Methods for the Measurement of Release of MWCNTs from MWCNT-Polymer Composites

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Summary

Multi-wall carbon nanotube (MWCNT)/polymer composites hold great promise as advanced materials for consumer and industrial applications. Throughout the life cycle of these composites, there is opportunity for release of MWCNTs or particles that contain them. A full understanding of the potential for exposure from release events requires validated sample collection, preparation, and analysis techniques. The current state-of-the-science on particle analysis is limited to adaptation of techniques that were originally developed for studies of micrometer-scale particles and none have been validated for MWCNT material. The most commonly utilized sample collection techniques involve capture of airborne particles on filters or other substrates such as transmission electron microscopy (TEM) grids. The exact sample preparation method is dependent upon the chosen analytical technique, though it is critical that any manipulation preserves the native properties of the MWCNTs as they were in the media to which they were released. Sample analysis is a two-step process. The first step involves detection – a qualitative method to answer (yes or no) whether MWCNTs are released from MWCNT-polymer composites. The second step involves quantification, which is an enumeration of the number or mass concentration of MWCNTs in or released from MWCNT-polymer composites per unit volume or area examined. Quantification of released MWCNTs, particularly those embedded in polymer fragments, is challenging. Available methods that can yield a qualitative measure of concentration are limited to electron microscopy. Quantitative electron microscopy is tedious and expensive and suffers from various limitations. Going forward, several critical research gaps need to be filled to enable accurate measurement of MWCNTs released from polymer composites. They include improved (or new) measurement methods, inter-laboratory studies of release scenarios, development of test and collection protocols, and standardization of methods.

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Introduction

Science-based risk assessment and management of engineered nanomaterial (ENM) release from consumer products is predicated on the availability of accurate and precise data on release mechanisms, physicochemical and human- and eco-toxicological properties of released ENMs, and exposure to released ENMs. In Section 1 of this monograph, the properties of multi-wall carbon nanotube (MWCNT)-polymer composites that may influence release due to various effects (e.g., mechanical, physical, chemical) were reviewed. This information is summarized by Kingston et al. (2013). In Section 2 of this monograph, possible scenarios under which such composites in the form of consumer products might be used and release materials to the environment were described. This information is summarized by Nowack et al. (2013). In the current section of this monograph, we review the available methods for detection and characterization of MWCNT released from composites into various media (e.g., air, solids, water and biological fluids) by different mechanisms linked to effects as a result of use.

MWCNTs consist of multiple concentric tubes of graphene. The nature of the atomic arrangements and bonding in CNTs provide these materials with exceptional mechanical properties (tensile strength, elastic modulus, and hardness), very good thermal conductivity, and structure-dependent electrical properties (metallic to semiconducting). These properties, along with low density and large aspect ratios, make CNTs attractive for myriad applications. Unlike single-wall CNTs, MWCNTs are produced commercially in large volume (more than 3 kilo-tons in 2012 [Future 2011]) and are widely used in products. Many processes for MWCNT synthesis require the use of metal catalysts, most commonly iron, nickel, and cobalt, to initiate tube growth. The presence of residual catalytic particles in MWCNTs can alter MWCNT properties; however, methods have been developed to purify MWCNTs and several companies can produce sufficient quantities of purified material for commercial use. Other forms of carbon, such as horns, cups, and amorphous material are typically

present in MWCNT material, though for our purposes only detection and characterization of MWCNT is considered herein.

Products that contain MWCNTs are manufactured from composite materials wherein MWNCTs are incorporated in a polymer matrix. The review in Section 1 of this monograph (see also Kingston et al., 2013) identified five polymeric materials for such composites that are commercially relevant and representative of a range of polymer characteristics: epoxy (EP), polyamide (PA), polyurethane (PU), polyethylene (PE) and polycarbonate (PC). Polymers used in products are typically formulations of the polymer and additives designed to modify the physico-chemical characteristics of the polymer, particularly its mechanical properties (strength, toughness, and wear resistance), chemical, physical, and durability. In Section 2 of this monograph, an evaluation of potential scenarios for release of MWCNTs from MWCNT-polymer composites was presented for nine different release scenarios (see also Nowack et al., 2013). Release scenario and release mechanism are intimately related, and determine the form of released material. Hence, methods for sampling released material for subsequent measurement are dependent on these considerations.

Forms of released material

This paper considers two general release scenarios for the polymer/use combinations outlined in Sections 1 and 2 of this monograph:

(1) The polymer is not degraded (*i.e.*, molecular structure unaltered) and the MWCNTs are embedded in the matrix. Material is released in the form of polymer fragments that may or may not contain MWCNTs, and possibly in the form of individual or agglomerated MWCNTs. Here only fragments are considered, as published studies report a preponderance of fragments in material released by this scenario.

(2) The polymer is chemically degraded, by a number of mechanisms, including chain scission, crosslinking, and oxidation, or their combination. As a result, polymer-free (unbound), partially-covered by the polymer, or polymer-embedded MWCNTs are released into the surrounding medium, or form a tangled network at the surface of the composite where the polymer has degraded away.

In scenario (1), for released fragments that contain MWCNTs, the MWCNTs may be physically attached to the surface of a polymer fragment (by weak van der Waals forces), protrude from the surface of the polymer fragment, or be fully embedded in a polymer fragment. A combination of all types of MWCNT-polymer associations is also possible (see Figure 1 below). For simplicity, loosely adhered MWCNTs will no longer be considered in this paper. Mechanical driving forces (*e.g.*, abrasion) are the most likely cause of release for this scenario. Note that this scenario has also been described as release by "high energy processes." For scenario (2), there is potential release of MWCNTs from the tangled networks that are revealed on polymer surfaces by subsequent agitation, wear, or fluid flow. Chemical, optical, biological, and thermal driving forces could cause release for this scenario. Note that this scenario has also been described as release by "low energy processes."

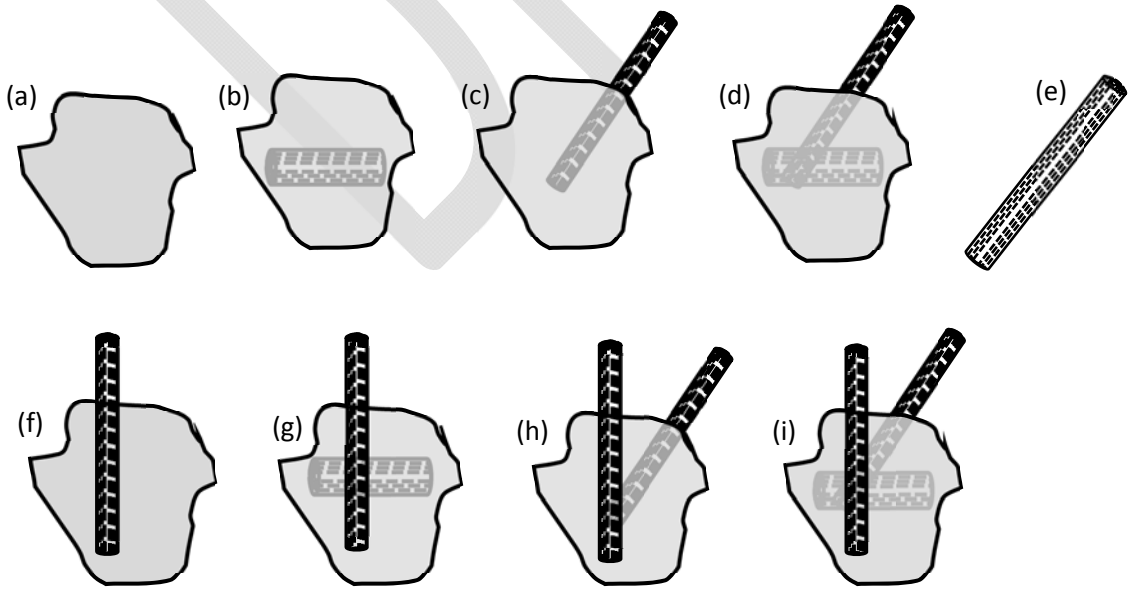

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Figure 1. Various forms of material from a scenario (1) release event: polymer fragments containing: (a) no MWCNTs; MWCNT bonded to the polymer matrix: (b) fully encased or (c) protruding from the polymer surface; (e) idealized unbound MWCNT; (f) MWCNT loosely adhered to the composite surface; (d), (g), (h), and (i): combinations of (b), (c), and (f). For simplicity, MWCNTs are portrayed as straight tubes, rather than as curved, twisted, and intertwined tubes typically observed in actual MWCNT-polymer composites.

