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STRATEGY FOR USING METAL IMPURITIES AS CARBON NANOTUBE TRACERS

Series on the Safety of Manufactured Nanomaterials
No. 79

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OECD Environment, Health and Safety Publications

Series on the Safety of Manufactured Nanomaterials

No. 79

**STRATEGY FOR USING METAL IMPURITIES AS CARBON
NANOTUBES TRACERS**

IOMC

INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS

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**Environment Directorate
ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT
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The Organisation for Economic Co-operation and Development (OECD) is an intergovernmental organisation in which representatives of 35 industrialised countries in North and South America, Europe and the Asia and Pacific region, as well as the European Commission, meet to co-ordinate and harmonise policies, discuss issues of mutual concern, and work together to respond to international problems. Most of the OECD's work is carried out by more than 200 specialised committees and working groups composed of member country delegates. Observers from several countries with special status at the OECD, and from interested international organisations, attend many of the OECD's workshops and other meetings. Committees and working groups are served by the OECD Secretariat, located in Paris, France, which is organised into directorates and divisions.

The Environment, Health and Safety Division publishes free-of-charge documents in 11 different series: **Testing and Assessment; Good Laboratory Practice and Compliance Monitoring; Pesticides; Biocides; Risk Management; Harmonisation of Regulatory Oversight in Biotechnology; Safety of Novel Foods and Feeds; Chemical Accidents; Pollutant Release and Transfer Registers; Emission Scenario Documents; and Safety of Manufactured Nanomaterials.** More information about the Environment, Health and Safety Programme and EHS publications is available on the OECD's World Wide Web site (www.oecd.org/chemicalsafety/).

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The Inter-Organisation Programme for the Sound Management of Chemicals (IOMC) was established in 1995 following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. The Participating Organisations are FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

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FOREWORD

The OECD Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology (the Joint Meeting) held a Special Session on the Potential Implications of Manufactured Nanomaterials for Human Health and Environmental Safety (June 2005). This was the first opportunity for OECD member countries, together with observers and invited experts, to begin to identify human health and environmental safety related aspects of manufactured nanomaterials. The scope of this session was intended to address the chemicals sector.

As a follow-up, the Joint Meeting decided to hold a Workshop on the Safety of Manufactured Nanomaterials in December 2005, in Washington, D.C. The main objective was to determine the “state of the art” for the safety assessment of manufactured nanomaterials with a particular focus on identifying future needs for risk assessment within a regulatory context.

Based on the conclusions and recommendations of the Workshop [ENV/JM/MONO(2006)19] it was recognised as essential to ensure the efficient assessment of manufactured nanomaterials so as to avoid adverse effects from the use of these materials in the short, medium and longer term. With this in mind, the OECD Council established the OECD Working Party on Manufactured Nanomaterials (WPMN) as a subsidiary body of the OECD Chemicals Committee in September 2006. This programme concentrates on human health and environmental safety implications of manufactured nanomaterials (limited mainly to the chemicals sector), and aims to ensure that the approach to hazard, exposure and risk assessment is of a high, science-based, and internationally harmonised standard. It promotes international co-operation on the human health and environmental safety of manufactured nanomaterials, and involves the safety testing and risk assessment of manufactured nanomaterials.

This document describes practical and cost-effective monitoring approaches for using metal impurities in carbon nanotubes (CNTs) as indicators of their presence in the workplace. Its goal is to supplement the “toolbox” of existing methods available for risk assessment and exposure mitigation. Advice on sampling techniques and off-line analysis will assist the occupational hygienist to identify situations where metal impurities in CNTs can be applied as a sensitive but qualitative indicator of the presence of process-related releases of CNTs in the workplace.

This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and Working Party on Chemicals, pesticides and Biotechnology of the OECD.

STRATEGY FOR USING METAL IMPURITIES AS CARBON NANOTUBE TRACERS

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EXECUTIVE SUMMARY

1. The purpose of this document is to describe the use of metal impurities as CNT indicators in the workplace and to discuss sampling and analytical techniques that will assist the occupational hygienist to identify situations where the CNT tracer strategy can be applied. Limitations are identified which place the CNT tracer strategy in the category of a sensitive but qualitative (semi-quantitative) indicator of the presence of process-related releases of CNTs in the workplace. Qualitative evidence provided by monitoring CNT tracers may be useful to evaluate the effectiveness of clean-up and engineering control measures, and to assist in distinguishing CNTs from non-CNT sources of elemental carbon (such as diesel soot). Thus, the CNT strategy is considered suitable for Tier 2 assessments under the OECD Harmonized Tiered Approach (OECD, 2015).

2. The literature review summarizes published studies that have used residual catalyst impurities as indexes or tracers for carbon nanotubes (CNTs) for various purposes. These include identifying CNTs in aerosols, settled dust, and surface sediments; differentiating airborne CNTs from other aerosols; estimating workers' personal air exposures; quantifying CNT adherence to workers' gloves after handling CNTs; and development of next-generation detectors. Earlier studies used ICP-AES as the instrumental method to determine metal impurities in collected filter samples, according to standard OSH protocols. Recent studies use ICP-MS because it has the greater sensitivity (lower detection limits) required to determine residual metal catalysts in the concentration range currently found in commercial MWCNTs and SWCNTs (typically 1000 to 5000 mg/kg). ICP-MS is also suitable for determining metal impurities in CNTs collected on variety of sample substrates (e.g., filter substrates, surface wipes and wet electrostatic precipitation samples).

3. Selection of an appropriate CNT tracer requires knowledge of the metal impurities in the CNT powder being handled, and knowledge of potential interferences from background sources (i.e., non-CNT sources) of metals in the workplace. Different combinations of metal elements are used as catalysts for production of CNTs, and the catalyst formulation and concentration are unique to each manufacturing process. Concentrations of metal impurities in CNTs are obtained by providing a few milligrams of the CNT powder to a commercial laboratory for multi-element analysis. Downstream users who are applying the CNT tracer method should monitor the metal content of the CNT powder being used, to track batch-to-batch or lot-to-lot consistency, and to identify possible changes in catalyst formulation over time. Possible changes to the metal content during the work process should be investigated by comparing samples of the released CNT with the original parent CNT material.

4. The selection of an appropriate sampling method is situation-dependent. Most studies seek adaptations of well-established protocols and approaches that are familiar to the occupational hygienist and fall within typical OH&S budgets. In each situation, it is important to determine whether the sample mass collected will be adequate for reliable determination of the CNT metal tracer. In the case of air sampling, it may be too difficult to collect sufficient particle mass on filter substrates using traditional pump flow rates (typically 2-4 L/min) and sampling durations (typically ≤ 8 hr). A case study of a CNT manufacturing plant (where elemental carbon concentrations exceeded the NIOSH $1 \mu\text{g}/\text{m}^3$ limit) showed that airborne CNT metal tracers were measurable using traditional sampling parameters. However, the airborne metal impurities did not correlate with airborne CNTs (determined using ICP-MS and thermal-optical carbon analysis respectively). The high variability was attributed to the very small CNT sample masses obtained

on the filter substrate, and interferences from non-CNT sources. While the case study showed that measurement of airborne metal catalysts was feasible and provided useful supplementary information, a lesson learned was that airborne metals were a qualitative indicator of the presence of airborne CNTs, not a quantitative metric.

5. Wipe sampling emerges as a useful and cost-effective qualitative technique suitable for Tier 2 assessments under the Harmonized Tiered Approach. Due to the relatively low cost, wipe sampling is suitable for representative mapping of spatial variations of surface metal contamination within a workplace. The method is considered qualitative when applied in the CNT tracer strategy, and provides an additional tool to detect workplace releases of CNTs (and potential exposures), evaluate housekeeping or clean-up procedures, and monitor the effectiveness of engineering controls. In the above case study, collocated floor wipe samples collected in four areas of a CNT manufacturing plant displayed strong correlations between CNT metal impurities and CNTs. This finding was supported by passive accumulation samples in the same four areas, which also displayed strong metal-CNT correlations and enabled the calculation of deposition rates. A lesson learned about wipe sampling was that these correlations were observed only in areas of active particle deposition, and not inactive surfaces with contamination of varying ages and sources. Other approaches for area monitoring of CNT releases include air sampling of metal impurities using wet electrostatic precipitation at high flow rates, which is useful for capturing transient changes in CNT air concentrations.

6. The final section discusses analytical methods for determining CNT tracers in collected samples. It covers advantages and challenges associated with using ICP-MS; issues related to quality control during sampling, handling and analysis; and the interpretation of elemental ratios and isotopic ratios from published studies. The greater sensitivity of ICP-MS (compared to ICP-AES) requires greater care to avoid inadvertent contamination of the sample, including rigorous monitoring of field blanks and media blanks. The advantage of ICP-MS is its capability to determine a wide range of elemental impurities, which facilitates the use of elemental and isotopic ratios to discriminate between CNTs and non-CNT particles.

INTRODUCTION

Purpose

7. This document describes practical and cost-effective monitoring approaches for using metal impurities in carbon nanotubes (CNTs) as indicators of their presence in the workplace. Its goal is to supplement the “toolbox” of existing methods available for risk assessment and exposure mitigation. Advice on sampling techniques and off-line analysis will assist the occupational hygienist to identify situations where metal impurities in CNTs can be applied as a sensitive but qualitative indicator of the presence of process-related releases of CNTs in the workplace.

Literature review

8. Table 1 summarizes published studies which use metal impurities in CNTs as indicators or tracers to identify CNT releases or estimate exposures. The CNT tracer approach makes use of the fact that metals such as Fe, Co, Ni, Mo or Y which are commonly used as catalysts during the CNT manufacturing process, typically remain as residual impurities in the CNT product in the parts-per-million or percentage concentration range.

