Quantification of carbon nanotubes in environmental matrices: Current capabilities, case studies, and future prospects

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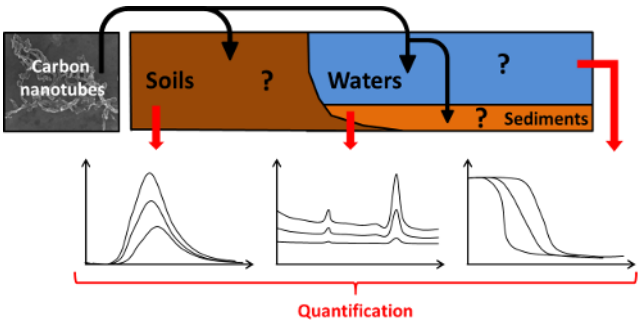

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ABSTRACT

Carbon nanotubes (CNTs) have numerous exciting potential applications and some that have reached commercialization. As such, quantitative measurements of CNTs in key environmental matrices (water, soil, sediment, and biological tissues) are needed to address concerns about their potential environmental and human health risks and to inform application development. However, standard methods for CNT quantification are not yet available. We systematically and critically review each component of the current methods for CNT quantification including CNT extraction approaches, potential biases, limits of detection, and potential for standardization. This review reveals that many of the techniques with the lowest detection limits require uncommon equipment or expertise, and thus, they are not frequently accessible. Additionally, changes to the CNTs (e.g., agglomeration) after environmental release and matrix effects can cause biases for many of the techniques, and biasing factors vary amongst the techniques. Five case studies are provided to illustrate how to use this information to inform responses to real-world scenarios such as monitoring potential CNT discharge into a river or ecotoxicity testing by a testing laboratory. Overall, substantial progress has been made in improving CNT quantification during the past ten years, but additional work is needed for standardization, development of extraction techniques from complex matrices, and multi-method comparisons of standard samples to reveal the comparability of techniques.

Introduction

The steady increase in potential applications[1] and production[1,2] of carbon nanotubes (CNTs) and their inevitable release during the life cycle of products has raised questions regarding their potential impact on humans and the environment.[3,4] CNTs can be conceptually understood as rolled up graphitic sheets of hexagonally arranged carbon atoms with sp^2 hybridization. These materials have exceptional mechanical strength as well as thermal and electrical conductivity properties that make them ideal for a myriad of potential applications (e.g. construction, environmental, optical, electronic, and biomedical).[5-8] The annual production capacity of CNTs reached 2 x 10^6 kg (2.25 ktons) yr^{-1} in 2011 with an estimated production capacity of 5 x 10^6 kg (4.5 ktons) yr^{-1}; this change was a 10-fold increase since 2006.[1] With increasing production volume, it is important to determine the potential for biological exposures to CNT during the production, usage, and disposal of CNT-enabled products. The necessary linchpin to quantifying potential CNT exposure, and any risks from it, is the availability of robust analytical methods for quantifying CNTs in complex environmental matrices.[9] These methods are critical for the assessment of potential CNT exposure, toxicity testing on the potential risks that may occur after exposure, and determination of the environmental fate of CNTs.[10]

Analytical techniques to quantify CNTs usually rely on unique physicochemical properties of CNTs that differentiate them from other compounds in relevant media. These approaches leverage the structural, thermal, and electrical properties of CNTs and include spectroscopic,[11,12,13,14,15] optical,[16,17] and thermal[16,14,18] techniques used individually or in combination.[9,15] Importantly, techniques used for analysis of traditional organic and inorganic toxic chemicals are often not applicable for the following reasons: a) unlike most organic pollutants, CNTs have a distribution of

lengths and diameters rather than a single molecular structure and, therefore, mass spectrometry methods, a key tool in current organic analytical methods, generally cannot be used; the large molecular weight of CNTs could potentially challenge mass spectrometric methods too; b) most techniques cannot distinguish between CNTs and naturally occurring black carbon allotropes (e.g., soot or charcoal), which are present at much higher concentrations in the environment than those modeled for CNTs[19]; c) several other carbon forms are often present in samples (e.g., natural organic matter; NOM) which may interfere with CNT quantification in the sample matrix; and d) the wide range of shapes, sizes, diameters, functional groups, and agglomeration states make it difficult to develop a universal analytical method for quantifying all types of CNTs. In addition, commercially manufactured CNTs may also contain substantial concentrations of metal catalysts, amorphous carbon, and graphitic (non-CNT) nanoparticles (NPs) which may cause biases with some analytical techniques, but are essential for other techniques.[20-22]

While there have been numerous analytical techniques used to quantify CNTs in various matrices,[4,14-16,23-38] for each technique there have only been a limited number of studies, often made by a single laboratory, and thus the robustness of the methods is unknown. In particular, relevant experimental parameters including comprehensive characterization of the CNTs and quantities used for testing and calibration procedures are not always reported. Moreover, failed attempts to apply new methods and techniques or to replicate approaches described in previous studies are often not published, and thus, the limitations of each technique such as potential biases for various matrices (e.g., water or soil with natural (NOM) or soil organic matter (SOM)) are often unclear. Overall, while some recent review papers have focused in part on CNT quantification,[4,39,40] many critical topics (e.g., interferences in key matrices

(environmental, biological, synthetic polymers), and the potential biases with CNT quantification from changes to the CNTs (e.g., oxidation)) related to the development of robust, precise, and reproducible CNT quantification methods have not yet been critically evaluated.

This manuscript reviews CNT quantification techniques and evaluates their applicability for different key matrices (water, soil/sediment, tissue) and different types of CNTs (i.e., single-wall carbon nanotubes (SWCNTs) or multiwall carbon nanotubes (MWCNTs)). We report a critical evaluation and comparison among the advantages and limitations of each technique including biases for relevant matrices, biases from physicochemical changes to CNTs in those matrices (i.e., oxidation/degradation, wrapping with organic molecules, and agglomeration), detection limits in various matrices, the potential for standardization, and the types of CNTs that can be analyzed. In addition, methods for extraction or separation of CNTs from different matrices, which may be necessary for sample preparation for some techniques, are enumerated. These quantification, separation, and extraction techniques may also be relevant for quantifying CNT loading in consumer products but the focus of this paper will be on scenarios relevant for assessing the potential environmental risks and fate of CNTs. For example, potential quantification techniques for representative scenarios related to environmental release and potential ecotoxicological effects are discussed. Future research topics to elucidate and improve the analytical performance of these techniques and CNT quantification in general are also highlighted. This paper is intended to serve as a reference to guide scientists in the area of CNT quantification through the selection of an appropriate technique given a type of CNT, sample matrix, and CNT concentration. Given the substantial literature on physicochemical properties and characterization of CNTs,[41,42] basic background information on these subjects is not

provided. While CNTs are also widely known to cause artifacts in many nanotoxicology assays such as by adsorbing key reagents,[26,43-45] this manuscript will focus on biases related to quantification of CNTs and not biases in the measurements of their potential toxicological effects.

Extraction and Separation Procedures for CNTs

Numerous techniques have been investigated to extract or separate CNTs from different matrices to overcome quantification limits in complex biological and environmental media (Table 1). In this manuscript, we define "extraction" as the isolation of analytes from a matrix by their physical transition from one phase into another. In contrast, separation means the isolation of analytes from themselves (e.g. differently sized CNTs), or from a matrix within a given phase (e.g. a mobile phase in chromatography or field flow fractionation). Successful extraction methods usually involve the suspension of CNTs in a specific media in which interfering compounds are less soluble, but the converse approach can also be utilized: removing the matrix while leaving the CNTs. However, most reported separation or extraction methods have only been used by a single research group in one or a small number of studies to partly or fully separate CNTs from an environmental matrix (e.g., asymmetric flow field flow fractionation (AF4), matrix digestion, and sonication with surfactants).[15,23,46] Other techniques have not yet been utilized with environmental and biological matrices (e.g., density gradient centrifugation, gel permeation chromatography, capillary electrophoresis, two-polymer phase extraction), but instead have been successfully applied to simpler matrices (e.g., deionized water) or have been used for CNT purification.[47-49] These techniques may be valuable for use with environmental and biological matrices and are also listed in Table 1. Conversely, there has been more

progress with extraction and analysis of fullerenes, another carbon nanomaterial, from complex matrices.[50-56]

Currently, many challenges remain in CNT extraction and separation strategies. First, it is unclear to what extent many of these techniques would be applicable for both MWCNTs and SWCNTs given the different properties of these two classes of CNTs, as most methods have only been applied to one or the other. This thought may be extended beyond the number of walls, to include any change in physicochemical properties (e.g., length, internal or external diameter, number of walls, or functional groups). Nevertheless, we expect that separation and extraction techniques may have to be tailored for a specific physicochemical property. For example, a method that can isolate short CNTs from a matrix could be ineffective when used against a population of long, highly entangled CNTs. Second, separation or extraction methods have not yet been applied to CNTs as utilized in potential consumer applications such as in polymer nanocomposite matrices. Given that CNTs will be released into the environment from consumer products, it is important to quantify the release of CNTs from these products after environmental stresses. It may also be important to quantify the concentration of CNTs in the consumer products, such as CNT-containing nanocomposites, to determine the potential quantity that could be released. Given challenges related to collecting and quantifying CNTs released from polymeric nanocomposites, one approach to estimate the quantity of CNTs released is to use a mass balance approach by quantifying the CNT concentration in a product before and after environmentally relevant degradation processes. For example, established methods are needed to extract CNTs from CNT-containing nanocomposites before and after the weathering and degradation processes (e.g., due to UV degradation and abrasion) to enable quantification of CNT concentrations.[57-60] This will allow scientists to more fully address the complete life

cycle of nano-enabled consumer products. Finally, extraction or separation procedures may change the physicochemical properties of the CNTs, potentially impacting the reliability of results from analytical methods. One such example is the matrix digestion approach described by Doudrick et al.,[23] which was suitable for subsequent analysis using thermal optical transmittance (TOT), but is potentially unsuitable for spectroscopic quantification by Raman scattering, because of concerns that the Raman spectra (e.g., ratio of D to G band) may be altered by the digestion procedure. Overall, although encouraging results have been obtained for a limited number of studies, the overall development of extraction and separation methods for CNTs from matrices for quantitative analyses is still a relatively new area of research.