Methods for Sampling Released Material

Any combination and distribution of free polymer fragments, free MWCNTs, and polymer fragments with MWCNTs (see Figure 1) may be released into air, water, solids (*e.g.*, soils), and biological fluids throughout the lifecycle of an article. Hence, the choice of method(s) for sampling released materials may differ depending upon the media of interest (*e.g.*, air versus water). Note that the choice of sampling method is not independent of an overall measurement strategy. Rather, the choice of sampling method, subsequent preparation (if any), and analysis are inter-dependent and all these measurements steps are critical for obtaining meaningful data on material release. To date, most efforts to evaluate MWCNT release from composites have focused on release to air. This emphasis on airborne release is, in part, because of interest in protecting workers who are often at risk of exposure early in a nanomaterial's lifecycle (*e.g.*, during masterbatch production). Attention is also given to potential for ecological consequences (release into water, soils, and biological tissues) at the end of an article's lifecycle.

Sampling material released into air

Generally, methods for sampling aerosols can be divided into two categories, real-time instruments and time-integrated samplers. Real-time instruments have the advantage of providing instantaneous measures of particle characteristics such as number, mass, size, or surface area concentration in air. However, all existing real-time instruments are non-specific and cannot differentiate an ENM of interest from an incidental aerosol particle (*e.g.*, diesel soot) with nanoscale size. Time-integrated samplers involve collection of aerosol particles onto a substrate for subsequent off-line analysis. These

sampling methods offer the advantage of permitting identification of an ENM of interest by off-line analysis, but often require additional preparation steps prior to analysis. In this paper, only time-integrated approaches to sample collection are discussed as they can be used to specifically identify MWCNT released from composites.

Numerous time-integrated samplers are available for aerosol collection and some are capable of separating particles by size (aerodynamic, thermodynamic, etc.) while others are not. In general, time-integrated samplers collect particles with sizes well above the nanoscale though it is widely recognized that many ENMs form micrometer-scale agglomerates in air or may be adhered to, or contained in, larger particles that become airborne (*e.g.*, polymer fragments) making these methods appropriate for exposure assessment. Size-selective aerosol samplers include dichotomous and multi-stage samplers. Dichotomous samplers such as the respirable cyclone have traditionally been used for occupational exposure assessment because they separate particles into two fractions, respirable (aerodynamic diameter less than 4 μm) and non-respirable. In these samplers, the respirable fraction is deposited onto a filter for subsequent off-line analysis. The respirable aerosol fraction has size small enough to permit penetration and deposition into the alveolar (gas exchange) region of the lung is of interest for chronic lung disease and is often used to sample for airborne ENMs (Bello et al., 2008, 2009; Pfefferkorn et al., 2010; Huang et al. 2010; Birch et al., 2011; Dahm et al., 2012, Curwin and Burtke, 2011). Analogous samplers developed for environmental monitoring of ambient particulate matter (PM) include PM_{10}, $PM_{2.5}$, and PM_1 samplers which collect particles with aerodynamic diameters of less than 10 μm, 2.5 μm, and 1 μm, respectively (Kuhlbusch et al., 2004; Pfefferkorn et al., 2010). Multi-stage aerosol samplers are capable of separating particles into multiple size fractions and include low-pressure impactors such as the Berner sampler (8 size fractions from 16000 nm to 60 nm) and electrical low-pressure impactor (12 size fractions from 6800 nm to 30 nm) (Brouwer et al., 2004;

Golanski et al., 2011; Golanski et al., 2012); wide-range aerosol sampling system (12 size fractions from 20000 nm to 2 nm) (Bello et al., 2010; Pfefferkorn et al., 2010); micro-orifice uniform deposit impactor (10 size fractions from >18000 nm to 56 nm) (Huang et al., 2010; Curwin and Burtke, 2011; Birch et al., 2011; Raynor et al., 2012); and Sioutas cascade impactor (5 size fractions from 10000 nm to 250 nm) (Birch et al., 2011; Ono-Ogasawara et al., 2013).

Time-integrated samplers that are not particle size-specific but still frequently used to collect airborne ENMs include plastic cassette samplers and conductive cowl samplers (Maynard et al., 2004; Han et al., 2008; Fujitani et al., 2008; Methner et al., 2008, 2010, 2012; Bello et al., 2008, 2009, 2010; Cena and Peters, 2010; Lee et al., 2010; Birch et al., 2011; Dahm et al., 2012; Johnson et al., 2010; Huang et al., 2012; Hirth et al., 2013; Ogura et al., 2013; Ono-Ogasawara et al., 2013). Plastic cassette samplers can be operated using a 'close-faced' configuration through which air is drawn into the sampler through a 4-mm inlet or in an 'open-faced' configuration in which the top section of the cassette with the inlet has been removed. Conductive cowl samplers utilize an open-faced configuration and were originally developed for sampling asbestos fibers but are being used to collect high aspect ratio ENMs such as MWCNTs and carbon fibers.

Regardless of the type of time-integrated sampler, the choice of filter used as collection substrate is a critical consideration because it must be amenable to the desired analytical technique (discussed below). For example, track-etched polycarbonate filters have a smooth surface appearance and are used to collect particles for subsequent electron microscopy analysis, polytetrafluoroethylene filters have good chemical resistance and weight stability and are used to collect particles for gravimetric determination of mass, mixed cellulose ester and polyvinyl chloride filters are easily digested in acids and are used to collect particles for determination of elemental mass by atomic spectroscopy, and quartz fiber filters possess good stability at elevated temperatures and are used to collect carbonaceous

nanomaterials such as MWCNT for thermal optical (TO) transmittance analysis (organic/elemental carbon). Additionally, sufficient sample must be collected onto a filter to meet analytical detection limits. Several options exist for increasing material collection. For example, in laboratory studies of MWCNT release from composites, investigators may construct a chamber or enclosure around an experimental system to concentrate the emitted aerosol for sampling (Guiot et al., 2009; Vorbau et al., 2009; Gohler et al., 2010, 2013; Hagendorfer et al. 2010; Koponen et al., 2011; Wohlleben et al., 2011; Golanski et al., 2012; Huang et al., 2012; Raynor et al., 2012; Schlagenhauf et al., 2012; Hirth et al., 2013; Sachse et al., 2012). For samplers such as cassettes and asbestos cowls, typical sample collection rates are 1 to 2 liters of air per minute when performing full work shift sampling but these flow rates can be increased to tens of liters per minute to increase collection. Note that increasing the air collection flow rate for samplers that separate particles by size will alter the performance of the sampler, *i.e.*, increasing flow rate will lower the aerodynamic size cut-offs of impactor stages.