9. Each of the studies in Table 1 uses a combination of analytical approaches to characterize the collected CNT samples. Three use inductively coupled plasma - atomic emission spectroscopy (ICP-AES) to determine metal concentrations according to NIOSH method 7300 (Maynard et al., 2004; Baron et al., 2008; Birch et al., 2011) and five use inductively coupled plasma - mass spectroscopy (ICP-MS) (Rasmussen et al., 2013; 2015; Olson et al., 2014; Schierz et al., 2014; Boonruksa et al., 2015). Three studies describe emerging instrumental approaches which are at the proof-of-concept stage but not yet fully developed for workplace applications (Neubauer et al., 2013; R'mili et al., 2011; Reed et al., 2013). Neubauer et al. (2013; Table 1) are working to develop new infrared sensor methods that could use metal impurities to identify CNTs as they are released in the workplace (*in situ*). Two other examples of next-generation instrumental approaches for using metal tracers to identify CNT releases are: laser-induced breakdown spectroscopy (R'mili et al., 2011) and single-particle ICP-MS (Reed et al., 2013).

10. Three studies in Table 1 explore the possibility of using metal impurities to detect CNT releases to the environment (Olson et al., 2014; Schierz et al., 2014; Boonruksa et al., 2015). Schierz et al. (2014) used the catalyst elements Mo and Co to fingerprint CNT occurrence in sediment cores after a simulated spill into an outdoor wetland mesocosm. Olson et al. (2014) used filter-based air sampling to quantify a variety of water soluble metals in CNT materials that were aerosolized in a controlled environmental chamber, and compared the results to ambient air samples. Boonruksa et al. (2015) used multi-element signatures of CNT impurities to test for release of CNTs during injection molding and recycling of CNT-polypropylene composites.

11. This report will discuss successes and limitations associated with using metal impurities as CNT indicators in the workplace, as reported in the peer-reviewed studies listed in Table 1. These studies show that multiple approaches are often needed to adequately characterize CNT releases. Most capture the CNT aerosol on a filter substrate for subsequent “off-line analysis” of elemental carbon and metals in the particles loaded on the filter. “Off-line analysis” requires submitting samples collected in the workplace to one or more laboratories specializing in physical and chemical characterization of CNTs, most commonly transmission electron microscopy (TEM), scanning electron microscopy (SEM) with energy dispersive X-ray spectrometry (EDX), thermal-optical analysis for elemental carbon, near-infrared fluorescence spectroscopy, ICP-AES, and ICP-MS.

Table 1. Summary of published studies (in chronological order) which have used metal impurities as indicators of carbon nanotube (CNT) or carbon nanofiber (CNF) releases.

REFERENCE	PURPOSE	TYPE OF SAMPLE	ANALYTICAL METHOD FOR METALS	COMMENTS	CNT IMPURITY
Maynard et al. 2004	Exposure monitoring at 4 CNT manufacturing facilities	Personal air samples using 25 mm mce filters; cotton glove samples	Nitric/perchloric acid extraction followed by ICP-AES (NIOSH 7300)	Fe and Ni used to estimate personal air CNT concentrations	Fe and Ni catalysts used as CNT index; Fe/Ni ratios
Baron et al. 2008	Characterization of CNT inhalation exposure for mouse experiments	MOUDI cascade impactor for collecting CNTs on MCE filters soaked with oleic acid	ICP-AES (NIOSH 7300)	CNT proxy provided size distribution information required to estimate mass dosage	Fe catalyst used as CNT proxy
Birch et al. 2011	Area and personal air monitoring in different areas of CNF manufacturing facility	Air sampled on quartz fibre and mixed cellulose ester	Nitric/perchloric acid extraction followed by ICP-AES (NIOSH 7300).	No correlation between Fe and EC concentrations due to non-CNF sources of Fe in the plant	Fe catalyst did not uniquely identify CNFs
R'mili et al. 2011	Characterization of particle releases during transfer of CNTs	CNT particles collected on grids	Laser-induced breakdown spectroscopy (LIBS) in combination with SEM-EDX and TEM	Currently qualitative; method under development; Goal is to develop quantitative real-time elemental characterization method.	C, Al and Fe in released CNTs
Rasmussen et al. 2013	Characterization of aerosolized CNTs generated by mechanical agitation	Wet electrostatic precipitation	Ultrasonic nitric acid digestion followed by ICP-MS	Useful for monitoring transient changes in airborne CNT impurities	Ni and Y catalysts used as CNT indicator; Ni/Fe, Y/Al ratios

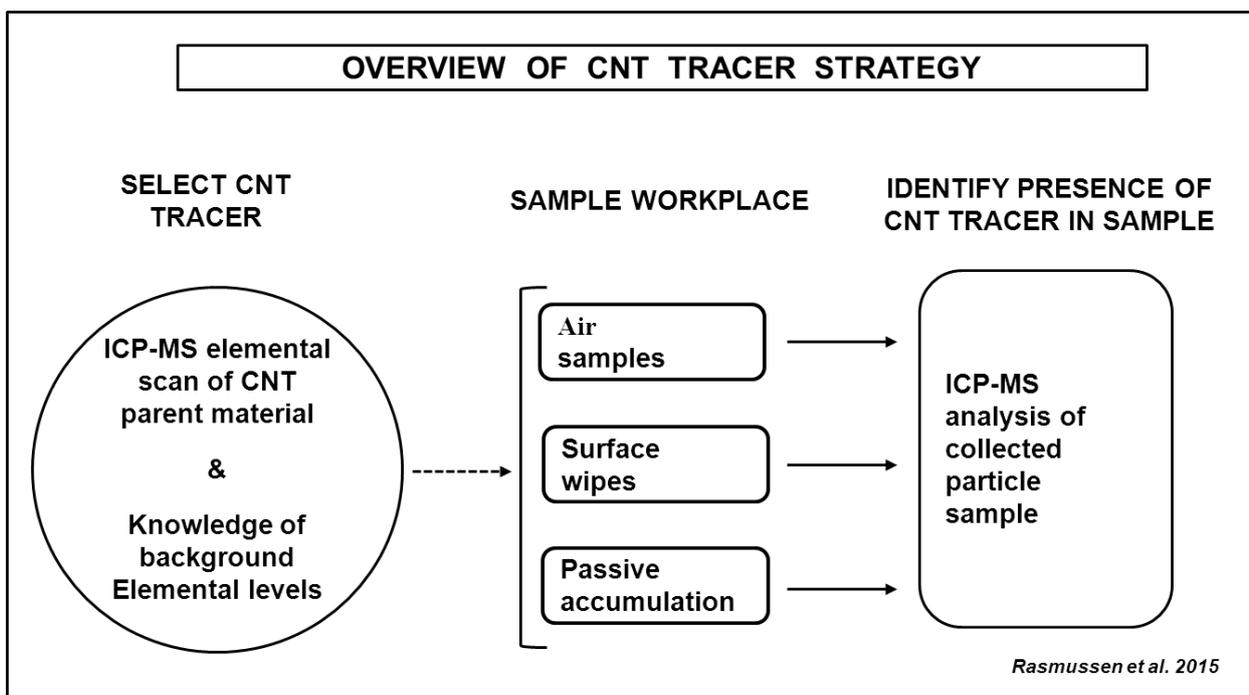
REFERENCE	PURPOSE	TYPE OF SAMPLE	ANALYTICAL METHOD FOR METALS	COMMENTS	CNT IMPURITY
Reed et al. 2013	Detect CNTs released to solution from CNT/polymer nanocomposites	CNT/chitosan composite allowed to sit for 7 days in DI water	Dispersant added prior to detection using single particle-inductively coupled plasma-mass spectrometry (sp-ICPMS)	Currently qualitative/semi-quantitative due to instrumental limitations; method under development	Co and Y used as indicators of CNTs in solution at low ng/L concentrations
Neubauer et al 2013	Detection of laboratory generated CNT	Air sampled on 25-mm quartz fibre filters	Development of next-generation <i>in situ</i> selective detection using IR sensor	Ni content must be known precisely; future development of method for other metals.	Ni catalyst in CNTs
Olson et al. 2014	Characterization of aerosolized CNTs in controlled environmental chamber	47 mm Teflon substrates from the PM2.5 cyclone sample	Water-soluble metals quantified by ICP-MS	CNTs have higher estimated Y contribution than Denver CO ambient air.	Y and other impurities (Ti, Fe, Cu, Zn, As, Cd, Pb and Ag)
Schriez et al. 2014	To fingerprint CNT in sediment cores after a simulated spill into a wetland mesocosm.	Sediment cores were sliced and dried	Acid digestion of sediment; extracts analyzed by ICP-MS	Estimated CNT content using metal impurities agreed with near-infrared fluorescence spectroscopy	Co and Mo (CoMoCat) catalyst in CNTs; Co/Mo ratios
Rasmussen et al. 2015	Area monitoring of CNT releases in CNT manufacturing plant and downstream users laboratory	Surface wipe samples, air samples using quartz filters, and passive accumulation samples	Microwave/hot block or ultrasonic assisted acid digestion followed by ICP-MS	Correlation between metal impurities and CNTs in floor wipes; distinct Pb isotope signatures in CNTs in lab environment.	Co catalyst and Ni trace impurities in CNTs; stable Pb isotopes; Co/Fe ratios
Boonrukka et al. (2015)	Testing for released CNTs during injection molding/recycling of CNT polypropylene composite	Emissions of < 250 µm CNT - pp captured on Teflon filter using Nano-ID sampler	Metals determined using Sector Field ICP-MS	Indirect confirmation of conclusion (from TEM and SEM) that no free CNTs were released	Mo, Al and Fe, plus signature trace impurities W, Sb, Sn, Mn and Co

Overview of the CNT tracer strategy

12. **Figure 1** provides an overview of the guidance provided in this document, which is divided into three components:

1. selection of an appropriate CNT tracer, which requires knowledge of the metal content of the CNT parent material and possible changes during processing, and knowledge of background sources of metals;
2. sampling in the workplace where CNTs may be released; and
3. off-line analysis of the collected particle sample using ICP-MS to identify the CNT tracer.