Quantification techniques

A broad range of techniques have been developed to quantify or identify CNTs in environmentally and biologically relevant matrices (Table 2). In general, the techniques can be sorted into four groups: those that rely on the unique spectroscopic and thermal characteristics of the CNTs (that enable them to be distinguished from the matrix), those that utilize the presence of metal catalyst impurities (associated with the CNTs from the synthesis process), those that require isotopically enriched or depleted CNTs (e.g., with carbon-14 or carbon-13), and finally, microscopic techniques. There are large differences in the sensitivities and applicability of these techniques. Some thermal processes produce detectable gases (CO, CO_2), while others measure radiative heating of a sample. For example, the microwave method involved irradiating CNT containing samples with microwave radiation, wherein the carbon nanotubes absorb the microwave radiation, and the increase in temperature is proportional to the CNT concentration for a given matrix.[61,62] When comparing different studies, even those

using the same quantification technique, there is substantial diversity in the characteristics of the CNTs utilized.

It is evident from Figure 1 that, while some instruments used in the CNT quantification techniques are commercially available (e.g., UV/vis/NIR spectroscopy and Raman spectroscopy), most of the techniques require uncommon equipment that need to be partially or wholly custom built (e.g., microwave method, photoacoustic and photothermal imaging) or expertise that is not readily available. The use of uncommon instruments in these techniques also poses challenges for commercial ecotoxicity testing facilities to fulfill guidelines for standard methods related to maintaining a consistent exposure concentration.[63] While some analytical instruments that can be used to quantify CNTs are widely available (e.g., UV/vis spectrophotometry), some of them have significant potential interferences as will be discussed in detail in subsequent sections. To provide one example, challenges related to the use of UV/vis spectrophotometry have recently been described including absorption coefficients dependent on the CNT structure distribution and dispersion method, as well as decreasing absorption coefficients with CNT agglomeration and uncertainty in determining non-CNT from CNT contributions.[64,65] The lack of robust and widely available analytical methods likely contributes to the exclusive use of nominal concentrations to describe the exposure concentration and the absence of reported changes in CNT concentrations during experiments in many nanoecotoxicology studies.

Microscopic techniques can provide unambiguous identification of the CNTs in a complex matrix (e.g., transmission electron microscopy (TEM) analysis using electron energy loss spectroscopy or high resolution TEM),[27,66] but low or uneven distributions of CNTs on microscopy samples hamper the conversion of the number of

CNTs detected on (several) images to the number/mass concentration of CNTs in a sample. These limitations can be overcome, for matrices without substantial interferences, by using a centrifugation-based method to capture the CNTs from a known volume onto a microscopy sample holder (e.g. TEM grid). Under these conditions, frequency data (number of CNTs per area) can be converted into particle number and mass concentration metrics.[67-69] However, when one considers projected environmentally relevant concentrations of CNTs (typically ng to µg kg^{-1} solids),[70] the likelihood that one captures a CNT onto a microscopy grid with µg-sized environmental samples is exceedingly small. Overall, due to limitations related to the sample preparation issues (low CNT concentration especially compared to other solids, overlapping particles, and uneven distribution of CNTs onto the sample holders), results from electron microscopic techniques remain mainly on a qualitative level, and are currently of limited utility for quantitation.

While electron microscopic techniques are very helpful to confirm the identity of CNTs in a matrix if the CNT loading is sufficiently high, reliable controls of the sample matrix without CNTs, the CNTs alone, the sample holder, and any other interferences are needed to avoid false positive or false negative results, but these controls are rarely available for environmental samples. In addition, the amount of time required for sample preparation depends on the samples matrix and greatly varies among techniques. For example, obtaining TEM images suitable for automated image analyses may require that individual CNTs are evenly distributed on a TEM grid and do not overlap with other particles. This often requires elaborate and tailored extraction, dispersion and deposition techniques that are very time intensive to develop. In contrast, sample preparation for hyperspectral imaging microscopy is usually very fast, as liquid samples can be directly cast onto a microscopy slide and subsequently imaged.

246 However, the current commercial setup lacks the possibility for automated image
247 acquisition as well as suitable measures to determine the deposited sample volume,
248 which hampers its quantitative capabilities.

249 Due to the similarities between CNT structure and that of atmospheric soot or
250 carbon black, many analytical techniques that have been used for their extraction or
251 isolation from air, soil, or sediment have been also used to quantify CNTs (e.g., thermal
252 optical transmittance (TOT), chemothermal oxidation at 375 °C (CTO-375),
253 thermogravimetric analysis (TGA), and total organic carbon (TOC)).[14,16,18,71] While
254 TOT can measure CNTs, custom temperature ramping programs are required for CNTs
255 that differ from standard National Institute for Occupational Safety and Health
256 (NIOSH) methods used for soot analysis on atmospheric samples.[16] Similar
257 modifications may also help improve CNT quantification by other thermal techniques
258 such as CTO-375. Sampling of soot in air requires separation from the air, and usually
259 involves filters, impactors or centrifugal separation. Airborne CNTs would likely also
260 be captured by these techniques.[72-76]

261 All of the quantification techniques are critically assessed in subsequent
262 sections for the potential impact of matrix interferences or interferences from changes
263 that may occur to the CNT in different test systems or the natural environment. For
264 example, the impact of CNT degradation, as has been shown to occur enzymatically
265 and due to interactions with cells and bacteria,[77-83] and oxidation on the performance of
266 different analytical methods are evaluated. In addition, the limits of detection (LODs)
267 for these techniques in different media are compared and used to assess the potentially
268 relevant techniques for five case study scenarios. The potential for these techniques to
269 be standardized, a critical issue for regulatory agencies, is also discussed.

270 *Evaluation of potential matrix interferences for quantification procedures*

Perhaps the principal reason that quantification of CNTs in environmentally relevant matrices is challenging is because of matrix interferences, namely difficulties associated with detecting carbon in a carbon background, especially at modeled average environmental CNT concentrations.[70,84-86] The matrix characteristics that are most likely to cause interferences are described in detail in Table S1. Overall, natural waters and cell media (e.g., in studies with fish or human cells) have significantly fewer matrix interferences compared to biological tissues, soil/sediment, and released material from nanocomposites. For most spectroscopic measurements, while molecules and suspended particles in natural waters and cell media can potentially scatter incoming/outgoing light thus potentially biasing measurements, methods that account for these effects are generally available; in contrast, separation from the matrix is often needed prior to CNT quantification in tissues, soil/sediment, and fragments released from nanocomposites. For inorganic elemental analysis, having a constant and relatively low background metallic content of the matrix of the same element as the catalyst(s) of the CNTs is most important for all relevant matrices to achieve a low LOD and accuracy. Additionally, the multi-isotopic capability of the inorganic elemental analysis may enable qualitative and/or quantitative isotopic analysis when the isotopic ratios of the catalyst particles differ from those typically observed in the environmental matrix. For single-particle inductively coupled plasma-mass spectrometry (spICP-MS) analysis of CNTs, the background metallic content in nanoparticulate form in matrices is similarly important with regards to the accuracy of the measurement, while low background metallic content in dissolved form is necessary for achieving a low LOD. However, spICP-MS instruments operating at microsecond dwell times can only perform nanoparticle isotopic analysis for detection of two elements, a capability which nevertheless can be used to distinguish naturally occurring NPs from their engineered

296 counterparts.[87] While spICP-time of flight (ToF)-MS has recently shown the capacity
297 for multi-element analysis,[88,89] the size limit of detection was larger for gold and silver
298 NPs compared to quadrupole-based instruments.[89] Given the expected small amounts
299 of the catalysts associated with individual CNTs and challenges associated with
300 determining the background cut off level for SWCNT analysis using spICP-MS,[22] it is
301 unclear if spICP-ToF-MS will work well for CNT quantification.

302 Thermal techniques often do not show interferences with natural waters and cell
303 media, although there were technique-specific chemicals in these matrices (e.g.,
304 peptone in the media for TGA analysis)[90] that could impact the results. Two key
305 considerations for many of the thermal techniques are whether components in the
306 matrix can change the thermal stability of the CNTs and if there is the potential for
307 overlap in the oxidation temperatures of CNTs and combustible components of the
308 matrix. Thermal techniques could generally work in all matrices but the detection limit
309 will be higher in matrices with more interferences as will be discussed in a subsequent
310 section. Lower LODs may be achievable by first extracting the CNTs or decreasing the
311 bias from other forms of organic carbon.

312 Quantification of CNTs (and other carbon nanomaterials[91-95]) via isotopic
313 labelling generally has fewer interferences than the other techniques, but obtaining
314 isotopically enriched CNTs is typically challenging and/or expensive. Furthermore, this
315 approach is only relevant for laboratory studies, not for detecting CNTs released into
316 the environment. A related strategy, labeling CNTs with coatings containing a
317 radioisotope, was used in many early biodistribution studies in the biomedical field,[96-
318 98] but has not been used in environmental or ecotoxicological studies. The challenge
319 with this approach is that the accuracy of any measurement is contingent upon the
320 radioactive tracer remaining associated with the CNTs.

Natural abundance, stable isotopic measurements (e.g., carbon-13)[21] face similar limitations in that they require a CNT-free sample to which one can compare the isotopic composition in order to deploy the technique quantitatively. In laboratory studies, this is possible and more economically viable than radiolabeling techniques, but one has to carefully select CNT-free controls for quantifying CNTs in environmental samples. Furthermore, while the initial label is more expensive, the analytical techniques required to trace a carbon-14 label (i.e., liquid scintillation counting) are facile compared to the expert preparatory and analytical equipment required to trace natural-abundance isotopes (i.e., much lower levels of either carbon-14 or carbon-13 require accelerator mass spectrometers and isotope ratio mass spectrometers, respectively, and each with closed-tube-combustion preparation upstream). Nevertheless, the carbon source for SWCNTs produced using the high pressure carbon monoxide (HiPco) process is usually biomethane,[21] which has a strong naturally depleted carbon-13 signature, and such CNTs would be good candidates for using natural abundance, stable isotopic measurements.[78]

Evaluation of potential bias from changes to the CNTs

In addition to interferences from different environmentally and biologically relevant matrices, changes that may occur to CNTs while in these matrices can also cause interferences for many of the quantification techniques (Table S2). The extent to which agglomeration, degradation, and wrapping by other molecules occurs depends on the physicochemical properties of the CNTs and of the matrix. It is well known that CNTs will agglomerate in waters with sufficient ionic strength if they are not stabilized through, for example, a surfactant and that CNTs have a large capacity to adsorb natural organic matter.[99-102] With regards to CNT agglomeration, while most techniques are sensitive to this change (e.g., most thermal techniques, Raman, NIRF, UV/vis/NIR

absorbance, and spICP-MS), some are not impacted by it (e.g., inorganic element analysis) or may even be enhanced (e.g., hyperspectral imaging). Potential interference from CNT agglomeration may result in, for example: a) changes to the intensity or peak wavelengths in the spectrophotometry signals; b) shifts in the thermal stability of the CNTs, which could prevent separation from other components in the matrix, such as black carbon soot; or c) hindering uniform distribution on a filter prior to analysis by TOT. Agglomeration may also increase the heterogeneity and affect representativeness of the subsamples in a matrix, which could lead to increased uncertainty. However, larger subsamples could help lower the uncertainty when feasible.

