Metal impurities provide useful tracers for identifying exposures to airborne single-wall carbon nanotubes released from work-related processes

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Abstract. This study investigated the use of metal impurities in single-wall carbon nanotubes (SWCNT) as potential tracers to distinguish engineered nanomaterials from background aerosols. TEM and SEM were used to characterize parent material and aerosolized agglomerates collected on PTFE filters using a cascade impactor. SEM image analysis indicated that the SWCNT agglomerates contained about 45% amorphous carbon and backscatter electron analysis indicated that metal impurities were concentrated within the amorphous carbon component. Two elements present as impurities (Y and Ni) were selected as appropriate tracers in this case as their concentrations were found to be highly elevated in the SWCNT parent material (% range) compared to ambient air particles (µg/g range), and background air concentrations were below detection limits for both elements. Bioaccessibility was also determined using physiologically-based extractions at pH conditions relevant to both ingestion and inhalation pathways. A portable wet electrostatic precipitation system effectively captured airborne Y and Ni released during sieving processes, in proportions similar to the bulk sample. These observations support the potential for catalysts and other metal impurities in carbon nanotubes to serve as tracers that uniquely identify emissions at source, after an initial analysis to select appropriate tracers.

1. Introduction

There is an urgent need for practical and cost-effective methods to distinguish process-related nanoparticle emissions from background aerosols in the workplace.$^1$ While direct readings of particle number, mass, size distribution, and surface area concentrations are recommended as a first step in identifying workplace exposures, these instruments do not provide the specific information (e.g. morphology or chemical composition) needed to distinguish nanomaterials from background.$^2,3$ Wide spatial and temporal variability of background aerosols make it especially difficult and time consuming to identify process-related emissions using direct-reading instruments.$^4$ Elemental carbon methods have been recommended$^6$ for measurement of occupational exposures to carbon nanotubes and carbon nanofibers (using NIOSH Method 5040 or an equivalent method), but such methods have potential limitations in situations where the background aerosol is also largely composed of elemental carbon (e.g., diesel soot).

The present study investigated the use of metal impurities in single-wall carbon nanotubes (SWCNT) as airborne tracers to distinguish process-related emissions from background. Electron
microscopy and elemental analyses provided morphological and chemical information about the bulk parent material and aerosolized samples, which supported the concept of using catalysts and other impurities as airborne tracers of carbon nanotubes for workplace monitoring. Electrostatic precipitation and filter-based approaches were used for sampling aerosolized SWCNT particles. Estimates of metal bioaccessibility, a relevant parameter in risk assessment, were calculated for both inhalation and ingestion pathways.

2. Materials and Methods
The SWCNT material used in this study (Aldrich no. 698695; batch MKBB3788) was purchased from Sigma-Aldrich Co. (Gillingham, UK). This product was manufactured by arc discharge, with 40-60 wt% carbon content, 30-35% metal content, average diameter 1.4 nm, and bundled dimensions of 2 to 10 nm in diameter and 1 to 5 µm in length (information provided by Sigma-Aldrich).

2.1. Physical characterization using TEM and SEM
Test samples of SWCNT dispersed in 99% ethanol were placed on 300 mesh copper grip coated with holey carbon film, then dried for 12 hr under vacuum (10^-8 torr) generated by a turbo pump (Varian V-81M Turbo Station). Diameter and cell wall thickness of aerosolized and parent SWCNT samples were measured using a JEM-2100F Transmission Electron Microscope (TEM; JEOL Industry) at high resolution (± 0.2 nm) and length was measured using a JSM-7500F Scanning Electron Microscope (SEM; JEOL Industry). Secondary electron imaging (SEI) was used to examine morphology of SWCNT agglomerates loaded on filter substrates (described below). Transmission electron diffraction (TED) mode was used for image analysis. Backscatter electron (BSE) mode was used to locate elements heavier than carbon (catalysts) in the amorphous carbon component of the agglomerates and x-ray energy dispersive spectrometry (EDS) was used to identify these heavier elements (>1%). SWCNT particles in an oil emersion were examined using a Carl Zeiss polarizing microscope equipped with a 100 x 10 objective.