Figure 1. Overview of strategy to identify CNT releases in the workplace using metal impurities. (From Rasmussen et al., 2015)



13. The main body of this document is organized into three sections corresponding to each of the components in Figure 1. Complexities and limitations associated with each component will be illustrated by examples in the corresponding sections.

14. This guidance aims to address the need for practical and cost-effective approaches for detecting CNT releases that are within the scope and budget of typical occupational health and safety investigations. Possible applications of the CNT tracer strategy include: identifying the major sources of CNT releases in a workplace, monitoring the effectiveness of control measures, and/or distinguishing CNTs released in the workplace from background particles.

Scope and limitations

15. The scope of this document is limited to guidance on identifying CNT releases in the workplace, suitable for a Tier 2 assessment in the context of the hierarchical 3-tiered risk assessment method described by OECD (2015). In the 3-Tiered Approach, Tier 1 focuses on data gathering, and Tier 2 includes simplified measurements to identify CNT releases and potential exposures. As described by OECD (2015), Tier 2 measurements also include an assessment of background. Determination of elemental carbon (e.g. NIOSH Method 5040) does not distinguish between CNT and non-CNT sources of elemental carbon such as soot or diesel exhaust. Therefore, in situations where elevated background exposure to non-CNT sources of elemental carbon is possible, the detection of catalyst metals in samples can assist in identifying CNTs (OECD 2015).

16. Wipe sampling, which is suitable for identifying CNT tracers in settled dust, is presented in this document as an example of a Tier 2 sampling approach. Results from Tier 2 may yield evidence that an extended, expert exposure assessment is required, in which case Tier 3 approaches become necessary. Tier 3 involves quantitative estimates of worker exposure that require expanded situation-specific data collection procedures that are beyond the scope of this project. The scope of this document is limited to semi-quantitative approaches suitable for area sampling, which would provide evidence of CNT releases and potential exposures. Techniques for quantitative exposure assessments (including personal breathing zone sampling) are outside the scope of this document.

17. Currently, there are no benchmark criteria for settled dust containing CNTs (or for dermal and ingestion pathways). The only existing benchmark for CNTs relates to the inhalation pathway and is based on elemental carbon measurements using thermal-optical methods (e.g., NIOSH, 2013; Ono-Ogasawara and Myojo, 2011).

SECTION 1. SELECTING AN APPROPRIATE CNT TRACER

18. The first component of the strategy outlined in Figure 1 is the selection of CNT impurities which will prove useful as tracers in a given workplace environment. The concentrations of metal impurities that are present in the CNTs being handled must be known at the outset. Equally important is finding out whether there are any significant background sources of those metals in the workplace environment.

1.1. Background sources of metals

19. The term “background source” in the present context refers to all sources of the candidate tracer other than the impurities in the CNTs being produced or handled. Even if a metal impurity is present at high concentrations in a CNT powder, it will not be a useful CNT tracer if there are also high environmental concentrations of that metal from other (non-CNT) sources. The presence of interfering sources complicates the interpretation of analytical results, because the metal tracer does not uniquely identify the presence of CNTs. If background sources of the metal are not properly identified, the use of that metal as a tracer is likely to yield false positives and to misidentify CNT sources.

20. For example, Birch et al. (2011) found that it was not possible to use the Fe catalyst as an indicator of CNFs in a CNF manufacturing plant, because the Fe aerosol was dominated by hot Fe emissions from the furnace. In indoor workplace environments, microscopic particles of metals such as Fe, Al and Cu are commonly emitted from equipment such as engine parts, brakes and wheels, electrical wiring, and electronic components, especially when heated. Any significant background sources of a candidate tracer need to be identified. Metals that are commonly used as pigments, stabilizers, or drying agents (e.g., Pb, Zn, Cd) tend to accumulate to elevated concentrations in indoor dust (Rasmussen et al., 2013) and therefore may not be useful CNT tracers. Possible sources of these metals include plastic components, floors and floor coverings, ceiling tiles and wall surfaces. Metals may enter the indoor environment from outdoor sources, as components of outdoor air particles that penetrate the building through crevices or open windows. Soil or street dust particles containing metals may be tracked inside on footwear or machinery.

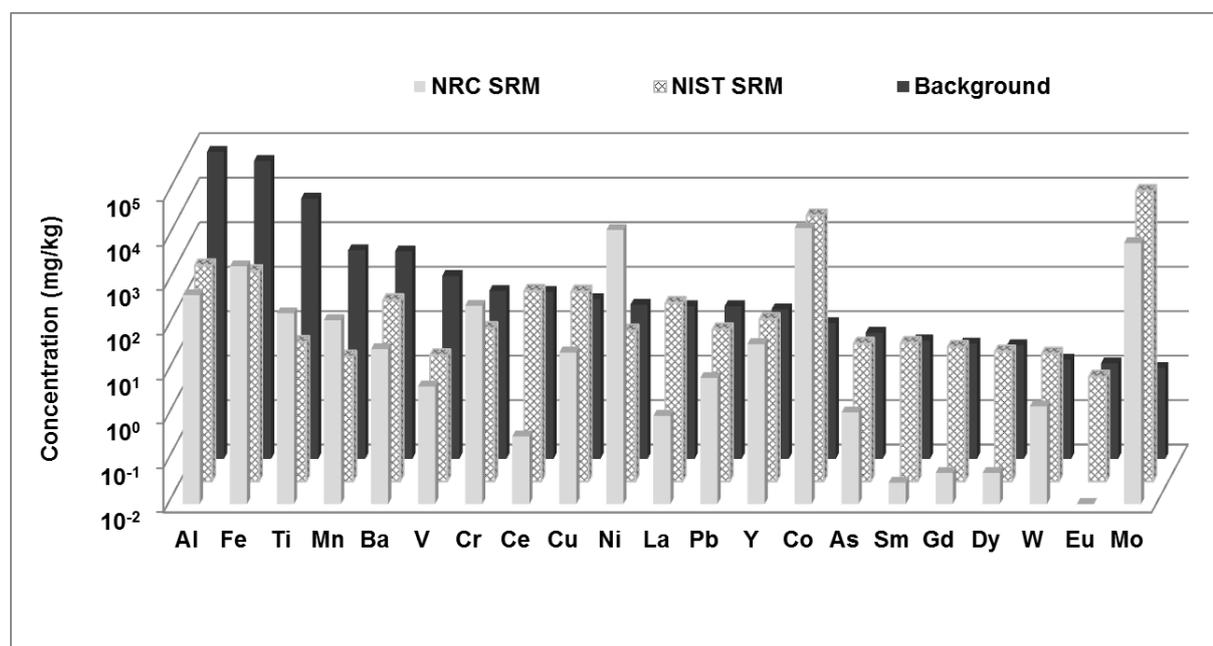
21. Approaches for assessing background sources are situation-specific. Characterizing background in a downstream user workplace (such as a clean research laboratory) may be quite simple compared to a large CNT manufacturing plant, where significant sources of non-CNT metal particles may be present. Section 2 discusses sampling approaches in different types of background situations, considering both CNT production sites and downstream user environments.

1.2. Which impurities are likely to be useful CNT tracers?

22. The most common catalyst elements are transition metals, such as Fe, Ni, Mo, Y, Co, Cu, and Cr (Bello et al., 2009; Ge et al., 2011) but many other elements may be present as trace impurities. Examples are shown in Figure 2 which compares elemental concentrations determined in two CNT standard reference materials (SRMs) with global average elemental concentrations in soils. Global soil averages (Kabata-Pendias, 2011) provide useful order-of-magnitude estimates of environmental background concentrations.

23. The impurities that are most likely to be useful tracers are those that exist as trace elements in the environment (low mg/kg range) but are elevated in the CNTs (1000 mg/kg and higher), such as Co, Ni and Mo in Figure 2. Each of the horizontal lines in Figure 2 represents an order-of-magnitude increase in concentration due to the log scale representation. Concentrations of Co (15,900 ppm), Mo (7,300 ppm) and Ni (14,400) in the National Research Council SRM (NRC SWCNT-1, Canada), and concentrations of Co (9,630 ppm) and Mo (34,060 ppm) in the National Institutes of Standards and Technology SRM (NIST SWCNT-2483, USA) exceed global background by two to three orders of magnitude (Figure 2).

Figure 2. Comparison of elemental concentrations of CNT standard reference materials (SRMs) with background elemental concentrations (represented by global soil averages). Histogram adapted from Rasmussen et al. (2015).



24. Figure 2 illustrates how potential CNT tracers may be identified, by comparing their CNT concentrations with those in background materials collected from the workplace (e.g. soil, indoor settled dust or aerosols). For the CNT reference materials in Figure 2, Co, Ni and Mo emerge as potentially useful tracers because their concentrations are 100s to 1000s of times higher in the CNTs than in the environmental background.

25. Note that the opposite is true for Fe and Al. Figure 2 shows that while Co, Mo and Ni are higher in the CNT SRMs than in soil, Fe and Al concentrations are much higher in soil than in CNTs. Fe and Al are considered “major elements”, not trace elements, because they are naturally present in percentage-level concentrations in the earth’s crust and surface environment (typically 3.5% for Fe in soil and 8% for Al in soil). In addition there are likely to be many anthropogenic sources of Fe and Al in aerosols, soil and dust in a workplace environment.

26. Thus, even if Fe and Al are enriched in CNTs, they are not likely to be the best CNTs tracers due to their prevalence in background particles. Residual catalyst concentrations of Ni, Co, Cu Mo, Mn and Cr, which typically occur in the 1000-5000 mg/kg range in commercially relevant MWCNTs and SWCNTs (Bello et al., 2009; Hseih et al., 2013; Bello personal communication 2015), are more likely to be useful tracers. Regardless of which CNT tracer is selected, if background sampling demonstrates no other

significant sources of the tracer in and around the workplace, then detection of elevated concentrations of that tracer will reliably identify CNT releases.