The literature shows variable results on the degradation of CNTs in environmental matrices. In some studies, degradation of carbon-14 labeled CNTs by enzymes or bacteria has been shown to be slow or not detectable [77,78,103] except under specific situations with a special microbial consortium.[77] In contrast, studies assessing the degradation of non-carbon-14 labeled CNTs have often shown substantial degradation.[82] The cause of this discrepancy is unclear. Studies on the photodegradation of CNTs have shown significant modifications to their surface structure or the loss of fluorescence under some experimental conditions.[104,105] Thus, it is reasonable to assume that some degree of degradation could occur with CNTs in surface waters if they stay suspended for a sufficiently long period. Almost all quantification techniques are sensitive to CNT degradation and oxidation, although the degree of oxidation needed before it impacts quantification varies among techniques. One exception is carbon-14 analysis, which is not impacted by oxidation. In contrast, the degree of oxidation can directly impact CNT thermal properties and potentially the capacity to differentiate between CNTs and other forms of carbon present in the matrix using many of the thermal based techniques.

371 Wrapping of organic molecules around CNTs, such as proteins or NOM, may
372 also impact most quantification techniques. Many of the potential changes that could
373 cause biases, such as decreased signal intensity of a spectroscopic measurement or a
374 change in the thermal stability of CNTs for thermal measurements, are similar to those
375 discussed for degradation. However, the reason behind these changes is from the impact
376 of the coating on the CNT properties rather than a change to the core CNT material
377 itself as would occur during degradation. One challenge in discussing the potential bias
378 from organic molecules wrapping around CNTs, and also agglomeration and
379 oxidation/degradation, is that the magnitude of the bias relates partly to the degree of
380 agglomeration, oxidation, and the quantity of organic molecules associated with the
381 CNTs. It is possible to foresee examples when these changes in the environmental
382 matrices could have a bias, but it is challenging to quantify the magnitude of the
383 expected bias without information about the sample system (e.g., aqueous phase NOM
384 concentrations can range between 5 mg/L and 50 mg/L) or the extent of oxidation. This
385 information about the sample system or magnitude of likely changes could allow one
386 to account for biases.
387 Being aware of the potential biases present in a sample from these changes to
388 the CNTs and/or carrier matrix will support researchers in determining to what extent
389 these factors may impact their measurements. However, it might be challenging to get
390 this kind of information from samples with low CNT concentrations when there is a
391 low signal to noise ratio. Environmentally-relevant information on the rate of CNT
392 modifications (e.g., oxidation) by environmental processes is limited,[77,103,106-108] and
393 systematic studies of those processes would be an enormous benefit to parallel efforts
394 to quantify CNTs in the environment. While leaching of metal catalysts from the CNTs
395 in environmental matrices is not explicitly covered in the above changes to the CNTs,

396 it could dramatically impact analyses using spICP-MS or elemental analysis. The
397 potential for changes in the catalyst particles associated with the CNTs in
398 environmental matrices is the primary reason that these techniques are not more broadly
399 used despite their low LODs.

400 *Detection limits of quantification techniques*

401 The LOD for CNT quantification is one of the most critical performance metrics
402 required to compare the various techniques. However, the definition of the LOD
403 depends partly on how the CNT mass in a given sample is determined. The most
404 common approach is for the whole sample, including CNTs, catalyst particles, and any
405 carbonaceous impurities, to be included in the CNT mass used. It is possible instead to
406 only use the CNTs themselves, at least for SWCNTs where, after purification
407 procedures, the properties are more clearly distinguishable and high quality separation
408 techniques exist.[109] While additional metrics such as number or surface area
409 concentrations are highly desired,[63,110] the LOD values provided here are for mass
410 concentrations.

411 There are two different approaches for determining the necessary LOD for
412 quantifying contaminants in the environment. The first requires that the LOD is
413 adequate for quantification of the contaminant at concentrations that may have harmful
414 effects. An alternative requirement is for the analytical techniques to quantify the
415 contaminant at the concentration that it is determined or estimated to be present in the
416 environment. We have compared the LODs for the various analytical techniques using
417 both approaches through comparing the LODs to a species sensitivity distribution for
418 CNT acute toxicity to pelagic organisms (Figure 2) and to modeled environmental
419 concentrations (Figure 3). Several trends are evident from reviewing these figures.
420 First, the LODs in water span several orders of magnitude with some techniques only

capable of quantifying CNTs in samples with concentrations greater than 10 mg/L (e.g., gravimetric measurements), while the most sensitive techniques can detect concentrations between 0.1 µg/L and 1 µg/L (e.g., spICP-MS) (Figure 3). Second, the lowest LOD values are for pristine water samples and increase with higher amounts of potential interferences in the matrix. Higher LODs are observed when NOM is present in waters, and even higher LODs are typically achieved when using CNT quantification techniques in soils, sediments, and biological tissues. Third, multiple techniques appear capable of quantifying CNTs at concentrations relevant for stock suspensions (e.g., 10 mg L^{-1} to 100 mg L^{-1}) that could be used for pelagic aquatic toxicity testing (Figure 2). As discussed in more depth in a case study, some techniques could also be used to quantify the initial exposure concentration for ecotoxicity testing and the concentration after the experiment concludes. Fourth, the LODs are often orders of magnitude higher than the average modeled environmental concentration, but some are within the range of modeled sediment concentrations despite the lower LODs for CNT quantification in sediments. This suggests that it may be feasible to quantify CNTs in the environment under certain conditions. Overall, these figures can be used to assess which methods may offer suitable techniques for an intended purpose, as is described in more detail in the case studies. Alternatively, extraction or separation techniques (see above) may be necessary to selectively isolate and concentrate the CNTs prior to analysis.

Potential for standardization

There are numerous reference materials (RM; e.g., UV/vis spectroscopy calibration standards) and standard methods that can support the standardization of CNT quantification techniques (Table S3). In addition, there are multiple CNT RMs and representative test materials (Table S4); RMs have assigned values for certain properties, whereas representative test materials are only guaranteed to be stable and

446 homogeneous with respect to one or more specified properties but may be used in the
447 development of test methods which assess properties other than those for which
448 stability and homogeneity have been shown.[111] Currently, three RMs are available for
449 SWCNTs, while MWCNTs are only available as representative test materials. The
450 careful characterization of the CNT RMs may be useful for the standardization of
451 numerous techniques, given the wide range of properties that have been certified (i.e.,
452 the sources of uncertainty are thoroughly understood and the certified values have
453 meaningful metrological traceability) or for which information values are provided
454 (i.e., the sources of uncertainty are not fully understood or a limited number of analyses
455 were performed). Standardized methods are also already available for characterization
456 of CNTs (e.g., Raman spectroscopy and NIR fluorescence characterization) which
457 could be modified to develop standard methods for CNT quantitation.[42,112-118] In
458 addition, a modified version of a NIOSH standard method for use of TOT for elemental
459 carbon analysis (NIOSH Method 5040) could potentially be used for CNT
460 quantification. However, the robustness of this method for CNTs will still need to be
461 evaluated for different matrices. Extraction and separation procedures also need to be
462 standardized but are not addressed in this section due to the limited number of studies
463 on this topic. Research topics that would support the standardization of these techniques
464 are described in the Future Research Topics section.

465 **Case studies**

466 In this section, five case studies will be used to illustrate how the quantitative
467 methods described in this manuscript could be utilized to address hypothetical
468 situations requiring CNT quantitation. The scenario for the first two case studies is that
469 scientists are asked to determine whether the concentration of CNTs in a stream
470 receiving effluent from a treatment plant where CNTs may be released is above 500 μg

L^{-1}; this concentration was chosen because it is approximately 50 % of the lowest LC_{50} value of the species sensitivity distribution shown in Figure 2. This scenario will be discussed in the context of whether the CNT characteristics (e.g., SWCNT or MWCNT, catalyst materials, and thermal properties) are known *a priori* or not. In the third case study, scientists will be trying to measure the exposure concentration to organisms during a laboratory ecotoxicity experiment in a water only system with an organism that has an EC_{50} value (the concentration at which 50 percent of the organisms are affected) of 10 mg L^{-1} and the lowest concentration tested is 1 mg L^{-1}. In the fourth case study, CNTs with known characteristics are accidentally released into a lake, and scientists are asked to determine the concentration in the lake sediment. In the fifth case study, "OECD Test 305: Bioaccumulation in fish: aqueous and dietary exposure" is performed using a known type of CNTs and the scientists need to quantify the concentration in the fish tissues.

