

Original Article

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% confidence interval (CI): 0.59–0.92] with an absolute mean difference of $59 \mu\text{g m}^{-3}$. 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 $570 \mu\text{g m}^{-3}$ 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 over-

estimated. Combining carbon analysis and SEM/EDX is the most suitable for quantitative exposure assessment of MWCNTs in an actual workplace situation.

Keywords: carbon analysis; ICP-MS; multi-walled carbon nanotubes; scanning electron microscopy

Introduction

Carbon nanotubes (CNTs) are nanoscale cylinders of carbon (essentially consisting of 'rolled' sheets of graphene) with very large aspect ratios (NIOSH, 2013; Hedmer *et al.*, 2014). 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 (Invernizzi, 2011; Upadhyayula *et al.*, 2012). There is a growing body of toxicological research indicating a potential health risk of CNTs (Hubbs *et al.*, 2009; Porter *et al.*, 2009; Wolfarth *et al.*, 2009; Porter *et al.*, 2010; Pauluhn, 2010; Mercer *et al.*, 2011; Castranova *et al.*, 2013). Especially certain types of multi-walled carbon nanotubes (MWCNTs) may have the potential for pulmonary toxicity due to their morphological similarity to asbestos (Poland *et al.*, 2008; Mercer *et al.*, 2010; Porter *et al.*, 2010; Sargent *et al.*, 2014). 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; Grosse *et al.*, 2014). 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 (Brouwer *et al.*, 2012). Direct-reading instruments (DRIs) and/or filter-based sampling methods have been used (Dahm *et al.*, 2013; Bello, 2009; Bello *et al.*, 2010; Lee *et al.*, 2010; Birch *et al.*, 2011; Ono-Ogasawara and Myojo, 2011; Brouwer *et al.*, 2012; Dahm *et al.*, 2012; Mattenklott and Thomas, 2012; Hashimoto *et al.*, 2013; Ogura, 2013; Reed *et al.*, 2013; Hedmer *et al.*, 2014). 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 (Birch *et al.*, 2011; Dahm *et al.*, 2012; Hashimoto *et al.*, 2013; NIOSH, 2013). For exposure to CNTs, a filter-based sampling technique is considered to be the most suitable method (Brouwer *et al.*, 2012; Dahm *et al.*, 2012). 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 (i) (scanning) electron microscopy/energy dispersive X-ray spectroscopy (SEM/EDX), (ii) carbon analysis, and (iii) inductive coupled plasma mass spectrometry (ICP-MS; Ogura, 2013). SEM is a technique for physico-chemical qualitative characterization of CNTs; however, objective criteria are lacking for quantitative counting CNTs (Brouwer *et al.*, 2012). In addition, due to heterogeneity in size, shape, and composition of CNTs quantification remains difficult and time consuming (Mattenklott and Thomas, 2012). 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 (NIOSH 2013; Ono-Ogasawara and Myojo, 2011). 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 (Reed *et al.*, 2013).

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.* (2015). Exposure measurements were performed during two periods in 2013 at the production facility. Four-to-eight hour shift-based personal breathing zone (PBZ) samples were collected inside the production area (3 and 4 days in respectively May without any synthesis activities and November during a period of full-scale synthesis), the R&D area (2 days in May), and offices (2 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 (0.4 μm and 25 mm, Nuclepore). Due to high air resistance of this filter the flow rate was set at 0.7 l min^{-1} (normally operating at 2 l min^{-1}). Sampling for the carbon analysis and ICP-MS analysis sampling was performed with pre-heated (2 h at 800°C) quartz fiber filters (QMA 25mm, Whatmann) at a flow rate of 2 l min^{-1} .