1.3. Stability and homogeneity of metals in CNTs

27. CNTs are extremely stable structures, containing metal nanoparticles that are sheathed by graphene sheets, and possible encapsulation of metals in defect structures (ISO 2011, Pumera 2011). Metals tend to be so firmly embedded in CNTs that residual catalysts persist even after lengthy acid purification treatments at high temperatures (ISO 2011, Pumera 2011). Provided that CNT reference materials are handled and stored appropriately (at room temperature in the original bottle and package, and protected from intense direct light or ultraviolet radiation), certified metal contents remain valid for many years within the specified measurement uncertainty. Monitoring of the stability of trace metal contents of NRC SWCNT-1 over an 18-month period indicated no discernible change in mass fraction for any certified element (NRC 2013). Uncertainty components for long- and short-term stability were therefore considered negligible and were not included in the uncertainty budget for trace metals (NRC 2013). Similarly, certified metal concentrations in NIST 2483 SWCNT reference material are valid for five years.

28. If there is reason to suspect that the metal content of CNTs may be altered due to the work process or due to environmental conditions, then the extent of the alteration should be ascertained by comparing samples of the released CNT with the original parent CNT material. For example, near-infrared fluorescence (NIFR) and ICP-MS have been used to investigate the long-term stability of CNTs under environmental conditions (Schriez et al., 2014). Using these techniques, metal catalysts in SWCNT-contaminated sediment in a wetland mesocosm were determined to be stable over a period of 10 months (Schriez et al., 2014). This study enhanced confidence in using metal catalysts to identify CNTs in contaminated sediments, where the metal content of the original CNT parent material is known (Schriez et al., 2014). Likewise, before applying the CNT tracer strategy in the workplace, the end-user should verify that the metal contents in the CNT product received from the manufacture are the relevant concentrations for detection in the workplace.

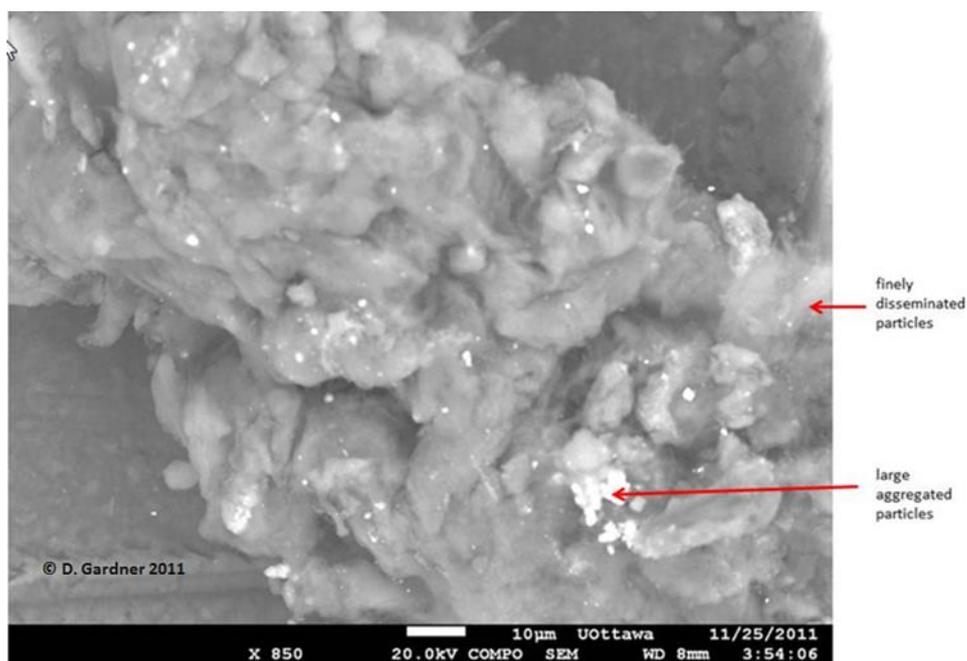
29. ISO Technical Specification 16195:2013 (ISO 2013) provides general guidelines on evaluating the stability and homogeneity of engineered nanomaterials that are used as reference materials. Certificates of analysis are a good source of CNT-specific information for reference materials, but workplace sample sizes are almost always smaller than the sample mass specified in certified uncertainty budgets, and quality control information may be absent for commercial CNT products. In such cases, the magnitude of heterogeneity should be estimated using the relative standard deviation of replicate subsample analyses. For example, ICP-MS analysis of 2 mg subsamples of NIST 2483 Single Wall CNT digested with blank wipes yielded relative standard deviations (%RSD) of 7-14% for Co and 5-11% for Mo (n=5 replicates; Avramescu et al., 2014). These RSDs were higher than the certified RSDs provided in the NIST 2483 certificate of analysis (2% for Co and 1% for Mo). The greater heterogeneity was attributed to the small sample mass used in the wipe study (2 mg compared with 25-40 mg recommended in the certificate of analysis) and to the uncertainty associated with the blank wipe correction (Avramescu et al., 2014). In the case of NRC-SWCNT-1, the certificate of analysis indicates that this reference material is homogeneous for certified trace metal contents at the level of 1 mg based on solid sample testing (NRC 2013). (If within-bottle and bottle-to-bottle contributions are included in the expanded uncertainty calculation, NRC-SWCNT-1 is considered homogeneous at a subsampling level of 25 mg.)

30. The above example shows that, as the CNT subsample mass decreases, the %RSD associated with metal analysis can be expected to increase due to the influence of heterogeneity. Microheterogeneity on smaller sample quantities is particularly problematic with microscopy measurement methods (NIST 2013). Figure 3 shows an example of spot analysis using SEM-EDX identify metals associated with individual CNT particles. In this image the Co catalyst appears as bright spots in particles of CNT standard

reference material produced by National Research Council in Canada (NRC SWCNT-1). SEM-EDX is useful for determining the identity of metals in CNT samples, but obtaining a quantitative measure of metal impurities using EDX is difficult due to the heterogeneous distribution of metals within and between CNT agglomerates.

Figure 3. Heterogeneous distribution of cobalt in carbon nanotubes.

Metal impurities (in this case Co and Ni and occasionally Fe, as identified by EDX) appear as bright spots. Photomicrograph of NRC SWCNT-1 standard reference material using SEM in backscatter mode, from Rasmussen et al (2015; Supporting Information).



31. To select a candidate tracer, knowledge of the metal impurities in the CNT product being handled should be obtained by submitting a minimum of 3 replicates of the powder to a commercial laboratory for quantitative multi-element analysis (usually by ICP-MS). The analytical laboratory likely will report the trace metal impurities in CNTs in units of micrograms of metal per gram of CNT powder ($\mu\text{g/g}$, ppm and mg/kg are synonymous). Metals present at higher concentrations may be reported as percentages. Heterogeneity should be estimated by calculating relative standard deviation (std dev/mean expressed as %). Instrumental neutron activation analysis (INAA) is widely used for characterization of metals in standard reference materials, but the relatively large sample mass required for INAA can be an obstacle to using this method. INAA typically requires 100s of milligrams of CNT powder, whereas ICP-MS analysis requires only a few milligrams of CNT powder. More information about multi-element analysis is provided in Section 3.

1.4. Metals in “purified” CNTs

32. CNTs are produced using various manufacturing processes which involve metal catalysts of varying types, concentrations and combinations. Removing the metal catalysts after production is a challenge: residual metals persist even after washing with nitric acid at elevated temperatures (ISO 2011; Pumera 2007). Other elemental impurities may enter the product during manufacturing (for example from the anode material) or even during purification (for example from metal components of the acid bath). A

survey by Ge et al. (2011) found that “purified” CNTs contained up to 3% residual metals, likely due to the difficulty of completely purifying CNTs using conventional acid reflux procedures. Furthermore, the term “purification” does not always refer to elimination of metals: in some contexts it may mean eliminating the amorphous carbon from the CNT product. Therefore even so-called “purified” CNTs are likely to contain a variety of metals at different concentrations, which may be useful as CNT indicators.

1.5. Changes in catalyst formulation

33. Downstream users who are applying the CNT tracer method are not likely to be informed of changes in catalyst formulation. For example the manufacturer may substitute a different catalyst metal, add another metal to the catalyst mixture, or change the concentration ratios of the metal catalyst mixture. Therefore it is prudent for downstream users to monitor the consistency of CNT metal concentrations from batch to batch. Where CNT products are purchased from a supplier, periodic follow-up analyses (after the initial analysis) will identify any changes in the metal catalyst, as the CNT supplier may obtain its CNTs from different manufacturers over time. The supplier may also combine CNTs from different manufacturers into one CNT product without informing the downstream user. Thus, if monitoring is to occur over or after an extended period of time, where there may be several lots/batches of CNT materials, then the lot-to-lot variability should be incorporated in the analysis and used to gauge the uncertainty of the CNT tracer methodology.

1.6. Summary of Section 1

34. Knowledge of the metal content of the CNT being handled can be obtained by submitting a few milligrams of the CNT powder to a commercial ICP-MS laboratory.

35. Residual catalyst concentrations of Ni, Co, Cu Mo, Mn and Cr typically occur in the 1000-5000 mg/kg range in commercially relevant CNTs, including both MWCNT and SWCNT. Because these metals are found in very low concentrations in environmental background samples they tend to be the most useful CNT tracers. In contrast, Fe and Al are not likely to be useful as CNT tracers due to their greater prevalence in environmental background samples.

36. Metal impurities introduced during CNT manufacturing processes are firmly embedded in the CNT structure, with the result that residual metals persist after acid washing at high temperatures. Therefore even so-called “purified” CNTs are likely to contain a variety of metals at different concentrations, which may be useful as CNT indicators.