Case I: CNTs with known characteristics are released into a river

First, identify the techniques that may have LODs better than 500 μg L^{-1} using Figure 3: UV/vis spectroscopy, inorganic elemental analysis, spICP-MS, NIRF, Raman spectroscopy, TOT, and carbon-14 labeling. Electron microscopy should, in principle, be able to detect CNTs at these concentrations, but it may be challenging to identify CNTs amidst the other particulate matter, and quantification will be challenging as discussed above. Of particle risk is the ability to collect a representative sample where the TEM thin section actually contains a statistically significant number of CNTs. Nevertheless, electron microscopy could be used for a qualitative assessment or to confirm the presence/absence of CNTs based on results from the quantitative analysis. Among the quantitative techniques, the choice of which technique to employ first would depend on numerous factors such as their availability and if the unique properties of the

CNTs of interest may eliminate some of the analytical techniques from consideration (e.g., quality assurance (QA), techniques only applicable for SWCNTs would not be relevant for MWCNT quantification). For example, carbon-14 labeling would not be relevant for field measurements, while NIRF would only be applicable for SWCNTs.[15] In addition, Raman spectroscopy analysis would require preconcentration of the sample to yield the desired LOD which may be challenging.[13] Next, the properties of the river water prior to the discharge location (e.g., thermal profile, elemental composition and organic matter concentration of the water) could be evaluated to assess what biases may be encountered during CNT quantification for various techniques. If it is possible to obtain the CNTs of interest, a next step would be to prepare a CNT dispersion, mix the dispersion with stream water prior to the location of discharge, and then analyze the water using the quantification technique(s) to determine relevant QA/quality control (QC) characteristics such as the LOD, reproducibility, bias, signal to noise ratio, and linearity of calibration curve. It may also be important to test the stability of the CNT in the water prior to the discharge location to assess if agglomeration or oxidation of the CNT could cause a bias; if agglomeration causes a significant bias, it may be possible to disperse the samples such as by adding a surfactant or sonicating the sample. If the QA/QC characteristics are sufficient to provide the needed level of statistical significance for the quantification measurement, the final step would be to analyze the test samples.

Case II: CNTs with unknown characteristics are released into a river

The process is substantially more complicated if characteristics of the CNT to be detected are unknown. First, it would be helpful to obtain water samples before and after the point source discharge location. It would then be possible to do some measurements to try to determine if characteristics of the river water reflective of CNT

characteristics are changed. For example, an elemental analysis or spICP-MS analysis of the river waters could be conducted to assess if uncommon elements (e.g., yttrium) or ratios of elements (e.g., cobalt to molybdenum) often used for CNT catalysts are present at different concentrations before and after the location of discharge; measuring these samples before and after filtering could reveal if the metals are associated with particles such as CNTs. One distinct advantage of the metal analysis techniques is that the LODs for many of these elements are orders of magnitude better than the limit of detection needed for the CNTs (Figure 3). This information supported by other characterization techniques (e.g., TEM analysis to assess if SWCNTs or MWCNTs can be identified) could help determine the type of CNT being used. An alternate first step would be to obtain a sample directly at the discharge location and conduct these analyses. The advantage of this approach is that there would not be dilution of the CNTs, but the matrix may be substantially more complex (e.g., wastewater treatment plant effluent). A next step is to spike known concentrations of the specific CNT if identified, or alternatively RM SWCNTs and representative test material MWCNTs, into the river water prior to the discharge location and determine the QA/QC characteristics for the selected techniques and the extent to which agglomeration or oxidation could influence the results. If acceptable results can be obtained with the specific CNT (if identified) or the RM CNTs, then analysis can be conducted on the river sample after the location of discharge.

Case III: Laboratory Ecotoxicity Study

The third case study involves a laboratory ecotoxicity experiment during which the concentration remaining suspended during the experiment needs to be quantified. Depending upon what organism is tested, there may be interferences such as algae or bacteria which remain suspended and have CNTs associated with them. If it is

straightforward to separate the test organisms from the media with suspended CNTs, numerous techniques may be applicable for quantifying the initial CNT concentration in suspension ($\geq$ 1 mg L^{-1}) (see Figure 2). The techniques available to determine the change in concentration during the experiment depend on the LOD needed for these measurements. For example, if it is unlikely that the CNT will settle during the experiment, numerous techniques would enable measurements to show that the concentration remained within 20 % of the initial concentration, the desired maximum concentration loss indicated in many OECD tests.[63] However, if substantial settling occurs, it is necessary to determine the lowest detection limit needed (e.g., 0.1 mg L^{-1} to quantify a loss in concentration of 90 % of the initial concentration). When measuring the CNT concentration dispersed in tests with suspended unicellular organisms or small multicellular organisms (e.g., *Tetrahymena thermophila*), the cells themselves may cause biases or require the extraction of the CNTs. It is also unclear if CNTs that are suspended but associated with cells should be counted as part of the total suspended concentration. Nevertheless, many techniques could likely still be used to quantify the total suspended concentration but control experiments to test for potential biases from the cells and the matrix would need to be conducted prior to starting the experiment.

Case study IV: Quantification of CNTs with known characteristics in lake sediment

Quantifying CNTs in sediments is substantially more difficult than in water samples. As shown in Figure 3, the LODs for most techniques are at least an order of magnitude higher in soils and sediments compared to in waters. To quantify CNTs in sediments, a first step would be to obtain "clean" sediment from another water body ideally with similar sediment characteristics. Because the CNT type is known in this case study, it is possible to spike this clean sediment with CNTs and then assess the quality of the analytical results (*e.g.*, linearity, LOD, etc.). The suitable techniques for

571 this analysis will depend upon instrument availability, the type of CNT (e.g., NIRF after
572 CNT extraction has been shown to be a valuable technique for analysis of SWCNTs in
573 sediments[15] but is not applicable to MWCNTs), and the estimated range of probable
574 CNT concentrations in the sediment. If satisfactory LODs are not available for the
575 available techniques in the reference sediment, it may be necessary to investigate
576 extraction or separation methods to decrease the LOD (e.g., [15,46]). Given the low
577 detection limits obtained using NIRF after extraction (62 µg/kg),[15] challenges with
578 obtaining a better LOD are likely only to be problematic for MWCNTs unless the
579 SWCNTs are oxidized or modified to the extent that NIRF is not applicable or NIRF is
580 not available for sample analysis.
581 *Case study V: Quantification of CNT in fish after a standard toxicity test*
582 Assessing potential bioaccumulation of chemicals in organisms is an important
583 component of risk assessment of chemicals. One frequently used test is OECD method
584 305: Bioaccumulation in fish: aqueous and dietary exposure.[119] Again, the LODs for
585 quantifying CNTs in organism tissues are greater than those in water, yet similar to the
586 LODs for soils and sediments (Figure 3). While the whole fish is usually analyzed in
587 this method, it may be beneficial to test the CNT biodistribution in addition to the total
588 concentration in the fish. This is important because CNT translocation across the gut
589 tract is rarely observed in ecotoxicological studies.[27,31,120,121] If the biodistribution of
590 SWCNTs is evaluated, then the technique with the best LOD is NIRF microscopy
591 which has been reported to detect individual SWCNTs.[31,121] If this instrument is not
592 available, Raman microscopy and electron microscopy can be used to assess
593 biodistribution of CNTs in organisms although it is important to carefully avoid
594 artifacts;[27,43,122,123] however, one should note that G/D ratios are strongly influenced by
595 any sp^2 or sp^3 hybridized carbons present in the organism for Raman microscopy

596 analysis. Other microscopic approaches such as photothermal/photoacoustic imaging
597 have also been successfully used to assess the distribution of CNTs in plants, yet are
598 infrequently available (Figure 1).[24] To quantify the total concentration of CNTs in the
599 fish, it is possible to use NIRF microscopy for SWCNTs,[31] but extraction from the fish
600 tissue will likely be needed for MWCNTs. An extraction procedure has been published
601 for MWCNTs in rat lungs followed by quantification using TOT,[23] but this approach
602 has not yet been used in tandem with other quantification techniques or with fish tissues.
603 If carbon-14 labeled CNTs are available, assessing uptake by and biodistribution in fish
604 through carbon-14 labeling is a viable approach.[124] The microwave method has also
605 shown promise for detecting MWCNTs in biological samples (e.g., earthworms) but
606 requires custom built equipment.[62,125]

607 **Future Research Topics**

608 The analysis that we present here on the current state of the science with regards
609 to quantification of CNTs in matrices relevant for nanotechnology environmental health
610 and safety measurements also reveals several key future research topics to move this
611 field forward. First, most of the quantification techniques developed for aqueous
612 environments will have potential biases or a higher LOD in complex matrices such as
613 soils and biological tissues. Thus, the continued development of CNT extraction and
614 separation procedures for environmental and biological matrices is a critical topic for
615 additional research. Nevertheless, addressing the quality of the CNT separation depends
616 in part on the robustness and precision of the subsequent analytical techniques, which
617 also need to be improved. Second, sensitivity analyses of techniques can provide
618 relevant information regarding the robustness of an experimental procedure to minor
619 changes to a protocol and the contributions of various steps to the total uncertainty of
620 the result. This approach and related approaches such as cause-and-effect analysis can

621 highlight which steps of a protocol need to be carefully followed to ensure a reliable
622 result and which steps are less critical.[126] Third, interlaboratory comparisons, where
623 multiple laboratories use the same protocol, are needed to standardize the more mature
624 techniques and extraction and separation procedures. While it is necessary to assess
625 many topics related to analytical precision of a single laboratory (e.g., within and
626 between operator variability, instrument to instrument variability, day-to-day
627 variability, all contributing to the within-laboratory repeatability), interlaboratory
628 comparisons can provide unique information about the comparability of results among
629 laboratories (i.e., between-laboratory reproducibility) and potential factors in the
630 protocols that need to be controlled to standardize the procedure. Such information is
631 needed to provide estimates of the bias and precision of an analytical method. Fourth,
632 analyzing an individual or set of homogenized test samples using multiple techniques
633 will be helpful in highlighting method specific biases and the comparability of results
634 among methods (e.g., similarly to a black carbon quantification ring trial[127]). This
635 differs from interlaboratory comparisons in that a single sample is analyzed by multiple
636 techniques, as opposed to different laboratories using the same technique and test
637 method. Similar results among orthogonal techniques would lead to greater confidence
638 in the results of the methods while different results could yield insights into biases,
639 strengths, and limitations of different methods. For example, in a recent study on the
640 fate of SWCNTs in a mesocosm, an experimental setup designed to simulate the natural
641 environment that often includes multiple species and which has been used in several
642 nanotoxicity studies,[128,129] both NIRF and elemental analysis were used on the same
643 samples.[29] The agreement among these methods suggested that elemental analysis may
644 be a useful approach in these complex matrices if the catalysts used to synthesize the
645 CNTs are of an element with low concentrations in the matrix (e.g., Mo).[29] A similar

approach could be used to compare among different extraction or separation techniques with a single sample. Fifth, isotopically enriched or depleted CNTs[21,78] could be used to help develop other orthogonal techniques given that isotopic techniques often have the fewest biases for many of the matrices and changes that could occur to the CNTs in these matrices. Such an approach was used by Schierz et al. to develop the NIRF technique for quantification of SWCNTs in sediments after extraction by also testing the extraction procedure with carbon-14 labeled SWCNTs.[15] Sixth, using extraction and/or separation techniques in combination such as AF4 followed by capillary electrophoresis could be another promising avenue for future research. Lastly, almost all quantitative techniques require known CNTs to yield information about their characteristic information (e.g., thermal profile, metal catalyst, impurities, NIR spectra, and Raman signature). Additional work is needed to develop techniques for quantification of unknown CNTs in an environmental or biological matrix. Along these lines, the impact of CNT heterogeneities (e.g., different lengths) on their quantification could also be helpful.