2.2. Collection of SWCNT on filters using cascade impactor
A dry powder aerosol generator (TSI Model 3400A) was employed to aerosolize the SWCNT for subsequent size fractionation and collection on filters using a cascade impactor. Approximately 10 cc of SWCNT were required to fill the reservoir. Samples of SWCNT that had passed through the aerosolizer were collected from the inside wall of the elutriator for morphological analysis. A micro-orifice uniform deposit impactor (NanoMoudi-II, Model 125B, MSP Co., MN, USA), which uses inertial impaction to separate particles into discrete fractions according to their aerodynamic diameter, was equipped with pre-weighed PTFE filters (47 mm Teflo® filter with PMP support ring, Pall Corp.). The aerosolized SWCNT entered the impactor at a flow rate of 10 L/min, for three 2-hr runs and one 3.5-hr run, using 14 filters per run (13 stages plus an after filter) at nominal cut sizes ranging from 10 micron to 10 nm. PTFE filters were conditioned before pre-weighing and post-weighing for 24 hr in a controlled environmental chamber at a constant air temperature of 21°C (± 0.5°C) and constant relative humidity (RH) of 40% (± 0.2%), using Health Canada’s buoyancy-corrected gravimetric analysis facility6 (Archimedes M3™). The filters were weighed using a Mettler UMX2 microbalance (readability of ± 0.1 µg) after removing static electricity by passing each filter between two polonium-210 strip deionizers.

2.3. Collection of SWCNT using wet electrostatic precipitation
An Aerosol-to-Liquid Particle Extraction System (ALPXS; Meinhard Glass Products, Golden, CO) was used to monitor airborne SWCNT released from a sieve shaker. After entering the ALPXS air intake (~300 L/min), particles are first negatively charged by a fine ionization wire (~8,000V DC) and then collected and concentrated in de-ionized water that continuously re-circulates around a counter electrode (~8,000V DC).7 A mechanical sieve shaker (Ro-Tap RX-94; W.S.Tyler) and the ALPXS were placed inside a self-ventilating fume hood, with the ALPXS air sampling intake located about 25
cm from the source. A mass of about 10 mg SWCNT was maintained on the sieve pan throughout the shaking tests. The rate of shaking was increased incrementally, with 30 min monitoring at each increment, for a total of 4 monitoring tests. The ALPXS aqueous samples were then prepared for elemental analysis (described below). Before sieving was initiated, background air concentrations of tracer elements in the laboratory and other areas of the building were below limits of quantification (≤ 12 ng/m³ for Ni and ≤ 7 ng/m³ for Y).

2.4. Elemental Analysis using ICP-MS

Elemental concentrations were determined in the sample digests using a Perkin Elmer Sciex Elan DRC II ICP-MS operated in standard mode and equipped with a Meinhard Concentric Quartz Nebuliser, cyclonic spray chamber, and platinum skimmer/sampler cones. Internal standards were Germanium, Indium and Rhenium. Total elements were determined in bulk parent material using two approaches: microwave digestion using 20 mg test samples in nitric acid and 2-hr ultrasonic digestion using 2 mg test samples in nitric/hydrofluoric acid mixture based on a method previously developed for air filter samples. Bioaccessibility was estimated using two physiologically-based extraction techniques (PBET): a weak hydrochloric acid extraction (0.07 M; pH 1.5) to simulate gastric acid, and an ammonium acetate buffer (0.01 M; pH 7) to simulate the neutral lung environment. The extraction tubes were placed in a covered hot water bath at body temperature (37°C) for 2 hr (1 hr with agitation and 1 hr without agitation) for both PBET methods. Samples collected by ALPXS were pre-concentrated by evaporation on a hot block, and then digested using nitric acid and ultrasonic digestion. High purity acids (Seastar Chemical Inc, Sidney BC, Canada) and high purity standard stock solutions (High-Purity Standards, SC, USA) were used for all stages of sample preparation and analysis.