37. Downstream users who are applying the CNT tracer method should monitor the metal content of the CNT powder being used, to track batch-to-batch or lot-to-lot consistency, and to identify possible changes in catalyst formulation. Possible changes during the work process should be investigated by comparing samples of the released CNT with the original parent CNT material.

38. As sample size decreases the variability of analytical results can be expected to increase due to heterogeneity of the CNT metal content (especially for samples below 1 mg). Heterogeneity should be calculated by submitting a minimum of 3 replicates and calculating relative standard deviation.

SECTION 2. CONSIDERATIONS IN SAMPLING FOR CNT TRACERS

39. The second component of the strategy outlined in Figure 1 is the selection of appropriate sampling approaches for monitoring CNT tracers in a workplace environment. OECD (2015) describes real-time measurement strategies suitable for a basic exposure or release assessment (at a Tier 2 level) using a limited set of easy-to-use, portable direct-reading instruments. A disadvantage of direct reading instruments currently used for monitoring CNTs in the workplace is that they do not have the capacity to distinguish among different sources of particles (Dahm et al., 2013; 2015). Research is underway to develop real-time methods for monitoring released CNTs using residual metal catalysts (e.g., R'mili et al., 2011; Neubauer et al., 2013). At the time of writing, however, there are no commercially available direct-reading instruments for metals that could be used for Tier 2 measurements.

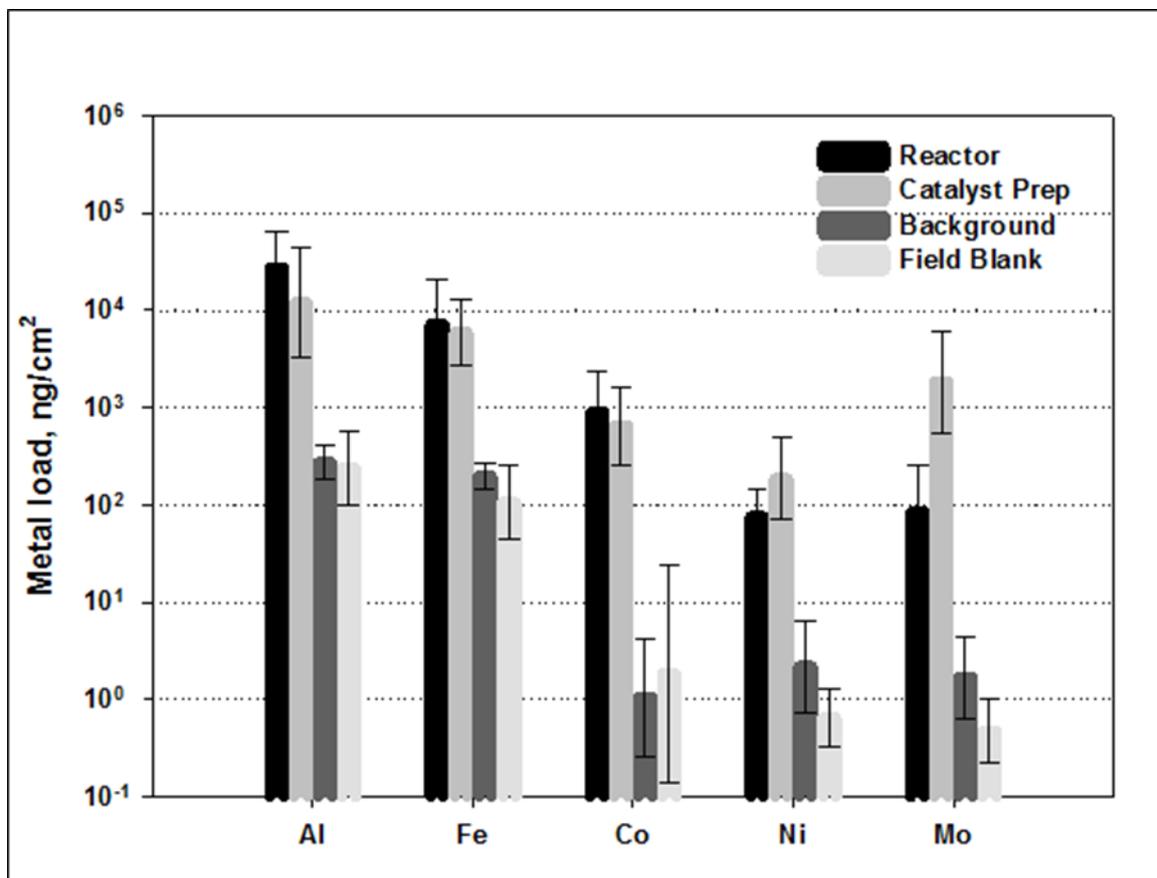
40. Several well-established protocols and approaches that are familiar to the occupational hygienist, which involve off-line analysis for metals, were previously recommended for workplace monitoring of engineered nanomaterials by OECD (2009) and would be suitable for Tier 2 assessments. These include surface sampling using wipes, and air sampling using simple single-filter cassettes. The studies listed in Table 1 involved efforts to modify both approaches (with varying degrees of success) for the purpose of measuring CNT tracers, as described in this section.

2.1. Surface wipes for area sampling

41. One of the key goals of surface sampling protocols is to determine the effectiveness of engineering controls and housekeeping actions (Ashley et al., 2011). Surface sampling is particularly relevant in nanotechnology workplaces, where hygienists can no longer rely on obvious visible dust sources to recognize the potential for exposures (Ramachandran et al., 2011). Re-suspension of carbon-based nanomaterials from workplace surfaces creates the potential for both secondary inhalation exposure and dermal exposure (Hedmer et al., 2015). NIOSH (2009) provides guidance on collecting surface wipe samples in nanotechnology applications, using a pre-moistened substrate such as Ghost Wipe™ towelettes.

42. Surface wipes were found to be useful for mapping levels of candidate elemental tracers in various locations in a CNT manufacturing facility. Preliminary metal concentration mapping (conducted during production downtime) indicated the presence of metal contamination in two work process sites (the catalyst preparation area and the vicinity of the reactor), which displayed significantly higher metal loadings than observed in the administrative area that was selected to represent “background” (Figure 4). The difference was most pronounced for Co, which was the catalyst element used in this facility. Only metals (and not CNTs) were analyzed in this preliminary mapping exercise, and a variety of floors and shelves were sampled in each location. This preliminary survey provided a forewarning that there were non-CNT sources of metals that needed to be considered, based on the observation of surface metal contamination in the catalyst preparation area (Figure 4).

Figure 4. Preliminary mapping of five metals in settled dust collected using wipe sampling of three areas of a CNT manufacturing plant: the vicinity of the reactor, the catalyst preparation area, and an administrative office (background site)



Error bars show standard deviation about the mean of five wipe samples and three field blanks. From Rasmussen et al. (2015).

43. A follow-up study was conducted later in the same CNT facility, when the reactor was in full production. Duplicate floor wipe samples were collected this time: one for ICP-MS and the other for thermal-optical carbon determination (Rasmussen et al., 2015). The collocated floor wipe samples were collected in the vicinity of the reactor, the packaging area, the catalyst preparation area, and an administrative office representing background (Table 2). Floor wipe samples showed a strong correlation between Co and CNT levels (Table 2). A similarly strong correlation was observed between Ni (a trace impurity) and CNTs in the same samples (Table 2).

44. The follow-up survey also indicated the presence of non-CNT sources of Co catalyst as well as non-CNT sources of elemental carbon in the facility. Table 2 shows Co loadings in the catalyst preparation area in the range of 39 to 141 ng/cm², whereas the CNT loadings were low (background-level) in this area. This finding confirmed the suggestion from the preliminary survey that there were non-CNT sources of Co contamination in the facility (namely Co reagents used to prepare the catalyst). However, Co floor loadings in the catalyst preparation area were 14 to 60 times lower than Co floor loadings in the vicinity of the reactor, reflecting the greater prevalence of CNT sources of Co in the reactor area during production (Table 2). Airborne elemental carbon measurements in the administrative background area were six-fold higher than the NIOSH limit (Rasmussen et al., 2015), suggesting the presence of non-CNT sources of

elemental carbon. Non-CNT sources of elemental carbon may explain the elevated CNT loadings in one of the background sites (Table 2).

Figure 5. Correlations between CNTs and metal impurities (Co and Ni) in settled dust.

Floor Loadings	CNT $\mu\text{g}/\text{cm}^2$	Cobalt ng/cm^2	Nickel ng/cm^2
Background area	3.2	1.0	1.9
Background area	<0.1	1.6	1.2
Catalyst prep area	0.2	141	3.0
Catalyst prep area	0.7	39.2	10.5
Packaging area	41.5	165	7.1
Packaging area	3.9	37.0	4.5
Reactor area	220	2254	115
Reactor area	170	2079	102
Correlation with CNT		$R^2 = 0.97$ ($p < .001$)	$R^2 = 0.97$ ($p < .001$)

Collected using wipe samples, from floors in four areas of a CNT manufacturing plant: an administrative office representing background, the catalyst preparation area, the packaging area, and the vicinity of the reactor. Co is a residual catalyst and Ni is a trace impurity in the CNTs. From Rasmussen et al., 2015.

45. In the above study, CNT and metal tracer analyses provided mutually supportive evidence that floor contamination in the vicinity of the reactor was 3-4 orders of magnitude higher than floor contamination in the background area, indicating the presence of CNT releases in the reactor area and the need for exposure mitigation measures. The study concluded that surface wipes could identify unintended releases of CNTs in the workplace using metal impurities, and that both Co (a residual catalyst in the CNTs) and Ni (a trace impurity in the CNTs) were informative environmental tracers (Rasmussen et al., 2015). However, it is important to note that a lack of correlation between CNTs and metals was reported for wipe samples from unused (inactive) shelves in the same four areas of the facility. This negative result indicated that inactive surfaces contaminated with particles of varying age should be considered separately from active surfaces loaded with fresh particles (floors in this case).