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 (PM) on these filters consisted of inorganic PM (Fig. 1B and C: white dots), organic carbonaceous matter (Fig. 1B), and soot (Fig. 1A). Automated detection of inorganic PM, organic carbonaceous matter, and of total PM 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 and 50 nm (Fig. 1A), which makes them easy to distinguish from other types of particles, including MWCNT aggregates (Fig. 1C–E). An indirect approach was developed to quantify the MWCNT concentration, by both using automated and manual particle analyses according the following equation:

$$\text{MWCNT concentration} = \text{total (automated)} - \text{inorganic (automated)} - \text{organic carbonaceous matter (automated)} - \text{soot (manual)}$$

A Tescan MIRA-LMH FEG-SEM microscope was used at an accelerating voltage of 15 kV, working distance 10 mm, spot size 5 nm. 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

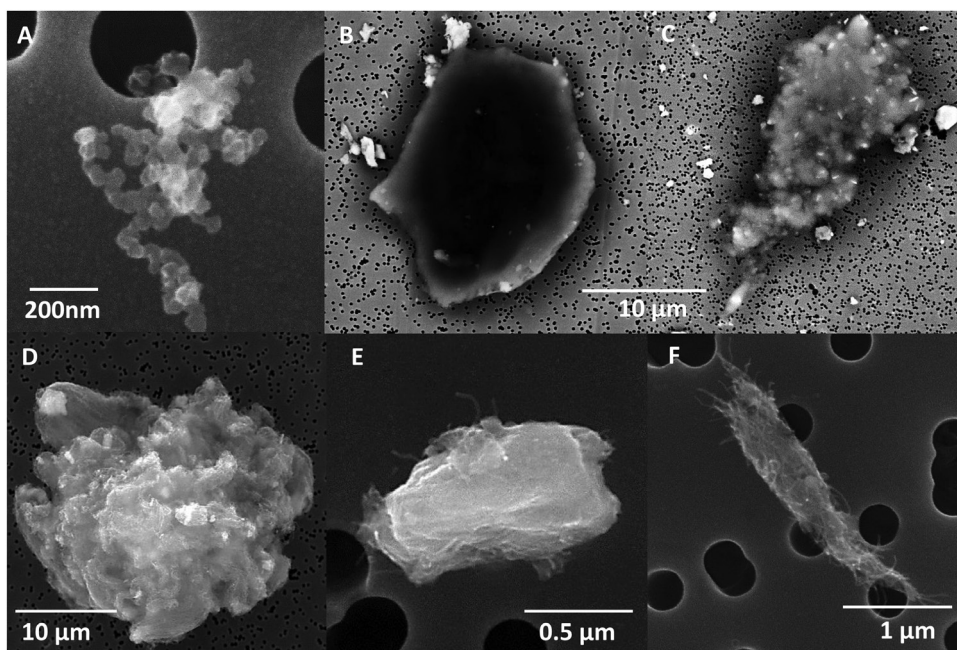


Figure 1. SEM images (SE-mode) of a diesel exhaust particle (A) and different inhalable MWCNT containing structures collected in the production facility: MWCNT hairball (D), MWCNT composite particles (E) and MWCNT bundle (F) and SEM images (BE-mode) of an organic carbonaceous particle (B: black flake),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

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 PM (Fig. 1B: black particle) and inorganic particles (Fig. 1B and C: white dots). In the BE-mode, inorganic particles and organic carbonaceous particles were distinguished from MWCNTs (Fig. 1C: grey particle), based on the grey scale of the particles.

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 $\times 200$, $\times 1.000$, $\times 5.000$, and $\times 25.000$. These magnifications cover in total 18 size bins: 25–40, 40–65, 65–100, 100–160, 160–250, 250–400, 400–650, 650–1000 nm 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 65–100 μm . Soot structures were manually counted at magnifications $\times 5.000$ and $\times 25.000$ covering the size bins between 25 nm and 2.5 μm . Per size bin a minimum of 50 particles were detected with in total ~ 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 (ISO, 2002). Per size bin the mean diameter and the standard deviation (SD) [95% confidence interval (CI)] was calculated on the basis of the Poisson distribution.