Another type of time-integrated sampler is precipitators. There are two main types of precipitators, electrostatic (ESP) and thermophoretic (TP), and both are frequently used to sample for airborne ENMs (Bello et al., 2008, 2009, 2010; Vorbau et al., 2009; Plitzko, 2009; Jankovic et al., 2010; Pfefferkorn et al., 2010; Hagendorfer et al., 2010; Gohler et al., 2010, 2013; Fleury et al., 2011; Koponen et al., 2011; R'mili et al., 2011; Wohlleben et al., 2011; Leppänen et al., 2012; Schlagenhauf et al., 2012; Golanski et al., 2012; Sachse et al., 2012). Precipitators have an important advantage over samplers that use filters because they collect particles directly onto grids (for electron microscopy analysis) or flat mica substrates (for atomic force microscopy analysis), thereby eliminating the need for any subsequent sample preparation. With an ESP, aerosol is pulled through the sampler and particles are simultaneously charged in a very high voltage electrical field and collected using a flat surface onto which a sampling substrate (grid) has been mounted as the cathode. For a TP, air is

drawn between two parallel plates having a temperature difference and particles move by diffusion away from the hot plate and are collected onto a grid located on the cold plate (Thayer et al., 2011). A spin-off of the direct particle-to-grid sampling approach of precipitators is to tape an electron microscopy grid to a filter held in a cassette sampler (Tsai et al., 2008a,b; Tsai et al., 2009; Cena and Peters, 2011; Birch et al., 2011); however, the performance characteristics of such a sampling approach are unknown.

Sampling material released into water, solids, and biological fluids

Studies of MWCNT release from composites into water are limited to a single study and no information is available for release into solids or biological fluids. Busquets-Fité et al. (2013) subjected ethyl vinyl acetate-, polypropylene- and PA-MWCNT composites to accelerated aging (UV light) and weathering (simulated rain episodes) in a chamber using ISO 4892: Plastics - Methods of exposure to laboratory light sources - Fluorescent UV lamps. Following exposures, analysis of the rain water using transmission electron microscopy (TEM) identified composite fragments but not MWCNTs. A few studies exist on the release of metal and metal oxide nanoparticles from paints into water. Golanski et al. (2011) evaluated titanium dioxide nanoparticle release from paints into water using an Elcometer 1720 abrader and abrasion conditions specified in ISO 11998: Paints and varnishes - Determination of wet-scrub resistance and cleanability of coatings. This device pumps an aqueous abrasive solution onto a coated surface and the abrasive buffer is linearly rubbed on the sample to generate dynamic friction and released particles are collected in the liquid. Analysis of the abrasive liquid after testing revealed the presence of titanium dioxide nanoparticles in paint particles. Kaegi et al. (2010) applied paint containing silver nanoparticles to a façade that was exposed to ambient weather. Rain water that contacted the painted panel was collected in a gutter mounted below the panel and subsequent analysis using TEM identified silver nanoparticles in paint particles.

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Studies on the fate of MWCNTs in water (Hyung et al., 2007; Holbrook et al., 2010; Zhang et al., 2011), solids (Petersen et al., 2008, 2011a), and biological tissues and fluids (Petersen et al., 2008, 2011a, 2011b) have been published and provide useful insights for sample collection in composite release scenarios. These studies have demonstrated that MWCNTs dispersed in waters containing natural organic matter (NOM) remain well dispersed (Hyung et al., 2007; Holbrook et al., 2010; Zhang et al., 2011). In contrast, Holbrook et al. (2010) demonstrated that the presence of kaolin and alginates in waters promoted MWCNT coagulation and Zhang et al. (2011) reported that MWCNTs were readily sorbed by sediments in hard or sea water. Hence, the presence of certain water constituents in release scenarios has important implications for sample collection. Specifically, if only NOM is present, MWCNTs will likely be well dispersed in the suspension and a representative sample may be drawn from anywhere in the water. In contrast, if kaolin or alginates are present (fresh water) or sediments (sea water), the MWCNTs will coagulate or sorb to sediment particles which may require sample collection from the bottom of the water and/or additional sample preparation steps (*e.g.*, centrifugation to separate solids from liquids). With regard to solids, Petersen et al. (2008) fortified river sediment containing peat with MWCNTs and by tumbling were able to disperse the tubes homogeneously throughout the matrix. A similar protocol was used in a subsequent study by Petersen et al. (2011a) to homogeneously disperse MWCNTs in soil. Studies of MWCNT uptake to tissues, *e.g.*, by fish (Petersen et al., 2011b), aquatic worms (Petersen et al., 2008), and earthworms (Petersen et al., 2011a), simply involves harvesting the organisms from the media using a net or sieve.

Methods for Preparing Samples of Released Material

Sample preparation is the second step of an overall measurement strategy. The exact preparation method will depend on the analytical technique to be employed. As with collection, it is critical that

the sample preparation step preserve the properties of the MWCNTs in the same state as in the matrix into which they were released.

Preparation of air samples

As noted above, collection of aerosol particles directly onto carbon-backed TEM grids (not graphene or lacey carbon support because they have the same characteristics as CNTs) or mica substrates using precipitators does not require any sample preparation. The substrate is simply removed from the sampling device and placed in the TEM or atomic force microscope (AFM) instrument sample holder. Hence, this section only addresses manipulation of filter samples for off-line analyses. The most common sample preparation steps described for filters were for subsequent analysis using TEM or scanning electron microscopy (SEM). For electron microscopy, sample preparation is relatively simple as long as the sample is vacuum compatible. In SEM, an electron beam is rastered across a sample, so the sample must be electrically conductive to prevent charging which results in blurred images. For SEM substrates with a smooth surface (*e.g.*, polycarbonate filters or aluminum foils), a piece is sectioned using a scalpel, mounted on a SEM stub using double sided carbon tape or other conductive adhesive, and sputter coated with a conductive material such as carbon, platinum, gold, and/or palladium (Brouwer et al., 2004; Han et al., 2008; Bello et al., 2010; Lee et al., 2010; Wohlleben et al., 2011; Huang et al., 2012; Schlagenhauf et al., 2012; Hirth et al., 2013; Ogura et al., 2013; Wohlleben et al., 2013).

In TEM, electrons are transmitted through a specimen to generate high-resolution images of the specimen. Therefore TEM samples must be thin enough (100 nm or less) to be electron transparent. If the substrate has a thick uneven surface, such as mixed cellulose ester (MCE) filters, additional preparation steps (collapsing and clearing) are necessary because particles may deposit and become hidden from view in the furrows and pores. Most collapsing and clearing procedures for MCE filters are a variation of NIOSH Method 7402 – Asbestos by TEM and are used to prepare samples for TEM

analysis (though SEM may also be used if the sample is coated with a conductive material). In this procedure, a section of the MCE filter is placed on a standard 3-mm TEM grid and collapsed and made translucent using acetone (Dahm et al., 2012; Johnson et al., 2010; Methner et al., 2010, 2012), chloroform (Han et al., 2008), or 50% water/35% dimethylformamide/15% glacial acetic acid (Peters et al., 2009) vapor. Another method reported to prepare samples for TEM analysis was to immerse polycarbonate filters in acetone, disperse the particles by ultrasonic agitation, and either dip a TEM grid into the suspension (Huang et al., 2012) or deposit a drop of the suspension onto a grid (Hirth et al., 2013). Methods to prepare bulk samples of MWCNT-polymer composites for electron microscopy analysis include cryo-fracturing (Koerner et al., 2005; Chen et al., 2006; Meng et al., 2009) and microtoming (Gojny et al., 2005; Song and Youn, 2005; Chen et al., 2006; Ono-Ogasawara et al., 2013; Vilar, 2013). In cryo-fracturing, a composite sample is immersed in liquid nitrogen then broken into pieces and the surfaces inspected by microscopy. In microtoming, a composite sample is embedded in a resin and cut into ultrathin slices (*ca.* 50 nm to 80 nm) using a blade made of diamond or other hard substance, and mounted on a TEM grid (or a SEM stub and coated with a conductive material). These methods are generally used to evaluate MWCNT dispersion in a composite, but could have application to release scenarios if large fragments or sufficient amounts of smaller particle were removed during a test.

