase 1 was 0.82 (IQR: 0.63, 0.93)and in phase 2 0.95 (IQR: 0.83, 0.98). Median ICC_{rep} was 0.51 (IQR: 0.36, 0.66). The median Pearson correlation between immunological markers measured among controls in phase 1 and phase 2 was 0.62.

In phase 1 we observed trends in immune marker concentrations with exposure to MWCNTs for C-X-C motif chemokine 11 (CXCL11) [upwards; increasing with increasing exposure], C-C motif ligand 20 (CCL20) [upwards], Interleukin 16 (IL-16) [downwards], eskine and cutaneous T-cell-attracting chemokine (CTACK) [downwards], basic fibroblast growth factor (FGF-BASIC) [upwards], and soluble IL-1 receptor II (sIL-1RII) [upwards] (Table 2).

Table 2: Difference in immunological marker concentration (ng/mL) between workers exposed to multi walled carbon nanotubes and controls in phase 1[a].

Marker[b]	Lab low[c]	Lab high[c]	Operators[c]	Trend ranking[c]	Trend GM[c]
BCA-1	-0.0681 (p=0.6138)	0.2883 (p=0.0609)	-0.0161 (p=0.9108)	0.0268 (p=0.5385)	0.0001 (p=0.9815)
CCL19 MIP3B	-0.0420 (p=0.7467)	0.1589 (p=0.2834)	-0.0030 (p=0.9826)	0.0160 (p=0.6974)	0.0002 (p=0.9536)
CCL20 MIP3A	-0.0794 (p=0.5880)	**0.3318 (p=0.0470)**	**0.3986 (p=0.0106)**	**0.1309 (p=0.0051)**	**0.0093 (p=0.0087)**
CCL21 6CKINE	-0.1033 (p=0.4037)	-0.2435 (p=0.0844)	-0.0538 (p=0.6830)	-0.0461 (p=0.2399)	-0.0012 (p=0.6801)
CRP	-0.2522 (p=0.2333)	**1.1658 (p<0.0001)**	-0.2775 (p=0.2179)	0.0583 (p=0.4706)	-0.0044 (p=0.4665)
CTACK	**-0.2101 (p=0.0440)**	-0.2223 (p=0.0616)	-0.1769 (p=0.1112)	**-0.0781 (p=0.0186)**	-0.0037 (p=0.1477)
CXCL11 I-TAC	0.2126 (p=0.2480)	**0.5221 (p=0.0128)**	0.2867 (p=0.1432)	**0.1396 (p=0.0169)**	0.0064 (p=0.1550)
CXCL6 GCP-2	0.1220 (p=0.4473)	0.1408 (p=0.4417)	0.1100 (p=0.5197)	0.0485 (p=0.3352)	0.0023 (p=0.5403)
CXCL9 MIG	0.0875 (p=0.5335)	**0.3569 (p=0.0258)**	-0.0296 (p=0.8430)	0.0386 (p=0.3951)	-0.0005 (p=0.8931)
EGF	**0.6687 (p=0.0357)**	0.0332 (p=0.9272)	0.5705 (p=0.0927)	0.1718 (p=0.0951)	0.0113 (p=0.1462)
ENA-78	**0.4597 (p=0.0415)**	0.3475 (p=0.1765)	0.1031 (p=0.6675)	0.0864 (p=0.2334)	0.0017 (p=0.7561)
EOTAXIN	-0.1145 (p=0.4003)	0.0503 (p=0.7457)	0.0061 (p=0.9663)	0.0021 (p=0.9602)	0.0004 (p=0.8975)
EOTAXIN-2	-0.2825 (p=0.2786)	-0.0101 (p=0.9729)	-0.0899 (p=0.7459)	-0.0356 (p=0.6642)	-0.0014 (p=0.8173)
FGF_BASIC	0.0375 (p=0.8268)	0.3667 (p=0.0607)	0.2635 (p=0.1487)	**0.1079 (p=0.0458)**	0.0061 (p=0.1381)
G-CSF	0.0300 (p=0.8554)	0.3018 (p=0.1078)	0.2278 (p=0.1936)	0.0914 (p=0.0774)	0.0053 (p=0.1801)
GRO	0.3358 (p=0.1017)	0.0834 (p=0.7215)	0.0986 (p=0.6516)	0.0487 (p=0.4549)	0.0016 (p=0.7469)
IL-16	-0.1685 (p=0.2722)	-0.2769 (p=0.1134)	-0.2707 (p=0.0973)	**-0.1051 (p=0.0288)**	-0.0059 (p=0.1077)
IL-1RA	0.1324 (p=0.5452)	0.2403 (p=0.3354)	0.3976 (p=0.0878)	0.1296 (p=0.0579)	0.0088 (p=0.0907)
IL-29 IFNL1	0.1917 (p=0.5333)	-0.3770 (p=0.3128)	-0.1891 (p=0.5761)	-0.0813 (p=0.4096)	-0.0052 (p=0.4868)
IL-7	0.0166 (p=0.9349)	0.1763 (p=0.4466)	0.3140 (p=0.1465)	0.0968 (p=0.1279)	0.0071 (p=0.1396)
IL-8	-0.0601 (p=0.8720)	0.3083 (p=0.4684)	0.2514 (p=0.5266)	0.0938 (p=0.4225)	0.0060 (p=0.4968)
IP-10	-0.1318 (p=0.3611)	0.1967 (p=0.2317)	0.2459 (p=0.1092)	0.0761 (p=0.0979)	0.0059 (p=0.0882)
MCP-1	**-0.2512 (p=0.0352)**	-0.1665 (p=0.2207)	0.1794 (p=0.1576)	0.0121 (p=0.7592)	0.0043 (p=0.1435)

MCP-2	0.0287 (p=0.7445)	0.0415 (p=0.6798)	-0.1015 (p=0.2791)	-0.0182 (p=0.5120)	-0.0023 (p=0.2780)
MCP-4	-0.2193 (p=0.0540)	-0.2444 (p=0.0578)	-0.0656 (p=0.5855)	-0.0543 (p=0.1355)	-0.0013 (p=0.6508)
MDC	-0.0519 (p=0.7382)	-0.0009 (p=0.9957)	0.2475 (p=0.1342)	0.0570 (p=0.2445)	0.0056 (p=0.1280)
MIP-1B	-0.1368 (p=0.4452)	-0.0390 (p=0.8487)	0.0110 (p=0.9539)	-0.0081 (p=0.8851)	0.0005 (p=0.9100)
MIP-1D	0.0936 (p=0.4826)	0.0062 (p=0.9676)	-0.0826 (p=0.5600)	-0.0149 (p=0.7229)	-0.0020 (p=0.5241)
SAA	-0.2739 (p=0.4100)	0.5399 (p=0.1542)	0.0338 (p=0.9239)	0.0589 (p=0.5803)	0.0019 (p=0.8180)
SAP	0.0403 (p=0.6538)	**0.2173 (p=0.0340)**	0.0140 (p=0.8833)	0.0306 (p=0.2880)	0.0005 (p=0.8365)
SDF-1A+B	-0.0737 (p=0.3750)	**-0.1970 (p=0.0374)**	0.1084 (p=0.2201)	-0.0004 (p=0.9889)	0.0023 (p=0.2513)
SEGFR	0.0500 (p=0.2851)	-0.0111 (p=0.8346)	-0.0059 (p=0.9055)	-0.0004 (p=0.9762)	-0.0002 (p=0.8279)
SGP130	-0.1161 (p=0.1567)	-0.0692 (p=0.4588)	-0.1378 (p=0.1140)	-0.0465 (p=0.0714)	-0.0029 (p=0.1412)
sIL-4R	-0.0785 (p=0.1391)	**0.1472 (p=0.0149)**	0.0246 (p=0.6634)	0.0195 (p=0.2696)	0.0009 (p=0.5266)
sIL-6R	0.0206 (p=0.8363)	**0.2607 (p=0.0217)**	-0.0370 (p=0.7268)	0.0225 (p=0.4866)	-0.0006 (p=0.8090)
sIL-1RII	**0.1835 (p=0.0344)**	**0.2191 (p=0.0268)**	**0.2646 (p=0.0042)**	**0.0975 (p=0.0004)**	**0.0057 (p=0.0079)**
STNFRI	0.0692 (p=0.5348)	0.2394 (p=0.0595)	-0.0596 (p=0.6151)	0.0168 (p=0.6390)	-0.0012 (p=0.6548)
STNFRII	-0.0055 (p=0.9757)	0.2001 (p=0.3312)	-0.1556 (p=0.4182)	-0.0142 (p=0.8039)	-0.0032 (p=0.4532)
SVEGFR2	0.0825 (p=0.2400)	0.0404 (p=0.6135)	-0.0827 (p=0.2685)	-0.0114 (p=0.6124)	-0.0020 (p=0.2444)
SVEGFR3	0.1844 (p=0.3147)	**0.4586 (p=0.0282)**	0.1397 (p=0.4739)	0.0956 (p=0.1019)	0.0032 (p=0.4768)
TARC	0.1456 (p=0.5135)	0.4358 (p=0.0862)	0.0929 (p=0.6952)	0.0799 (p=0.2575)	0.0022 (p=0.6808)
TGF-A	0.0084 (p=0.9716)	0.2802 (p=0.2973)	0.3768 (p=0.1333)	0.1237 (p=0.0940)	0.0086 (p=0.1238)
TNF-B	0.0992 (p=0.7507)	0.2064 (p=0.5621)	0.4822 (p=0.1468)	0.1445 (p=0.1389)	0.0107 (p=0.1474)
TNFA_1	-0.0963 (p=0.5149)	0.1677 (p=0.3199)	0.0715 (p=0.6497)	0.0324 (p=0.4882)	0.0019 (p=0.5816)
TRAIL	-0.0297 (p=0.7787)	-0.1501 (p=0.2123)	0.1841 (p=0.1012)	0.0253 (p=0.4580)	0.0040 (p=0.1163)
VEGF	-0.0722 (p=0.7619)	0.1909 (p=0.4823)	0.2390 (p=0.3461)	0.0764 (p=0.3064)	0.0056 (p=0.3185)

[a] Estimates from Tobit regression, corrected for age, BMI, sex, and smoking, [b] Markers were specified to be lognormally distributed in the Tobit model, [c] Lab low: Laboratory personnel performing tasks with relatively low exposure; lab high: laboratory personnel performing tasks with relatively high exposure; operators: operators working with the reactor; trend ranking: trend estimate based on a ranking of the exposure categories; trend GM: trend estimate based on assigned EC exposure estimates (µg/m^3). The bolded values are the p-values that are significant (p<.05).

For CCL20 and sIL-1RII estimates from trend analysis were significant regardless of the approach for exposure assessment (ranking: β=0.1309; p=0.0051 and β=0.0975; p=0.0004, respectively, and assignment: β=0.0093; p=0.0087 and β=0.0057; p=0.0079, respectively). For these two markers we also observed significant differences in blood concentrations between operators or lab-workers and controls (with the exception of a non-significant decrease in the concentration of CCL20 among low-exposed lab workers). We observed no consistent association between exposure to MWCNT and the two inflammation scores (Supplemental Material, table S1), though we did observe significant elevation of the inflammation score among higher exposed lab workers (lab high). After log-transformation the inflammation score reported in Shiels et al.[15] was no longer significantly elevated among higher exposed lab workers.

Among the markers that were significantly associated with MWCNT exposure in phase 1, we observed significant trends in blood concentration with exposure to MWCNT (either assigned exposure ranking, or assigned GM MWCNT) for CCL20 (upwards; blood concentrations among operators also significantly elevated), CTACK (upwards; blood concentrations among operators also significantly elevated), FGF-BASIC (upwards; blood concentrations among operators and higher exposed lab personnel also significantly elevated) in phase 2 (Supplemental material Table S2). The effect for CTACK was in the opposite direction of what was observed during phase 1. For sIL-1RII we observed a non-significant upward trend with exposure to MWCNT and we observed significantly elevated blood concentrations among operators and higher exposed lab personnel.

In Figure 1 we show boxplots of the distributions of the markers that were most robustly associated with MWCNT exposure in phase 1 and 2 (CCL20, sIL-1RII, and FGF-BASIC). The effect of exposure to MWCNT on blood concentrations of sIL-1RII was robust (both in direction of effect and statistical significance) to all sensitivity analyses (Supplemental Material, Table S3). The effect of exposure to MWCNT on blood concentrations of CCL20 was generally stable across sensitivity analyses. While the direction of the effect for the three exposure categories remained unchanged in each sensitivity analysis, the effect for the highest exposure category 'operator' lost its formal statistical significance after correction for alcohol use, previous exposure to particulates, and when we excluded females and smokers. Results for FGF-BASIC were robust to the sensitivity analyses in terms of the direction of the effect. The effects among higher exposed lab workers and operators became stronger after correction for educational level and when we excluded females and smokers, now reaching statistical significance. Our findings for sIL-1RII, CCL20, and FGF-BASIC were not (partially) explained by a strong correlation between these markers (Supplemental material, Figure S1). While FGF-BASIC was strongly correlated to several other immunological markers, the correlations with sIL-1RII and CCL20 were low in phase 1 (0.21 and 0.31, respectively) and slightly higher in phase 2 (0.25 and 0.64, respectively).

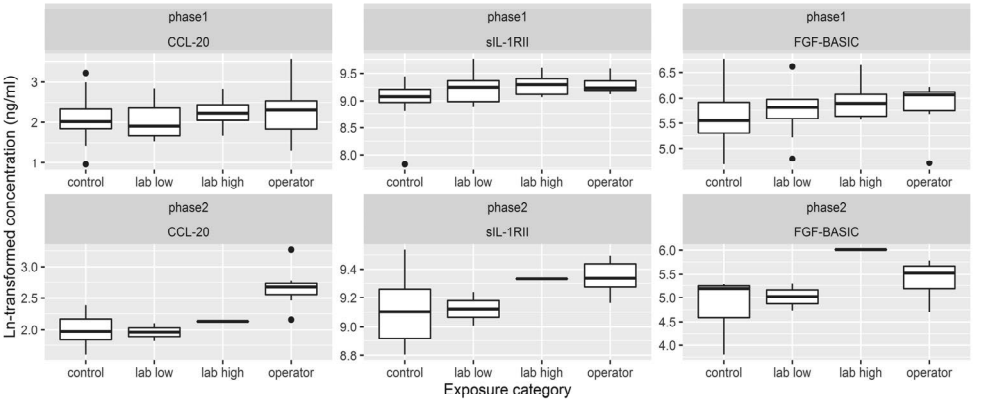

Figure 1: Boxplots showing the distribution of Ln-transformed concentrations of CCL20, sIL-1RII, and FGF-BASIC during phase 1 and phase 2, by exposure category. Kruskal-Wallis rank sum test p-values for phase 1 were 0.6172, 0.02232, 0.1527, for CCL20, sIL-1RII, and FGF-BASIC, respectively. Wilcoxon rank sum test p-values (operators versus controls) for phase 1 were 0.4352, 0.01087, 0.1188. Kruskal-Wallis rank sum test p-values for phase 2 were 0.018, 0.2097, 0.1018, for CCL20, sIL-1RII, and FGF-BASIC, respectively. Wilcoxon rank sum test p-values (operators versus controls) for phase 2 were 0.0047, 0.1375, 0.0734.

Median ICC_{rep} for the 23 parameters that were measured as part of the CBC was 0.69 (IQR: 0.54, 0.79). We observed significant depression in neutrophils and significant elevation in monocytes, mean platelet volume, immature platelet fraction, and immature reticulocytes fraction with increasing exposure to MWCNT in phase 1 (Table 3) and phase 2 (Supplemental Material, Table S4). For neutrophils, monocytes, and mean platelet volume the results were robust to all sensitivity analyses (Supplemental Material, Table S5), with the exception of a correction for previous exposure to nanoparticles which resulted in a loss of formal significance for mean platelet volume (p=0.1134). The result for immature platelet fraction and immature reticulocytes fraction was not robust in the sensitivity analyses. Neutrophils and white blood cells were elevated among the higher exposed lab workers in phase 1, but this effect was not replicated in phase 2. In phase 2 we observed several significant associations that were not observed in phase 1.

Table 3: Difference in complete blood cell counts between workers exposed to multi walled carbon nanotubes and controls in phase 1[a].