Based on the numerical concentration (N_{1-18}) 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_{pa1-18}), and volumetric shape factor (S_v) (Wagner and Leith, 2001; Ott *et al.*, 2008), by applying the following equation:

$$\text{Total mass concentration}_{SEM} = \sum_{i=1}^{18} M_p \text{ with } M_p = \left(\left(\frac{\pi}{6} \right) \times \rho_p \times \left(\frac{d_{pa1-18}}{S_v} \right)^3 \right) \times N_{1-18}$$

For carbon-based particles, like soot, MWCNTs, and other carbonaceous PM, an average ρ_p of 1.5 g cm^{-3} was used. The density was based on literature; for soot and other carbonaceous particles: McMurry *et al.* (2002), Slowik *et al.* (2004), Park *et al.* (2004), Hofmann *et al.* (2009) and for MWCNTs: Ku *et al.* (2006), Kim *et al.* (2009), Laurent *et al.* (2010). For inorganic non-carbonaceous particles, 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} (Wagner and Leith, 2001). 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: Wagner and Leith (2001), Ott *et al.* (2008), McMurry *et al.*, (2002), for soot: Slowik *et al.* (2004), Park *et al.* (2004), Hofmann *et al.* (2009) and for MWCNTs: Ku *et al.* (2006), Sturm (2015).

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 (NIOSH, 2006). A modified IMPROVE protocol (specific for MWCNTs) was used for the temperature and atmospheric gas settings (Ono-Ogasawara and Myojo, 2011, 2013). 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 EC and OC 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 ionisation. 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) (Ono-Ogasawara and Myojo, 2011, 2013).

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 (Ogura, 2013). Beside the CNT characteristics, the temperature also depends on the filter load of other PM. This is acknowledged in literature (Ono-Ogasawara and Myojo, 2013; Dahm, 2015; Birch, 2016) 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 in the online Supplementary Material, available at *Annals of Work Exposures and Health*). 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 PM decreases the oxidation temperature of MWCNTs, resulting in a shift to EC₂, but not EC₁. Based on these results, the sum of EC₂ and EC₃ 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 in the online Supplementary Material, available at *Annals of Work Exposures and Health*; ISO 5725 (1994). For MWCNTs, the limit of detection was 0.3–0.5 µg cm⁻² corresponding to an inhalable MWCNT concentration of 1.5 µg m⁻³ (8-h sampling period with flow rate 2 l min⁻¹), 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 (Na and Cocker, 2005). 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 (EC₂ + EC₃) 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 EC₂ + EC₃ and soot (SEM/EDX soot):

$$\text{Total mass concentration}_{\text{Corrected}} = \text{EC}_2 + \text{EC}_3 - \text{Soot}$$

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 (Reed et al., 2013). 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; Birch et al., 2011; Rasmussen et al., 2013). 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 $\leq 0.002 \mu\text{g}/\text{filter}$. Indoor concentrations of the metal proxy ($0.02\text{--}1.0 \mu\text{g}/\text{filter}$) were corrected for background metal proxy levels outdoors ($0.003\text{--}0.007 \mu\text{g}/\text{filter}$). Sensitivity of the filter sampling technique in combination with analysis with HR-ICP-MS was $\sim 2.5 \text{ ng m}^{-3}$ for an 8-h sampling period with a flow rate of 2.0 l min^{-1} .

Statistical analysis

Performance characteristics of the three analytical methods were determined in accordance with ISO 5725 (1994). 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 (I-Kuei Lin, 1989).

Reproducibility was determined with the corresponding 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% CI 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 (ISO 5725, 1994).

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–E). Overall particle size of these inhalable structures varied between ~ 0.25 and $100 \mu\text{m}$ and no single MWCNTs were detected.

Besides MWCNTs also soot structures, inorganic particles, and organic PM 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 PM consisted of biological particles (plant fragments, textile fibers), but the majority of these particles were production-related carbonaceous structures with large particle sizes ($5\text{--}200 \mu\text{m}$).