Another common filter analysis technique is thermal-optical transmittance (TO-transmittance) for organic carbon (OC) and elemental carbon (EC) content. In TO-transmittance, particles that were collected on quartz fiber filters (QFF) are incrementally heated to oxidize the carbon constituents. In this technique, the EC fraction is used as marker for MWCNTs based on the assumption that CNTs have negligible OC content (Kuhlbusch and Fissan, 2006; Methner et al., 2007, 2010, 2012; Birch et al., 2011; Dahm et al., 2012; Doudrick et al., 2012; Ono-Ogasawara et al., 2013). Typically, a 1.5 cm^2

section of a filter is removed for analysis; however, if the particles were not evenly distributed across the filter surface during collection, multiple sections of the filter or the entire filter must be analyzed to accurately determine EC content (Birch et al., 2011). Most TO-transmittance analyses are variations of NIOSH Method 5040 - Diesel particulate matter (as elemental carbon).

If a weight-stable filter was used to collect a sample it can be pre- and post-weighed under identical temperature- and humidity-controlled conditions to determine the total mass of particles captured during a release test. The composite sample itself can also be pre- and post-weighed in this same manner to determine the mass of material that was released during a test (Nguyen et al., 2011; Wohlleben et al., 2011, 2013). Subsequent analysis of filter samples by atomic spectroscopy is a sensitive method for determination of low levels of metals in aerosols (Bello et al., 2010; Methner et al., 2010; Pfefferkorn et al., 2010; Huang et al., 2010; Birch et al., 2011; Curwin and Burtke, 2011). Atomic spectroscopy has been used to quantify masses of airborne metal oxide (e.g., titanium dioxide) nanoparticles or masses of residual catalyst contamination (e.g., nickel or cobalt) as a marker of CNTs. Prior to quantification of metals, the entire mixed cellulose ester or polyvinyl chloride filter sample is subject to dissolution using acids and/or thermal treatment such as a hotblock or microwave. The exact choice of acids and or thermal treatment is specific to the metal of interest, though commonly used standard methods include U.S. EPA Method 3051 – Microwave assisted acid digestion of sediments, sludges, soils, and oils, NIOSH Method 7300 – Elements by ICP (nitric/perchloric acid ashing), and NIOSH Method 7300 – Elements by ICP (hot block/HCl/HNO_3 digestion). For composite release scenarios, if MWCNTs could be tagged with a unique metal, analysis of air filters could be used as a qualitative indicator of release.

The filter preparation procedures outlined above are used for direct analysis of material collected on the substrate. However, with additional processing, material can be analyzed by analytical

techniques including colloid counting and analytical ultracentrifugation (AUC). For example, a filter can be placed in an appropriate liquid and subjected to ultrasonic agitation to remove particles (fragments and free MWCNTs) from the substrate and disperse them in the liquid for subsequent sizing by laser diffraction or AUC (Wohlleben et al., 2011, 2013) or dynamic light scattering (Li et al., 2006).

In some cases the masses of fragments released to air may be relatively high (*e.g.*, sanding in scenario (1)) or occur over such a long period of time (e.g., UV weathering in scenario (2)) that collection onto a filter is not feasible. In these cases, investigators have recovered materials from the surfaces of the enclosure surrounding a sample (Wohlleben et al., 2011, 2013) or used settling plates (Nguyen et al., 2011). When sufficient quantities of released material are available, the loose powder can be pressed into a sample holder for analysis using x-ray photoelectron spectroscopy to identify MWCNT protrusions from polymer fragments using the C(1s) spectra of tubes to discriminate from the polymer background (Wohlleben et al., 2011 supplement).

Preparation of waters, solids, and biological fluids samples

With appropriate sample preparation, many of the same analytical techniques used to analyze air samples are also applicable to waters, solids, and biological fluids. For electron microscopy analysis of materials released into waters, an aliquot of suspension is directly pipetted onto a substrate such as a SEM stub (Li et al., 2006; Holbrook et al., 2010; Golanski et al., 2011; Busquets-Fité et al., 2013) or a TEM grid (Tagmatarchis et al., 2005; Hyung et al., 2007, Kaegi et al., 2010, Busquets-Fité et al., 2013) and allowed to dry in air or in an oven. If an aqueous sample contains a wide range of particle sizes, additional sample preparation may be necessary before depositing the suspension onto a substrate. For example, Kaegi et al. (2010) used a two-step centrifugation process to isolate silver nanoparticles in rain water. First an aliquot of water was centrifuged at a low *g*-force to remove large particles followed by a second centrifugation at a higher *g*-force to collect nanoparticles onto TEM grids placed

in the bottom of the centrifuge tube. Alternatively, Tagmatarchis et al. (2005) separated MWCNTs from amorphous material in a buffered suspension by flow field-flow fractionation and used the eluted MWCNT suspension to prepare a grid samples for TEM analysis. Numerous other techniques exist for pre-fractionation of nanoparticle from large particles in aqueous suspensions, soils and biological (*e.g.*, milk) fluids using capillary electrophoresis and variations of chromatography (hydrodynamic, ion exchange, size exclusion, etc.). These pre-fractionation techniques can be used in tandem with analytical methods such as ultraviolet-visible spectroscopy, atomic spectroscopy, etc. and the reader is referred to the review articles by Bandyopadhyay et al. (2013) and Tiede et al. (2008) for more information on these procedures. Finally, if it is desired to analyze wet or hydrated samples, specialized sample holders (Tiede et al., 2008) and specialized variable pressure SEMs (Echlin, 2009) may be used for measurement of MWCNTs in water and biological fluids and tissues.

TO-transmittance is a robust method that can be used to detect EC in not only air, but also water, soil, and biological fluids (Hyung et al., 2007; Doudrick et al., 2012; Wohlleben et al., 2013). In a methods development study, Doudrick et al. (2012) prepared suspensions of MWCNT in waters (surface, municipal tap, waste), solids (river sediment), and biological fluids and tissues (lyophilized bacteria, synthetic urine, human serum, lung tissue, and commercially available cow's milk), deposited the materials onto QFF, dried, and successfully used TO-transmittance analysis (along with Raman spectroscopy) to quantify masses of EC in samples.