Marker[b,c]	Lab low[d]	Lab high[d]	Operators[d]	Trend ranking[d]	Trend GM[d]
Hemoglobine (g/dL)	0.0151 (p=0.6691)	0.0640 (p=0.0943)	0.0419 (p=0.2402)	0.0183 (p=0.0830)	0.0010 (p=0.2306)
Hematocrit (%)	0.0020 (p=0.9556)	0.0482 (p=0.2051)	0.0494 (p=0.1639)	0.0176 (p=0.0914)	0.0011 (p=0.1521)
RBC (10 12/L)**	0.0194 (p=0.5768)	0.0507 (p=0.1793)	0.0264 (p=0.4532)	0.0132 (p=0.2053)	0.0006 (p=0.4462)
MCV (fL)	-0.0176 (p=0.2014)	-0.0026 (p=0.8633)	0.0229 (p=0.1010)	0.0044 (p=0.2914)	0.0005 (p=0.0894)
MCH (pg)	-0.0044 (p=0.7390)	0.0132 (p=0.3623)	0.0158 (p=0.2415)	0.0052 (p=0.1948)	0.0004 (p=0.2192)
MCHC (g/dL)	0.0132 (p=0.1968)	0.0161 (p=0.1479)	-0.0073 (p=0.4829)	0.0007 (p=0.8202)	-0.0002 (p=0.4800)
RDW (%)	-0.0096 (p=0.4689)	0.0020 (p=0.8903)	-0.0048 (p=0.7222)	-0.0013 (p=0.7372)	-0.0001 (p=0.7691)
Reticulocytes (109/L)**	0.0740 (p=0.5448)	0.0256 (p=0.8468)	-0.1006 (p=0.4159)	-0.0180 (p=0.6226)	-0.0023 (p=0.3986)
IRF (%)	0.0545 (p=0.7856)	0.2744 (p=0.2065)	0.3552 (p=0.0800)	**0.1201 (p=0.0439)**	0.0080 (p=0.0762)
Ret-He (pg)	0.0133 (p=0.2844)	-0.0019 (p=0.8892)	0.0023 (p=0.8537)	0.0009 (p=0.8066)	0.0000 (p=0.9220)
WBC (109/L)**	0.0724 (p=0.3566)	**0.2847 (p=0.0008)**	-0.0739 (p=0.3532)	0.0189 (p=0.4597)	-0.0015 (p=0.4451)
Neutrophils (%)	0.0105 (p=0.8736)	0.0242 (p=0.7345)	**-0.1692 (p=0.0111)**	-0.0374 (p=0.0620)	-0.0037 (p=0.0117)
Neutrophils (109/L)**	0.0897 (p=0.4340)	**0.3191 (p=0.0103)**	**-0.2338 (p=0.0441)**	-0.0148 (p=0.6910)	**-0.0050 (p=0.0678)**
Eosinophils (%)	-0.3551 (p=0.1914)	-0.2615 (p=0.3751)	0.3226 (p=0.2414)	0.0314 (p=0.7056)	0.0075 (p=0.2305)
Eosinophils (109/L)**	-0.7728 (p=0.0810)	0.0985 (p=0.8350)	0.3826 (p=0.3818)	0.0716 (p=0.5885)	0.0098 (p=0.3233)
Basophils (%)	-0.1708 (p=0.4653)	-0.3039 (p=0.2056)	0.0893 (p=0.6908)	-0.0207 (p=0.7575)	0.0020 (p=0.6945)
Lymphocytes (%)	-0.0507 (p=0.6234)	-0.0926 (p=0.4088)	0.2040 (p=0.0513)	0.0360 (p=0.2552)	0.0045 (p=0.0547)
Lymphocytes (109/L)**	0.0214 (p=0.8561)	0.1912 (p=0.1357)	0.1163 (p=0.3313)	0.0514 (p=0.1457)	0.0027 (p=0.3114)
Monocytes (%)	-0.0172 (p=0.8369)	0.0473 (p=0.6014)	**0.2174 (p=0.0102)**	**0.0571 (p=0.0232)**	**0.0049 (p=0.0093)**
Monocytes (109/L)**	0.1027 (p=0.2600)	**0.3369 (p=0.0007)**	0.1407 (p=0.1274)	**0.0779 (p=0.0056)**	0.0033 (p=0.1384)

Plateletes (109/L)**	**-0.0234 (p=0.7547)**	**-0.0388 (p=0.6322)**	**-0.1086 (p=0.1517)**	**-0.0317 (p=0.1543)**	**-0.0024 (p=0.1530)**
MPV (fL)	-0.0296 (p=0.2857)	0.0233 (p=0.4371)	**0.0799 (p=0.0044)**	**0.0207 (p=0.0148)**	**0.0018 (p=0.0035)**
IPF (%)	0.0203 (p=0.9069)	0.2086 (p=0.2674)	**0.3488 (p=0.0471)**	**0.1093 (p=0.0343)**	**0.0079 (p=0.0439)**

[a] Estimates from Tobit regression, corrected for age, BMI, sex, and smoking, [b] Markers were specified to be lognormally distributed in the Tobit model, [c]RBC (red blood cells), MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), MCHC (mean corpuscular hemoglobin concentration), RDW (red blood cell distribution width), IRF (immature reticulocytes fraction), Ret-He (reticulocyte hemoglobin equivalent), WBC (white blood cells), MPV (mean platelet volume), IPF (immature platelet fraction), [d] Lab low: Laboratory personnel performing tasks with relatively low exposure; lab high: laboratory personnel performing tasks with relatively high exposure; operators: operators working with the reactor; trend ranking: trend estimate based on a ranking of the exposure categories; trend GM: trend estimate based on assigned EC exposure estimates (μg/m^3).

We observed significantly lower FENO among operators compared to controls during phase 1 (Table 4). This effect was robust (both in direction of effect and statistical significance) to all sensitivity analyses (Supplemental Material, Table S6), with the exception of a correction for previous exposure to nanoparticles, which resulted in a loss of formal significance (p=0.069). We observed significant trends based on assigned GM MWCNT mass concentrations in the full population as well as among male non-smokers. Trends based on exposure ranking were in the same direction, but were not significant.

Table 4: Difference in fractional exhaled nitric oxide (ppb) between workers exposed to multi walled carbon nanotubes and controls in phase 1[a,b].

Exposure category[c]	Total study population	Among non-smoking males
Lab low	0.2478 (p=0.4019)	-0.2407 (p=0.5331)
Lab high	0.0841 (p=0.7943)	0.1070 (p=0.7517)
Operators	-0.8816 (p=0.0115)	-0.9674 (p=0.0081)
Trend ranking	-0.1768 (p =0.0809)	-0.2053 (p=0.0576)
Trend GM	-0.0204 (p=0.0086)	-0.0201 (p= 0.0123)

[a] Estimates from Tobit regression, corrected for age, BMI, sex, and smoking, [b] Fractional exhaled nitric oxide concentration was specified to be lognormally distributed in the Tobit model, [c] Lab low: Laboratory personnel performing tasks with relatively low exposure; lab high: laboratory personnel performing tasks with relatively high exposure; operators: operators working with the reactor; trend ranking: trend estimate based on a ranking of the exposure categories; trend GM: trend estimate based on assigned EC exposure estimates (μg/m^3).

Acceptable spirometry data was collected from 55 individuals. We observed some indication for a larger (percentage of the predicted) FVC among operators compared to controls, but observed no significant trends with FVC, FEV1 and FEV1/FVC with exposure to MWCNTs, regardless of the approach for exposure assessment that we used (Table 5). Results were similar among non-smoking males (Supplemental Material, Table S7).

Table 5: Difference in lung function between workers exposed to multi walled carbon nanotubes and controls in phase 1[a].

Exposure category[b]	FEV1[c]	FEV1%[c,f]	FVC[d]	FVC%[d,f]	FEV1/FVC[e]	FEV1/FVC%[e,f]
Lab low	0.0093 (p=0.9700)	0.1611 (p=0.9768)	-0.0280 (p=0.9274)	-0.7952 (p=0.8730)	1.3312 (p=0.6484)	0.6232 (p=0.8550)
Lab high	0.0717 (p=0.7647)	0.3231 (p=0.9511)	0.1236 (p=0.6790)	0.5312 (p=0.9110)	0.1141 (p=0.9678)	-0.8263 (p=0.8000)
Operators	0.3294 (p=0.1954)	6.7278 (p=0.2274)	0.6075 (p=0.0585)	8.1841 (p=0.1060)	-2.5288 (p=0.3977)	-1.7899 (p=0.6010)
Trend ranking	0.0859 (p=0.2356)	1.5690 (p=0.3172)	0.1558 (p=0.0895)	1.8980 (p=0.1837)	-0.5245 (p=0.5392)	-0.5054 (p=0.5990)
Trend GM	0.0073 (p=0.1817)	0.1485 (p=0.2180)	0.0136 (p=0.0494)	0.1832 (p=0.0944)	-0.0593 (p=0.3600)	-0.0421 (p=0.5700)

[a] Estimates from linear regression, corrected for age, BMI, sex, and smoking. Analyses of 'percentage of predicted values' were corrected smoking only, [b] Lab low: Laboratory personnel performing tasks with relatively low exposure; lab high: laboratory personnel performing tasks with relatively high exposure; operators: operators working with the reactor; trend ranking: trend estimate based on a ranking of the exposure categories; trend GM: trend estimate based on assigned EC exposure estimates (µg/m^3), [c] Forced expiratory volume in 1 second (L), [d] Forced vital capacity (L), [e] Ratio forced vital capacity and forced expiratory volume in 1 second, [f] Percentage of predicted values calculated using European Respiratory Society equations.[20]

ICC_{dup} for the pneumoproteins was high (>0.94) and ICC_{rep} for CC16, SP-A, and SP-D was 0.81, 0.77, and 0.48, respectively. We observed no significant trends in blood concentrations of the pneumoproteins with exposure to MWCNTs in phase 1, regardless of the approach for exposure assessment that we used (Table 6). This observation was confirmed by visual assessment of the variation in pneumoprotein concentrations across exposure categories (Figure 2). We observed similar results when we restricted the dataset to male non-smokers and in phase 2 (results not shown).

Table 6: Difference in pneumoproteins (ng/mL) between workers exposed to multi walled carbon nanotubes and controls in phase 1[a].

Marker[b]	Lab low[c]	Lab high[c]	Operators[c]	Trend ranking[c]	Trend GM[c]
CC16	-0.2080 (p= 0.1876)	0.0939 (p= 0.5987)	-0.1767 (p= 0.2910)	-0.0409 (p= 0.4073)	-0.0034 (p= 0.3618)
SP-A	0.4344 (p= 0.0799)	0.2476 (p= 0.3753)	0.1227 (p= 0.6368)	0.0783 (p= 0.3094)	0.0021 (p= 0.7191)
SP-D	-0.0287 (p= 0.8811)	-0.1077 (p= 0.6229)	0.0975 (p= 0.6335)	0.0095 (p= 0.8720)	0.0021 (p= 0.6362)

[a] Estimates from Tobit regression, corrected for age, BMI, sex, and smoking, [b] Markers were specified to be lognormally distributed in the Tobit model, [c] Lab low: Laboratory personnel performing tasks with relatively low exposure; lab high: laboratory personnel performing tasks with relatively high exposure; operators: operators working with the reactor; trend ranking: trend estimate based on a ranking of the exposure categories; trend GM: trend estimate based on assigned EC exposure estimates (µg/m^3).

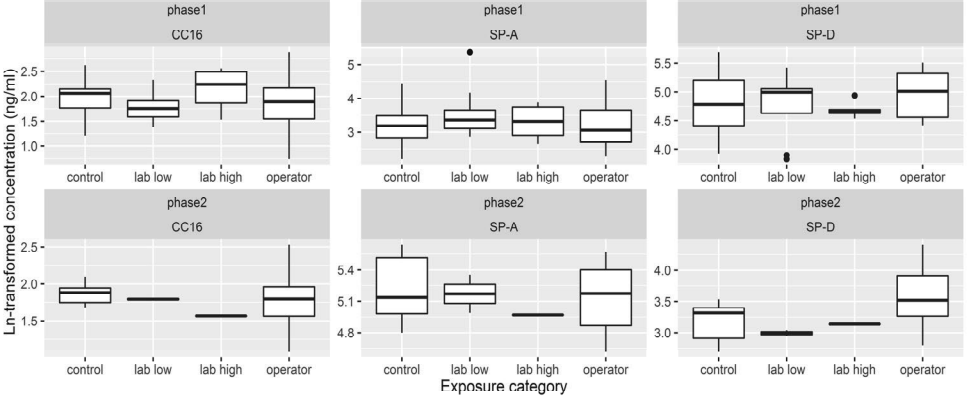

Figure 2: Boxplots showing the distribution of Ln-transformed concentrations of CC-16, SP-A, and SPD during phase 1 and phase 2, by exposure category. Kruskal-Wallis rank sum test p-values for phase 1 were 0.1388, 0.4333, 0.706, for CC-16, SP-A, and SPD, respectively. Wilcoxon rank sum test p-values (operators versus controls) for phase 1 were 0.4, 0.8576, 0.4529. Kruskal-Wallis rank sum test p-values for phase 2 were 0.5381, 0.2996, 0.7483, for CC-16, SP-A, and SPD, respectively. Wilcoxon rank sum test p-values (operators versus controls) for phase 2 were 0.5338, 0.2343, 0.6282.

Discussion

We observed an indication for an effect of exposure to MWCNTs on selected immune markers (CCL20, sIL-1RII, FGF-BASIC), FENO, and selected blood parameters (neutrophils, monocytes, and mean platelet volume) in a small scale cross-sectional study in a population occupationally exposed to MWCNT.

The observed increase in CCL20, sIL-1RII, and FGF-BASIC may be indicative of an inflammatory reaction. CCL20 is a chemokine involved in antimicrobial activity that has been reported to be negatively associated with emphysema in patients with chronic obstructive pulmonary disease.[22, 23] CCL20 production and secretion was shown to be suppressed when cells were exposed to cigarette smoke.[23] Although the literature is scarce, these reports contradict our finding of elevated levels of CCL20 among MWCNT exposed workers, for which we would expect the effect to be in the same direction as for cigarette smoke. SIL-1RII is a decoy receptor that binds proinflammatory interleukin 1 (IL-1), reducing its activity.[24] The observed increase in SIL-1RII might therefore be indicative of an IL-1 mediated response as a result of exposure to MWCNT. While we did not assess IL-1 in the current study, it has been reported to play a role in cigarette smoke induced inflammation.[25] FGF-BASIC is a member of a family of proteins with growth, anti-apoptotic, and differentiation promoting activity.[26] Tumor cell expression of FGF-BASIC has been reported as marker for cancer prognosis,[27, 28] but we are not aware of any literature relating exposure to environmental agents to changes of FGF-BASIC in peripheral blood. Considering the small number of identified markers we refrain from the formal identification of enriched pathways. Two of the inflammatory markers included in our study were also measured in serum by Fatkhutdinova et al.[11]: IL-8 and TNF-α. We did not replicate the significant association between exposure to MWCNT and TNF-α reported in that study, though the non-significant effect we observed was in the same direction (upregulation).[11] Eleven of the immune markers we assessed in our study have been reported to be predictive of lung cancer risk (CRP, SAA, sTNFRII, IL-1RA, IL-7, TGF-A, ENA 78/CXCL5, MIG/CXCL9, BCA-1/CXCL13, TARC/CCL1, MDC/CCL22).[15, 18] These markers and two risk scores based on these markers were not consistently associated to exposure to MWCNTs in our study. We therefore did not observe indirect evidence of a potential increased risk of lung cancer due to exposure to MWCNTs. CRP is also an established marker of acute phase inflammation and a risk factor for cardiovascular disease. CRP has been reported to be significantly affected by ambient particle exposure (including nano-sized particles).[29] We did not replicate this finding in our study.

Depression in FENO as result of exposure to MWCNT would suggest that MWCNT exposure may have an inhibitory effect on NO synthase in the airways.[30] Depression of FENO has been reported in response to cigarette smoke (a source of particulate exposure).[30] However, this effect might be related to the high concentrations of NO in cigarette smoke itself (inducing a negative feedback loop resulting in downregulation of NO synthase),[30] an effect that we

would not expect as result of exposure to MWCNTs. Our results are in contrast with the study by Wu et al.,[7] in which no significant effect of exposure to carbon nanotubes (type not further specified) on FENO was observed, while exposure to nanosized titanium dioxide significantly increased the risk of elevated FENO levels (> 35 ppb).[7] Other literature generally reported elevation in FENO (e.g. in response to air pollution [31, 32]) as potential indicator of eosinophilic inflammation. In our study, eosinophil concentrations measured in peripheral blood in phase 1 were non-significantly elevated in operators compared to controls and laboratory workers, and therefore did not corroborate our finding for FENO. The clinical relevance of our finding for FENO is unclear. A FENO greater than 50 ppb has been suggested as a clinically relevant cut point to indicate that eosinophilic inflammation is likely, while levels smaller than 25 ppb are considered an indication that eosinophilic inflammation is less likely.[33] In our study five individuals had a FENO greater than 50 ppb (none of them were exposed to high levels of MWCNTs), while the majority (n=51) had levels lower than 25 ppb. While all blood counts were within clinical reference ranges, we observed significant depression of neutrophils and significant elevation of monocytes and mean platelet volume among operators compared to controls. Neutrophils play a role in inflammation and have been reported to increase after exposure to particulates.[34] Decreases in neutrophil counts can originate from viral infections, drug use, and exposure to certain solvents, among other causes,[35] but have not been reported to be lower in relation to exposure to particulates and are therefore not likely explained by exposure to MWCNTs. Lee et al. [6] reported that no noticeable abnormalities were observed in hematology and blood biochemical marker measurements among workers exposed to MWCNT, though did not analyze subclinical changes in these parameters. Interestingly, neutrophils are involved in the production of NO in the airways [36] which corresponds to our observation of decreased FENO. Monocytes also play a role in in inflammation and have been shown to increase after exposure to particulates,[37-39] providing a suggestion of biological plausibility of this finding. Elevated mean platelet volume would indicate a high number of larger, younger platelets in the blood, resulting from upregulated bone marrow production and release of platelets into circulation and has been suggested as marker of platelet activation.[40] Platelet activation has been associated in the literature with exposure to particulates in the form of ambient air pollution, but the direction of the effect has been inconsistent.[41, 42]

We observed no effect of exposure to MWCNT on lung function. The non-significantly increased FVC among operators compared to controls might be attributable to insufficient correction for the effect of sex on FVC (though we did correct for sex both the regression models and in the predicted values). Our study did not have sufficient statistical power to detect subtle effects of MWCNT on lung function.