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%), inor-

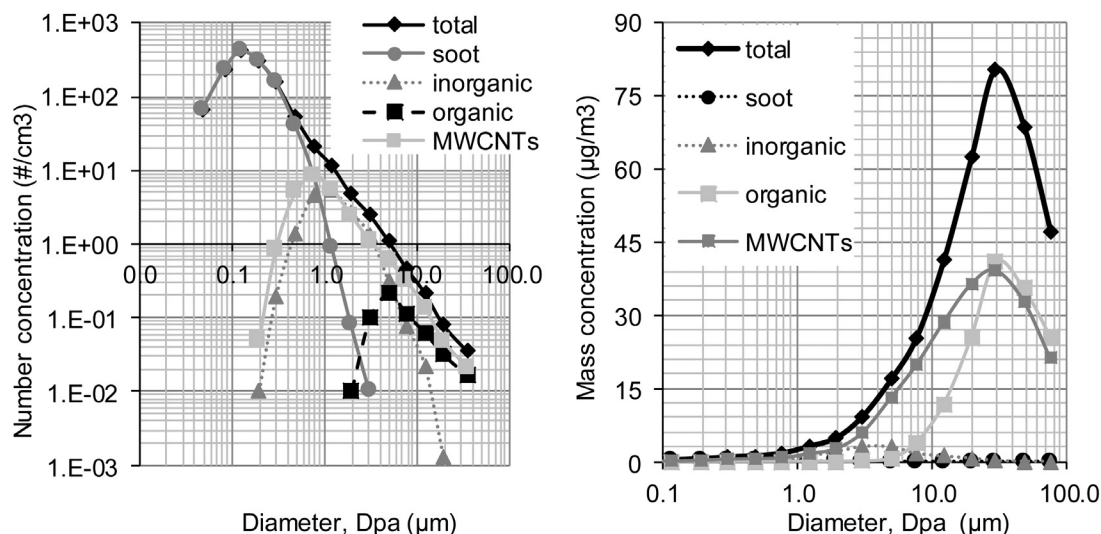


Figure 2. Particle number size distribution ($\# \text{ cm}^{-3}$) (A) and particle mass size distribution ($\mu\text{g m}^{-3}$) (B) of total particles, soot structures, inorganic particles, organic carbonaceous particles, and MWCNTs determined with SEM/EDX.

ganic particles (1.6%), and organic PM (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 PM (39.7%), while the contribution of inorganic particles (3.8%) and soot (0.4%) is only minor (Fig. 2B).

To date, there is no standardized electron microscopy-based method for counting CNTs (Brouwer *et al.*, 2012; Schulte *et al.*, 2012; Hedmer *et al.*, 2014). Besides counting procedures, also instrument settings of the electron microscope, like magnification(s), are not specified. Ogura *et al.* (2011) used two magnifications of $\times 1,000$ and $\times 10,000$, while Hedmer *et al.* (2014) reported an image field of $9000 \mu\text{m}^2$, corresponding to a single magnification of about $\times 2,000$. Mattenklott and Thomas (2012) suggest 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 ($\times 200$, $\times 1,000$, $\times 5,000$, and $\times 25,000$).

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 (Fig. 1E and 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 PM (especially organic carbonaceous particles), which makes automated particle analysis feasible. In general, the method is not limited by the particle size of PM 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% CI based on a Poisson probability distribution for SEM/EDX). With the subtraction technique, the concentration was 6.0 ($4.2\text{--}8.7$) structures cm^{-3} and with manual counting the concentration was 1.4 ($0.7\text{--}3.1$) structures cm^{-3} . After conversion to a mass concentration the difference was less, but still significant: 26.1 ($18.3\text{--}37.8$) $\mu\text{g m}^{-3}$ versus 12.2 ($6.0\text{--}23.2$) $\mu\text{g m}^{-3}$ 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 $19 \mu\text{g m}^{-3}$.