Atomic spectroscopy is also amenable to analysis of waters, soils, and biological fluids (Kaegi et al., 2010). As with air filters, samples must first be digested using various chemical and thermal treatments, though there are many standard methods available for this purpose (*e.g.*, EPA 3051). As with air samples, the applicability of atomic spectroscopy to detection of MWCNTs, either tagged with a metal or containing residual catalysts, in waters, soils, and biological fluids will be limited to

qualitative information. An alternative to metals analysis by atomic spectroscopy is scintillation counting of radiolabeled MWCNTs. Petersen et al. (Petersen et al. 2008, 2011a,b; Zhang et al., 2011) have demonstrated the sensitivity and utility of scintillation counting for detection and quantification of labeled MWCNTs in waters, soils (sediment, peat), and biological fluids (earthworm gut contents), and tissues (fish, earthworms, aquatic worms). For waters, sample preparation consists of diluting the specimen with scintillation cocktail before counting. For solids and biologicals, samples were freeze dried, combusted in a biological oxidizer, and mixed with scintillation cocktail before counting. While scintillation counting is a robust method that is applicable to a wide range of matrices, a potential disadvantage of this approach is the use of radioactive materials and the generation of radioactive waste from testing.

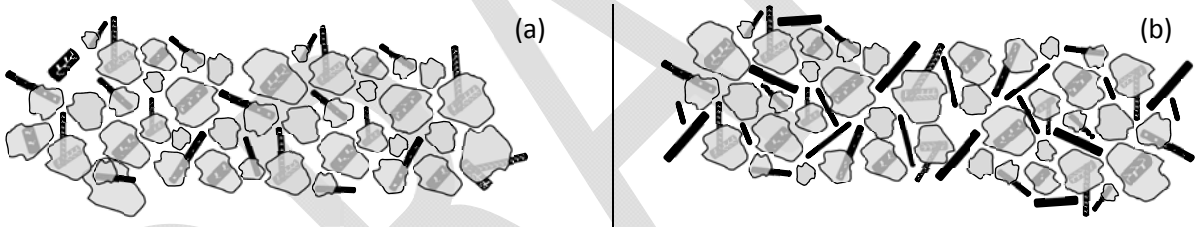
Measurement Methods for Released Material

The requisite first step in designing a measurement study is the prioritization and selection of what is to be measured. This selection will dictate the choice of sample collection and preparation methods and the choice of measurement methods and instruments (and potentially identify the need for additional measurement capabilities). Measurement of material released from MWCNT-polymer composites is exceptionally challenging for numerous reasons. The foremost reason is the chemical and physical complexity of the released material. This complexity is illustrated by the two scenarios mentioned previously:

(1) The released material is likely composed of polymer fragments which may or may not contain MWCNTs. Unbound MWCNTs may also be present. These two types of samples, one containing only fragments and the other containing both fragments and unbound MWCNTs, are illustrated in Figure 2. The sample characteristics of interest are presented below each type of sample. To simplify the scope of subsequent measurement methods discussions in this paper,

samples of material released in scenario (1) that contain only fragments (no unbound MWCNTs) will be considered.

(2) The released or potentially released material is likely in the form of unbound MWCNTs. A schematic illustration of such an entangled network is illustrated in Figure 3a. The measurement sample will contain many MWCNTs as shown schematically in Figure 3b. In this scenario some of the sample characteristics of importance include the presence of MWCNTs in the sample (detection), number or mass concentration of MWCNTs in the sample, and the physico-chemical characteristics or properties of MWCNTs in the sample, *e.g.*, average size (diameter and length), size distribution, shape, surface composition and surface reactivity.



• Presence of MWCNTs in fragments (detection) • Number or mass concentration of MWCNTs in fragments • Physico-chemical characteristics or properties of MWCNTs in fragments, *e.g.*, average size (diameter and length), size distribution, spatial distribution (*e.g.*, well-dispersed or poorly dispersed), and surface composition • Relative amounts of fragments that contain MWCNTs vs. fragments that do not contain MWCNTs (by number or mass) • Average size and size distribution of the fragments • Shape of fragments	• All of the same in Figure 2a, and • Presence of MWCNTs in sample (detection) • Relative amounts of fragments vs. unbound MWCNTs in sample • Number or mass concentration of unbound MWCNTs in sample • Physico-chemical characteristics or properties of unbound MWCNTs in sample, *e.g.*, average size (diameter and length), size distribution, surface composition and surface reactivity

Figure 2. Types of samples released under release scenario (1)

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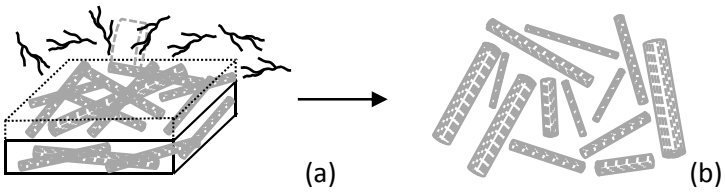

Figure 3. Types of samples released under release scenario (2). Released MWCNTs are portrayed as straight tubes and degraded polymer particles are shown as branched structures.

Measurement challenges

Based upon existing data, it is reasonable to assume that the sample of interest from a given release scenario is a collection of many entities (fragments, unbound MWCNTs, or both) as opposed to a single fragment or MWCNT and that there are a plethora of possible characteristics of a sample that could be measured (size distribution, etc.). In addition to the complexity of the released sample itself, there are several challenges associated with measurement of MWCNTs in complex matrices and these are discussed below in the order in which they would be encountered in a measurement strategy.

- **Surface modification and transformations**: The large surface area and reactivity of the various MWCNT chirality types may promote adsorption of contaminants from the surrounding media following release. Such surface modification may result in physical and chemical transformations that affect measurements. The type of modification and resultant transformations will vary depending on the phase of the product life cycle. For example, it is widely recognized that surfaces of MWCNTs are hydrophobic so they are functionalized (*e.g.*, with carboxylic acid functional groups) to promote dispersion or chemical interactions in masterbatches of polymer during production. If released to air during use, MWCNTs (individually or protruding from polymer fragments) can be oxidized thereby changing surface reactivity. If released to aqueous matrices during the end-of-life phase of the life cycle, the presence of organic matter, alginates, and other water constituents will alter the surface

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chemistry and the behavior of MWCNTs in suspension (Hyung et al., 2007, Holbrook et al., 2010, Zhang et al., 2011).

- **Sample collection artifacts:** Proper choice of a sampler and the collection conditions are the first opportunity in a measurement strategy for ensuring that a representative sample is obtained for analysis. For example, if the measurement goal is to obtain information about particle physical dimensions using electron microscopy, then the area of the filter sectioned and mounted for analysis must be representative of the whole filter. Sample bias is likely when sectioning if the particles were not uniformly deposited across the filter by the sampler. One approach to obtaining a representative filter section is to use a conductive cowl sampler that is designed to promote uniform fiber deposition across the filter surface.
- **Applicability of a measurement method for a given release media**: MWCNT-polymer composites and products and material released from composites and products will come into contact with various media throughout the product lifecycle. Such media may be environmental, *e.g.*, air, water, and mixed solids such as soil; biological, *e.g.,* saliva, blood, and tissue; and man-made, *e.g.*, chemicals and other products in landfills. The release scenario will determine the media. For example, in the case of grinding or sanding of MWCNT-polymer composites to manufacture a product, material will most likely be released into air. In the case of "weathering" of a product, optical (UV radiation) as well as chemical (water) driving forces may result in polymer degradation and subsequent release of MWCNTs, most likely into surrounding water or soil. It is essential that the chosen analytical method does not alter the properties of the MWCNT or matrix from their state at the time of sample collection. For example, TEM uses a high energy electron beam to interrogate a specimen and the beam itself

can cause changes in nanoparticle properties such as size by beam-induced swelling (Latham et al., 2006).