The null effect of exposure to MWCNTs on pneumoproteins CC16, SP-A, and SP-D that we observe in our current study contradicts with previously published in vitro studies that demonstrated an effect of exposure to MWCNTs on the production of these

pneumoproteins.[43, 44] Potential explanations include limited statistical power in our study (a false negative finding), differences between the in vitro studies and ours with regards to the levels and patterns of exposure that lung cells incurred and the types of MWCNTs that were used.

Strengths of our study include a relatively high contrast in exposure to MWCNTs [45] within the study population, quantitative exposure assessment, and detailed assessment of potential confounding factors. Even though our study is currently the largest that has evaluated the biological effects of MWCNT exposure in a human population, an important limitation is still its modest sample size. We conducted a series of sensitivity analyses to assess the influence of confounding on the noteworthy findings in the main analysis. To avoid identifying too many false positive findings, we did not explore the impact of negative-confounding on the markers for which we did not observe an association with MWCNT exposure in the main analysis.

Replication of our findings in an independent study population exposed to MWCNT is crucial. Setting up sufficiently large studies to allow the assessment of subtle health effects due to exposure to MWCNT is a challenge. In an inventory of engineered carbonaceous nanomaterial manufactures in the USA, the average number of workers per company that handled engineered carbonaceous nanomaterial was 10.[46] Furthermore, the type of MWCNTs that are produced across companies, and within companies over time varies considerably. Therefore, large-scale collaborations between research groups and companies are needed to be able to study the early health effects of MWCNT exposure with sufficient statistical precision.

In conclusion, in this molecular cross-sectional study we observed some indications of early biological perturbations associated with exposure to MWCNTs. We view this as an exploratory study and therefore a false discovery rate correction was not conducted. We have assessed the robustness of our findings in a series of sensitivity analyses and by conducting a repeat assessment of selected markers among the highest exposed workers. However, considering our modest sample size and our cross-sectional study design, influence of selection biases cannot fully be excluded. Our findings warrant follow-up in other MWCNT exposed populations incorporating personal exposure estimates. In addition, studies are needed that assess the potential impact of exposure to MWCNT on human health including outcomes such as non-malignant respiratory disease, oxidative stress, and cardiovascular disease.

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Supporting information

Description of the methodology for MWCNT exposure assessment

Exposure measurements

The collection of the exposure measurements that formed the basis of the MWCNT exposure assignment is described in detail.[1] Personal breathing zone measurements were performed during 3 days in May 2013 without any synthesis activities (handling period) and 4 days in November 2013 during a period of full-scale synthesis of MWCNTs in the reactor (synthesis period). During the handling period, exposure measurements were performed in the production area and in the R&D area (2 days) while during the synthesis period measurements were performed in the production area and in the office (2 days). In the production area and the R&D area, every available worker (present between 6:00h and 22:00h) was assessed resulting in repeated measurements for individuals (with a maximum of 6), while in the office a random selection of the workers was measured based on availability.[1]

Approach for the assessment of the level and nature of inhalable MWCNTs

The approach for the assessment of the level and nature of inhalable MWCNTs is described in detail in.[2] Personal breathing zone samples were analyzed with carbon analysis, inductively coupled plasma mass spectrometry (ICP-MS) and scanning electron microscopy / energy dispersive X-ray spectroscopy (SEM/EDX). Analytical methods were optimized for carbon analysis and SEM/EDX. Methods were developed for background correction using carbon analyses and SEM/EDX, CNT structure count with SEM/EDX and subsequent mass conversion based on both carbon analyses and SEM/EDX. The analysis of elemental carbon (EC) and organic carbon (OC) was based on the thermal optical method varying in treatment temperature and atmosphere composition (with Helium and Oxygen) resulting in three OC stages and three EC stages as described in the American Standard Method NIOSH 5040.[2, 3]

Appendix 1

Additional results

Table SI-1: Difference in inflammation scores between workers exposed to multi walled carbon nanotubes and controls in phase 1[a].

Inflammation score	Lab low[h]	Lab high[h]	Operators[h]	Trend ranking[h]	Trend GM[h]
Shiels 2013[b]	-0.5893 (p=0.5202)	**2.2490 (p=0.0353)**	0.5881 (p=0.5465)	0.3775 (p=0.2008)	0.0165 (p=0.4613)
Shiels 2013 + WBC[c]	-0.2961 (p=0.7711)	**3.4525 (p=0.0044)**	0.3348 (p=0.7572)	0.4708 (p=0.1649)	0.0115 (p=0.6548)
Shiels 2013 log transformed[d]	-0.0698 (p=0.8297)	0.4256 (p=0.2530)	0.0603 (p=0.8612)	0.0611 (p=0.5436)	0.0019 (p=0.8015)
Shiels 2015[e]	-0.2013 (p=0.8084)	**1.9684 (p=0.0420)**	-0.6113 (p=0.4900)	0.0743 (p=0.7820)	-0.0111 (p=0.5836)
Shiels 2015 + WBC[f]	0.0919 (p=0.9190)	**3.1720 (p=0.0034)**	-0.8645 (p=0.3712)	0.1677 (p=0.5864)	-0.0160 (p=0.4897)
Shiels 2015 log transformed[g]	-0.0249 (p=0.8776)	**0.4658 (p=0.0146)**	-0.1623 (p=0.3483)	0.0144 (p=0.7890)	-0.0031 (p=0.4489)

[a] Estimates from linear regression, corrected for age, BMI, sex, and smoking, [b] Inflammation score calculated as the sum of Z scores of CRP, BCA-1/CXCL13, MDC/CCL22, and IL-RA based on Shiels et al. 2013[4], [c] Inflammation score calculated as the sum of Z scores of CRP, BCA-1/CXCL13, MDC/CCL22, IL-RA, and white blood cell count, [d] Inflammation score calculated as the natural log of the sum of Z scores of CRP, BCA-1/CXCL13, MDC/CCL22, IL-RA, and a constant to avoid negative values, [e] Inflammation score calculated as the sum of Z scores of CRP, SAA, CXCL9, and sTNFRII based on Shiels et al. 2015[5],[f] Inflammation score calculated as the sum of Z scores of CRP, SAA, CXCL9, sTNFRII, and white blood cell count, [g] Inflammation score calculated as the natural log of the sum of Z scores of CRP, SAA, CXCL9, sTNFRII, and a constant to avoid negative values, [h] Lab low: Laboratory personnel performing tasks with relatively low exposure; lab high: laboratory personnel performing tasks with relatively high exposure; operators: operators working with the reactor; trend ranking: trend estimate based on a ranking of the exposure categories; trend GM: trend estimate based on assigned EC exposure estimates (µg/m^3).

Table SI-2: Difference immunological marker concentration (ng/mL) between workers exposed to multi walled carbon nanotubes and controls in phase 2[a].

Marker[b]	Lab low[c]	Lab high[c]	Operators[c]	Trend ranking[c]	Trend GM[c]
BCA-1	**-0.5625 (p=0.0091)**	-0.6922 (p=0.2021)	**-0.2414 (p=0.0465)**	-0.0701 (p=0.1109)	-0.0027 (p=0.2266)
CCL19 MIP3B	-0.187 (p=0.5034)	0.0292 (p=0.9668)	0.0334 (p=0.8317)	0.0082 (p=0.8696)	0.0007 (p=0.7852)
CCL20 MIP3A	-0.1885 (p=0.4231)	-0.4741 (p=0.4232)	**0.4154 (p=0.0017)**	**0.1579 (p=0.0004)**	**0.0083 (p=0.0001)**
CCL21 6CKINE	0.3332 (p=0.4141)	**-2.6267 (p<0.0001)**	0.3211 (p=0.1616)	0.1545 (p=0.0633)	0.0074 (p=0.0776)
CRP	-0.5312 (p=0.5561)	-2.721 (p=0.2308)	-0.3683 (p=0.4678)	-0.0497 (p=0.7626)	-0.0017 (p=0.8384)
CTACK	-0.1468 (p=0.6346)	-0.32 (p=0.6805)	**0.3717 (p=0.0323)**	**0.1383 (p=0.0127)**	**0.0072 (p=0.0078)**

CXCL11 I-TAC	**-0.9202 (p=0.0013)**	**-1.5536 (p=0.0309)**	0.0099 (p=0.9511)	0.0404 (p=0.539)	0.0034 (p=0.2888)
CXCL6 GCP-2	-0.2987 (p=0.4195)	1.7137 (p=0.0656)	0.3274 (p=0.1155)	0.0562 (p=0.5014)	0.0032 (p=0.4388)
CXCL9 MIG	**-0.9307 (p=0.0013)**	**-1.5878 (p=0.0295)**	-0.209 (p=0.2)	-0.0353 (p=0.5837)	-0.0004 (p=0.8975)
EGF	0.3976 (p=0.6541)	1.9368 (p=0.3861)	0.3718 (p=0.4571)	0.0746 (p=0.6393)	0.0032 (p=0.6918)
ENA-78	-0.0284 (p=0.9706)	1.0839 (p=0.5753)	0.8192 (p=0.0581)	0.252 (p=0.0661)	0.0128 (p=0.0609)
EOTAXIN	**0.7639 (p=0.0085)**	1.2636 (p=0.0837)	0.3098 (p=0.0577)	0.0786 (p=0.1838)	0.0029 (p=0.3445)
EOTAXIN-2	0.664 (p=0.256)	-1.9482 (p=0.1853)	0.2169 (p=0.5094)	0.1467 (p=0.2476)	0.0066 (p=0.3041)
FGF_BASIC	0.762 (p=0.0783)	**2.9619 (p=0.0065)**	**0.7363 (p=0.0025)**	**0.174 (p=0.0495)**	0.0076 (p=0.0922)
G-CSF	0.1532 (p=0.7224)	1.1009 (p=0.3103)	0.4508 (p=0.063)	0.1249 (p=0.1076)	0.0061 (p=0.1181)
GRO	-0.2191 (p=0.695)	0.2217 (p=0.8747)	0.39 (p=0.2146)	0.1265 (p=0.2042)	0.0067 (p=0.1739)
IL-16	0.0849 (p=0.8229)	-1.0875 (p=0.2544)	0.0068 (p=0.9746)	0.038 (p=0.5986)	0.0018 (p=0.6093)
IL-1RA	0.6514 (p=0.2361)	**3.8831 (p=0.005)**	**0.9692 (p=0.0017)**	0.2276 (p=0.0561)	0.0105 (p=0.083)
IL-29 IFNL1	**-2.2374 (p<0.0001)**	**5.171 (p<0.0001)**	-0.3123 (p=0.0776)	-0.0942 (p=0.135)	-0.0039 (p=0.2386)
IL-7	0.2764 (0.4625)	1.4822 (0.1173)	0.4536 **(p=0.0322)**	0.1161 (p=0.1004)	0.0055 (p=0.1264)
IL-8	-0.2757 (p=0.5748)	0.5419 (p=0.6612)	**1.0662 (p=0.0001)**	**0.3523 (p=0.0001)**	**0.0182 (p<0.0001)**
IP-10	**-1.0605 (p=0.0204)**	-2.072 (p=0.0717)	-0.1913 (p=0.4568)	-0.0155 (p=0.8673)	0.0008 (p=0.865)
MCP-1	0.2532 (p=0.2546)	0.6904 (p=0.217)	**0.3602 (p=0.0039)**	**0.1076 (p=0.0068)**	**0.0051 (p=0.0125)**
MCP-2	0.3227 (p=0.3708)	1.4682 (p=0.1056)	0.3135 (p=0.122)	0.0675 (p=0.3167)	0.0029 (p=0.3898)
MCP-4	**0.9896 (p=0.0066)**	**2.3373 (p=0.0133)**	**0.573 (p=0.0033)**	**0.1293 (p=0.0394)**	0.0053 (p=0.106)
MDC	**1.0374 (p<0.0001)**	**2.5998 (p<0.0001)**	**0.6512 (p<0.0001)**	**0.1594 (p=0.0033)**	**0.0065 (p=0.0262)**
MIP-1B	-1.0747 (p=0.155)	0.3234 (p=0.5345)	**0.5579 (p=0.0452)**	0.1373 (p=0.0512)	**0.0069 (p=0.0463)**
MIP-1D	-0.2012 (p=0.4033)	-0.4824 (p=0.4258)	0.0848 (p=0.5308)	0.0423 (p=0.3321)	0.0024 (p=0.2564)
SAA	-0.2914 (p=0.77)	2.034 (p=0.4174)	-0.3944 (p=0.4863)	-0.209 (p=0.2658)	-0.0103 (p=0.2749)

SAP	**-0.4151 (p=0.0163)**	**-1.6164 (p=0.0002)**	-0.1012 (p=0.2977)	0.0103 (p=0.805)	0.0012 (p=0.5729)
SDF-1A+B	**0.5188 (p=0.0002)**	**0.8073 (p=0.0214)**	**0.3041 (p=0.0001)**	**0.0878 (p=0.0046)**	**0.0037 (p=0.026)**
SEGFR	**0.4303 (p<0.0001)**	0.2818 (p=0.056)	**0.1099 (p=0.0009)**	0.0353 (p=0.1202)	0.0012 (p=0.3214)
SGP130	**0.3931 (p<0.0001)**	0.4605 (p=0.0546)	**0.1514 (p=0.0047)**	0.0437 (p=0.0601)	0.0016 (p=0.176)
sIL-4R	-0.2376 (p=0.0698)	0.7450 (p=0.0239)	0.0559 (p=0.4482)	-0.0073 (p=0.8415)	-5.6862e-05 (p=0.9751)
sIL-6R	0.0067 (p=0.9679)	-0.5102 (p=0.2264)	0.0229 (p=0.8082)	0.0243 (p=0.4444)	0.0012 (p=0.4342)
sIL-1RII	0.3183 (p=0.0720)	**1.3019 (p=0.0034)**	**0.2896 (p=0.0036)**	0.0643 (p=0.0840)	0.0028 (p=0.1457)
STNFRI	0.322 (p=0.0618)	**1.3067 (p=0.0026)**	**0.3779 (p=0.0001)**	**0.0951 (p=0.0084)**	**0.0043 (p=0.0206)**
STNFRII	**-0.266 (p=0.0396)**	**-0.8745 (p=0.0072)**	**0.1722 (p=0.0178)**	**0.0844 (p=0.0041)**	**0.0047 (p=0.0007)**
SVEGFR2	**0.3847 (p=5.72e-07)**	**1.1710 (p=1.45e-09)**	**0.1614 (p=1.90e-04)**	0.0245 (p=0.3106)	0.0007 (p= 0.5932)
SVEGFR3	0.4966 (p=0.1686)	1.4642 (p=0.1067)	**0.4414 (p=0.0295)**	0.1147 (p=0.0857)	0.0051 (p=0.1361)
TARC	-0.2792 (p=0.6573)	1.0726 (p=0.4981)	0.2504 (p=0.479)	0.0498 (p=0.6683)	0.0029 (p=0.6183)
TGF-A[d]	--	--	--	--	--
TNF-B	0.7991 (p=0.4291)	5.0390 (p=0.0547)	**1.3407 (p=0.0127)**	0.2912 (p=0.0778)	0.0137 (p=0.0933)
TNFA_1	**-0.3124 (p= 0.0384)**	-0.6268 (p=0.0987)	**0.1662 (p=0.0499)**	**0.0739 (p=0.019)**	**0.0042 (p=0.0048)**
TRAIL	-0.2787 (p=0.3974)	-1.1018 (p=0.1836)	0.0054 (p=0.9768)	0.0331 (p=0.5879)	0.0021 (p=0.4865)
VEGF	**2.3277 (p=0.0064)**	**6.8677 (p=0.0018)**	**1.6458 (p=0.0004)**	**0.372 (p=0.0229)**	0.0157 (p=0.0649)

[a] Estimates from Tobit regression, corrected for age, BMI, sex, and smoking, [b] Markers were assumed to be lognormally distributed, [c] Lab low: Laboratory personnel performing tasks with relatively low exposure; lab high: laboratory personnel performing tasks with relatively high exposure; operators: operators working with the reactor; trend ranking: trend estimate based on a ranking of the exposure categories; trend GM: trend estimate based on assigned EC exposure estimates (µg/m^3), [d] Regression model did not converge.