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 (Laurent *et al.*, 2010), 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 and 0.17 g cm^{-3} (Mattenklott and Thomas, 2012) while compact composite MWCNT material have densities similar to the skeletal density, reported to be 2.1 g cm^{-3} . Lehman *et al.* (2011) and Kim *et al.* (2009) have reported a mean density of $1.74 \pm 0.16 \text{ g cm}^{-3}$ for two different samples of MWCNTs (outer diameters of 15 nm and 22 nm), while Laurent *et al.* (2010) reported mean values between 1.1 and 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 $\sim 25\%$.

For MWCNTs, there is no information about shape factors. Ku *et al.* (2006) 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 (2015), 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. \(2008\)](#) 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 ([Davies, 1979](#); [Wagner and Leith, 2001](#)), 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 ~15%. The counting method of MWCNT structures with SEM/EDX results (similar as for soot) in an estimated CV (95% CI) of 30%. The overall uncertainty in MWCNT mass concentration determined with SEM/EDX is estimated at ~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^{-1}) than prescribed due to practical reasons (lower resistance). Deviations from the specified flow rate of 2.0 l min^{-1} 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 through

the design of the sampler's head ([Sanchez Jimenez et al., 2012](#)). This is supported by a study of [Zhou and Cheng \(2010\)](#), where the IOM sampler was found to behave similar when sampling at a higher flow rate (10.6 l min^{-1}), except for particles $>80 \mu\text{m}$, for which a 20% lower sampling efficiency was measured. In addition, [Vincent \(1989\)](#) and [Sansone and Bernard \(1976\)](#) 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 [Fig. 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 five times higher than in May, indicating that a major

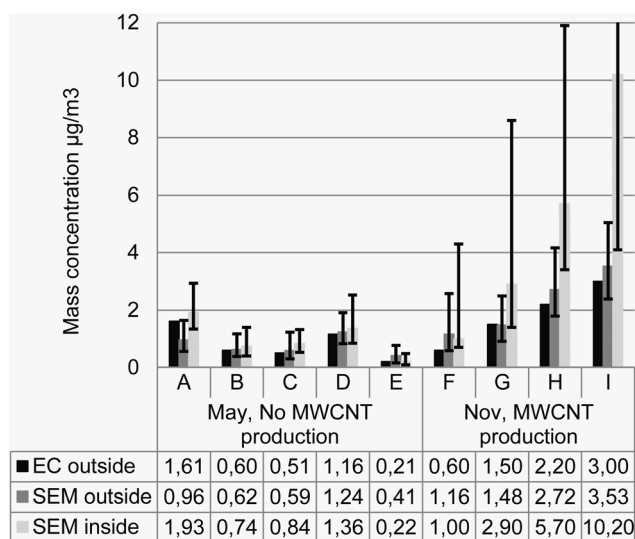


Figure 3. EC outside concentration ($\mu\text{g m}^{-3}$) determined with carbon analysis ($\text{EC}_2 + \text{EC}_3$) and SEM/EDX inside and outside (soot). Static air samples were collected outside the production facility in the field campaign of May 2013 (samples A–C in the production area, D–E in 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.

Table 1. Comparison soot (a) and MWCNT (b) determined with the different analytical methods: carbon analysis (EC₂ + EC₃), SEM/EDX and ICP-MS (metal proxy), and locations

Analytical method	Parameter	Location (reactor) ^a	AM ± SD concentration (µg m ⁻³)	Ratio ± SD (%) ^b	Concordance correlation coefficient (95% CI) ^a
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 # cm ⁻³	28 ± 43%	0.001 (0–0.04)

Arrhythmic mean (AM) and ratios taking carbon analysis as the reference.

^aReactor on/off: with/without production of MWCNTs.

^bRatio in AM concentration between carbon analysis (CA), ICP-MS, and SEM/EDX, calculated from the nine individual measurements as follows: (SEM1/CA1 + SEM2/CA2 + ... SEM9/CA9)/9.

source of EC inside the facility is the MWCNT synthesis itself which was only performed in November.