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Supporting Information

Tables describing potential matrix interferences and interferences from changes to the CNTs on selected CNT quantification techniques, standard reference material carbon nanotubes, and standards and references materials related to standardization of carbon nanotube quantification techniques. This information is available free of charge via the Internet at http://pubs.acs.org.

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Technique	Overview	Strengths	Limitations
Asymmetric Flow Field-Flow Fractionation [46,47,130-133]	Flow-assisted separation technique based on particle diffusion against a hydrodynamic field in the absence of a stationary phase	Enables separation of well dispersed CNTs by length, reduces sample polydispersity, possibility for online/offline coupling with a variety of analytical techniques can yield complementary information	Time-consuming and laborious operation/method development, high sample dilution during the analysis, possibility of strong particle-membrane interactions may result in low recoveries, separation less efficient (low number of theoretical plates) than with e.g., capillary electrophoresis
Capillary Electrophoresis[134-137]	Based on the different electrophoretic mobilities of the species (on the basis of their charge/size ratio) through an electrolyte contained in a fused silica capillary when an electrical field is applied; a suspension of CNTs, usually in a surfactant, is subjected to an electric current	Potential separation of bulk samples of CNTs according to the charge/size ratio, length sorting and separation according to bundled/non-bundled can occur; high theoretical plate number, thus potentially superior resolution power, due to the plug-like flow of the electroosmotic flow	Laborious sample preparation for controlled experiments, several important challenges still remain, including limited sensitivity, non-quantitative recoveries, and reproducibility problems; micellular electrokinetic chromatography cannot be used, as CNTs are too large to reside in the intramicellular region
Centrifugation[138]	Large suspended particles are removed first on basis of difference in sedimentation velocities	Potential isolation of CNTs from matrix, either in sediment or supernatant	Protocol will depend on CNT and matrix, further separation of the fraction is challenging without disturbing neighboring fractions
Density gradient centrifugation[139,140]	Particles will equilibrate to their isopycnic (equal bouyancy point) in a density gradient at sufficiently high applied acceleration	Can enable extraction of specifically modified subpopulations, resolves aggregate states	Low processing quantity, kinetic and transport non-idealities can occur, different aggregation states have different buoyant densities.
Size exclusion chromatography[48,141-147]	A chromatographic method that separates analytes based on their size and shape by differential exclusion from the pores of the stationary phase; no interactions must exist between CNTs and the stationary phase	Relatively simple and inexpensive, good size separation for SWCNTs within a certain length limit and shape	It has mainly been used for short single-walled carbon nanotubes; it is unclear if this technique can separate larger SWCNTs or MWCNTs; prefiltration might be needed; agglomerates can get trapped within the chromatographic column or the prefilter; well dispersed suspensions are required; only for qualitative analysis; no environmental samples have been tested
Matrix Digestion[23]	Different chemicals or solutions are used to dissolve the matrix (e.g., tissues) to facilitate subsequent analytical techniques	Lowers detection limits and removes potential biases for many techniques	Different approaches will likely need to be developed for each type of matrix (e.g., tissue vs. sediment) and may need to be developed for different types of tissues
Micro-nanofiltration[13,148]	Use of micro and nanopore-sized filters to separate analytes based on their size	Very simple and inexpensive; at low CNT concentrations, can treat larger volumes than other techniques	Mainly used for CNT suspensions with very little interferences and at low concentrations to avoid clogging the filters; it is difficult to regenerate the CNT suspension for further characterization/quantification; there might be sample losses and filter interferences
Selective Oxidation[14,18,149]	Use of thermal or chemical oxidation to separate more refractory carbon fractions (CNTs) from more labile organic carbon	Allows for a cleaner (and easier) subsequent characterization or quantification	Not very reliable in the presence of interfering material or when the oxidation is not complete (e.g. coals, very rich organic carbon environments); recoveries might vary between different types of CNTs

Sonication with surfactant[15]	Use of a surfactant to create a stable CNT suspension that can then be separated from the remaining non CNT material that settles down at a different speed	Can extract CNTs with varying surface chemistry from sediment, no special equipment is necessary	Recoveries vary among SWCNTs with no recognizable pattern; repeatability varies, surfactants may interfere with quantitation procedure

Table 1: Extraction and separation techniques to isolate CNTs from environmental and biological matrices

Method	Overview	Strengths	Limitations
Spectroscopic			
Absorbance[65,109,150,151]	Measures absorbance of aqueous sample; can include ultraviolet, visible, or near infrared wavelengths	Readily available in many environmental laboratories	Interference from other sample components, relatively high detection limit, only applicable for aqueous samples
Near infrared fluorescence (NIRF) [15,29,31,152]	A specific emission spectra can be used as an identification tool of SWCNTs; the intensity of the fluorescence signal can be used for quantification of SWCNTs	Quantification/Detection at very low limits of detection	Limited to non-functionalized SWCNTs; semi-conducting SWCNTs but not metallic SWCNTs can be detected
Raman[13,122,123,153-164]	Measures radial breathing (SWCNT) , G, D and G' vibrational bands in dry and various solvent suspended samples, tissues	Minimal sample preparation, enables CNT characterization, compatible with *in vitro* and *in vivo* samples, can be used with a microscope, low detection limits achieved using resonance Raman conditions	Some matrices may produce interferences, sensitive to laser power, requires calibration for quantitative analysis
Spectrometric			
Inorganic Element Analysis[29,32]	Measures trace catalytic metallic elemental impurities intercalated in the CNT structure (Cr, Co, Cu, Fe, Mo, Ni, Y, Zn), analysis of bulk metal content; the applicability of this approach could be impacted by removal of the metal catalysts by purification but catalysts located within the CNTs often remain after purification processes	Multi-elemental capability and extreme sensitivity of ICP-MS allow an accurate and selective determination of metal impurities of CNT in a wide range of matrices at ngL^{-1} or sub ngL^{-1} levels, the rapid sample throughput of this method is attractive for routine screening	Carbon is generally not detectable with standard ICP-MS methods, quantitative sample dissolution is required prior to analysis; incomplete sample digestion, release of metal ions from the CNTs in the sample matrix, or elemental contamination from the sample digestion steps could lead to an important bias in the bulk metal content determination; the feasibility of using this technique could depend partly on if the metal contents of the CNTs are known *a priori*
Single particle inductively coupled plasma-mass spectrometry (spICP-MS)[22]	Metal catalyst impurities are used as proxies to detect and quantify CNTs; the applicability of this approach could be impacted by removal of the metal catalysts by purification but catalysts located within the CNTs often remain after purification processes	Potential capability for the size, size distribution, and particle number concentration determination of CNT; high selectivity to differentiate CNT at extremely low concentrations from naturally occurring carbon-containing species (i.e. cells, organic detritus, humic acid); very low detection limit	Size/length estimation requires the invalid assumption that metal content is homogeneous among the CNTs, very small particles cannot be separated from the background, leaching of catalysts in the sample matrix prior to spICP-MS analysis can bias the result, only applicable for aqueous samples; the feasibility of using this technique could depend partly on if the metal contents of the CNTs are known *a priori*
Microscopic			
Atomic Force Microscopy[109,165]	Measure the surface features of a sample by dragging a cantilever over the sample; the length of identifiable tubes can be determined by the movements of the cantilever	Most trusted technique for determining number and length	Deposition bias, measurement bias, and detection errors are all possible in most samples
Hyperspectral Imaging[166,167]	Measures reflectance spectra of NPs in a darkfield (visual near infrared /short-wave infrared spectral range), resulting in 2D-optical images with full spectral information that contain a full spectrum (400 nm to 1000 nm or 900 nm to 1700 nm, respectively) in each pixel; CNTs appear bright against a dark background	Easy sample preparation, provides optical (i.e. differentiation between single nanotube and nanotube-agglomerate) and spectral information, allows spatial localization of particles, can provide semi-quantitative information, short-wave infrared spectral range could be applicable for detection of SWCNTs	Currently long analysis times, visual near infrared not specific for CNTs, many potential analysis artifacts