46. The advantage of sampling fresh (active) particles was demonstrated by measuring active deposition rates for Co and CNTs in the same four areas of the CNT facility where the wipe samples were collected (Rasmussen et al., 2015). To calculate deposition rates, passive accumulation samples were collected by placing Ghost Wipe™ towelettes on petri dishes and exposing them to the air for 40 hr. Deposition rates of the Co tracer did show a strong and significant correlation with those of CNTs (adjusted $R^2 = 0.97$; $p = 0.01$), with deposition rates for both substances increasing incrementally from background, to the catalyst preparation area, to the packaging area and finally to the reactor area where the highest deposition rates occurred (Rasmussen et al., 2015). These results supported the correlations found in the floor wipe samples (Table 2), and confirmed that surfaces exposed to active deposition of CNTs yield the strongest correlations between the catalyst metal and CNTs.

47. Wipe sampling is a relatively simple and accessible method which enables sampling a number of locations throughout the workplace, and allows a more complete characterization of spatial variations in background and work process sites. Surface wipe sampling is well-suited for concentration mapping, and the relatively low cost of wipe sampling is an advantage in major facilities where large numbers of samples are required to obtain spatially representative data. Concentration mapping is an approach recommended by Ramachandran et al. (2011) to investigate spatial and temporal variability and identify contaminant sources in nanotechnology workplaces, or as a pre-survey tool to determine optimal sampling locations for subsequent measurements.

48. It is important to stress that wipe sampling for CNT metal tracers is only a qualitative indicator of the presence of CNTs, not a quantitative exposure metric. Even in traditional applications, wipe sampling is considered a semi-quantitative method (Lichtenwalner, 1992), because the percent recovery varies with the surface characteristics of the area being sampled (e.g., rough or smooth), the sampling medium and solvent, and the technique used to collect the sample. Measurement of the dimensions of the area being sampled can be another source of uncertainty. The area dimensions must be recorded accurately because the mass amount of tracer measured by the laboratory is used to determine the mass per unit area for the wipe sample. Added to these sources of uncertainty is the tendency of settled CNT particles to stick to surfaces, and the heterogeneity of very small masses of CNTs as discussed in Section 1.3.

2.2. Workplace air sampling

49. The main challenge in using metal impurities as tracers of airborne CNTs is the difficulty in accurately measuring low airborne metal concentrations. Most standard air monitoring protocols used in occupational health and safety applications are designed for determining metals in the range of occupational exposure limits, typically micrograms/cubic meter. The studies in Table 1 indicate that airborne concentrations of CNT metal impurities are likely to be significantly lower, requiring the ability to reliably measure airborne metals in the nanogram/ cubic meter range. This is accomplished by substituting ICP-MS instead of ICP-AES as the detector, as indicated by later studies in Table 1, and by paying rigorous attention to quality control parameters which are discussed in Section 3.

50. Maynard et al. (2004) were the first to employ CNT tracers to estimate personal exposures to CNTs in manufacturing environments, by determining Fe and Ni impurities using ICP-AES on personal air sample filters. This early study was facilitated by the high content of Fe and Ni (30% combined mass) in the CNT product being handled at the time. Now, concentrations of residual metal catalysts in current CNT products are about one hundred times lower than in the early CNTs (typically <0.5% mass in the CNT), which calls for more sensitive detection methods such as ICP-MS.

51. Contributing to the analytical challenge is the very small particle mass collected on filter substrates using the low pump flow rates which are typically used in occupational monitoring protocols. Pump flow rates of 4 L/min used for 24 hr personal air monitoring protocols typically yields less than 100 micrograms of particle mass on the filter substrate (Rasmussen et al., 2007). Proportionately smaller sample sizes may be expected for shorter duration occupational air samples, which are usually collected to represent an 8 hr shift (or less, if monitoring a specific work process is required). As discussed in Section 1.3, the smaller the sample mass, the greater the impact of CNT heterogeneity, resulting in higher variability of analytical results.

52. Altering the sampling approach (e.g., using a higher pump flow rate) may be necessary to ensure that an adequate particle mass is collected. For example, in a CNT production facility (where airborne CNT concentrations exceeded the NIOSH $1 \mu\text{g}/\text{m}^3$ limit) samples collected using a low pump flow rate (2 L/min) and short sampling duration (4-6 hr) displayed a lack of correlation between airborne CNTs and airborne Co, which meant that airborne Co was incapable of uniquely identifying CNTs (Rasmussen et al.,

2015). However, in the same facility, passive accumulation sampling (using Ghostwipes™ laid flat on petri dishes) showed a strong and significant correlation between Co and CNTs (Adjusted $R^2 = 0.97$; $p=.01$). By sampling particles that had accumulated over an extended time period (>40 hr accumulation period compared to <8 hr duration for air filter sampling), the passive accumulation method yielded a sample mass that was adequate to utilize the metal catalyst as an index for CNTs, whereas air sampling was not. The success of the passive accumulation method was attributed to the reduced impact of fluctuating air concentrations and the larger amount of particles collected using the passive accumulation method (Rasmussen et al, 2015). The lack of correlation in the air samples was attributed to heterogeneity within the very small CNT sample masses obtained on the filter substrate, and interferences from non-CNT sources.

53. The importance of being able to monitor transient increases in airborne carbon nanomaterials in a production environment has been documented previously by Evans et al. (2010). As an alternative to filter-based air sampling methods, a wet electrostatic precipitation method has been investigated for its ability to capture transient increases in airborne CNT metal impurities. In a laboratory environment (Rasmussen et al., 2013), concentrations of airborne catalyst elements Y and Ni were monitored using wet electrostatic precipitation for four sequential 30 min sampling periods, while CNTs were released from a mechanical sieve. The method was able to detect transient increases in airborne Y and Ni concentrations as the sieve shaking rate was increased incrementally. Measurements taken before operations began indicated that background concentrations of Y and Ni were below the limit of detection (LOD). The ability of the method to capture short-term fluctuations in air concentrations is related to the high air flow rate (300 L/min; Jayawardene et al., 2014), and it is likely that use of an equally high flow rate with a filter-based method would also be capable of detecting these transient changes.

54. Based on toxicity studies indicating that carbon and metal particles together can produce greater health impacts than either particle type alone (Bello et al., 2009; Hsieh et al., 2013), measuring airborne metal impurities in addition to airborne CNTs can provide extra information for health risk assessments. For example if exposure samples are being collected for off-line analysis of elemental carbon, then collocated samples can be collected in duplicate for elemental analysis as suggested previously (OECD 2009; 2015).

2.3. Spatial versus temporal assessment of background

55. “Background” may be defined temporally or spatially, and the sampling approach depends on operation-specific circumstances. For example, the effectiveness of housekeeping measures may be monitored by surface wipe sampling before and after clean-up. Monitoring temporal variability allows a comparison of background emissions (i.e. before and/or after operations) with those at the peak of operations. Quantifying background levels before the initiation of production or usage of the CNT may be advantageous, if the opportunity exists. However, if production/handling is already underway, samples of background dust or aerosols may be collected from offices or meeting rooms that are remote from CNT sources in the same building. Sampling for spatial variability allows a comparison of emissions close to the work process site with those at a background site (i.e., a location far from the work process site). Settled dust provides a useful indicator of “background” because dust acts as a sink for metals, and integrates the wide variety of indoor and outdoor sources of metals.

56. Where knowledge of background concentrations is needed for selecting a candidate tracer (as described in Section 1), indoor dust is useful because enough particle mass can be collected to ensure that results for most elements are above ICP-MS limits of detection (LOD). In contrast, filter-based air sampling often yields background values below LOD due to the small particle mass typically collected on filters in background areas. Settled dust particles may be collected using a vacuum or brush and dustpan, and submitted for ICP-MS analysis along with the CNT material being handled. In one study (Niu et al., 2010), aerosol particulate matter was collected from the building intake filtration system and analysed by ICP-MS to obtain workplace background concentration values in ppm.

2.4. Summary of Section 2

57. The selection of sampling approaches is situation-specific. Most studies seek adaptations of well-established protocols and approaches that are familiar to the occupational hygienist and fall within typical OH&S budgets. A minimum of two sampling locations is needed to compare the work process site with a background site (i.e., a location remote from the work process site). Mapping spatial variations at a number of sites within the workplace provides more detailed and representative information. Another approach is to monitor temporal variations by comparing samples collected before the work process begins with samples collected during the peak of the work process, or to compare surface contamination before and after clean-up.

58. Results of surface sampling in a CNT facility showed that the CNT tracer strategy worked best on active surfaces loaded with fresh particles (namely wipe sampling of CNT loading on floors, and Ghostwipes™ laid out on petri dishes to measure passive CNT accumulation on surfaces). Results of floor wipe samples were consistent with those of passive accumulation samples, in that the strongest correlations between the catalyst metal and CNTs were observed in areas of active deposition of CNTs. No correlations were found in samples collected from unused (inactive) surfaces where contamination accumulated over variable amounts of time. Preliminary mapping using wipe samples was useful to identify surfaces that were contaminated with non-CNT sources of metals, such as metal reagents used in catalyst preparation.

59. Wipe sampling for CNT metal tracers is considered a qualitative indicator of the presence of CNTs, not a quantitative exposure metric. As described by the OECD Tiered Approach (OECD, 2015), such qualitative assessments are useful for comparing particle concentrations at the emission source with background particle concentrations. Results of surface wipe sampling may be used qualitatively to identify violations of housekeeping standards and requirements for cleanliness of personal protective equipment (PPE). Qualitative assessments also help to determine whether existing measures are adequate for controlling CNT emissions or if additional controls are needed.