Table SI-3a: Difference in CCL20 (ng/mL) between workers exposed to multi walled carbon nanotubes and controls in a set of sensitivity analyses using phase 1 data[a,b].

Sensitivity analysis	Lab low[f]	Lab high[f]	Operator[f]
Corrected for alcohol use	-0.0412 (p=0.771)	0.2126 (p=0.206)	0.2624 (p=0.102)
Corrected for doctor diagnosed cardiovascular disease	-0.1268 (p=0.383)	0.317 (p=0.052)	**0.3631 (p=0.018)**
Corrected for doctor diagnosed chronic disease	-0.083 (p=0.576)	0.264 (p=0.156)	**0.3911 (p=0.015)**
Corrected for doctor diagnosed inflammatory disease	-0.0805 (p=0.587)	0.3325 (p=0.047)	**0.4003 (p=0.012)**
Corrected for doctor diagnosed metabolic disease	-0.0928 (p=0.508)	0.2096 (p=0.211)	**0.4277 (p=0.004)**
Corrected for educational level	0.0642 (p=0.68)	**0.4791 (p=0.005)**	**0.447 (p=0.011)**
Corrected for 'recent infection'	-0.0924 (p=0.531)	0.3237 (p=0.052)	**0.3907 (p=0.012)**
Corrected for white blood cell count	-0.1127 (p=0.435)	0.2005 (p=0.267)	**0.4263 (p=0.005)**
Corrected for previous exposure to chemicals	-0.0862 (p=0.564)	0.326 (p=0.053)	**0.3973 (p=0.011)**
Corrected for previous exposure to nanoparticles	0.0927 (p=0.667)	**0.5035 (p=0.028)**	**0.5564 (p=0.009)**
Corrected for previous exposure to particulates	-0.063 (p=0.649)	0.2903 (p=0.067)	0.2314 (p=0.146)
Without laboratory workers that potentially had previous exposure as operator	-0.0991 (p=0.475)	**0.3874 (p=0.028)**	**0.5525 (p<0.0001)**
Without 'manager operators'	-0.1038 (p=0.452)	**0.33 (p=0.035)**	**0.5564 (p<0.0001)**
Without controls A[c]	-0.0846 (p=0.571)	0.3278 (p=0.054)	**0.3907 (p=0.014)**
Without controls B[c]	-0.0797 (p=0.623)	0.316 (p=0.079)	**0.3925 (p=0.022)**
Without controls C[c]	-0.1535 (p=0.407)	0.3089 (p=0.121)	**0.4041 (p=0.027)**
Without controls D[c]	-0.0258 (p=0.863)	**0.3924 (p=0.022)**	**0.4022 (p=0.013)**
Among male non-smokers[d]	-0.1648 (p=0.315)	0.0953 (p=0.539)	0.2931 (p=0.062)
Among male non-smokers,corrected for 'recent infection'[d,e]	-0.1648 (p=0.315)	0.0953 (p=0.539)	0.2931 (p=0.062)
Among male non-smokers, corrected for white blood cell count [d]	-0.1786 (p=0.271)	0.0045 (p=0.979)	0.2832 (p=0.067)

[a] Estimates from Tobit regression, corrected for age, BMI, sex, and smoking, [b] Marker was specified to be lognormally distributed in the Tobit model, [c] Controls were selected from four different locations (A-D), controls on location A were working in the CNT factory, but where not exposed to CNTs, [d] Estimates from Tobit regression, corrected for age and BMI, [e] None of the male non-smokers were categorized as having had a recent infection, [f] Lab low: Laboratory personnel performing tasks with relatively low exposure; lab high: laboratory personnel performing tasks with relatively high exposure; operators: operators working with the reactor.

Table SI-3b: Difference in SIL-1RII (ng/mL) between workers exposed to multi walled carbon nanotubes and controls in a set of sensitivity analyses using phase 1 data[a,b].

Sensitivity analysis	Lab low[f]	Lab high[f]	Operator[f]
Corrected for alcohol use	**0.1939 (p=0.025)**	0.1866 (p=0.07)	**0.2274 (p=0.021)**
Corrected for doctor diagnosed cardiovascular disease	0.1682 (p=0.055)	**0.2143 (p=0.029)**	**0.2531 (p=0.006)**
Corrected for doctor diagnosed chronic disease	**0.2023 (p=0.016)**	**0.2433 (p=0.02)**	**0.2392 (p=0.008)**
Corrected for doctor diagnosed inflammatory disease	**0.2113 (p=0.012)**	**0.2019 (p=0.034)**	**0.2218 (p=0.014)**
Corrected for doctor diagnosed metabolic disease	**0.1772 (p=0.036)**	0.1616 (p=0.109)	**0.2782 (p=0.002)**
Corrected for educational level	**0.2964 (p=0.001)**	**0.3273 (p=0.001)**	**0.2687 (p=0.008)**
Corrected for 'recent infection'	**0.1856 (p=0.034)**	**0.2204 (p=0.026)**	**0.2658 (p=0.004)**
Corrected for white blood cell count	**0.1802 (p=0.04)**	0.2059 (p=0.06)	**0.2673 (p=0.004)**
Corrected for previous exposure to chemicals	0.1712 (p=0.052)	**0.2086 (p=0.036)**	**0.2623 (p=0.004)**
Corrected for previous exposure to nanoparticles	0.232 (p=0.071)	0.2674 (p=0.051)	**0.309 (p=0.015)**
Corrected for previous exposure to particulates	**0.1794 (p=0.037)**	**0.2295 (p=0.02)**	**0.3065 (p=0.002)**
Without laboratory workers that potentially had previous exposure as operator	**0.188 (p=0.032)**	0.2133 (p=0.056)	**0.2326 (p=0.019)**
Without 'manager operators'	**0.1886 (p=0.03)**	**0.2194 (p=0.027)**	**0.2322 (p=0.018)**
Without controls A[c]	**0.1885 (p=0.028)**	**0.2167 (p=0.026)**	**0.2742 (p=0.003)**
Without controls B[c]	**0.169 (p=0.018)**	**0.2086 (p=0.008)**	**0.1676 (p=0.025)**
Without controls C[c]	0.1392 (p=0.162)	**0.2706 (p=0.011)**	**0.3163 (p=0.001)**
Without controls D[c]	**0.1968 (p=0.024)**	**0.2341 (p=0.018)**	**0.2812 (p=0.003)**
Among male non-smokers[d]	**0.3028 (p=0.001)**	**0.1789 (p=0.036)**	**0.3079 (p<0.0001)**
Among male non-smokers,corrected for 'recent infection'[d,e]	**0.3028 (p=0.001)**	**0.1789 (p=0.036)**	**0.3079 (p<0.0001)**
Among male non-smokers, corrected for white blood cell count[d]	**0.295 (p=0.001)**	0.1275 (p=0.183)	**0.3023 (p<0.0001)**

[a] Estimates from Tobit regression, corrected for age, BMI, sex, and smoking, [b] Marker was specified to be lognormally distributed in the Tobit model, [c] Controls were selected from four different locations (A-D), controls on location A were working in the CNT factory, but where not exposed to CNTs, [d] Estimates from Tobit regression, corrected for age and BMI, [e] None of the male non-smokers were categorized as having had a recent infection, [f] Lab low: Laboratory personnel performing tasks with relatively low exposure; lab high: laboratory personnel performing tasks with relatively high exposure; operators: operators working with the reactor.

Table SI-3c: Difference in FGF-BASIC (ng/mL) between workers exposed to multi walled carbon nanotubes and controls in a set of sensitivity analyses using phase 1 data[a,b].

Sensitivity analysis	Lab low[f]	Lab high[f]	Operator[f]
Corrected for alcohol use	0.01656 (p= 0.9229)	**0.43219 (p= 0.0335)**	0.33838 (p= 0.0814)
Corrected for doctor diagnosed cardiovascular disease	0.04616 (p= 0.7912)	0.36937 (p= 0.059)	0.26999 (p= 0.1422)
Corrected for doctor diagnosed chronic disease	0.04361 (p= 0.7928)	0.29387 (p= 0.1577)	0.17706 (p= 0.3243)
Corrected for doctor diagnosed inflammatory disease	0.07103 (p= 0.6771)	0.34598 (p= 0.0731)	0.2119 (p= 0.2483)
Corrected for doctor diagnosed metabolic disease	0.02453 (p= 0.8828)	0.24861 (p= 0.2116)	0.29162 (p= 0.1006)
Corrected for educational level	0.22164 (p= 0.1772)	**0.55706 (p= 0.0022)**	**0.53834 (p= 0.0036)**
Corrected for 'recent infection'	0.0244 (p= 0.8875)	0.35853 (p= 0.0666)	0.25555 (p= 0.1615)
Corrected for white blood cell count	0.03167 (p= 0.8547)	0.3436 (p= 0.1119)	0.26841 (p= 0.1434)
Corrected for previous exposure to chemicals	0.00617 (p= 0.9717)	0.34009 (p= 0.083)	0.25771 (p= 0.1552)
Corrected for previous exposure to nanoparticles	-0.16899 (p= 0.5026)	0.16064 (p= 0.5493)	0.07417 (p= 0.7655)
Corrected for previous exposure to particulates	0.04984 (p= 0.7663)	0.33545 (p= 0.0803)	0.13789 (p= 0.4745)
Without laboratory workers that potentially had previous exposure as operator	0.02891 (p= 0.868)	0.29188 (p= 0.1871)	0.2843 (p= 0.1471)
Without 'manager operators'	0.03509 (p= 0.8394)	0.36649 (p= 0.0628)	0.27926 (p= 0.1526)
Without controls A[c]	0.05455 (p= 0.752)	0.38089 (p= 0.0529)	0.28925 (p= 0.1164)
Without controls B[c]	0.16911 (p= 0.3411)	**0.41994 (p= 0.0332)**	0.2531 (p= 0.1757)
Without controls C[c]	-0.13785 (p= 0.4819)	0.32979 (p= 0.1176)	0.2268 (p= 0.2406)
Without controls D[c]	-0.02267 (p= 0.8899)	0.27993 (p= 0.1345)	0.19887 (p= 0.2637)
Among male non-smokers[d]	0.14979 (p= 0.4631)	**0.39126 (p= 0.0428)**	**0.43977 (p= 0.0242)**
Among male non-smokers,corrected for 'recent infection'[d,e]	0.14979 (p= 0.4631)	**0.39126 (p= 0.0428)**	**0.43977 (p= 0.0242)**
Among male non-smokers, corrected for white blood cell count [d]	0.14703 (p= 0.4726)	0.37317 (p= 0.0905)	**0.43779 (p= 0.0251)**

[a] Estimates from Tobit regression, corrected for age, BMI, sex, and smoking, [b] Marker was specified to be lognormally distributed in the Tobit model, [c] Controls were selected from four different locations (A-D), controls on location A were working in the CNT factory, but where not exposed to CNTs, [d] Estimates from Tobit regression, corrected for age and BMI, [e] None of the male non-smokers were categorized as having had a recent infection, [f] Lab low: Laboratory personnel performing tasks with relatively low exposure; lab high: laboratory personnel performing tasks with relatively high exposure; operators: operators working with the reactor.

Table SI-4: Difference in complete blood cell counts between workers exposed to multi walled carbon nanotubes and controls in phase 2[a].

Marker[b,c]	Lab low[d]	Lab high[d]	Operators[d]	Trend ranking[d]	Trend GM[d]
Hemoglobine (g/dL)	-0.0033 (p=0.9217)	-0.0091 (p=0.8762)	0.0027 (p=0.9116)	0.0012 (p=0.8773)	0.0001 (p=0.8332)
Hematocrit (%)	-0.0173 (p=0.6532)	-0.0034 (p=0.9598)	0.0014 (p=0.9606)	0.0010 (p=0.9112)	0.0001 (p=0.8340)
RBC (10 12/L)**	-0.0438 (p=0.3007)	-0.0778 (p=0.2882)	-0.0349 (p=0.2537)	-0.0097 (p=0.3483)	-0.0003 (p=0.5490)
MCV (fL)	**0.0265 (p=0.0312)**	**0.0742 (p=0.0005)**	**0.0361 (p<0.0001)**	**0.0107 (p=0.0018)**	**0.0004 (p=0.0303)**
MCH (pg)	**0.0401 (p=0.0041)**	**0.0691 (p=0.0042)**	**0.0380 (p=0.0002)**	**0.0110 (p=0.0043)**	0.0004 (p=0.0557)
MCHC (g/dL)	0.0149 (p=0.0827)	-0.0074 (p=0.6206)	0.0014 (p=0.8243)	0.0002 (p=0.9162)	0.0000 (p=0.9255)
RDW (%)	-0.0042 (p=0.8085)	**-0.0919 (p=0.0020)**	0.0063 (p=0.6125)	0.0042 (p=0.4226)	0.0003 (p=0.1675)
Reticulocytes (109/L)**	**-0.3917 (p=0.0111)**	-0.1210 (p=0.6502)	0.0093 (p=0.9337)	0.0163 (p=0.7050)	0.0018 (p=0.3770)
IRF (%)	0.1469 (p=0.4509)	0.5074 (p=0.1324)	**0.3885 (p=0.0058)**	**0.1241 (p=0.0082)**	**0.0053 (p=0.0220)**
Ret-He (pg)	-0.0149 (p=0.1983)	0.0086 (p=0.6663)	0.0142 (p=0.0893)	0.0053 (p=0.0753)	**0.0003 (p=0.0386)**
WBC (109/L)**	0.1151 (p=0.4423)	-0.2635 (p=0.3097)	-0.0673 (p=0.5343)	-0.0217 (p=0.5613)	-0.0010 (p=0.5735)
Neutrophils (%)	-0.0536 (p=0.5466)	-0.1746 (p=0.2565)	**-0.2553 (p=0.0001)**	**-0.0858 (p<0.0001)**	**-0.0040 (p=0.0001)**
Neutrophils (109/L)**	0.0600 (p=0.7774)	-0.4404 (p=0.2305)	**-0.3187 (p=0.0376)**	**-0.1062 (p=0.0413)**	**-0.0049 (p=0.0490)**
Eosinophils (%)	**0.9987 (p<0.0001)**	**1.4674 (p=0.0003)**	**1.2685 (p<0.0001)**	**0.3948 (p<0.0001)**	**0.0159 (p<0.0001)**
Eosinophils (109/L)**	**2.5987 (p<0.0001)**	**2.2319 (p=0.0001)**	**2.6425 (p<0.0001)**	**0.8284 (p<0.0001)**	**0.0320 (p=0.0005)**
Basophils (%)	0.8421 (p=0.0342)	0.1675 (p=0.8077)	0.3378 (p=0.2396)	0.0941 (p=0.3637)	0.0028 (p=0.5797)
Basophils (109/L)**	2.2898 (p=0.9304)	4.1470 (p=0.9797)	**4.3242 (p=0.0058)**	**1.4413 (p=0.0059)**	**0.0742 (p=0.0058)**
Lymphocytes (%)	0.0216 (p=0.8734)	-0.0258 (p=0.9127)	**0.2334 (p=0.0173)**	**0.0830 (p=0.0114)**	**0.0042 (p=0.0068)**
Lymphocytes (109/L)**	0.1247 (p=0.3976)	-0.3135 (p=0.2192)	0.1663 (p=0.1184)	0.0621 (p=0.1056)	0.0032 (p=0.0714)
Monocytes (%)	-0.0210 (p=0.8867)	**0.6566 (p=0.0102)**	**0.2690 (p=0.0117)**	**0.0831 (p=0.0363)**	0.0034 (p=0.0804)
Monocytes (109/L)**	0.1650 (p=0.2367)	0.4486 (p=0.0630)	**0.1980 (p=0.0492**)	0.0570 (p=0.1029)	0.0020 (p=0.2534)

Plateletes (109/L)**	0.1082 (p=0.1617)	-0.0630 (p=0.6375)	-0.0188 (p=0.7358)	-0.0084 (p=0.6711)	-0.0006 (p=0.5439)
MPV (fL)	0.0064 (p=0.8742)	0.0426 (p=0.5433)	**0.0614 (p=0.0356)**	**0.0208 (p=0.0304)**	**0.0010 (p=0.0330)**
IPF (%)	0.2017 (p=0.2981)	0.3061 (p=0.3617)	**0.3369 (p=0.0161)**	**0.1082 (p=0.0192)**	**0.0046 (p=0.0402)**

[a] Estimates from Tobit regression, corrected for age, BMI, sex, and smoking, [b] Markers were assumed to be lognormally distributed, [c]RBC (red blood cells), MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), MCHC (mean corpuscular hemoglobin concentration), RDW (red blood cell distribution width), IRF (immature reticulocytes fraction), Ret-He (reticulocyte hemoglobin equivalent), WBC (white blood cells), MPV (mean platelet volume), IPF (immature platelet fraction), [d] Lab low: Laboratory personnel performing tasks with relatively low exposure; lab high: laboratory personnel performing tasks with relatively high exposure; operators: operators working with the reactor; trend ranking: trend estimate based on a ranking of the exposure categories; trend GM: trend estimate based on assigned EC exposure estimates (µg/m^3).