3. Results and Discussion

All samples of SWCNT examined by SEM/TEM showed a mixture of bundled nanotubes (individual nanotubes were rare) and irregularly-shaped agglomerates consisting of nanotube structures tangled with amorphous carbon/catalyst (figure 1). Both SEM micrographs in figure 1 cover the same area of dry-mounted sample. BSE mode (right image) was used to investigate the location of elements heavier than carbon within the agglomerates. Elemental impurities (in this case Y and Ni, as identified by EDS) appeared as bright spots on the BSE micrograph, and were associated almost exclusively with the amorphous carbon component (figure 1).

Figure 1. Two SEM micrographs of same area of SWCNT sample (Aldrich). Left image in TED mode shows distribution of amorphous carbon/catalyst and bundled SWCNT. Right image in backscatter mode shows location of metal catalysts (Ni, Y) as bright spots associated with amorphous carbon. Scale bar = 100 nm.
3.1. Selection of Y and Ni as suitable tracers

Analysis of bulk SWCNT (Aldrich 698695) by ultrasonic digestion and ICP-MS confirmed elevated concentrations of Y (25,916 µg/g = 2.6%) and Ni (132,745 µg/g = 13%), as listed in table 1. The next highest concentrations of elemental impurities were Al (492 µg/g) and Fe (475 µg/g). The presence of Y and Ni at concentrations that were orders-of-magnitude higher other elemental impurities indicated that Y and Ni were used as catalysts in the manufacture of the Aldrich SWCNT.

Concentrations of the two potential tracer elements (Y and Ni) and other impurities (Al and Fe) were analyzed in bulk samples of outdoor air particles that had been collected in a previous study. Concentrations of Y (6.6 µg/g) and Ni (40 µg/g) in ambient air particles were thousands of times lower than in the SWCNT. In contrast, concentrations of Al (8500 µg/g) and Fe (17,700 µg/g) in ambient air particles were several times higher than in the SWCNT. Thus, elemental ratios in SWCNT, such as Y/Al (52), Y/Fe (54), Ni/Al (270) or Ni/Fe (279), provided unambiguous discrimination from the ambient air particles (in which these ratios are <0.01). Moreover, Y and Ni were undetectable in indoor air monitored in the sieving laboratory and two other locations within the building. Therefore, Y and Ni qualified as appropriate tracers to identify releases of Aldrich SWCNT for this monitoring situation.

Metallic components of inhaled particles have the capacity to generate an increase in reactive oxygen species in the respiratory system. As the agglomerated forms of SWCNT, containing nanotube bundles tangled with amorphous carbon and metal impurities (figure 1), are likely candidates for inhalation and/or ingestion exposure, estimates of the bioaccessibility of metal impurities in SWCNT are relevant for risk assessments. Bioaccessibilities of the catalyst elements were determined using physiologically-based extractions at pH conditions relevant to inhalation and ingestion pathways (table 1).

Table 1. Concentrations (mg/g) of yttrium (Y) and nickel (Ni) in Aldrich SWCNT determined using ICP-MS and three extraction techniques. Concentrations shown are the mean of three replicates for each test, expressed as dry wt. PBET = physiologically based extraction technique.

| Element | Total Extraction | Neutral PBET | Gastric PBET | Bioaccessibility |
|---------|------------------|--------------|--------------|-----------------|
|         |                  | Inhalation Pathway | Ingestion Pathway |
| $^{89}$Y | 25.9             | 4.50         | 12.7         | 17%             | 49%             |
| $^{60}$Ni| 133              | 14.4         | 51.7         | 11%             | 39%             |

The microwave digestion method resulted in an incomplete extraction (not included in table 1), attributed to inefficient contact between particles and acid caused by the higher solid:liquid ratio and absence of agitation in the microwave method.