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 $124\% \pm 45\%$.

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 $EC_2 + EC_3 - \text{SEM/EDX soot}_{\text{daily indoor concentration}}$. 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 (McMurry *et al.*, 2002; Slowik *et al.*, 2004; Park *et al.*, 2004; Hofmann *et al.*, 2009). 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 OC content (Slowik *et al.*, 2004) and hygroscopic growth (Hofmann *et al.*, 2009) 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 $\pm 15\%$. By

summing these CVs according to standard error propagation procedures (ISO 5725, 1994), the uncertainty in mass concentration is $\sim 45\%$. Additionally, the counting method of soot structures resulted in a CV (95% CI) of $\sim 30\%$. Therefore, in this study, the overall uncertainty of the SEM/EDX method to determine soot mass concentrations is $\sim 55\%$.

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 two in the online Supplementary Material (available at *Annals of Work Exposures and Health*).

Comparison quantitative results MWCNTs

Quantitative MWCNT results of the side-by-side PBZ samples per day are presented in Fig. 4 and AM results

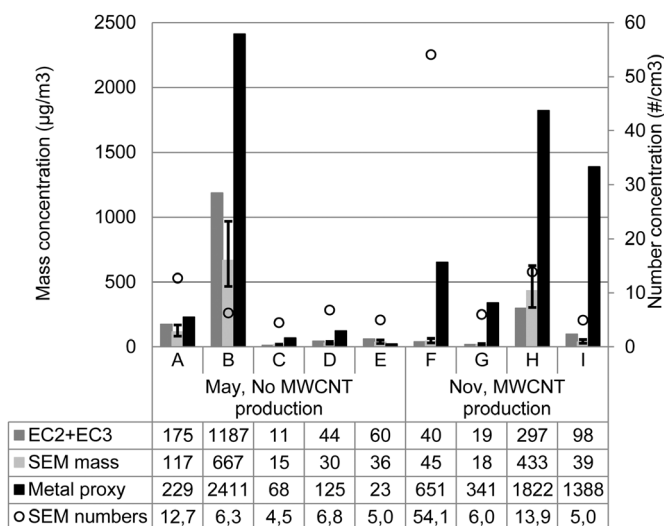


Figure 4. Mass concentration MWCNTs ($\mu\text{g m}^{-3}$) determined with carbon analysis ($\text{EC}_2 + \text{EC}_3$), SEM/EDX (both number and mass concentration) and mass concentration ICP-MS (metal proxy). Personal air samples were collected at the production facility in the field campaign of May 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.

are presented in Table 1b, comparing carbon analyses with the other three methods.

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.* (2012) and Hedmer *et al.* (2014) 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.* (2015) found a significant correlation for inhalable samples but with considerable data scatter explained due to measurement uncertainty (Dahm *et al.* 2015). Furthermore, three other studies (Lee *et al.*, 2010; Bello *et al.*, 2010; Bello, 2009) were using a standard method for asbestos fiber counting in accordance with ISO14966 (only fibers/structures with length $>5 \mu\text{m}$, width $<3 \mu\text{m}$, and length:width ratio $>3:1$), but did not consider the correlation. Hedmer *et al.* (2014) reported that 79% of the collected airborne CNTs did not fulfill the ISO fiber dimensions. In this study, the estimated percentage is even higher; $>90\%$ of the MWCNT containing particles have no typical fiber dimensions but consist of agglomerates with aspect ratios <3 . If only fibrous struc-

tures would be counted in this study, the MWCNT concentration would be <5 structures cm^{-3} .