Photoacoustic (PA)[24,168-170]	PA measures the acoustic response to the rapid volume change resulting from the absorption of an optical pump beam and the transfer of heat to the surrounding environment	Suitable for detection in liquids such as water and complex media such as plants, minimal sample preparation, can be quantifiable, excellent penetration depth enables samples > 100 µm, works equally well with metallic and semiconducting SWCNTs and MWCNTs, label free, unaffected by some complex media issues including carbon-on-carbon	Signal is dependent on absorption and heat transfer to material surrounding the CNTs, can be 10x lower sensitivity than PT, medium surrounding CNTs must be transparent to the beams, heating laser must overlap with absorbance of the CNTs, signal scales with size of CNT cluster, non-transparent media may cause detection issues, quantification may require diameter and length distributions
Photothermal (PT)[24,168,169]	PT measures the optical scattering response of a probe beam to the change in local environment refractive index that results from the absorption of an optical pump beam and the transfer of heat to the surrounding environment	Suitable for detection in liquids such as water and complex media such as plants, minimal sample preparation, can be quantifiable, penetration depth can handle samples up to 10 µm, works equally well with metallic and semiconducting SWCNTs and MWCNTs, label free, unaffected by some complex media issues including carbon-on-carbon, sensitivity down to single particle sensitivity, lower LOD than absorbance-based measurements	Same as Photoacoustic plus is limited to thin samples (< 100 µm)
Scanning Electron Microscopy and Scanning Transmission Electron Microscopy	Measures the interaction of a finely focused electron beam with the CNTs; secondary electrons, and transmitted electrons can be used for image formation	Provides detailed morphological properties (length, width, shape) of individual CNTs; individual CNTs can be localized in complex matrices based on morphological criteria	Labor intensive, often only qualitative information
Transmission Electron Microscopy (TEM)[27,66]	Illuminates a selected sample area (parallel electron beam) and detects the transmitted electron after passing through the samples	Provides detailed morphological properties (length, width, shape) of individual CNTs; high resolution can be used to distinguish between SWCNTs and MWCNTs; CNTs can be identified in energy filtered TEM images	Challenging sample preparation for tissues; it may be very hard to detect NPs in complex samples at low concentrations; low contrast (conventional TEM) due to reduced interactions between CNTs at the electron beam at high acceleration voltages
Thermal			
CTO-375[18]	Quantification of carbon that remains after combustion at 375 °C for 24 h under excess air sample and subsequent chemical oxidation	Particularly good for complex matrices such as soil and sediment	Not fully tested for suspensions, requires high concentrations of CNTs and low concentrations of interferences (e.g., soot interfering with MWCNTs or graphene with SWCNTs)
Thermal Gravimetric Analysis (TGA) [20,171,172]	Quantification of mass percentage of phases with distinct thermal stabilities under a variety of reactive atmospheres (usually air) and relatively rapid temperature programs (e.g., heating rates of 5 C/min to 20 C/min,; room temperature- ca. 950 °C); each sample takes 1 h to 2 h total	A rapid technique that allows the quantification of multiple phases in a single sample, good for complex matrices, no special sample preparation needed	Effect of thermal ramp rate and reactive atmospheres on apparent phase distribution is not well understood (and is largely ignored), detection limits are relatively high for solid matrices, potential for interferences between sample matrix (e.g., other carbon nanomaterials, soot, or black carbon) and CNT decomposition temperatures
Thermal Gravimetric Analysis-Mass Spectrometry (TGA-MS) [14]	TGA coupled with mass spectrometric detection of evolved gas fragments, typically in the 2 to 300 m/z range	Mass fragments can give insight into the chemical structure of the source material (e.g., C/H/O ratios or unique evolved fragments)	Current mass spectrometers have poor mass resolution (ca. 1 amu), relatively high detection limits, and low sampling rates relative to the chamber flush rate (i.e., consequently, only a small portion of the evolved mass is transferred to the MS); all reduce identification accuracy and increase detection limit

Total Organic Carbon (TOC) Analysis[71]	TOC analysis can be conducted on water or soil samples by oxidizing (chemical, heated catalyst, UV) carbon to carbon monoxide or dioxide which is detected by infrared or other detectors	TOC analysis of waters has been used to measure CNTs in stock solutions in water	Very little optimization of temperature or catalytic conditions have been examined; its application to CNT stock solutions have been consistent with prepared masses; any organics, such as natural organic matter, in solution or soils would interfere; this is a non-specific method and thus matrices that contain sufficiently high concentrations of other carbon nanomaterials (e.g., graphene), soot, or black carbons would impact the technique
Thermal Optical Transmittance (TOT) [16,23]	As the sample is analyzed under programmed temperature, the volatilized and combusted carbon travels to an oxidizing oven, where it is transformed into carbon dioxide (CO_2); the amount of elemental carbon is determined based on the CH_4 signal measured using a flame ionization detector; sample is first heated under inert conditions to remove volatile organic carbon, then oxidizing carrier gas is used for elemental carbon; the portion of TC that is organic carbon or elemental carbon is defined by the method, which determines where the organic carbon-elemental carbon split is placed post-analysis; this split can be automatic on the basis of automatic optical correction; the optical transmittance or reflectance is observed throughout analysis, and the split is placed where the transmittance/ reflectance returns to the initial reading; for samples in which optical correction does not work, a manual split defined by the analyst should be used	Very reliable technique for detecting elemental carbon in environmental matrices, this technique could differentiate between types of CNTs based on their thermal stability	Too much organic carbon in a sample causes peak overlapping between elemental and organic carbon which affects the accuracy; similar carbonaceous materials such as graphene and fullerene will be counted in the CNT peak if they exist in the sample; unless the peak from CNT is far enough from other carbonaceous material, it is difficult to exclude the other carbonaceous materials but adjusting the temperature program might help to some extent
Isotopic labeling			
Carbon-13 Labelling[21,32,78]	A measure of the ratio of ^{13}C to ^{12}C, applicable for all CNTs but works best for isotopically enriched or depleted CNTs	Instrumentation is readily available in many environmental laboratories	Highly dependent on matrix and large variability may be observed for CNTs that are not specifically ^{13}C enriched
Carbon-14 Labelling[15,26,30,99,124,152,173-181]	Measures beta emissions from carbon-14 emissions, can be used to quantify liquids after mixing with scintillation cocktail or any matrix after combustion in a biological oxidizer, autoradiography can provide spatial distribution of radioactivity	Provides definitive quantification of CNTs in complex matrices, can be used as an orthogonal technique to develop other analytical techniques, can be used to identify degradation products	High cost to synthesize radioactively labeled CNTs, safety concerns, limited availability of radioactively labeled CNTs

Other radioactive isotopes[96-98]	Measures release of emissions from a radioactive isotope that is associated (e.g., attached to a polymer wrapping the CNT) with the CNT	This approach can enable extremely low detection limits, can be used with a range of CNT surface functionalizations, non-destructive sample is possible for gamma emitters	Artifacts are possible if the radioactive isotope becomes separated from the CNT, it may be challenging or impossible to determine if this occurred in complex matrices without orthogonal CNT quantitation techniques
Additional Techniques			
Analytical Ultracentrifugation (AUC)[60,165,182-184]	Measurement of sedimentation velocity distribution, can be used to determine particle density or size/shape distribution	Can measure entire CNT population via absorbance or interference measurement, high resolution, little size bias	Finicky technique that requires well understood and controlled samples for robust analysis
Gravimetric[185]	The CNT concentration in suspension is estimated by drying a fraction of the suspension and weighing it, or by determining the fraction of CNTs not suspended during the dispersion process (e.g., by sonication) by weighing the mass of CNT particles at the bottom of the container	Uses readily available equipment	Limited to high CNT concentrations, only applicable for aqueous suspensions
Microwave Method[62,125,186,187]	Measures the temperature rise of a sample at a specific microwave energy within a specific timeframe	Straightforward method for CNT detection and quantification in biological tissue, low cost	Not commercially available; it still remains to be investigated for environmental samples if interferences arise from other carbon allotropes with similar behavior in the microwave field (e.g., carbon black, soot)[188]
aF4-MALS[46]	Measures a shape factor (ρ=radius of gyration/hydrodynamic radius) of particles present in a complex liquid sample (e.g. surface water, leachate, soil and sediment extract), which is indicative of the particle aspect ratio; comparing these results to a CNT-free sample can then be used for CNT detection	Allows for CNT detection in water, soils, and sediments; may be useful in exposure studies	Need for the baseline of a CNT-free sample, full quantitative use currently not straightforward, often low CNT recoveries for aF4

Table 2: Selected techniques for CNT Quantitation

Abbreviations: Asymmetric flow field flow fractionation with multi-angle light scattering (aF4-MALS), analytical ultracentrifugation (AUC), carbon nanotube (CNT), chemothermal oxidation at 375 °C (CTO-375), inductively coupled plasma-mass spectrometry (ICP-MS), near infrared fluorescence (NIRF), multiwall carbon nanotube (MWCNT), photoacoustic (PA), photothermal (PT), single particle inductively coupled plasma-mass spectrometry (spICP-MS), single-wall carbon nanotube (SWCNT), transmission electron microscopy (TEM), thermal gravimetric analysis (TGA), thermal gravimetric analysis-mass spectrometry (TGA-MS), total organic carbon (TOC), thermal optical transmittance (TOT).

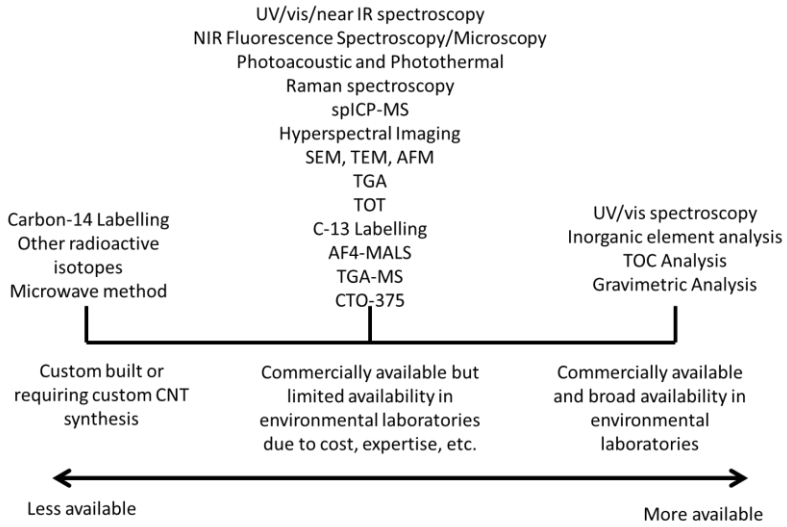

Figure 1: Availability of CNT quantification techniques.

Abbreviations: Asymmetric flow field flow fractionation with multi-angle light scattering (AF4-MALS), analytical ultracentrifugation (AUC), chemothermal oxidation at 375 °C (CTO-375), near infrared fluorescence (NIR), single particle inductively coupled plasma-mass spectrometry (spICP-MS), thermal gravimetric analysis (TGA), thermal gravimetric analysis-mass spectrometry (TGA-MS), total organic carbon (TOC), thermal optical transmittance (TOT), scanning electron microscopy (SEM), transmission electron microscopy (TEM), atomic force microscopy (AFM).