3.2. Characterization of aerosolized agglomerates

SWCNT particles that had passed through the aerosolizer (collected before entering the MOUDI) were characterized for morphology by SEM/TEM to determine if aerosolization had caused a change in morphology compared to the parent material. Post-aerosolization measurements (provided in table 2) indicated that aerosolization had no observable impact on morphology. Nanotube diameter of the
parent material (mean=1.9 ± 0.6 nm; n=15 measurements) was not significantly different from the aerosolized sample (mean=1.7 ± 0.2 nm; n=27 measurements). Likewise, there was no difference in wall thickness between the two samples (0.4 ± 0.1 nm for both aerosolized and non-aerosolized). Bundles of both parent material and aerosolized SWCNT ranged from 5-10 nm in diameter and were well-formed, typically containing from 3 to 20 individual nanotubes. All nanostructure measurements were normally distributed (both Kolmogorov-Smirnov and Shapiro-Wilk normality tests \( p > 0.2 \)).

**Table 2.** Physical characterization of SWCNT after aerosolization, using TEM to determine diameter and wall width and SEM to determine length (n= number of measurements).

|                  | Diameter (nm) | Wall Width (nm) | Length SEM (µm) |
|------------------|---------------|---------------|-----------------|
|                  | n=27          | n=27          | n=31            |
| Mean             | 1.7 (sd 0.2)  | 0.4 (sd 0.1)  | 2.13 (sd 1.08)  |
| Median           | 1.8           | 0.4           | 2.15            |
| Range            | 1.3 – 2.0     | 0.3 – 0.5     | 0.32 – 4.70     |

SEM micrographs of aerosolized SWCNT agglomerates collected on PTFE filter samples collected on two MOUDI stages (nominal cut sizes 1 µm and 3.2 µm) are shown in figures 2 and 3. These agglomerates appear as scattered clusters of bundled nanotubes and amorphous carbon/catalyst material against the backdrop of PTFE fibers. These micrographs confirm that aerosolized SWCNT agglomerates include amorphous carbon/catalyst material. Such evidence of the presence of amorphous carbon/catalyst material in aerosolized nanomaterials substantiates the concept of using catalyst elements as airborne tracers.

Overall, the aerosol generator-MOUDI system yielded very lightly loaded filter samples, required labour-intensive cleaning between runs to minimize carry-over, and resulted in inconsistent particle size distributions between runs. Gravimetric analysis detection limits (≈ 2 µg) were generally exceeded in filter samples from the 32 nm to 3.2 µm range, but many samples were below detection limits and between-run reproducibility was poor. These factors, combined with the impact of agglomeration on particle size distribution, suggested little advantage in using a MOUDI for monitoring SWCNT in the workplace, compared to simpler 37 mm cassettes recommended previously. However, the aerosol generator-MOUDI system did prove to be useful for the preparation of filter samples of SWCNT agglomerates suitable for examination by SEM (i.e., minimal particle overlap) as shown in figures 2 and 3.
3.3. Image analysis

Significant amorphous carbon/catalyst material was observed in all SWCNT agglomerates in all size ranges. Nano- to micro-scale SWCNT agglomerates were observed using SEM/TEM (figures 1 to 3), and larger agglomerates in the 10 to 100 µm size range (along the long axis) were observed using a light microscope. In order to quantify the proportion of amorphous carbon/catalyst compared to nanotube structures in the agglomerates, SEM images were classified into intervals corresponding to the grey-scale pixel value of individual points. Figure 4 shows the original SEM micrograph of an area of dry-mounted SWCNT sample, plus the classified image distinguishing nanotube structures, amorphous carbon / catalyst material, and background. The relative proportion of nanotube structures to amorphous carbon/catalyst was calculated by completing an area analysis for each of 19 images (including figure 4) randomly selected along transects of the mounted sample. Excluding background material, the average proportions were: 55% nanotube structures to 45% amorphous carbon/catalyst (in two-dimensional view).

Figure 2. Clusters of aerosolized SWCNT agglomerates collected on PTFE filter (MOUDI cut size 1 µm); scale bar = 10 µm; particle mass 51 µg.

Figure 3. Close-up of SWCNT agglomerate impacted on PTFE filter fibres (MOUDI cut size 3.2 µm); scale bar = 1 µm, particle mass 22 µg.