MWCNT mass concentrations by SEM/EDX

There was a high correlation between carbon analysis ($\text{EC}_2 + \text{EC}_3$ corrected for soot) and mass concentrations derived with SEM/EDX ($R_c = 0.81$, absolute mean difference = $59 \mu\text{g m}^{-3}$; Table 1b). The ratio between the MWCNT concentration determined with SEM/EDX and carbon analysis was $87\% \pm 38\%$ 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)] $35 \mu\text{g m}^{-3}$ (20–88) (Kuijpers *et al.*, 2015). 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 = $570 \mu\text{g m}^{-3}$). The MWCNT concentration based on catalyst metals as a proxy was seven times higher than determined with carbon analysis (Table 1b). Especially with MWCNT production (reactor on) metal concentrations were high (Fig. 4) and would greatly overestimate the inhalable MWCNT concentrations

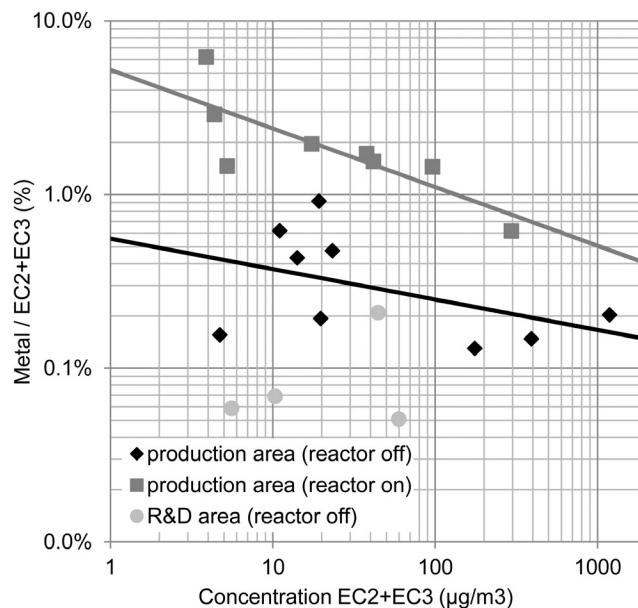


Figure 5. Correlation of the ratio between ICP-MS and carbon analyses (metal/ $EC_2 + EC_3$) and mass concentration $EC_2 + EC_3$ ($\mu\text{g m}^{-3}$) detected with carbon analyses in the production area with and without primary production (reactor on/off) and the R&D area. Additional samples were a random selection ($N = 21$) of residual quartz filters, already reported in the occupational exposure article.

if it would be used as a quantitative marker. 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 (Kuijpers *et al.*, 2015) were analyzed with ICP-MS. The correlation of the ratio between ICP-MS and carbon analyses (metal/ $EC_2 + EC_3$) and mass concentration ($\mu\text{g m}^{-3}$) detected with carbon analyses ($EC_2 + EC_3$) is presented in Fig. 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 (Fig. 1A) than MWCNT composite particles (Fig. 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 (Kuijpers *et al.*, 2015).

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 (Rasmussen *et al.*, 2013). 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 (Birch *et al.*, 2011; Rasmussen *et al.*, 2013). 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 (Reed *et al.*, 2013).

Conclusion

Exposure assessment approach for inhalable MWCNTs

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 (NIOSH, 2013; Birch, 2016), 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 (Birch *et al.*, 2011).

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 (Dahm, 2012; Birch, 2016; Dahm, 2015); 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 struc-

tures 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 of $1 \mu\text{g m}^{-3}$ (NIOSH, 2013), 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 $1.4 \mu\text{g m}^{-3}$ ($0.2\text{--}10 \mu\text{g m}^{-3}$); compared to the relatively high total MWCNT concentration this is ~5%. Although in this case relevancy is disputable, for respirable MWCNT concentrations the difference is substantial: ~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 ~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 PM.

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 (Brouwer *et al.*, 2012; NIOSH, 2013; Ogura, 2013) which include also oxidative damage (Hsieh *et al.*, 2012). 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.

Supplementary Data

Supplementary data are available at *Annals of Work Exposures and Health* online.

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Declaration

The authors declare no conflict of interest relating to the material presented in this Article. Its contents, including any opinions and/or conclusions expressed, are solely those of the authors.

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