Table SI-5a: Difference in neutrophils (%) between workers exposed to multi walled carbon nanotubes and controls in a set of sensitivity analyses using phase 1 data[a,b].

Sensitivity analysis	Lab low[f]	Lab high[f]	Operator[f]
Corrected for alcohol use	0.0082 (p=0.9001)	0.0506 (p=0.4963)	**-0.1424 (p=0.0422)**
Corrected for doctor diagnosed cardiovascular disease	0.0078 (p=0.908)	0.0233 (p=0.7442)	**-0.1701 (p=0.0108)**
Corrected for doctor diagnosed chronic disease	0.0004 (p=0.9954)	-0.025 (p=0.7398)	**-0.1976 (p=0.0024)**
Corrected for doctor diagnosed inflammatory disease	0.0186 (p=0.7672)	0.0104 (p=0.8789)	**-0.2015 (p=0.0019)**
Corrected for doctor diagnosed metabolic disease	0.0147 (p=0.8232)	0.0414 (p=0.5806)	**-0.1733 (p=0.0092)**
Corrected for educational level	0.0359 (p=0.6145)	0.0491 (p=0.5173)	**-0.2029 (p=0.0089)**
Corrected for 'recent infection'	0.008 (p=0.9042)	0.0226 (p=0.7519)	**-0.1708 (p=0.0104)**
Corrected for white blood cell count	-0.0004 (p=0.9945)	-0.0355 (p=0.6266)	**-0.1489 (p=0.0209)**
Corrected for previous exposure to chemicals	0.0187 (p=0.7852)	0.029 (p=0.6875)	**-0.1691 (p=0.011)**
Corrected for previous exposure to nanoparticles	0.1223 (p=0.2632)	0.1226 (p=0.2413)	-0.0827 (p=0.3818)
Corrected for previous exposure to particulates	0.0106 (p=0.872)	0.0238 (p=0.7394)	**-0.1708 (p=0.018)**
Without laboratory workers that potentially had previous exposure as operator	0.013 (p=0.8453)	0.0603 (p=0.4506)	**-0.1644 (p=0.0208)**
Without 'manager operators'	0.009 (p=0.8916)	0.024 (p=0.7379)	**-0.1619 (p=0.0228)**
Without controls A[c]	0.0089 (p=0.8918)	0.0196 (p=0.783)	**-0.1681 (p=0.0118)**

Without controls B[c]	0.0511 (p=0.4285)	0.0316 (p=0.6393)	**-0.1822 (p=0.0044)**
Without controls C[c]	-0.0273 (p=0.7151)	0.0136 (p=0.8616)	**-0.1987 (p=0.0056)**
Without controls D[c]	0.0393 (p=0.5706)	0.0447 (p=0.5502)	**-0.1656 (p=0.0203)**
Among male non-smokers[d]	0.118 (p=0.1699)	0.1205 (p=0.1009)	-0.143 (p=0.054)
Among male non-smokers,corrected for 'recent infection'[d,e]	0.118 (p=0.1699)	0.1205 (p=0.1009)	-0.143 (p=0.054)
Among male non-smokers, corrected for white blood cell count [d]	0.1117 (p=0.1881)	0.0761 (p=0.3564)	**-0.1478 (p=0.0432)**

[a] Estimates from Tobit regression, corrected for age, BMI, sex, and smoking, [b] Marker was specified to be lognormally distributed in the Tobit model, [c] Controls were selected from four different locations (A-D), controls on location A were working in the CNT factory, but where not exposed to CNTs, [d] Estimates from Tobit regression, corrected for age and BMI, [e] None of the male non-smokers were categorized as having had a recent infection, [f] Lab low: Laboratory personnel performing tasks with relatively low exposure; lab high: laboratory personnel performing tasks with relatively high exposure; operators: operators working with the reactor.

Table SI-5b: Difference in monocytes (%) between workers exposed to multi walled carbon nanotubes and controls in a set of sensitivity analyses using phase 1 data[a,b].

Sensitivity analysis	Lab low[f]	Lab high[f]	Operator[f]
Corrected for alcohol use	-0.0151 (p=0.8561)	0.0228 (p=0.8107)	**0.1924 (p=0.0316)**
Corrected for doctor diagnosed cardiovascular disease	-0.0508 (p=0.5442)	0.0365 (p=0.6809)	**0.2068 (p=0.0125)**
Corrected for doctor diagnosed chronic disease	-0.0215 (p=0.7944)	0.1014 (p=0.3022)	**0.2198 (p=0.0098)**
Corrected for doctor diagnosed inflammatory disease	-0.0222 (p=0.7888)	0.0558 (p=0.5354)	**0.2371 (p=0.0056)**
Corrected for doctor diagnosed metabolic disease	-0.0228 (p=0.785)	0.0247 (p=0.7956)	**0.2228 (p=0.0084)**
Corrected for educational level	-0.0045 (p=0.96)	0.0607 (p=0.5275)	**0.2 (p=0.0414)**
Corrected for 'recent infection'	0.0163 (p=0.8289)	0.0688 (p=0.3973)	**0.2388 (p=0.0017)**
Corrected for white blood cell count	0.0027 (p=0.9724)	0.1562 (p=0.0776)	**0.1803 (p=0.021)**
Corrected for previous exposure to chemicals	-0.0321 (p=0.7123)	0.0386 (p=0.6734)	**0.2171 (p=0.0101)**
Corrected for previous exposure to nanoparticles	0.0296 (p=0.8332)	0.0885 (p=0.5105)	**0.2535 (p=0.0371)**
Corrected for previous exposure to particulates	-0.0177 (p=0.8328)	0.0485 (p=0.5939)	**0.2224 (p=0.0153)**
Without laboratory workers that potentially had previous exposure as operator	-0.024 (p=0.7753)	0.0564 (p=0.5767)	**0.2572 (p=0.0042)**

Without 'manager operators'	-0.025 (p=0.7634)	0.0465 (p=0.6054)	**0.2579 (p=0.0038)**
Without controls A[c]	-0.0188 (p=0.8244)	0.0441 (p=0.6321)	**0.2166 (p=0.012)**
Without controls B[c]	-0.0436 (p=0.6328)	0.0372 (p=0.6964)	**0.2164 (p=0.0168)**
Without controls C[c]	0.0144 (p=0.8562)	0.032 (p=0.6986)	**0.2575 (p=0.0007)**
Without controls D[c]	-0.0359 (p=0.6786)	0.0447 (p=0.6327)	**0.2202 (p=0.0137)**
Among male non-smokers[d]	-0.0548 (p=0.6225)	0.0036 (p=0.9696)	**0.2124 (p=0.0269)**
Among male non-smokers,corrected for 'recent infection'[d,e]	-0.0548 (p=0.6225)	0.0036 (p=0.9696)	**0.2124 (p=0.0269)**
Among male non-smokers, corrected for white blood cell count [d]	-0.0293 (p=0.7531)	0.1811 (p=0.0457)	**0.2318 (p=0.0039)**

[a] Estimates from Tobit regression, corrected for age, BMI, sex, and smoking, [b] Marker was specified to be lognormally distributed in the Tobit model, [c] Controls were selected from four different locations (A-D), controls on location A were working in the CNT factory, but where not exposed to CNTs, [d] Estimates from Tobit regression, corrected for age and BMI, [e] None of the male non-smokers were categorized as having had a recent infection, [f] Lab low: Laboratory personnel performing tasks with relatively low exposure; lab high: laboratory personnel performing tasks with relatively high exposure; operators: operators working with the reactor.

Table SI-5c: Difference in mean platelet volume(fL) between workers exposed to multi walled carbon nanotubes and controls in a set of sensitivity analyses using phase 1 data[a,b].

Sensitivity analysis	Lab low[f]	Lab high[f]	Operator[f]
Corrected for alcohol use	-0.0308 (p=0.2561)	0.0392 (p=0.206)	**0.096 (p=0.001)**
Corrected for doctor diagnosed cardiovascular disease	-0.0375 (p=0.1847)	0.0207 (p=0.4862)	**0.0772 (p=0.0055)**
Corrected for doctor diagnosed chronic disease	-0.0294 (p=0.294)	0.0124 (p=0.71)	**0.0831 (p=0.004)**
Corrected for doctor diagnosed inflammatory disease	-0.0311 (p=0.2577)	0.0263 (p=0.3785)	**0.0866 (p=0.0022)**
Corrected for doctor diagnosed metabolic disease	-0.0326 (p=0.2358)	0.0112 (p=0.7204)	**0.0828 (p=0.0029)**
Corrected for educational level	-0.0417 (p=0.1689)	0.0122 (p=0.7049)	**0.0925 (p=0.005)**
Corrected for 'recent infection'	-0.0316 (p=0.2554)	0.022 (p=0.4625)	**0.0786 (p=0.005)**
Corrected for white blood cell count	-0.0315 (p=0.2536)	0.0123 (p=0.6984)	**0.0837 (p=0.0029)**
Corrected for previous exposure to chemicals	-0.0198 (p=0.4899)	0.0291 (p=0.3333)	**0.0801 (p=0.0039)**

Corrected for previous exposure to nanoparticles	-0.0505 (p=0.279)	0.005 (p=0.9116)	0.0638 (p=0.1134)
Corrected for previous exposure to particulates	-0.0297 (p=0.2847)	0.0236 (p=0.4347)	**0.0809 (p=0.0078)**
Without laboratory workers that potentially had previous exposure as operator	-0.0244 (p=0.3711)	0.0439 (p=0.1796)	**0.0626 (p=0.0315)**
Without 'manager operators'	-0.0266 (p=0.3319)	0.0236 (p=0.425)	**0.064 (p=0.0291)**
Without controls A[c]	-0.0309 (p=0.2706)	0.0222 (p=0.4649)	**0.0775 (p=0.0065)**
Without controls B[c]	-0.0086 (p=0.7274)	0.0278 (p=0.2768)	**0.0929 (p=0.0001)**
Without controls C[c]	-0.0483 (p=0.1111)	0.0272 (p=0.3898)	**0.0791 (p=0.0064)**
Without controls D[c]	-0.0422 (p=0.1267)	0.0142 (p=0.6333)	**0.0804 (p=0.0047)**
Among male non-smokers[d]	-0.0644 (p=0.0979)	0.0162 (p=0.6254)	**0.0854 (p=0.0109)**
Among male non-smokers,corrected for 'recent infection'[d,e]	-0.0644 (p=0.0979)	0.0162 (p=0.6254)	**0.0854 (p=0.0109)**
Among male non-smokers, corrected for white blood cell count [d]	-0.0652 (p=0.0939)	0.0103 (p=0.7855)	**0.0847 (p=0.0116)**

[a] Estimates from Tobit regression, corrected for age, BMI, sex, and smoking, [b] Marker was specified to be lognormally distributed in the Tobit model, [c] Controls were selected from four different locations (A-D), controls on location A were working in the CNT factory, but where not exposed to CNTs, [d] Estimates from Tobit regression, corrected for age and BMI, [e] None of the male non-smokers were categorized as having had a recent infection, [f] Lab low: Laboratory personnel performing tasks with relatively low exposure; lab high: laboratory personnel performing tasks with relatively high exposure; operators: operators working with the reactor.

Table SI-5d: Difference in immature platelet fraction (%) between workers exposed to multi walled carbon nanotubes and controls in a set of sensitivity analyses using phase 1 data[a,b].

Sensitivity analysis	Lab low[f]	Lab high[f]	Operator[f]
Corrected for alcohol use	0.0113 (p=0.947)	0.3127 (p=0.1064)	**0.4546 (p=0.0127)**
Corrected for doctor diagnosed cardiovascular disease	0.0113 (p=0.9495)	0.2057 (p=0.2751)	0.3459 (p=0.0494)
Corrected for doctor diagnosed chronic disease	0.0163 (p=0.9259)	0.1183 (p=0.5701)	0.3517 (p=0.0511)
Corrected for doctor diagnosed inflammatory disease	0.0148 (p=0.9318)	0.2179 (p=0.2469)	**0.3705 (p=0.0382)**
Corrected for doctor diagnosed metabolic disease	0.0229 (p=0.8954)	0.2192 (p=0.2691)	0.3462 (p=0.0495)
Corrected for educational level	0.0716 (p=0.7017)	0.2459 (p=0.2166)	0.2924 (p=0.1503)
Corrected for 'recent infection'	0.0261 (p=0.8811)	0.2123 (p=0.2597)	**0.3525 (p=0.0452)**
Corrected for white blood cell count	0.0054 (p=0.9751)	0.1268 (p=0.5232)	**0.3766 (p=0.0317)**

Corrected for previous exposure to chemicals	0.1445 (p=0.4021)	0.2818 (p=0.1207)	**0.3508 (p=0.0361)**
Corrected for previous exposure to nanoparticles	-0.0529 (p=0.8562)	0.1442 (p=0.6057)	0.2922 (p=0.2472)
Corrected for previous exposure to particulates	0.0187 (p=0.9142)	0.2128 (p=0.2596)	0.3668 (p=0.0539)
Without laboratory workers that potentially had previous exposure as operator	0.052 (p=0.7605)	0.3619 (p=0.0781)	0.2589 (p=0.1562)
Without 'manager operators'	0.0357 (p=0.8366)	0.2102 (p=0.2611)	0.2693 (p=0.1458)
Without controls A[c]	-0.0027 (p=0.9874)	0.1864 (p=0.3116)	0.3095 (p=0.0725)
Without controls B[c]	0.1462 (p=0.396)	0.2518 (p=0.1619)	**0.3841 (p=0.0246)**
Without controls C[c]	-0.0417 (p=0.822)	0.2916 (p=0.1317)	**0.4352 (p=0.0142)**
Without controls D[c]	-0.0506 (p=0.7669)	0.1318 (p=0.4741)	0.303 (p=0.0846)
Among male non-smokers[d]	0.1914 (p=0.4261)	0.3083 (p=0.1333)	0.224 (p=0.2802)
Among male non-smokers,corrected for 'recent infection'[d,e]	0.1914 (p=0.4261)	0.3083 (p=0.1333)	0.224 (p=0.2802)
Among male non-smokers, corrected for white blood cell count [d]	0.1808 (p=0.4506)	0.2346 (p=0.3143)	0.216 (p=0.296)

[a] Estimates from Tobit regression, corrected for age, BMI, sex, and smoking, [b] Marker was specified to be lognormally distributed in the Tobit model, [c] Controls were selected from four different locations (A-D), controls on location A were working in the CNT factory, but where not exposed to CNTs, [d] Estimates from Tobit regression, corrected for age and BMI, [e] None of the male non-smokers were categorized as having had a recent infection, [f] Lab low: Laboratory personnel performing tasks with relatively low exposure; lab high: laboratory personnel performing tasks with relatively high exposure; operators: operators working with the reactor.

Table SI-5e: Difference in immature reticulocyte fraction (%) between workers exposed to multi walled carbon nanotubes and controls in a set of sensitivity analyses using phase 1 data[a,b].