60. Measurement of airborne CNT metal tracers requires analytical methods with low detection limits capable of reliably quantifying nanogram/cubic metre air concentrations. The sampling method must yield adequate sample mass to overcome uncertainty associated with CNT heterogeneity. It may be necessary to use higher air flow rates to allow short duration air sampling, for example to capture emissions from a given work process or to capture transient changes in airborne CNT concentrations. Measurement of airborne metal impurities in addition to airborne CNTs can provide extra information for health risk assessments. For example if exposure samples are being collected for off-line analysis of elemental carbon, then collocated samples can be collected in duplicate for elemental analysis as suggested previously (OECD 2015).

61. Settled dust in a facility provides a useful indicator of “background” because dust acts as a sink for metals, and integrates the wide variety of indoor and outdoor sources of metals. Settled dust particles may be collected using a vacuum or brush and dustpan, and submitted for ICP-MS analysis along with the CNT material being handled.

SECTION 3. ANALYSIS OF PARTICLE SAMPLES FOR CNT TRACERS

62. The third component of the strategy outlined in Figure 1 is the analysis of the collected particle sample (settled dust or aerosol) to identify the presence of the CNT tracer. The most commonly used analytical methods for determining metals in CNTs are: Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Instrumental Neutron Activation Analysis (INAA), prompt-gamma neutron activation analysis (PGAA), Direct Solid Sampling Electrothermal Atomic Absorption Spectrometry, and Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). A variety of sample pretreatments have been employed for determination of metals in CNT samples, e.g., hot block acid digestion, microwave-assisted acid digestion, dry ashing combined with acid digestion, Carius tube digestion, microwave-induced combustion, and ultrasonic digestion. Proton-induced x-ray emission (PIXE) and X-ray fluorescence (XRF) have been used for determination of trace metals on wipe and filter sample substrates, and could be applied to CNT metal tracers on wipes and filters if detection limits prove adequate. This section discusses advantages and challenges associated with using ICP-MS (section 3.1), issues related to Quality Control (section 3.2), and the interpretation of elemental ratios and isotopic ratios from published studies listed in Table 1 (section 3.3).

3.1. Advantages and challenges of ICP-MS

63. As discussed earlier, substitution of ICP-MS for ICP-AES in standard air monitoring protocols provides the greater sensitivity required to monitor trace metal impurities at the concentrations found in current commercially available CNTs. Greater sensitivity also means a *wider range of elements* can be determined, which allows interpretations using elemental ratio techniques as discussed in Section 3.2.

64. A second advantage is that ICP-MS has become commonplace in fee-for-service laboratories all over the world. Many commercial laboratories are equipped with both types of ICP instruments, and use ICP-MS for samples containing lower metal concentrations and ICP-AES for samples containing higher metal concentrations.

65. A third advantage is the availability of published ICP-MS methods that have proven useful for the types of samples analyzed in the studies listed in Table 1 (various air samples and surface wipe samples). While multi-element instruments for determining trace elements in solid samples (such as instrumental neutron activation analysis, INAA) are excellent for determining metal impurities in CNTs, as indicated in Section 1, they have not (at the time of writing) been applied to filter and wipe samples in nanotechnology occupational health and safety applications.

66. The main challenge associated with using sensitive instrumentation such as ICP-MS is the greater care that is required to avoid contaminating the sample. Another challenge associated with both ICP-MS and ICP-AES, compared to INAA, is the need for an acid digestion step to extract the metal impurities from the CNTs and put them into solution. As metals tend to be firmly bound in the CNT matrix, it can be difficult to obtain 100% recovery. Both challenges are experienced commonly with environmental sampling for metals, and are discussed further in the next section, "Quality Control."

3.2. Quality Control

67. A semi-quantitative analysis may be appropriate in the case of a Tier 2 assessment, for example to identify presence/absence of the CNT tracer to evaluate the effectiveness of housekeeping. Depending on the circumstances, a fully quantitative determination of metal concentrations in CNT samples may be required. In either case, use of ICP-MS requires careful attention to quality control during sampling and analysis to ensure the reliability of analytical results. The first goal of the quality control program is to

guard against sample contamination throughout the entire chain of custody (Section 3.2.1). The second goal is to be able quantify the amount of contamination that was introduced despite all the precautions that were taken Sections 3.2.2 and 3.2.3). The final goal is to monitor the extraction efficiency (Section 3.2.4).

3.2.1 Steps to Guard against Sample Contamination and Losses

68. Gloves should always be worn for personal protection when handling CNTs, but when sampling for metals it is important to wear powder-free gloves to guard against contaminating CNT samples with trace metals. Adequate supplies of powder-free sampling gloves should be made available to enable personnel to adhere to a protocol of frequent glove changes during sampling. The gloves should be changed at least in every new sampling location (if not after every sample). Vinyl or nitrile gloves are alternatives for personnel who are allergic to latex.

69. Samples should be packaged for shipping using self-sealing /zipper-locking plastic bags. Double or triple bagging the samples before shipment is recommended to guard against cross-contamination which can occur when a high concentration sample lies next to a low concentration sample. Additional measures must be taken to avoid potential losses of sampled CNTs during handling, shipping and analysis. Having the trace metal laboratory provide the appropriate metal-free digestion vessels, each packaged inside a self-sealing plastic bag, at the outset of field sampling is the most effective means of protecting the sample. If the strategy involves shipping aqueous samples, acidification of the sample at the field site will be required to avoid loss of metal ions to vessel surfaces. If the samples being shipped consist of solid particulate matter, losses may occur if particles fall off the sampling medium (for example, the wipe or the filter). For this reason wipe sampling protocols typically prescribe a method of folding the wipe to protect the particles by keeping them on inside surfaces. Whether it is possible to fold the sampling medium or not, the practice of packaging the wipe or air filter sample directly inside the metal-free digestion tube is an effective safeguard against losses and contamination.

70. The above steps are critical to minimize losses and contamination, but even with such safeguards it is an unfortunate reality that small amounts of metals will be accidentally introduced from unexpected and unwanted sources during the sampling process. Therefore field blanks must be collected to be able to quantify metal contamination that was inadvertently introduced during sampling.

3.2.2 The importance of collecting field blanks

71. Field blanks are collected by subjecting the sampling medium (e.g. the filter or wipe) to precisely the same handling procedures that are applied to the real sample, in all the same locations as the real sample, but omitting the sample collection step. For example, if filter-based air samples are collected (using standard filter cassettes), the field blank filter is subjected to all the same procedures and environments as the sample filter (minus attaching the field blank cassette to the pump) such as conditioning, loading the cassette, shipping, exposing the field blank cassette to the sampling environment throughout the duration of sampling, and unloading the filter from the cassette for acid digestion and analysis by ICP-MS. Quality control programs typically indicate that field blanks should account for 10% of the total number of samples in order to estimate uncertainty (e.g. IRRST, 2013). In a small study, a minimum of three field blanks is required to calculate mean and standard deviation.

72. Figure 4 (in Section 2) shows how field blanks provide a frame of reference for the interpretation of results from a wipe sampling survey. This histogram shows average values for five metals determined in wipe samples from three areas in a CNT manufacturing plant: in the vicinity of the furnace where CNTs are produced, the catalyst preparation area, and an administrative room selected as the background site. One field blank was collected in each of the three sampling locations, and the average of the three field blank values is shown for each metal (Figure 4). Field blanks were subjected to precisely the same

treatment and handling as the real samples throughout all steps of packaging, shipping, sample preparation, digestion and ICP-MS analysis (Rasmussen et al., 2015).

73. Results for the two work process sites in Figure 4 can be reported with confidence because they are significantly higher (by more than an order of magnitude) than the field blanks and the background samples for each of the five metals. Note, however, that the average metal content of the field blanks was approximately equal to the average background for each of the five metals shown (Figure 4). This means that “background” for the five metals in Figure 4 cannot be distinguished from the contamination of the field blanks. This example shows the importance of monitoring field blanks; i.e., any interpretation of background data trends would be futile because such trends are within the same range as the field blanks.

3.2.3 Media blanks and limits of detection (LOD)

74. Media blanks are new filters or wipes taken directly from the packaging (unlike field blanks which are taken to the sampling site) and submitted to the laboratory along with field blanks and samples. Media blanks quantify trace metal contamination from two sources: the laboratory conducting the analyses, and the background trace metal contamination introduced into the sampling medium by the manufacturer. USEPA (1999) calls for analysis of one blank filter every ten filter samples to enable the calculation of an average media blank correction. Analytical results for aerosol samples and field blanks are corrected by subtracting the media blank. The calculation of the limit of detection (LOD) for airborne metals is based on the variability of measurement results obtained with blank filters (typically three times their standard deviation; EN 14902:2005).

75. Therefore, attention to both the mean and standard deviation of media blanks is extremely important to ensure that only reliable trace metal data are reported. Media blanks for a given element may be so elevated that subtraction from the sample yields a negative value, and highly variable media blanks negatively impact the signal-to-noise ratio. In either case, the occurrence of the element in air or surface wipe samples may not be reportable due to unacceptable uncertainty. This point is illustrated by the example of the CNT manufacturing facility provided in Figure 4, in which surface loadings for Al, Fe, Mo, Ni and Co were above LOD. Air concentrations in the same facility were below LOD for two of those five elements - Mo and Al. Mo was not detectable due to low concentrations in the air (only trace Mo was present in the CNTs). The reason was different for Al: the media blanks contained such elevated and highly variable Al concentrations, that the airborne Al signal was indistinguishable from the “noise” of the media blanks. Thus, airborne Al was reported as below LOD (due to the signal-to-noise ratio), despite higher surface loadings for Al compared to any other metal in Figure 4.