- **Sample preparation artifacts:** Proper sample preparation is critical for ensuring meaningful measurement results. Unfortunately, sample preparation is rarely described in sufficient detail in existing reports. When preparing samples for electron microscopy from aqueous suspensions, drying artifacts can occur if the surface tension of the water droplet pulls the particles into contact as the liquid evaporates. To avoid this problem, TEM and SEM substrates can be functionalized with a charged coating (positive or negative) opposite to the surface charge of the particles of interest. The charged coating on the substrate will hold the oppositely charged particles in place which helps to reduce drying artifacts and promotes well dispersed samples for analysis. Another example is preparation of particles that were collected onto a filter for colloidal counting. Often, a filter is immersed in a liquid and subjected to ultrasonic agitation to dislodge the particles; however, bias is likely with this dispersion method if particle removal efficiency from the filter is not complete for all sizes of particles. An alternative approach may be to collect the particles directly into the analytical medium using an impinger or similar device.
- **Capability of a measurement method:** For each property of interest, there are numerous possible measurement methods applicable to MWCNTs and each has unique advantages and disadvantages. Multiple complimentary and confirmatory measurement methods are needed to ensure a robust data set. For example, the size of fragments released in scenario (1) could vary from hundreds of nanometers to hundreds of micrometers in size which is very large compared to the diameters of individual MWCNTs (on the order of tens of nanometers). Few techniques can accurately measure across such a wide range of size scales. In one study, Wohlleben et al.

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(2011) reported the use of laser diffraction to measure the size distribution of abraded material released from a composite. This technique is not sensitive below 200 nm so a second complimentary technique, AUC (range 0.5 nm to 10,000 nm), was used to understand the size distribution of particles below 200 nm. Note also that if the MWCNTs in a polymer chunk are encased in the polymer matrix or protruding from the surface, measurement by surface-based methods such as microscopy will not detect all the MWCNTs in a fragment. For scenario (2), it is likely that MWCNTs will possess varying aspect ratios, making it difficult to describe the population in a sample using a single technique. In this case, pre-fractionation of samples using size exclusion chromatography or field flow fractionation in tandem with electron microscopy analysis may be required, though such approaches are tedious and time consuming.

- **Diversity of measurement methods**: Each measurement method will vary in key factors such as spatial resolution, detection limit, measurement media (*e.g.,* vacuum, ambient, liquid), availability, required level of expertise, etc. Hence, a unique cutting edge instruments might exist at a few specialized laboratories in the world; however, for purposes of inter-laboratory testing of release scenarios, such an instrument is not a practical choice because of its rarity and the level of expertise needed to operate such an instrument. Rather, a more common but less sophisticated instrument (*e.g.*, SEM) might be preferred as it will permit a broader range of participation and be more representative of real-world capabilities.
- **Representativeness of measurements:** The chosen measurement method must be capable of providing representative information on the sample that is being interrogated. The importance of this concept is highlighted by the extensive use of SEM for analysis of released particles. As noted above, SEM operates by rastering an electron beam across a sample surface and an image is formed from the scattered electrons. Hence, this technique only provides information on the

surface of a sample and the full lengths of MWCNTs protruding from a fragment cannot be ascertained from this technique. More importantly, SEM (and many other electron microscopy techniques) cannot detect MWCNTs encased in polymer fragments. This means that fragments that contain MWCNT cannot be discriminated from fragments that consist of polymer only. More recently, investigators have made progress in the use of electric force microscopy to characterize MWCNTs below the surface of composites (Jespersen, 2007; Zhao, 2010). Another limitation of electron microscopy techniques is obtaining sufficient measurements to ensure robust counting statistics. For example, a standard 37-mm filter has about 960 mm^2 of available collection surface area. Typically, a 3 mm^2 to 5 mm^2 piece of filter is sectioned for analysis and this represents only 0.3 % to 0.5 % of the available collection surface. In turn, typically a few fields of view, each on the order of 10 μm^2 are viewed in the electron microscope which corresponds to an area of about 10^{-6} % of the original sample. None of the papers we reviewed reported number of fields viewed or sufficiently described counting statistics from electron microscopy analysis.

- **Composition measurements**: Compositional analysis of MWCNT-polymer composites can be challenging because both MWCNTs and polymers are carbon-based. Additionally, the actual form of carbon nano-objects in products is not pure MWCNTs but also likely includes carbon cups, horns, etc. As noted previously, TO-transmittance is commonly used for the analysis of released particles and the EC fraction is used as marker for MWCNTs. To date, studies have not sufficiently evaluated the ability of this technique to distinguish among MWCNTs, cups, horns, and polymer matrices. Further complicating measurements is the fact that polymers are not pure but contain additives and stabilizers which may interfere with analyses.

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- **Polymer Stability**: Each type of polymer possesses unique properties which make it attractive for an application though these same properties may lead to instabilities (*e.g.*, dissolution or degradation) of a polymer matrix that vary with the phase of the product lifecycle, thus complicating measurement of released MWCNTs. This variation in polymer stability is especially important for scenario (2) where the released or potentially released material is likely in the form of unbound MWCNTs. Most composites are unlikely to be exposed to acids, bases, or solvents during use; however, at the end of its useful life, it is reasonable that a product could come into contact with liquids that degrade the polymer matrix in landfills, etc.
- **Commercial practices:** As with any emerging nanotechnology, competitive advantages are to be gained by those who can understand how to effectively disperse MWCNTs in a polymer matrix and control reproducibility of manufacturing procedures. Hence, intellectual property issues will present challenges for sharing measurement data. Additionally, the dynamic nature of the field of nanotechnology is such that products are continuously being improved and a polymer matrix used today may be obsolete in six months or a year because of changes in polymer formulations, processing methods, etc. making it difficult to identify a 'representative' composite for release testing and risk assessment.

Detection, Quantification, and Characterization of Released Materials

Detection is a qualitative method simply defined as the presence—yes or no—of MWCNTs released from MWCNT-polymer composites as free MWCNTs or in polymer fragments. The detectability limit in terms of number or mass concentration of MWCNTs will vary from method-to-method, and needs to be determined for each method using control samples (Schutz, 2010) with appropriate MWCNT concentrations. To the authors' knowledge, detectability limits for MWCNT by any measurement method descried herein have not been published. Quantification concerns the

number or mass concentration of MWCNTs in or released from MWCNT-polymer composites per unit volume or area of polymer matrix. Quantification of released MWCNTs, particularly those in polymer fragments, is challenging for the reasons described in the previous sections, and many methods such as electron microscopy can only yield a qualitative measure of concentration.

Characterization is the crucial first step in hazard and exposure studies but it is also the most complex step in the measurement hierarchy. It is well-documented that the physico-chemical characteristics and properties of MWCNTs determine the toxicity and fate of MWCNTs in humans and the environment; see, for example, Warheit et al. (2008) and Petersen et al. (2011c). In this section of the monograph, we address four characteristics or properties of MWCNTs that are of importance in released material: size (*i.e.*, MWCNT length and diameter) and size distribution of MWCNTs, both unbound and in polymer fragments; MWCNT surface chemistry; and spatial distribution of MWCNTs in polymer fragments. As for quantification, characterization of MWCNTs in polymer fragments is challenging because of the presence of embedded MWCNTs (see Figures 1 and 2).