Sensitivity analysis	Lab low[f]	Lab high[f]	Operator[f]
Corrected for alcohol use	0.0402 (p=0.8339)	**0.4391 (p=0.0451)**	**0.5225 (p=0.0114)**
Corrected for doctor diagnosed cardiovascular disease	0.0501 (p=0.8076)	0.2730 (p=0.2100)	0.3538 (p=0.0819)
Corrected for doctor diagnosed chronic disease	0.0659 (p=0.7459)	0.3161 (p=0.1914)	0.3760 (p=0.0725)
Corrected for doctor diagnosed inflammatory disease	0.0524 (p=0.7940)	0.2780 (p=0.2020)	0.3636 (p=0.0790)
Corrected for doctor diagnosed metabolic disease	0.0331 (p=0.8677)	0.1875 (p=0.4079)	0.3759 (p=0.0619)
Corrected for educational level	-0.0419 (p=0.8464)	0.1720 (p=0.4552)	0.2788 (p=0.2360)
Corrected for 'recent infection'	0.0663 (p=0.7418)	0.2820 (p=0.1944)	0.3628 (p=0.0739)

Corrected for white blood cell count	0.0499 (p=0.8036)	0.2494 (p=0.2818)	0.3637 (p=0.0754)
Corrected for previous exposure to chemicals	0.0660 (p=0.7519)	0.2812 (p=0.2012)	0.3554 (p=0.0797)
Corrected for previous exposure to nanoparticles	-0.0527 (p=0.8758)	0.1801 (p=0.5765)	0.2724 (p=0.3502)
Corrected for previous exposure to particulates	0.0411 (p=0.8333)	0.3095 (p=0.1456)	**0.5048 (p=0.0185)**
Without laboratory workers that potentially had previous exposure as operator	0.0777 (p=0.6998)	0.3453 (p=0.1546)	0.2690 (p=0.2123)
Without 'manager operators'	0.0703 (p=0.7258)	0.2761 (p=0.2027)	0.2737 (p=0.2020)
Without controls A[c]	0.0356 (p=0.8584)	0.2571 (p=0.2351)	0.3217 (p=0.1124)
Without controls B[c]	0.1753 (p=0.4156)	0.3568 (p=0.1129)	**0.5368 (p=0.0120)**
Without controls C[c]	-0.0356 (p=0.8678)	0.2269 (p=0.3101)	0.2048 (p=0.3178)
Without controls D[c]	0.0194 (p=0.9250)	0.2495 (p=0.2630)	0.3213 (p=0.1310)
Among male non-smokers[d]	-0.0482 (p=0.8566)	0.3530 (p=0.1215)	0.3379 (p=0.1423)
Among male non-smokers,corrected for 'recent infection'[d,e]	-0.0482 (p=0.8566)	0.3530 (p=0.1215)	0.3379 (p=0.1423)
Among male non-smokers, corrected for white blood cell count [d]	-0.0659 (p=0.8031)	0.2303 (p=0.3700)	0.3245 (p=0.1542)

[a] Estimates from Tobit regression, corrected for age, BMI, sex, and smoking, [b] Marker was specified to be lognormally distributed in the Tobit model, [c] Controls were selected from four different locations (A-D), controls on location A were working in the CNT factory, but where not exposed to CNTs, [d] Estimates from Tobit regression, corrected for age and BMI, [e] None of the male non-smokers were categorized as having had a recent infection, [f] Lab low: Laboratory personnel performing tasks with relatively low exposure; lab high: laboratory personnel performing tasks with relatively high exposure; operators: operators working with the reactor.

Table SI-6: Difference in fractional exhaled nitric oxide (ppb) between workers exposed to multi walled carbon nanotubes and controls in a set of sensitivity analyses using phase 1 data[a,b].

Sensitivity analysis	Lab low[f]	Lab high[f]	Operator[f]
Corrected for alcohol use	0.2214 (p=0.4479)	0.229 (p=0.4918)	**-0.7999 (p=0.0228)**
Corrected for doctor diagnosed cardiovascular disease	0.1724 (p=0.5636)	0.0673 (p=0.8336)	**-0.9336 (p=0.0076)**
Corrected for doctor diagnosed chronic disease	0.2346 (p=0.4247)	0.0114 (p=0.9742)	**-0.7455 (p=0.0378)**
Corrected for doctor diagnosed inflammatory disease	0.1774 (p=0.5293)	0.1169 (p=0.7033)	**-0.7059 (p=0.0357)**
Corrected for doctor diagnosed metabolic disease	0.2629 (p=0.3705)	0.1744 (p=0.6024)	**-0.8871 (p=0.0103)**

Corrected for educational level	0.134 (p=0.6729)	-0.0032 (p=0.9923)	**-0.7837 (p=0.0359)**
Corrected for 'recent infection'	0.3081 (p=0.2857)	0.1189 (p=0.7048)	**-0.8327 (p=0.0142)**
Corrected for white blood cell count	0.2467 (p=0.4051)	0.0778 (p=0.8178)	**-0.8815 (p=0.0117)**
Corrected for previous exposure to chemicals	0.2876 (p=0.3403)	0.1099 (p=0.7348)	**-0.8842 (p=0.0112)**
Corrected for previous exposure to nanoparticles	0.3528 (p=0.4128)	0.1766 (p=0.6776)	-0.7934 (p=0.0688)
Corrected for previous exposure to particulates	0.2402 (p=0.4127)	0.1408 (p=0.6632)	**-0.7394 (p=0.0443)**
Without laboratory workers that potentially had previous exposure as operator	0.2864 (p=0.3182)	0.3011 (p=0.3874)	**-1.1238 (p=0.0021)**
Without 'manager operators'	0.2684 (p=0.3535)	0.0819 (p=0.7954)	**-1.1234 (p=0.0023)**
Without controls A[c]	0.2552 (p=0.3961)	0.0872 (p=0.7906)	**-0.8822 (p=0.013)**
Without controls B[c]	0.2763 (p=0.3217)	0.1322 (p=0.6539)	**-0.7047 (p=0.0293)**
Without controls C[c]	0.1501 (p=0.6555)	-0.1167 (p=0.7479)	**-0.8859 (p=0.0177)**
Without controls D[c]	0.2311 (p=0.4782)	0.0964 (p=0.7862)	**-0.9922 (p=0.0136)**
Among male non-smokers[d]	-0.2407 (p=0.5332)	0.107 (p=0.7518)	**-0.9674 (p=0.0082)**
Among male non-smokers,corrected for 'recent infection'[d,e]	-0.2407 (p=0.5332)	0.107 (p=0.7518)	**-0.9674 (p=0.0082)**
Among male non-smokers, corrected for white blood cell count [d]	-0.2585 (p=0.5019)	-0.0176 (p=0.962)	**-1.0005 (p=0.0066)**

[a] Estimates from Tobit regression, corrected for age, BMI, sex, and smoking, [b] Marker was specified to be lognormally distributed in the Tobit model, [c] Controls were selected from four different locations (A-D), controls on location A were working in the CNT factory, but where not exposed to CNTs, [d] Estimates from Tobit regression, corrected for age and BMI, [e] None of the male non-smokers were categorized as having had a recent infection, [f] Lab low: Laboratory personnel performing tasks with relatively low exposure; lab high: laboratory personnel performing tasks with relatively high exposure; operators: operators working with the reactor.

Table SI-7: Difference in lung function between workers exposed to multi walled carbon nanotubes and controls in a set of sensitivity analyses using phase 1 data[a].

Exposure category[b]	FEV1[c]	FEV1%[c,f]	FVC[d]	FVC%[d,f]	FEV1/FVC[e]	FEV1/FVC%[e,f]
Lab low	-0.1697 (p=0.6712)	-4.9300 (p=0.5140)	-0.1494 (p=0.7968)	-3.0175 (p=0.6620)	-0.8170 (p=0.8500)	-2.2105 (p=0.5930)
Lab high	0.1437 (p=0.6276)	-4.2630 (p=0.4850)	0.3583 (p=0.4082)	-0.8842 (p=0.8740)	-2.7147 (p=0.3990)	-4.4105 (p=0.1940)
Operators	0.3812 (p=0.4268)	11.4870 (p=0.0930)	0.5429 (p=0.4362)	12.0658 (p=0.0560)	-0.9194 (p=0.8580)	-0.7105 (p=0.8460)

Trend ranking	0.1007 (p=0.3747)	1.8660 (p=0.3550)	0.1843 (p=0.2633)	2.5450 (p=0.1640)	-0.8765 (p=0.4690)	-0.8713 (p=0.4100)
Trend GM	0.0102 (p=0.3030)	0.2692 (p=0.0710)	0.0146 (p=0.3124)	0.2769 (p=0.0409)	-0.0275 (p=0.7960)	-0.0099 (p=0.9020)

[a] Estimates from linear regression, corrected for age, BMI, sex, and smoking. Analyses of 'percentage of predicted values' were corrected smoking only, [b] Lab low: Laboratory personnel performing tasks with relatively low exposure; lab high: laboratory personnel performing tasks with relatively high exposure; operators: operators working with the reactor; trend ranking: trend estimate based on a ranking of the exposure categories; trend GM: trend estimate based on assigned EC exposure estimates (µg/m3), [c] Forced expiratory volume in 1 second (L), [d] Forced vital capacity (L), [e] Ratio forced vital capacity and forced expiratory volume in 1 second, [f] Percentage of predicted values calculated using European Respiratory Society equation.[6]

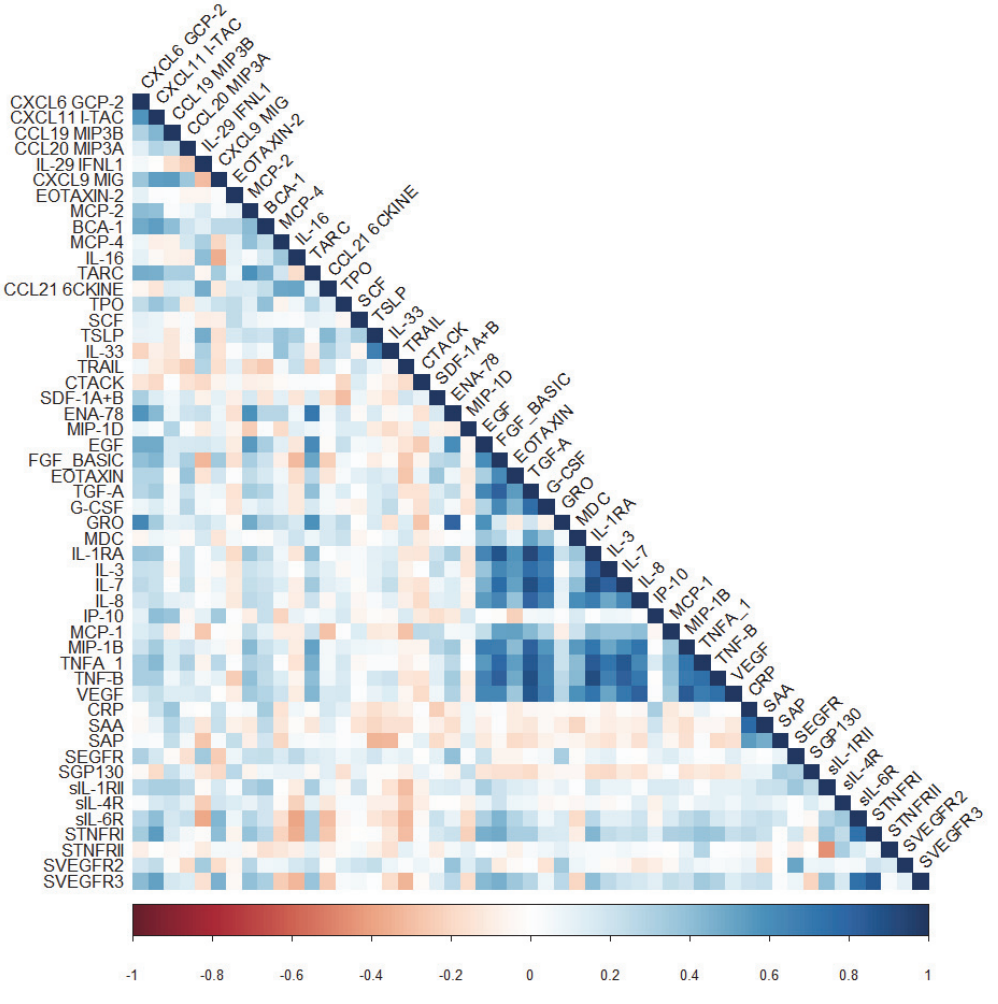

Figure SI-1a: Correlations between immune markers phase 1. Blue represents positive correlation, red represents negative correlation. Color intensity corresponds to Pearson correlation coefficient ranging from -0.6 to 0.9.

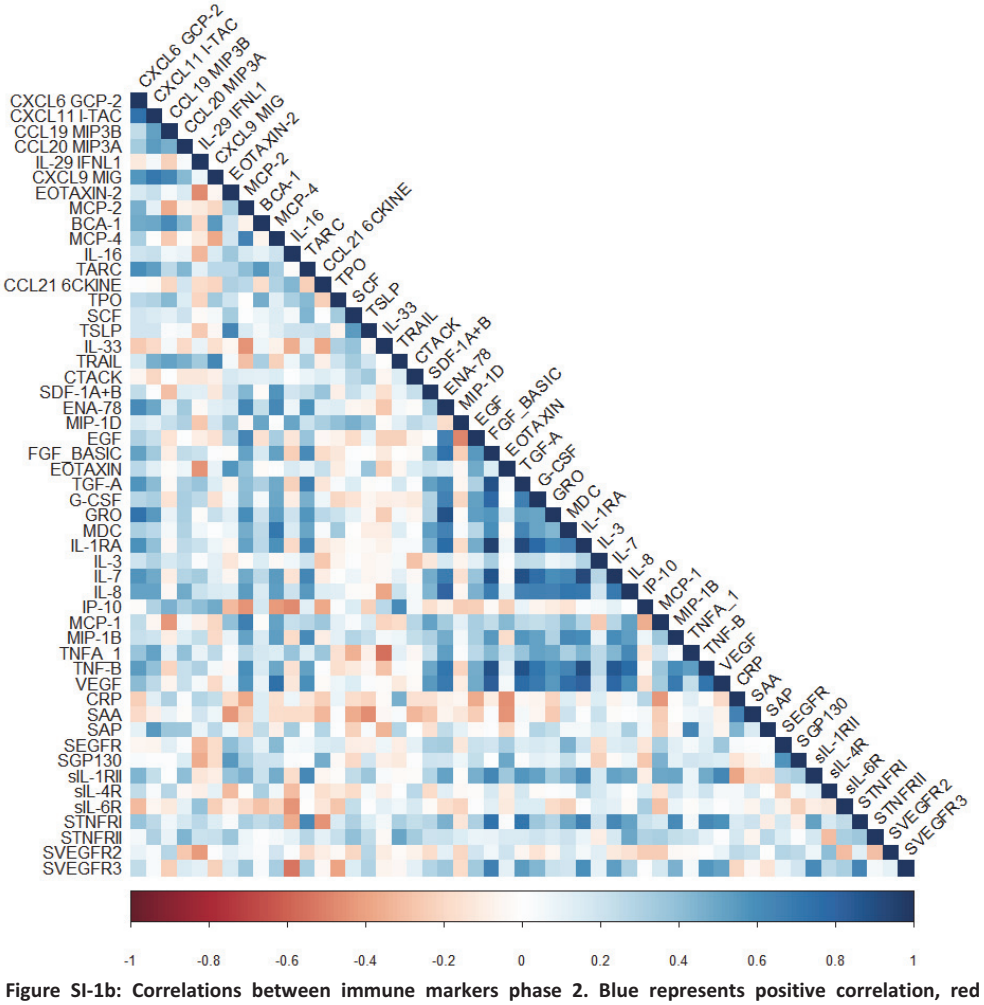

Figure SI-1b: Correlations between immune markers phase 2. Blue represents positive correlation, red represents negative correlation. Color intensity corresponds to Pearson correlation coefficient ranging from -0.6 to 1.