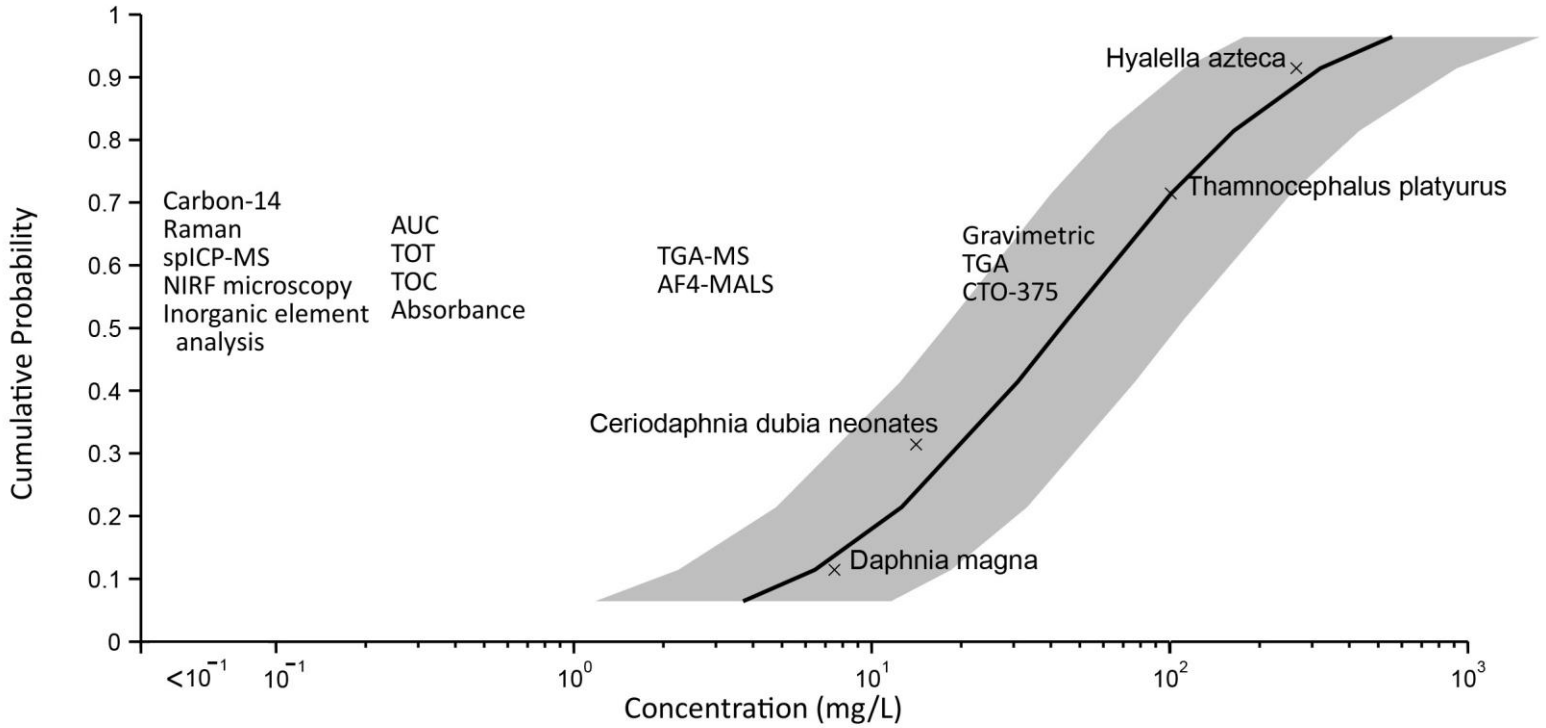

Figure 2: Comparison between detection limits for analytical techniques in a water-only media under optimal conditions juxtaposed with a species sensitivity distribution for CNTs for acute toxicity testing of pelagic organisms. For the species sensitivity distribution, the 95 % confidence for the LC_{50} values is shown by the gray shaded area around the curve. The detection limits for the techniques span a range of one order of magnitude (e.g., 1 mg/L to 10 mg/L). This figure is modified with permission from Garner et al.[189]

Abbreviations: Asymmetric flow field flow fractionation with multi-angle light scattering (aF4-MALS), analytical ultracentrifugation (AUC), chemothermal oxidation at 375 °C (CTO-375), near infrared fluorescence (NIRF), single particle inductively coupled plasma-mass spectrometry (spICP-MS), thermal gravimetric analysis (TGA), thermal gravimetric analysis-mass spectrometry (TGA-MS), total organic carbon (TOC), thermal optical transmittance (TOT).

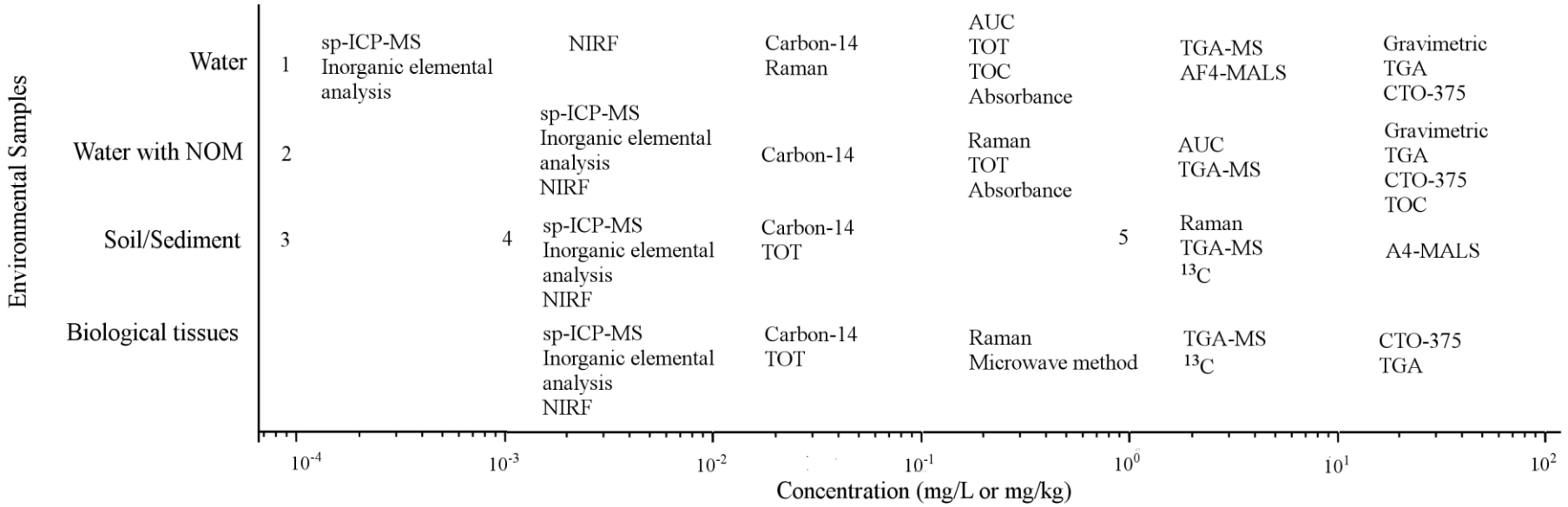

Figure 3: Detection limits for analytical techniques in various media under optimal conditions and modeled environmental concentrations (1[84], 2[85], 3[86], 4[70], 5[19]); modeled environmental concentrations are not available for biological matrices. The detection limits for individual techniques span a range of one order of magnitude (e.g., 1 mg/L to 10 mg/L).

Abbreviations: Asymmetric flow field flow fractionation with multi-angle light scattering (aF4-MALS), analytical ultracentrifugation (AUC), chemothermal oxidation at 375 °C (CTO-375), near infrared fluorescence (NIRF), single particle inductively coupled plasma-mass spectrometry (spICP-MS), thermal gravimetric analysis (TGA), thermal gravimetric analysis-mass spectrometry (TGA-MS), total organic carbon (TOC), thermal optical transmittance (TOT).

Supporting Information
Quantification of carbon nanotubes in environmental matrices: Current capabilities, case studies, and future prospects

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Summary of contents

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Method	Natural Waters	Cell Media	Biological Tissues	Soil/Sediment	Release from polymer nanocomposites
Spectroscopic					
Absorbance[65,109,150,151]	Scattering and absorbance of other components from the matrix may interfere with CNT quantification				
Near infrared fluorescence (NIRF)[15,29,31,152]	Scattering and absorbance of other components may interfere with CNT quantification; background fluorescence of the matrix may also impact measurements				
Raman[13,122,123,153-164]	Humic acid, other Raman active organic contaminants, and suspended particles (e.g., clays) could impact the detection method as could background fluorescence	This matrix may have background fluorescence	This matrix may have auto-fluorescence and may limit light penetration	Light scattering by large particulate material, may require separation prior to Raman analysis	Presence of the aromatic compounds at high concentration could influence the signal as could fluorescence
Spectrometric					
Inorganic Elemental Analysis[29,32]	The background metallic content in the matrix should be clearly defined before ICP-MS analysis and compared to the metals present in the CNTs; the elemental specificity and the high matrix tolerance, makes ICP-MS based techniques practically independent to most common environmental and biological interferences				
Single particle inductively coupled plasma-mass spectrometry (spICP-MS)[22]	Difficult to distinguish CNTs from other particulates containing the same metals, this is most likely for soils/sediments; extraction may be needed first				
Microscopic					
Atomic Force Microscopy[109,165]	For all matrices the presence of any other particulates depositing on the measurement substrate will require protocols for selective removal of all non-nanotube components; calculation of length distributions can be hindered by resolution issues (for short nanotubes) and observation bias (undercounting of long nanotubes) and/or the presence of aggregates				
Hyperspectral Imaging[166,167]	For all matrices, soot and other black particles could impact the detection of CNTs				