Figure 4. Two images from the same SEM micrograph of dry mounted SWCNT. Image analysis (right) shows how the TED micrograph (left) is classified as SWCNT (grey) and amorphous carbon/catalyst (black). Scale bar = 100 nm.
3.4. Monitoring airborne tracers using electrostatic precipitation

After electrostatic precipitation, SWCNT particles were visible as black supermicrometre clusters in the aqueous ALPXS sample. Figure 5 shows results of monitoring Y and Ni tracers of SWCNT released from the sieve shaker as the rate of shaking was increased incrementally. The particle release rate increased as the shaking rate increased, indicated by an increase in airborne Y concentration from 27 ng/m$^3$ to 1839 ng/m$^3$ (figure 5a), and in airborne nickel concentration from 151 ng/m$^3$ to 8794 ng/m$^3$ (figure 5b). These air concentrations are consistent with table 1 which indicates that Y and Ni occur as impurities in the SWCNT parent material in approximately the same proportion (total concentration Y:Ni ratio $\approx$ 1:5). It was established (figures 1 to 4) that a large percentage of aerosolized agglomerate is amorphous carbon/catalyst material. The observed increase in airborne Y and Ni concentrations (figure 5) is consistent with the release of amorphous carbon/catalyst material as the SWCNT agglomerates disaggregate.

Figure 5. Yttrium (5a) and nickel (5b) tracers of airborne SWCNT released in an R&D lab during sieving. Rate of shaking was increased incrementally with 30 min monitoring at each increment, using wet electrostatic precipitation and ICP-MS determination. Background air concentrations of both elements were below limits of quantification.

Determination of metal impurities in SWCNT (as shown in figure 5) as an alternative to elemental carbon (e.g. NIOSH Method 5040) could improve detection sensitivity significantly. Carbon content of the SWCNT parent material used in this study ranges from 40 to 60% and elemental impurities are present at 13% for Ni and 2.6% for Y. Based on upper limits of quantification (LOQ) for detecting airborne SWCNT using the above electrostatic precipitation method (12 ng/m$^3$ for Ni and 7 ng/m$^3$ for Y), the equivalent LOQ estimated for elemental carbon ranges from 0.04 to 0.16 µg/m$^3$. This is one- to two- orders of magnitude lower than that of NIOSH Method 5040 (7 µg/m$^3$). Note that LOQs for elemental tracer methods will vary depending on the particle sampling approach (e.g. filter media), sample handling, ICP-MS equipment and ICP-MS personnel.

4. Summary and Conclusions

The goal of this research is to develop practical monitoring techniques within the scope and budget of typical occupational health and safety investigations\(^4\). A promising strategy to identify process-related releases of carbon nanotubes is to monitor elemental tracers using electrostatic precipitation, filter cassettes, or possibly surface wipes, followed by elemental analysis of the collected samples by ICP-MS. Selection of appropriate tracers requires an initial analysis of (1) the bulk parent nanomaterial, as different elements (e.g. Co, Cu, Fe, W, Ta, Mo) are used as catalysts and anodes and these are unique to each manufacturing process, and (2) the background environment to ensure that the proposed tracer
does not arise from local sources. In this study, Y and Ni proved to be suitable tracers because they were undetectable in background air, and their concentrations in SWCNT were orders of magnitude higher than in background particles. Elemental ratios such as Y/Al, Y/Ni, Ni/Al, or Ni/Fe unambiguously distinguished SWCNT from ambient aerosols. Wet electrostatic precipitation was an effective method for capturing airborne Y and Ni released during sieving processes. SEM analysis indicated that metal catalysts occur predominantly in the amorphous carbon component, which constitutes about 45% of SWCNT agglomerates. Examination of SWCNT agglomerates captured on PTFE filters demonstrated that amorphous carbon/catalyst material remains a significant component after aerosolization. The above lines of evidence support the concept of using metal impurities as airborne tracers of carbon nanotubes for workplace monitoring.

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