A1

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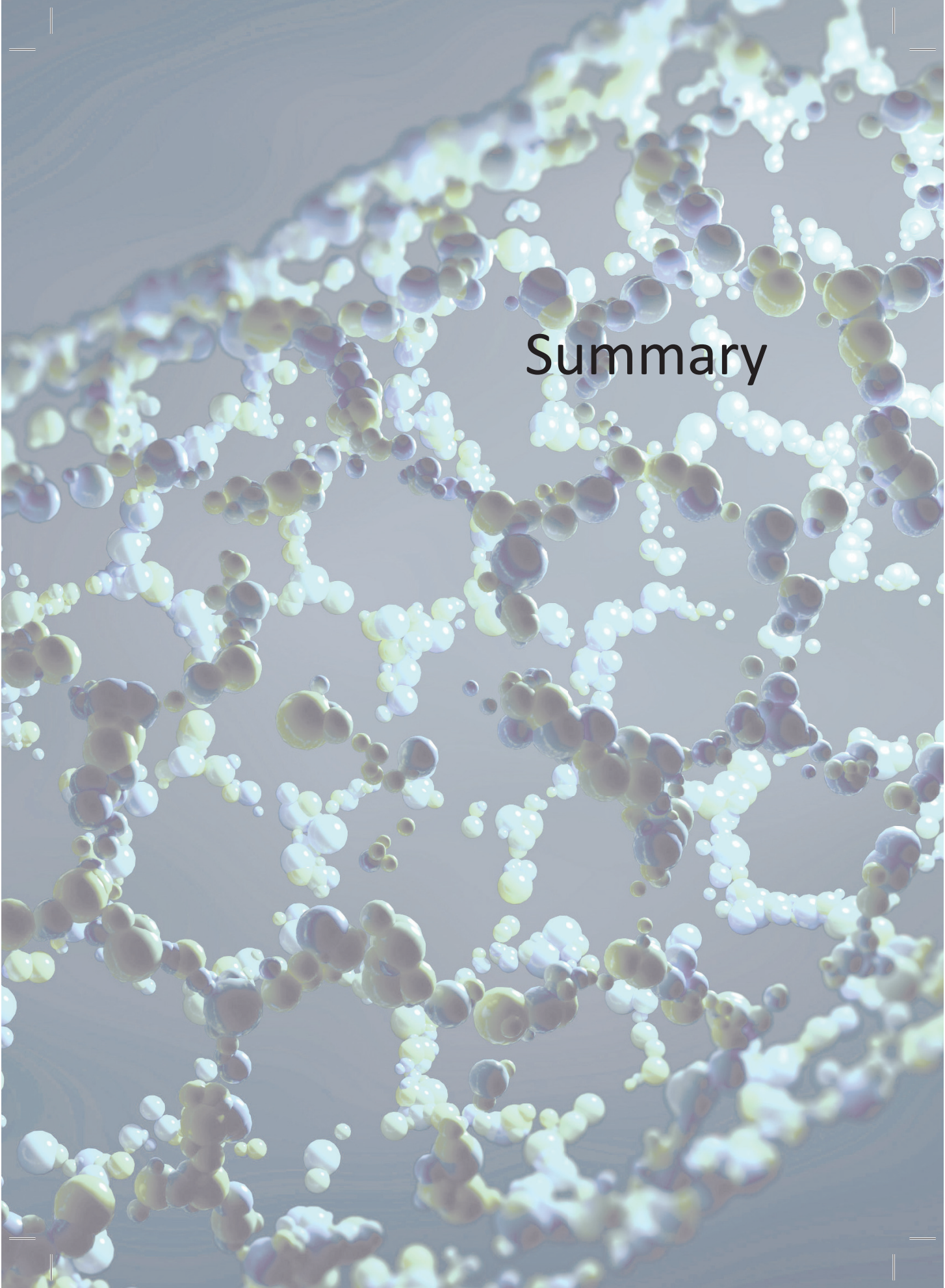


Summary

Introduction

Carbon nanotubes (CNTs) are hollow structures of one (single-walled CNT; SWCNT) or multiple (double-walled or multi-walled CNT; DWCNT, MWCNT) rolled graphene sheet(s) that offer society new opportunities to make materials effectively stronger, lighter, better electrically conductive, and more flexible. Although CNTs are already applied in many fields (e.g. automotive, electronics, energy production, transport and storage, sensors, sport goods, construction, oil and gas, and textiles), research organization have been raising growing concerns about the (human) health risks associated with exposure to different types of CNTs. With the expected increase in use of CNTs due to decreasing market prices and consequently the need to scale up production, workers and consumers will be increasingly exposed to CNTs, both in number and in terms of exposure levels if no measures are taken to mitigate exposure.

Uncertainty concerning the (occupational) risk assessment of CNTs has resulted in conservative and often worst-case regulatory frameworks to ensure the safety of workers. Both workers and society would benefit from the full potential of CNTs with more evidence-based risk assessment. Early exposure studies for CNTs used exposure methods (direct-reading instruments [DRIs] and filter-based gravimetrical methods) which are not sensitive and selective enough for detecting CNTs. Recently, three more refined and selective methods have been used for the assessment of CNT exposure, but a comprehensive approach that focuses on exposure measurements has still not been studied. Furthermore, with a growing CNT market and the increased application of CNTs in products, there is a need to obtain more insight into exposure determinants and activities of CNT exposure across the product life cycle. In addition, with only a few small-scale studies done on the association between CNT exposure and (early) health effects on workers, more large-scale cross-sectional studies are needed.

This thesis aims to 1) develop a method to measure (MW)CNT exposure based on evaluating and optimizing those available methods that are different and more selective, 2) study determinants and activities of MWCNT exposure across the product life cycle, and 3) evaluate the association between occupational exposure to MWCNTs and early cardiovascular effects in a cross-sectional epidemiologic study.

Methods and results

In order to identify available information on determinants and activities of exposure, and subsequently identify key information and data gaps for future research, the emission potential of nano-objects, and their aggregates and agglomerates (NOAA) including CNTs was systematically reviewed across the product life cycle (**Chapter 2**). During the synthesis phase, gas-phase production methods, which are normally used for the production of CNTs, resulted in relatively high emissions. In regard to the handling and transfer of bulk nanopowders,

harvesting (mainly in case of CNT exposure) and dumping were identified as activities with the highest emission potential. Spraying activities resulted in the highest emission for processed liquids contained NOAA. Most of the identified studies focused on the handling of nano-enabled products. These studies conducted experiments on the release of pristine nanomaterials from a matrix polymer; one study observed free agglomerates of CNTs due to abrasive activities. Furthermore, it was concluded that emission substantially differs due to intrinsic properties such as the shape of NOAA and the handled amount of material, which emphasizes the need to focus on CNT exposure across the product life cycle.

In order to develop an exposure measurement approach for the detection and quantification of (MW)CNTs in actual workplaces, a field survey was conducted in which three relatively more selective analytical methods were evaluated and optimized (**Chapter 3**). It was concluded that carbon analysis with the sum of elemental carbon thermal treatment stage 2 and 3 (EC2 and EC3) is a good quantitative estimate of (MW)CNT exposure. These carbon analyses need to be combined with scanning electron microscopy/energy-dispersive X-ray spectroscopy (SEM/EDX) for background correction of EC2, as soot is also present in this stage. The third method evaluated, inductively coupled plasma mass spectrometry (ICP-MS), was not found to be selective enough for CNT exposure, due to the catalyst particles present at the workplace. This evaluation of analytical methods was part of a study on occupational exposure and potential health effects at a commercial industrial MWCNT production facility, part of which is described in **Chapter 4a, Chapter 5** and **Appendix 1** of this thesis.

The personal exposure to MWCNTs of each worker in this production facility was assessed, as discussed in **Chapter 3**. During the synthesis and handling of these materials, results were linked to specific activities (**Chapter 4a**). Results via SEM/EDX showed only large agglomerates (200 nm – 100 µm) with tangled and bundled MWCNTs with catalyst metals attached. Personal elemental carbon (EC) exposure levels of workers in the production area were comparable during a period with full-scale synthesis (N=23, geometric mean [GM] 41 µg/m^3) and a period with only handling of MWCNTs (N=19; 43 µg/m^3). Exposure levels were significantly lower for workers in the research & development (R&D) (N=11; 5 µg/m^3) and the office (N=5; 7 µg/m^3). Bagging, maintenance of the reactor, powder conditioning (all activities in the production area) and handling of MWCNTs powder (R&D area) were associated with higher levels of exposure.

Twelve different experiments were performed to evaluate the particle concentrations during mechanical sawing and drilling in car bumpers containing MWCNT and nano-sized organic pigment (OP). The effect of general ventilation and machine settings on particle concentrations were studied (**Chapter 4b**). No pristine engineered nanoparticles (MWCNTs or OP) were observed during the experiments, and partly melted carbon-rich particles were identified with SEM/EDX only during sawing experiments. Significant effects on increased

particle concentrations (geometric mean ratio [GMR]) were observed in near field (NF) and/or far field (FF) for a higher sawing speed (NF: 58.73, FF: 22.07) and for car bumpers containing MWCNTs as compared to car bumpers with OP (FF: 0.45). Particle size distributions were significantly increased with a higher sawing speed (NF: 1.15, FF: 1.08) and decreased with the use of general ventilation (FF: 0.86) and in car bumpers containing MWCNTs as compared to car bumpers with OP (FF: 0.92).

The association between occupational exposure to MWCNTs (as discussed in **Chapter 4a**) and 12 cardiovascular blood biomarkers was studied in workers at the selected production facility. The biomarker measurements were repeated for a subpopulation of highly exposed workers after 5 months (**Chapter 5**). A significant upward trend in the GMRs of endothelial damage marker intercellular adhesion molecule-1 (ICAM-1) was observed with increased exposure to MWCNTs in both measurement periods (GMR=1.40 and GMR=1.37, respectively). Other cardiovascular markers were not significantly associated with MWCNT exposure. This indication of endothelial activation was further supported by increased inflammation in these workers, which was previously reported and discussed in **Appendix 1**. Moreover, early effects on lung health and the immune system were also observed in this study and were associated with exposure to MWCNTs (**Appendix 1**).

Reflection, future perspectives and considerations

Chapter 6 summarizes and reflects upon the main findings of this thesis in light of the state-of-the-art knowledge on CNT exposure and health effects; future perspectives are discussed.

Depending on the form(s) of CNT exposure, different measurement approaches will provide the most valid quantitative exposure results. When exposure to fibers may be expected, a combination of carbon analyses and SEM/EDX is the most selective. When CNTs are embedded in (more spherical) matrix particles, the use of direct reading instruments (DRIs) instead of carbon analyses provides valuable time-resolved data instead of time-integrated data, which can be used to identify sources of exposure.

In general, the evidence for potential adverse health effects in humans associated with exposure to different types of CNTs is not strong enough to draw firm conclusions. Although the international agency for research on cancer (IARC) concluded that the mechanistic evidence in animals is relevant to humans, information about responses in humans is limited to a few small-scale epidemiological studies focused on potential early effects. The cross-sectional study discussed in this thesis substantially contributes to this knowledge with an observed indication of endothelial activation and an increased inflammatory reaction, and provides a basis for future epidemiological research into CNT exposure. However, due to the

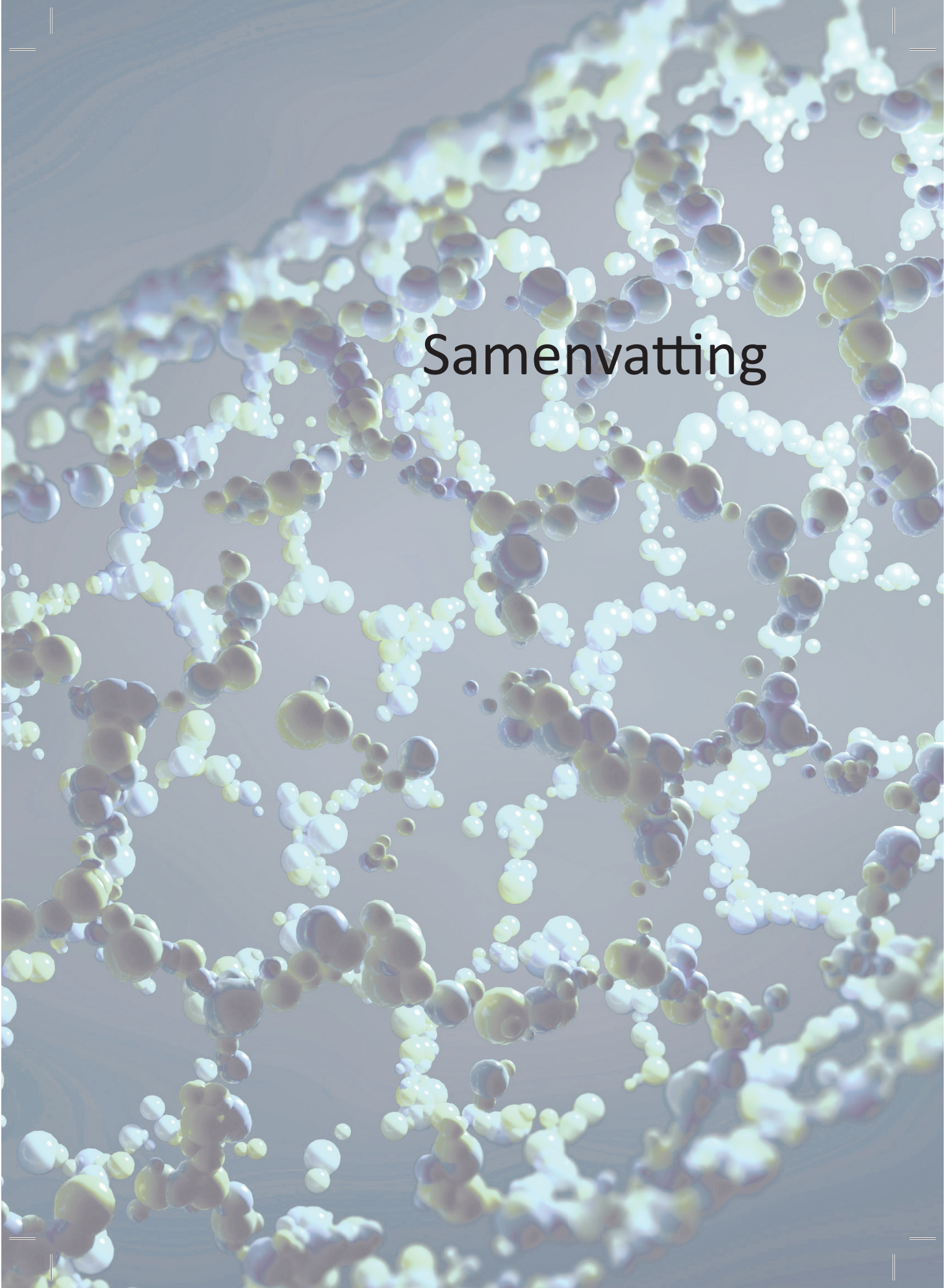
heterogenicity of CNTs and relatively small study populations, risk assessment will continue to be challenging.

Several occupational exposure limits (OELs) and recommended exposure limits (RELs) have been proposed and are largely based on health effects associated with asbestos or subacute/sub-chronic animal studies. Ongoing chronic animal studies will result in more health-based limit values for different types of CNTs, and as a result uncertainty in risk assessment will be reduced. As health effects of CNTs are still largely unknown and in order to ensure the safe use of CNTs, control measures should be considered according to the hierarchy of controls to reduce exposure to CNTs to as low as reasonable achievable.

The following future perspectives are discussed 1) read-across and grouping, 2) safe innovation, 3) risk governance, and 4) exposure registration and epidemiological research.

- Read-across and grouping approaches support more health-based risk assessment by optimally using existing hazard and exposure data to limit the amount of (animal) testing for different types of CNTs.
- Taking into consideration safety at an early stage, preferably before the product enters the market, is defined as safe innovation. The use of less hazardous functionalized forms of CNTs, which are identified based on physical and chemical characteristics of products, should be stimulated; this requires more sharing of information between regulators and innovators.
- Coping with uncertainty in current risk assessment and safety regulations while keeping pace with fast development requires innovating risk governance. Soft laws (soft regulatory agreements) are based on non-binding requirements, and when these agreements are conservative enough, this helps society adapt to the pace of innovation safely.
- A mandatory European exposure registration for the increasing number of workers exposed to CNTs is suggested as health effects are still largely unknown. The results collected with this registration can be used for both retrospective research using already available data on mortality and diseases and for new epidemiological research focused on potential early health effects.

In conclusion, this thesis contributes to the safe(r) use of CNTs and provides scientific knowledge concerning 1) a comprehensive exposure assessment method for MWCNTs, 2) the identification of activities and exposure determinates which significantly contributes to MWCNTs exposure across the product life cycle and 3) the observation of an indication of endothelial activation and an increased inflammatory reaction associated with MWCNT exposure.



Samenvatting

Inleiding

Koolstofnanobuisjes (CNT's) zijn holle structuren van één (enkelwandige CNT; SWCNT) of meerdere (dubbelwandige of meerwandige CNT; DWCNT, MWCNT) opgerolde grafeen structuren, die de samenleving nieuwe mogelijkheden biedt om materialen effectief sterker, lichter, beter elektrisch geleidend en flexibeler te maken. Hoewel CNT's al in verschillende sectoren en producten worden toegepast (o.a. in de auto-industrie, elektronica, energieproductie, transport en opslag, sensoren, olie en gas, sportartikelen, bouw en textiel), wordt er door onderzoekers groeiende bezorgdheid geuit over de humane gezondheidsrisico's geassocieerd met blootstelling aan verschillende soorten CNT's. Met de verwachte verdere toename van het gebruik van CNT's worden werknemers en consumenten meer blootgesteld aan CNT's als geen maatregelen worden genomen om de blootstelling te reduceren.

Onzekerheid over de (beroeps)risico's van blootstelling aan CNT's heeft geleid tot conservatieve risicobeoordelingen om de veiligheid van werknemers te waarborgen. Echter, een meer onderbouwde risicobeoordeling is nodig om te profiteren van het volledige potentieel van CNT's. In de eerste blootstellingsstudies voor CNT's werd gebruik gemaakt van directe meetinstrumenten (DRI's) en op filter gebaseerde gravimetrische methoden, die niet gevoelig en selectief blijken te zijn voor het detecteren van CNT's. Onlangs zijn drie meer selectieve methoden toegepast voor de beoordeling van CNT-blootstelling, maar een alomvattende benadering die zich richt op persoonlijke blootstellingsmetingen ontbreekt. Bovendien is er met een groeiende CNT-markt en de toegenomen toepassing van CNT's in producten behoefte aan meer inzicht in blootstellingsdeterminanten en activiteiten die leiden tot een hogere blootstelling aan CNT's, gedurende de gehele levenscyclus van het product. Daarnaast zijn er met slechts een paar kleinschalige studies over de associatie tussen CNT-blootstelling en (vroege) gezondheidseffecten voor werknemers, meer grootschalige cross-sectionele studies nodig.