Finally, it is important to note that measurement methods may be *qualitative*, defined here as measurements that provide a result ranging from "the sample does or does not contain MWCNTs" to "the sample contains about 50 % MWCNTs" per unit area or volume examined. For qualitative methods, the relative uncertainty in the result is large or cannot be defined adequately because all sources of error are not known or quantifiable. A *quantitative* measurement produces a numerical result such as "the diameter of individual MWCNTs ranges from 100 nm to 200 nm" with knowledge of the sources of error that contribute to relative uncertainty for the MWCNT population. The relative uncertainty in a numerical result obtained from a quantitative measurement is much smaller than that obtained from a qualitative measurement.

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Measurement instrumentation for MWCNTs in or released from MWCNT-polymer composites may be grouped into three categories based on the availability and practicality:

(1) Commercial instruments that are broadly available and widely and easily used in many industry, government, and university laboratories, such as scanning electron and atomic force microscopes.

(2) Expensive commercial instruments that are frequently not available in industry, government, and university laboratories and often require expert users, such as TEMs. A number of commercial organizations offer fee-based measurements on such instruments.

(3) Non-commercial and often world-class instruments available at one or a few organizations, such as synchrotron spectroscopy at a U.S. Department of Energy User Facility.

The choice of the type of instrument depends on the type of measurement (*e.g.*, detection, quantification, and characterization) needed by the user and the required level of accuracy and precision for the particular scenario.

Applicable Measurement Methods

A comprehensive review article (Tiede et al., 2008) describes, in detail, measurement methods for the physico-chemical characterization of ENMs. The information in this article includes spatial resolution, detection limit, advantages, disadvantages, and measurable characteristics and properties for 42 methods. Most of the measurement methods in published release studies on MWCNT-polymer composites are included in the review article (Tiede et al., 2008); additionally, the article includes methods of potential applicability for release studies. In addition, there is an excellent review article by Petersen et al. (2011c) that focuses solely on methods to detect, quantify, and characterize MWCNTs in environmental media. Only some of the numerous reported measurement methods for

the quantification and characterization of MWCNTs are discussed below, as the methods are well-documented elsewhere.

Published studies on the evaluation of release or potential release of MWCNTs from MWCNT-polymer composites are summarized in Table 1. In addition to measurement methods, this table includes the form of released or potentially released material, methods used to generate released material, polymer type[1], and media in which material was released. (Note that potentially released material refers to exposed MWCNTs, in the form of entangled masses on the surface of a composite, which could be released as unbound MWCNTs by agitation, wear, or fluid flow.) For ease of discussion, the published studies listed in the Table have been grouped by the release scenarios (1) and (2) described in a previous section. There are 21 published release studies presented in this Table: twelve on release scenario (1) and twelve on release scenario (2). Three of the studies covered both scenarios (1) and (2) (Wohlleben et al., 2011, 2013; Hirth et al., 2013). A total of 15 different measurement methods were collectively reported in these 21 manuscripts. Microscopy-based methods, notably SEM and TEM, were the most widely used measurement methods for release studies. The frequent use of these techniques reflects their utility in detection and characterization of MWCNT and/or MWCNT fragments released from composites. Specifically, microscopy methods can provide information on the presence or absence of MWCNT in released material and, if detected, possible information on the length and/or diameter of MWCNTs (unbound and/or protruding from, polymer fragments) and limited information on spatial distribution of MWCNTs in polymers. Though information on chemistry can be discerned from EDX analysis, to elucidate MWCNT surface chemistry requires independent analysis using XPS or other appropriate technique.

[1] Note that the polymer types include the four of the five considered in Section 1 of this monograph (epoxy, PA, PC, and PU), polyoxymethlene, and poly(methyl methacrylate).

Table 2. Published MWCNT-polymer composite release studies

Published Study	Release Scenario	Form of released or potentially released material	Generation of released material	Polymer[1]	Media	Measurement methods[2]
Bello et al., 2009	(1): Release due to mechanical driving forces. MWCNTs strongly bonded to polymer and no polymer degradation.	fragment	sawing, drilling	EP	Air	TEM SEM
Bello et al., 2010		fragment	drilling	EP	Air	TEM-EDX SEM ICP-MS
Cena and Peters, 2011		fragment	sanding	EP	Air	TEM
Schlagenhauf et al. 2012		fragment; unbound MWCNT	abrading	EP	Air	SEM TEM
Huang et al., 2012		fragment; unbound MWCNT	sanding	EP	Air	TEM SEM
Golanski et al., 2012		fragment; unbound MWCNT	abrading, grinding	EP, PA, PC	Air	SEM TEM-EDX
Hirth et al., 2013		fragment; unbound MWCNT	sanding	EP, PU, POM	Air	TEM SEM
Fleury et al., 2011		fragment; unbound MWCNT	mixing, grinding	PA	Air	TEM-EDX
Wohlleben et al., 2013		fragment	sanding, abrading	PU	Air	XPS LD AUC SEM
Samuel et al., 2009		fragment	machining	PC	Air	SEM
Wohlleben et al., 2011		fragment	sanding, abrading	POM	Air	XPS

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Published Study	Release Scenario	Form of released or potentially released material	Generation of released material	Polymer[1]	Media	Measurement methods[2]
						TOF-SIMS LD AUC SEM
Wan et al., 2008		fragment	micromachining	PMMA	Air	SEM
Nguyen et al., 2011	(2): Release or potential release due to photolytic, hydrolytic, chemical, biological, thermal driving forces, or a combination of the above. Polymer degradation resulting in release of unbound MWCNTs or an entangled network of MWCNTs on the composite surface that may subsequently be released.	unbound MWCNT	photolysis by accelerated exposure	EP	Air	FE-SEM-EDX
Asmatulu et al., 2011		unbound MWCNT	photolysis by extended exposure	EP	Air	AFM
Busquets-Fité et al., 2013		unbound MWCNT	photolysis by extended exposure	PA	Air	Gravimetric TEM SEM
Vilar et al., 2013		unbound MWCNT	photolysis by extended exposure	PA	Air	TEM
Pillay et al., 2009		unbound MWCNT	photolysis by extended exposure	PA	Air	SEM
Hirth et al., 2013		unbound MWCNT	photolysis by extended exposure	PU	Air	SEM
Wohlleben et al., 2013		unbound MWCNT	photolysis by extended exposure	PU	Air	XPS AUC SEM
Wohlleben et al., 2011		unbound MWCNT	photolysis by extended exposure	POM	Air	XPS TOF-SIMS LD AUC

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Published Study	Release Scenario	Form of released or potentially released material	Generation of released material	Polymer[1]	Media	Measurement methods[2]
						SEM
Deka et al., 2010		fragment?	biological by	PU		SEM
Li et al., 2006		fragment ?	chemical by	PC	Organic solvent	SEM DLS Zeta
Schartel et al., 2005		MWCNT	thermal by	PA	Air	SEM
Ribeiro et al., 2012		MWCNT	thermal by	PA	Air	TGA XRD

[1] EP = epoxy, PA = polyamide, PU = polyurethane, PC = polycarbonate, POM = polyoxymethlene, PMMA: Poly(methyl methacrylate)

[2] TEM = transmission electron microscopy, SEM = scanning electron microscopy, FE-SEM = field emission SEM, EDX = energy dispersive x-ray spectrometry, ICP-MS = inductively coupled plasma mass spectrometry, XPS = x-ray photoelectron spectroscopy, LD = laser diffraction, AUC = analytical ultracentrifugation, TOF-SIMS = time of flight-secondary ion mass spectrometry, DLS = dynamic light scattering, Zeta = zeta potential, XRD = x-ray diffraction

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Gaps and Needs

In the preceding sections we reviewed the properties of MWCNTs-polymer composites, described scenarios and driving forces that influence release, and reviewed available methods to collect, prepare, and analyze samples of materials released to environmental and biological media throughout the lifecycle of a nano-enabled product. While progress has been made in the last decade to understand MWCNT release from composites, there are critical gaps and needs that need to be addressed so that we can better understand risk. These gaps and needs include improved (or new) measurement methods, inter-laboratory studies (ILS) of release scenarios, development of test protocols, and standardization of methods.