76. The amount of trace metal contamination found in media blanks is element-dependent and varies with different sampling media. Thus, attention to media blanks is also important to ensure the appropriate sampling medium is selected for the metals being studied, to ensure the highest possible signal-to-noise ratio is achieved. Ashley et al. (2010) reported significantly elevated media blanks for Al, As, Ag, Cr, Cr, Fe, Mg, Mn, Ni, Se and Zn in mixed-cellulose ester (MCE) filters. Rasmussen et al. (2015) reported significantly elevated media blanks for Al, Ba, B, Fe and Zn in quartz fiber filters. Avramescu et al. (2015) and McDonald et al. (2011) reported significantly elevated media blanks for Al, B, Cu, Fe, Mn, V, and Zn in Ghost Wipes (polyvinyl chloride polymer). Rasmussen et al. (2007) reported elevated media blanks for Al, Cr, Mn and Zn in PTFE (Teflon) filters.

77. Attention to potential variability in media blanks is also important for elemental carbon determination. Doudrick et al. (2012) describe some modifications to thermal-optical carbon methods that may be required for determining CNTs in different environmental matrices. Thus, where it is necessary to use sampling media other than quartz fiber filters (e.g. PTFE, MCE, or polyvinyl chloride polymer) it may be necessary to conduct experimental work to optimize the elemental carbon analyses.

3.2.4 Monitoring extraction efficiency

78. For CNT metal impurity determinations using either ICP-MS or ICP-AES, samples are pre-treated using an acid digestion procedure to extract the metals into solution, so that the digestion solution can then be aspirated into the inductively-coupled plasma. Achieving quantitative extraction of metals (i.e. where 100% of the metal is put into solution) is a common challenge for all environmental samples, and studies show that widely accepted industry standards for air quality monitoring using ICP typically yield incomplete recoveries (Hassan et al., 2007). The same applies to CNT samples - metal impurities tend to be firmly embedded in the CNT structure and therefore lengthy multi-step digestion protocols may be required to ensure the quantitative extraction of metal impurities from CNTs (ISO 2011; Avramescu et al., 2015; Grinberg et al., 2015).

79. Extraction efficiency is a parameter that can be evaluated using certified reference materials for CNTs, unlike other sources of laboratory and sampling uncertainty which are not as easily constrained (e.g. weighing errors caused by static electricity, spatial and temporal variability). To assess the efficiency of the extraction, CNT standard reference materials (such as NIST 2483 and NRC SWCNT-1 in Figure 2) can be submitted to the laboratory along with the CNT powder and collected samples. The observed values (reported for the reference materials by the laboratory) can then be compared with the certified values (reported on the Certificates of Analysis provided with purchase of standard reference materials) in order to calculate percent recovery.

80. For surface sampling in a CNT manufacturing plant using wipes, Rasmussen et al. (2015; Supporting Information) reported that a short ultrasonic nitric-HF extraction (Niu et al., 2013) performed as well as a lengthy multi-step hot-block microwave extraction (Avramescu et al., 2015). The existence of short practical digestion protocols, which can provide acceptable, reproducible results, facilitates the use of ICP-MS for routine identification of workplace releases of CNTs and evaluating the efficiency of control measures.

3.2.5 Stability of Tracer Concentration

81. The stability of the elemental concentrations and elemental ratios in the CNTs is a prerequisite for the CNT tracer method. Therefore, the tracer concentration must be checked for stability if the CNTs are further processed, transported or exposed to aging processes. An example is the work of Schriez et al. (2014) who investigated the long-term stability of CNTs under environmental conditions, as discussed in Section 1.3.

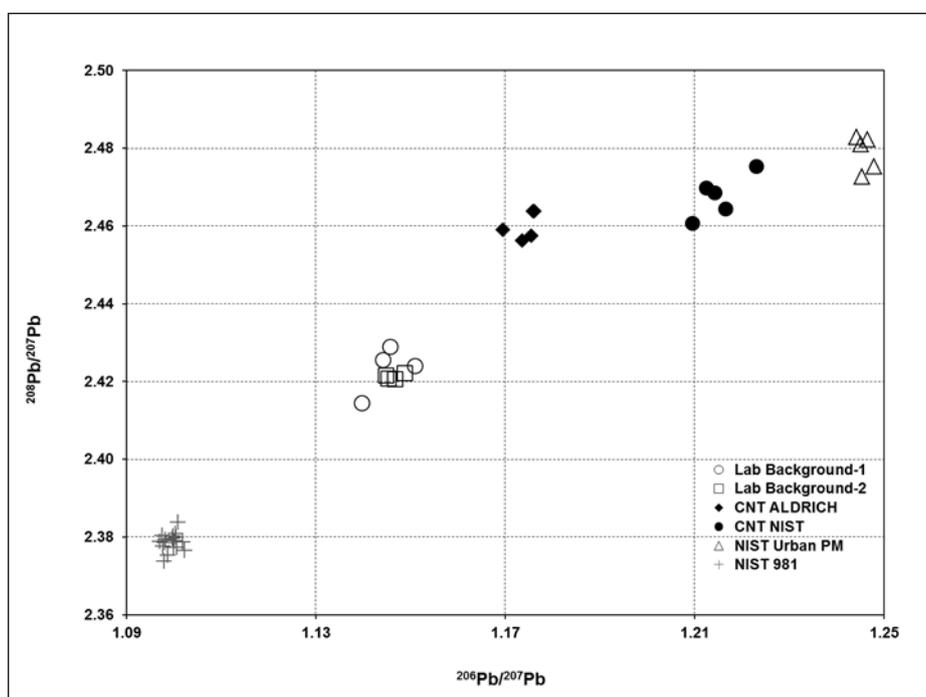
3.3 Elemental and isotopic ratios as aids to interpretation

82. A number of studies in Table 1 made use of two or more metal impurities in CNTs to assist in the interpretation through elemental ratio techniques. Maynard et al. (2004) analyzed samples of workers' gloves for Fe and Ni as surrogates for total CNT mass, and used Fe/Ni elemental ratios to estimate CNT loading on the gloves (between 0.2 and 6 mg per hand). To distinguish aerosolized CNTs from background aerosols in a research laboratory environment, Rasmussen et al. 2013 reported that elemental ratios in the CNTs, such as Y/Al (52:1), Y/Fe (54:1), Ni/Al (270:1) and Ni/Fe (279:1), provided unambiguous discrimination from the ambient air particles (in which all these ratios were <0.01). Co/Mo elemental ratios were used by Schriez et al. (2014) to confirm long-term stability of CNTs in sediment cores 10 months after a simulated spill of CoMoCat SWCNT into an outdoor wetland mesocosm. Estimates of the CNT concentrations in the sediment cores using Mo as a surrogate for CNT agreed well with estimates using near-infrared fluorescence spectroscopy (Schriez et al., 2014). Rasmussen et al. (2015) used Co/Fe elemental ratios in surface wipe samples to distinguish CNT from non-CNT sources of Co in a CNT manufacturing plant. Plotting Co/Fe against Co identified contaminated areas where

workplace industrial hygiene practices needed to be improved, specifically the shelves in the catalyst preparation area and the floor in the vicinity of the reactor (Rasmussen et al., 2015).

83. The discovery that different CNTs each have distinctive stable Pb isotope signatures (Figure 5), opens up the possibility of using Pb isotopic ratios such as $^{208}\text{Pb}/^{207}\text{Pb}$ and $^{206}\text{Pb}/^{207}\text{Pb}$ as unique tracers of CNTs released into the environment or into the workplace. Pb impurities are unintentionally introduced into CNTs during production, from exposure to contaminated airborne dust or from contact with metal alloys (such as solder) during processing and handling. For this reason, Pb isotopes are not likely to be useful for identifying CNT releases within a CNT manufacturing environment, because the CNT signature is likely to be the same as the local indoor background signature (Rasmussen et al., 2015). In downstream user environments and in outdoor environments, the unique Pb isotopic signatures of CNTs will be a useful aid in distinguishing CNTs from background particles, as demonstrated in Figure 5 for a laboratory setting.

Figure 6. Stable Pb isotope signatures of two aerosolized CNT materials (NIST SWCNT 2483 and Sigma-Aldrich SWCNT)



In a downstream user environment (research laboratory) are distinct from those of indoor background aerosols, and are also distinct from NIST 1648a Urban Particulate Matter. Aerosols were sampled using wet electrostatic precipitation; NIST 981 is a SRM for ICP-MS quality control. From Rasmussen et al. (2015).

3.4 Summary of Section 3

84. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) has become the most commonly used method for determining metals in collected particle samples (settled dust or aerosol) to identify the presence of CNT tracers. This is primarily because it is more sensitive than ICP-AES, and also because it is more widely available than INAA. A third advantage is the availability of published ICP-MS methods for the various air samples and surface wipe samples that have proven useful for CNT metal tracers.

85. The main challenge associated with using sensitive instrumentation such as ICP-MS is the greater care that is required to avoid contaminating the sample. Field blanks must be collected in order to quantify metal contamination that was inadvertently introduced during sampling. Quantification of metal contamination (mean and standard deviation) found in media blanks is important to ensure the appropriate sampling medium is selected for the metals being studied, to calculate limits of detection, and to make appropriate blank corrections.

86. Metal impurities tend to be firmly embedded in the CNT structure and therefore lengthy multi-step digestion protocols may be required to ensure the quantitative extraction of metal impurities from CNTs. If shorter extraction techniques are preferred, extraction efficiency should be evaluated using certified reference materials for CNTs. Modifications to existing thermal -optical carbon methods will be required to optimize the elemental carbon analyses for sampling media other than quartz fiber filters (e.g. PTFE, MCE, or polyvinyl chloride polymer).

87. Monitoring two or more metal impurities in CNTs permits the use of elemental ratio techniques to assist in distinguishing CNTs from non-CNT sources. Examples from the literature include Fe/Ni and Y/Al ratios. There is published evidence that CNTs are characterized by distinct stable Pb isotopic ratios. This opens up the possibility of using Pb isotopes as unique tracers of CNTs released into the environment or into the workplace.

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