Dit proefschrift beoogt 1) een methode te ontwikkelen voor het meten van (MW)CNT-blootstelling op basis van het evalueren en optimaliseren van bestaande selectieve methoden, 2) determinanten en activiteiten van MWCNT-blootstelling identificeren gedurende de levenscyclus van het product, en 3) het verband evalueren tussen beroepsmatige blootstelling aan MWCNT's en vroege cardiovasculaire effecten in een cross-sectioneel epidemiologisch onderzoek.

Methoden en resultaten

Het emissiepotentieel van nano-objecten en hun aggregaten en agglomeraten (NOAA) inclusief CNT's zijn systematisch beoordeeld voor de gehele levenscyclus van producten, op basis van beschikbare wetenschappelijke informatie (**Hoofdstuk 2**). Tijdens de productie van

nanomaterialen resulteerden gasfaseproductie methoden, die veelal worden gebruikt voor de productie van CNT's, in relatief hoge emissies. Voor activiteiten met bulk nanopoeders, werden oogsten (voornamelijk van CNTs) en storten van poeder geïdentificeerd als activiteiten met het hoogste emissiepotentieel. Spray activiteiten resulteerden in de hoogste emissie voor vloeistoffen die NOAA bevatten. De meeste van de geïdentificeerde onderzoeken waren gericht op bewerking van producten met NOAA die veel voorkomen tijdens de gebruikersfase en het einde van de levenscyclus van een product. Deze studies voerden experimenten uit om het vrijkomen van primaire nanomaterialen uit een matrixpolymeer te bestuderen; één studie observeerde vrije agglomeraten van CNT's als gevolg van schuren. Bovendien werd geconcludeerd dat de emissie aanzienlijk kan verschillen door intrinsieke eigenschappen van het product, zoals de vorm van NOAA en de verwerkte hoeveelheid materiaal.

Om een blootstellingsmeetmethode te ontwikkelen voor de detectie en kwantificatie van (MW)CNT's op de werkplek, werd een veldstudie uitgevoerd waarin drie relatief meer selectieve analysemethoden werden geëvalueerd en geoptimaliseerd (**Hoofdstuk 3**). Er werd geconcludeerd dat koolstofanalyse met het totaal elementaire koolstof thermische behandelingsfase 2 en 3 (EC2 en EC3) een goede kwantitatieve schatting is van de blootstelling aan (MW)CNT. Deze koolstofanalyses moeten worden gecombineerd met scanning-elektronenmicroscopie / energiedispersieve röntgenspectroscopie (SEM / EDX) voor achtergrondcorrectie van EC2, aangezien roet ook in deze fractie aanwezig is. De derde geëvalueerde methode, inductief gekoppelde plasmamassaspectrometrie (ICP-MS), bleek niet selectief genoeg te zijn voor CNT-blootstelling, vanwege de aanwezige metalen gebruikt als katalysator voor de productie van CNT's op de werkplek. Deze evaluatie van analysemethoden was onderdeel van een onderzoek naar beroepsmatige blootstelling en mogelijke gezondheidseffecten bij een commerciële MWCNT-productiefaciliteit, waarvan een deel wordt beschreven in **Hoofdstuk 4a**, **Hoofdstuk 5** en **Appendix 1** van dit proefschrift.

De persoonlijke blootstelling aan MWCNT's van werknemers in deze productiefaciliteit werd beoordeeld, zoals methodologisch voorgesteld in **Hoofdstuk 3**. Tijdens de productie en verwerking van deze materialen zijn de resultaten van werknemers gekoppeld aan specifieke activiteiten (**Hoofdstuk 4a**). Resultaten m.b.v. SEM / EDX toonden alleen grote agglomeraten (200 nm - 100 µm) met gebundelde MWCNT's waaraan metalen waren bevestigd. Persoonlijke blootstellingsniveaus voor elementaire koolstof (EC) van werknemers in het productiegebied waren vergelijkbaar tijdens een periode met volledige productie (N = 23, geometrisch gemiddelde [GM] 41 µg / m^3) en een periode met alleen activiteiten waarbij MWCNT's werden gebruiken (N = 19; 43 µg / m^3). Het blootstellingsniveau was aanzienlijk lager voor werknemers in de onderzoeksafdeling (R & D) (N = 11; 5 µg / m^3) en het kantoor (N = 5; 7 µg / m^3). Poeder storten, onderhoud van de reactor, poederconditionering (allen in

het productiegebied) en werken met het poeder van MWCNT's (R&D gebied) zijn geassocieerd met hogere blootstellingsniveaus.

Twaalf verschillende experimenten werden uitgevoerd om de deeltjesconcentraties te evalueren tijdens mechanisch zagen en boren in autobumpers die MWCNT en organisch pigment (OP) bevatten. Het effect van algemene ventilatie en machine-instellingen op deeltjesconcentraties werd bestudeerd (**Hoofdstuk 4b**). Tijdens de experimenten werden geen vrij voorkomende bewust toegevoegde nanodeeltjes (MWCNT's of OP) waargenomen en werden enkel deels gesmolten koolstofrijke deeltjes geïdentificeerd met SEM / EDX voor de zaagexperimenten. Significante effecten op verhoogde deeltjesconcentraties (geometrisch gemiddelde ratio [GMR]) werden waargenomen in de nabijheid van de activiteit (NF) en / of verder van de activiteit vandaan (FF) voor een hogere zaagsnelheid (NF: 58,73, FF: 22,07) en voor autobumpers die MWCNT's bevatten in vergelijking met autobumpers met OP (FF: 0,45). De deeltjesgrootteverdelingen waren significant verhoogd met een hogere zaagsnelheid (NF: 1,15, FF: 1,08) en daalden met het gebruik van algemene ventilatie (FF: 0,86) en voor autobumpers die MWCNT's bevatten in vergelijking met autobumpers met OP (FF: 0,92).

De associatie tussen beroepsmatige blootstelling aan MWCNT's (zoals besproken in **Hoofdstuk 4a**) en 12 cardiovasculaire biomarkers werd bestudeerd bij werknemers in dezelfde productiefaciliteit. Deze biomarker metingen werden herhaald voor een subpopulatie van hoog blootgestelde werknemers na 5 maanden (**Hoofdstuk 5**). Een significante stijgende trend in de GMR's van endotheel schade marker intercellulaire adhesiemolecuul-1 (ICAM-1) werd waargenomen bij verhoogde blootstelling aan MWCNT's in beide meetperioden (GMR = 1,40 en GMR = 1,37, respectievelijk). Andere cardiovasculaire markers waren niet significant geassocieerd met blootstelling aan MWCNT. Deze indicatie van endotheel activatie werd verder ondersteund door verhoogde ontstekingswaarden bij deze werknemers, die eerder werd gerapporteerd en beschreven in **Appendix 1** van dit proefschrift. Bovendien werden vroege effecten op de longgezondheid en het immuunsysteem waargenomen in deze studie en werden geassocieerd met blootstelling aan MWCNT's (**Appendix 1**).

Reflectie, toekomstperspectieven en overwegingen

Hoofdstuk 6 vat de belangrijkste bevindingen van dit proefschrift samen en vergelijkt deze kennis met ander onderzoek over blootstelling aan CNT's en gezondheidseffecten. Verder worden aanbevelingen besproken. Afhankelijk van de vorm(en) van CNT-blootstelling, resulteren andere meetbenaderingen tot de meest geldige kwantitatieve blootstellingsresultaten. Wanneer blootstelling aan vezels mag worden verwacht, is een combinatie van koolstofanalyses en SEM / EDX het meest selectief. Wanneer CNT's zijn ingebed in (meer bolvormige) matrixdeeltjes, biedt het gebruik van directe

meetinstrumenten (DRI's) in plaats van koolstofanalyses waardevolle tijd specifieke gegevens in plaats van tijd geïntegreerde gegevens, die bijvoorbeeld kunnen worden gebruikt om bronnen van blootstelling eenvoudiger te identificeren.

In het algemeen is het bewijs voor mogelijke nadelige humane gezondheidseffecten geassocieerd met blootstelling aan verschillende soorten CNT's niet sterk genoeg voor harde conclusies. Hoewel het internationale agentschap voor onderzoek naar kanker (IARC) concludeerde dat het mechanistische bewijs bij dieren relevant is voor mensen, is de informatie over reacties bij de mens beperkt tot enkele kleinschalige epidemiologische onderzoeken gericht op mogelijke vroege effecten van CNT's. De cross-sectionele studie in dit proefschrift draagt substantieel bij aan deze kennis met een geobserveerde endotheel activatie en een verhoogde ontstekingsreactie geassocieerd met blootstelling aan MWCNT's, en biedt een basis voor toekomstig epidemiologisch onderzoek naar CNT-blootstelling. Vanwege de heterogeniteit van CNT's en de relatief kleine onderzoekspopulaties blijft de risicobeoordeling echter een uitdaging.

Inmiddels zijn verschillende grenswaarden voor beroepsmatige blootstelling (OEL's) en aanbevolen blootstellingslimieten (REL's) voorgesteld, maar deze waarden zijn grotendeels gebaseerd op gezondheidseffecten voor asbest of op basis van subacute / subchronische dierstudies voor CNT's. Lopende onderzoeken naar chronisch blootgestelde dieren resulteren in meer gezondheid gerelateerde grenswaarden voor verschillende soorten CNT's, waardoor de onzekerheid in de risicobeoordeling zal afnemen. Aangezien effecten op de gezondheid van CNT's nog grotendeels onbekend zijn en om een veilig gebruik van CNT's te garanderen, moeten beheersmaatregelen worden overwogen volgens de hiërarchie van beheersmaatregelen om de blootstelling aan CNT's zo veel mogelijk te verminderen.

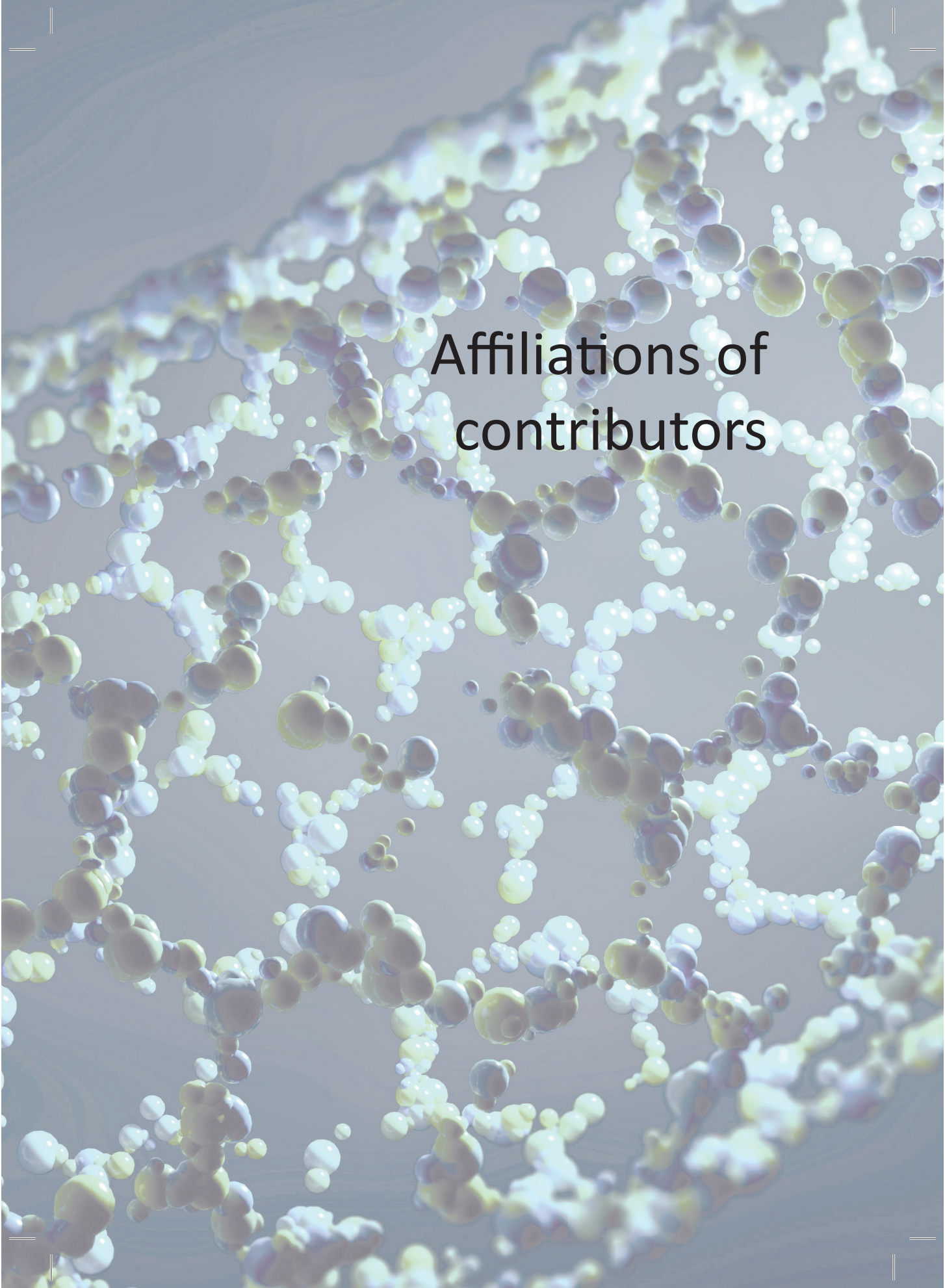
De volgende aanbevelingen worden besproken 1) read-across en groepering, 2) veilige innovatie, 3) risicobeheer, en 4) blootstellingsregistratie en epidemiologisch onderzoek.

- Read-across en groepering dragen bij aan een meer op gezondheid gebaseerde risicobeoordeling door optimaal gebruik te maken van bestaande gevaren- en blootstellingsgegevens. Tevens reduceert dit het aantal dierstudies voor nog niet onderzochte CNT's.
- Rekening houden met veiligheid in een vroeg stadium, bij voorkeur voordat het product op de markt komt, wordt gedefinieerd als veilige innovatie. Het gebruik van minder gevaarlijke vormen van CNT's, die kunnen worden geïdentificeerd op basis van fysieke en chemische kenmerken van producten, moet worden gestimuleerd; dit vereist meer informatie-uitwisseling tussen regelgevers en innovators.
- Omgaan met onzekerheid in de huidige risicobeoordeling en tegelijkertijd gelijke tred houden met snelle ontwikkeling vereist innovatie in risicobeheer. Zachte wetten (zachte regelgevende overeenkomsten) zijn gebaseerd op niet-bindende vereisten, en

wanneer deze overeenkomsten conservatief genoeg zijn, helpt dit de samenleving om zich sneller aan het tempo van innovatie aan te passen.

- Een verplichte Europese blootstellingsregistratie voor het toenemende aantal werknemers blootgesteld aan CNT's wordt gadviseerd omdat de gevolgen voor de gezondheid nog grotendeels onbekend zijn. De met deze registratie verzamelde resultaten kunnen worden gebruikt voor zowel retrospectief onderzoek met behulp van reeds beschikbare gegevens over sterfte en ziekten van werknemers, als voor nieuw epidemiologisch onderzoek gericht op de identificatie van mogelijke vroege gezondheidseffecten.

Concluderend draagt dit proefschrift bij tot het veilig(er) gebruik van CNT's en biedt het wetenschappelijke kennis met betrekking tot 1) een uitgebreide methode voor blootstellingsbeoordeling voor MWCNT's, 2) de identificatie van activiteiten en blootstellingsdeterminanten die in aanzienlijke mate bijdragen aan blootstelling aan MWCNT gedurende de levenscyclus van het product en 3) de waarneming van een indicatie van endotheel activatie en een verhoogde ontstekingsreactie geassocieerd met blootstelling aan MWCNT.



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About the author

Eelco was born in Boxtel, the Netherlands, on 26 October 1985 and grew up in Drunen. After completing secondary school (d'Oultremontcollege, Drunen) in 2005 he moved to Nijmegen to study Biomedical Sciences at the Radboud University. In order to obtain his degree, he performed an internship at the Institute of Occupational Medicine in Edinburgh, United Kingdom, aiming to perform a risk assessment for dimethyl fumarate (DMF) which was applied in several consumer products to prevent mould growth. In 2011, he received his MSc degree in Occupational and Environmental Health. After graduating, he started working as a research scientist at TNO and was mainly involved in the exposure assessment of chemicals including nanomaterials. In 2016, he started his PhD at the Institute for Risk Assessment Sciences (IRAS) at Utrecht University in cooperation with TNO. His project involved the evaluation of human health risks of exposure to carbon nanotubes, as described in this thesis. Currently, Eelco is still working at TNO where he is involved in the characterization of the exposome, which include the evaluation of new sensor applications both at the workplace and in the environment.

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