Potential Improved/New Methods

As summarized in Table 1, electron microscopy methods have most often been used to interrogate samples of released materials because these instruments can provide information on the presence of MWCNTs and/or information on characteristics of these tubes. However, to obtain quantitative information on specific properties (*e.g.*, size distribution) with these techniques is time-consuming and expensive. As such, there is a critical need for automated microscopy-based methods (SEM, TEM, and AFM) for detection, quantification, and characterization of MWCNTs in polymer fragments to greatly accelerate measurements. Automated methods are particularly important for quantitative MWCNT size distribution and number concentration measurements.

For purposes of simplicity, we limited the scope of scenario (1) to release of polymer fragments (see Figure 2). These fragments may consist of any combination of MWCNTS that are fully encased or protruding from the polymer surface as well as fragments that consist of polymer only (see Figure 1). Discrimination of polymer fragments that contain fully encased

MWCNT from polymer only is critical. The presence of polymer only fragments that have the same appearance in the electron microscope as fragments with encased MWCNTs increases background 'noise' and precludes accurate assessment of exposure potential. Hence, a sample preparation procedure or pre-separation technique that yields a higher purity sample (fewer polymer only fragments) would decrease analysis time and improve measurement accuracy.

Inter-laboratory studies

An inter-laboratory study (ILS) is a testing scheme that involves multiple laboratories wherein samples from the same batch are measured by the participants and the results are analyzed with the goal of providing a precision (uncertainty) statement. There are numerous purposes for conducting an ILS, among them is to establish the effectiveness of new test methods (ASTM1995; ISO/IEC1997). In the context of MWCNT-polymer composites, new test methods could be procedures to reproducibly generate materials for a release scenario, protocols for sample preparation, and procedures for analytical measurements. The results of an ILS are often used to support development of a standard method.

Reference materials (RMs) are a means to assure or improve measurement quality and are a critical tool in any ILS scheme. Generally, reference materials are produced as certified RMs (CRMs) or as non-certified RMs. A RM is a material that is sufficiently homogeneous and stable with respect to one (or more) properties which has been established to be acceptable for its intended measurement use (ISO, 2009). RMs may be used as benchmark materials for ILS studies of new test methods or protocols. A sub-set of RMs is CRMs whose properties are certified and are metrologically traceable, *i.e.*, to a specified reference system and accompanied by an uncertainty value derived from an uncertainty budget covering all significant uncertainty contributions (ISO, 1992). CRMs are used for calibration purposes or for method testing. A third class of materials that are relevant for development of test methods is representative test

materials (RTMs) which is defined as a material from a single batch which is sufficiently homogeneous and stable with respect to one (or more) specified properties and is assumed to be fit for its intended use (Roebben et al., 2013). It is important to note the differences among these types of materials and their appropriate uses in the development of test methods. For example, a RTM does not carry an uncertainty budget so it is inappropriate to use as a calibration standard but may be adequate for testing a protocol to generate materials during a release scenario. At the opposite extreme, a CRM carries a full uncertainty budget which is necessary for instrument calibration to ensure accuracy and precision of measurement data but unnecessary for testing a protocol to generate materials during a release scenario.

Another concept related to measurement quality that is important for ILS is measurement validation. Validation of a measurement is defined in the Vocabulary of Metrology (VIM, 2008) as verification, wherein the specified requirements are adequate for an intended use. Validation is a subset of verification, which is provision of objective evidence that a given item fulfills specified requirements. There are several examples of a validated measurement result. One example is the case wherein results on a given sample measured by more than one method are consistent. Another example is measurement validation through the use of a model that incorporates actual data.

Prior to conducting an ILS, a protocol must be developed that outlines the purpose and scope of the procedure and the desired outcome (*e.g.*, a measurement result). Experience with gold nanoparticle RMs sized by dynamic light scattering, TEM, SEM, and AFM (ASTM ILS #206) and testing hemolysis (ASTM ILS #211) and cytotoxicity (ASTM ILS#202) protocols indicates that it is good practice to perform a pilot study with a modest number of organizations on the intended sample to refine a protocol before conducting a full ILS (ASTM Research Report RR:

E56–1001). A pilot evaluation of a protocol permits identification of steps that might be confusing to participants, opportunities for improvement, and allows participants to practice the procedure. Note that a video demonstration of the protocol may also be helpful to participants. Once a protocol is agreed upon, a full-scale ILS can be conducted in accordance with recognized standards (ASTM1995; ISO/IEC1997).

Protocols and Assays

The absence of standardized test methods for generation, collection, and analysis of MWCNTs released from composites is a major barrier to release testing (see section 6.4 below). As such, researchers have taken methods that were originally developed for some other purpose and adapted them to evaluate MWCNT release from composites. One common example is use of a Taber abrader, which is a well-established method to quantify wear resistance of polymer coatings and paints, and is specified in ISO 5470-1: Rubber- or plastics-coated fabrics – Determination of abrasion resistance – Taber abrader and ASTM D4060-95: Standard test method for abrasion resistance of organic coatings by the Taber abraser, to evaluate MWCNT release from composites. The adaptation of other standards essentially results in the development of a test protocol (defined here as a list of the steps to be followed in the test). It is important to note that a test protocol prescribes a procedure but does not carry the same level of confidence as a standard method. In the early phases of research, such as with ENM material release studies, test protocols are an invaluable means to begin to address critical measurement approaches and serve as starting points for evaluation of these approaches.

Opportunities for standardization of methods

The development of consensus-based standard test methods (*i.e.*, protocols that produce a measurement result) for quantification or characterization of MWCNTs by a standards organization such as ISO or ASTM International is predicated on the availability of a well-

defined, validated protocol for the method of interest. As noted above, a goal of an ILS is to provide data to calculate a precision (uncertainty) statement. ASTM requires that all test methods be accompanied by a precision and bias statement though ISO does not. Given the current state-of-the-science, it is not possible to develop standard test methods for quantification or characterization of MWCNT-polymer materials released from composites at this time. However, it may be possible to develop standards in the form of guides, technical reports, and specifications (i.e., protocols that do not produce a measurement result) for sample preparation approaches for SEM, TEM, or AFM. Such procedures would be an essential precursor to the eventual development of standard test methods. There are a number of standard technical reports and specifications from the ISO Technical Committee (TC) 229 on Nanotechnologies on how to measure free SWCNT characteristics and properties (but no standard test methods that yield a numerical result with an uncertainty determination). There is only one document related to free MWCNTs, Technical Report 10929 - Nanotechnologies — Characterization of multiwall carbon nanotube (MWCNT) samples. ISO Technical Committee TC 229 has established a study group to explore the feasibility of developing a standard for the determination of MWCNT size by TEM. As a first step, a pilot study involving a few organizations has been initiated. The intention is to conduct a more extensive ILS and develop a standard if the ILS is successful. This ILS is concerned only with the measurement of discrete MWCNTs, not MWCNTs released from polymer matrices.

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