Photoacoustic (PA) and photothermal (PT)[24, 168-170]	Water is a good PA/PT solvent, anything else in the sample that absorbs or scatters the beam(s) would decrease signal or increase background	PA/PT works well in tissues transparent to beam(s); PT sensitivity drops in non-transparent tissue	No reports in literature; would be a difficult matrix to detect CNT with a lot of scattering and absorption of the beam(s)	A polymer matrix does not inhibit CNT detection; as long as there is a thermal response in the matrix, PT/PA can detect a signal
Scanning Electron Microscopy, Scanning Transmission Electron Microscopy, Transmission Electron Microscopy (TEM)[27,66]	Biopolymers, low concentration of CNTs compared to other particles leading to overlapping particles on the samples holder	Other fibrillar particles; low contrast between CNTs and biological tissue	Other fibrillar particles; low concentration of CNTs compared to other particles leading to overlapping particles on the samples holder	
Thermal				
CTO-375[18]	Very little interference in this matrix except for high N, organic carbon, or black carbon content waters	Matrices with high N or organic C content can char and form higher stability materials that, together with high BC concentrations, can interfere with the analysis of CNTs; conversely some matrices can produce "catalytic" effects that reduce the oxidation temperature of recalcitrant carbons	Sample specific oxidative strength (protective or catalytic) leading to variable recoveries of spiked CNTs; high organic C can char and high BC content can interfere with the CNT analysis	Some tested polymers (e.g., gamma-poly caprolactone) have lower thermal stability than CNTs which makes this a promising approach
Thermal Gravimetric Analysis (TGA) [20,171,172]	Very little interference expected except for waters with levels of BC that are approximately equal in concentration to the CNTs, NOM can stabilize CNTs	Isolated test materials show little interference, full matrix testing needed, peptone also binds to CNTs and may change oxidation temperature	Isolated test materials show little interference; full matrix testing needed, major	Interferences are unclear, tested epoxies have overlapping thermal stabilities with CNTs, and seem to influence the

				challenge in sample size (typically > 10 mg) and overlap of oxidation temperatures may hinder detection of CNTs	burn temperature of one another; overlap of oxidation temperatures or changes in thermal stability of CNTs in this matrix can hinder CNT quantification; in some cases, CNTs can be extracted from the matrix to give clear ratios of matrix:CNT composition
Thermal Gravimetric Analysis-Mass Spectrometry (TGA-MS) [14]	Very little interference; interference with BC can be reduced by MS peak deconvolution	Interferences with this matrix are unknown, but the unique chemical composition of cell media is promising for lower interferences	Isolated test materials show little interference and the unique chemical composition of biological tissues suggests low interferences, major challenge in sample size and overlap of oxidation temperatures may hinder detection of CNTs	Few direct interferences, but can raise background levels and raise the detection limit	The unique chemical compositions of most polymers suggests low interferences, evolved gases should be distinct for CNTs as compared to the polymer matrix
Total Organic Carbon (TOC) Analysis[71]	Interference exists from any organic matter (natural organic matter, soil organic matter, cellular material, serum or other organic compounds, organic polymers, etc.)				

Thermal Optical Transmittance (TOT) [16,23]	Typically little interference	Interferences should be minimal, but may arise if cell material chars into optically absorptive or thermally stable material	Interferences can be minimized by preparatory digestions (demonstrated for mouse lung)	Few direct interferences, unless the soil or sediment has a high non-CNT organic load	Potentially interfering, as many polymers will exhibit poor degradability under the inert atmosphere utilized in the first phase of this method; This could cause charring and confound the measurement of EC once the oxidative atmosphere is introduced
Isotopic labeling					
Carbon-13 Labelling[21,32,78]	Very little interference expected	Very little interference expected	Separation of CNTs from tissues is advised as accumulation of CNTs may be tissue dependent; background $\delta^{13}C$ signatures are necessary for each tissue type	Sulfates may interfere with the preparation of pure CO_2; CNT-free background required for comparative $\delta^{13}C$ signatures	CNT-free background require for comparative $\delta^{13}C$ signatures
Carbon-14 Labelling[15,26,30,99,124,152,173-181]	The potential biases depend on how the carbon-14 is quantified; some compounds may interfere with scintillation cocktails adsorbing beta emissions and could lead to autofluorescence; these issues would not be expected if the sample is combusted using biological oxidation		It may be possible to sonicate the tissue in liquid scintillation fluid, but there may be incomplete dispersion of the CNTs or quenching of the radioactivity; interferences are	Interferences have not been observed in previous studies with biological oxidation of the samples; good recovery was also found when sonicating SWCNTs with sodium dodecyl sulfate and using	There may be interference from quenching if the sample is added to liquid scintillation cocktail, but interferences would not be expected for biological oxidation

		unlikely with biological oxidation	liquid scintillation counting
Other radioactive isotopes[96-98]	This would depend to some extent on the radioactive isotope added and quantification used but generally interferences would not be expected for these different matrices; however, the stability of the radioactive tracer may be impacted by the dispersion process in the matrix or metabolic processes in tissues		
Additional Techniques			
Analytical Ultracentrifugation (AUC) [60,165,182-184]	Measurement of sedimentation requires homogenous dispersions with measureable viscosities and densities; significant light scattering from suspended particles from the matrix will additional likely complicate all but the most rigorous experimental protocols		
Gravimetric[185]	Measurement of CNTs in these matrices would encounter significant biases depend on the mass of other compounds that would be deposited when drying samples except at very high CNT concentrations		This technique is not applicable for these matrices
Microwave Method[62,125,186,187]	Other carbon forms such as soot may cause interferences; this interference would be most likely for soils and sediments		
aF4-MALS[46]	No known interferences, but theoretically other low density fibre-like/high aspect ratio particles may interfere; if these particles exhibited lower thermal stability compared to the CNTs, oxidation could potentially be used to selectively remove them		

Table S1: Potential Matrix Interferences for Selected Techniques for CNT Quantitation

Abbreviations: Asymmetric flow field flow fractionation with multi-angle light scattering (aF4-MALS), analytical ultracentrifugation (AUC), black carbon (BC), carbon nanotube (CNT), chemothermal oxidation at 375 °C (CTO-375), inductively coupled plasma-mass spectrometry (ICP-MS), near infrared fluorescence (NIRF), photoacoustic (PA), photothermal (PT), single particle inductively coupled plasma-mass spectrometry (spICP-MS), single-wall carbon nanotube (SWCNT), thermal gravimetric analysis (TGA), thermal gravimetric analysis-mass spectrometry (TGA-MS), total organic carbon (TOC), thermal optical transmittance (TOT).

Method	Impact of CNT agglomeration	Impact of CNT Oxidation/Degradation	Wrapping with Organic Molecules (proteins, NOM)
Spectroscopic			
Absorbance[65,109,150,151]	Measured absorbance signal per unit mass typically decreases above a threshold level of nanotube aggregation, especially for the intrinsic nanotube optical transitions, although apparent absorbance in the UV and visible regions may broadly increase due to increased light scattering by larger particles; for intrinsic optical transitions, the transition wavelength will typically red shift and peak intensities will decrease with any reduction from individualized dispersion	Absorbance of intrinsic optical transitions typically decreases monotonically above very low levels	In the absence of changes in agglomeration state, the adsorption of material to the nanotube interface generally will affect the absorbance mostly through red/blue shifts in intrinsic optical transition wavelengths by modification of the local dielectric environment; changes to the surface accessibility of the bulk solvent can also affect optical transition intensities
Near infrared fluorescence (NIRF)[15,29,31,152]	Peak shifts and intensity decrease for SWCNTs could occur for either of these changes		Variable
Raman[13,122,123,153-164]			
Absorbance[65,109,150,151]	Not a significant factor but G and D band ratio may be sensitive to sample agglomeration	Raman spectra are very sensitive to oxidation or degradation	Vibrational features are sensitive to structural stress which may be caused by wrapping with organic molecules or polymers
Spectrometric			
Inorganic Elemental Analysis[29,32]	Minimum impact on elemental analysis when a complete sample digestion is performed	Any loss of metals intercalated in CNTs before the elemental analysis would lead to biased results	Minimum impact when the wrapping does not alter the elemental composition of CNT
Single particle inductively coupled plasma-mass spectrometry (spICP-MS)[22]	Severe undercounting effect on actual CNT concentrations since each agglomerate may only be counted as one single pulse depending on the dwell time	Important influence on sizing and counting results because of the increasing contribution of smaller CNTs containing metal masses below instrument detection limit	Wrapping would affect physical transport of the CNT in introduction system, increasing the uncertainty on the size and number concentration determination
Microscopic			
Atomic Force Microscopy[109,165]	Agglomeration or oxidation/degradation may impact apparent size distribution and hinder analysis		Variable

Hyperspectral Imaging[166,167]	Better optical visibility due to enhanced scattering from agglomerates	Potential changes in spectral profiles from oxidation/degradation or wrapping with organic molecules	
Photoacoustic (PA) and Photothermal (PT)[24, 168-170]	Will follow the same changes that affect Absorbance; anything that changes the absorption of the CNTs would affect the PT/PA signal causing shifts in peak wavelength and changes in absorption cross-section; degradation would certainly affect the PT/PA signal; the effect of agglomeration, oxidation, and the addition of physisorbed or chemisorbed ligands would be case-by-case		
Scanning Electron Microscopy, Scanning Transmission Electron Microscopy, and Transmission Electron Microscopy[27,66]	CNTs will still be detected if investigated manually, but automated analysis may fail to identify CNT in agglomerates	Change in the size distribution, depending on the extent of degradation	May reduce the image resolution due to contamination effects (resulting from the volatilization and redeposition of the organic material under the electron beam)
Thermal			
CTO-375[18]	CNT agglomeration will slightly increase thermal stability, but not to an extent discernable by CTO-375	Oxidation and degradation reduce CNT thermal stability, which would enhance separation from BC but require a different cut off temperature to quantify SWCNTs; MWCNTs will still be interfering with BC	Organic coatings should be resolved (e.g., more labile than CNTs) by CTO-375 and not affect the measurement; however, proteins can char and cause interference

Thermal Gravimetric Analysis (TGA) [20,171,172]	CNT thermal stability will increase measurably; SWCNTs may no longer be resolved from BC or soot; MWCNTs should still have higher thermal stability than BC; higher temperature shoulders on oxidation peaks occurs with bundling, changes in oxidation temperature of material when bundled vs not	Oxidation and degradation of CNTs will reduce the thermal stability, which should help resolve SWCNTs from soot, but will likely not change the MWCNT thermal stability to such an extent that it interferes with BC	Organic coatings can influence the thermal stability of the CNTs, where lower onset temperatures and broader mass loss events have been observed, increasing potential interferences; proteins can char and cause interference
Thermal Gravimetric Analysis-Mass Spectrometry (TGA-MS) [14]	CNT thermal stability will increase measurably, no anticipated change in MS signal	Oxidation of CNTs will reduce the MS-derived advantages, which leverage the low-oxygen content of CNTs; potential for changes in decomposition products	Organic coatings can change CNT thermal stability and should increase the O and/or N content of the diluting matrix; thus, CNT-derived depletions in O would become easier to observe with organic matter coatings
Total Organic Carbon (TOC) Analysis[71]	Unlikely to be impacted by aggregation	Oxidation of CNTs would likely improve detection, as TOC analysis relies upon complete conversion to gaseous carbon mon- or di-oxides	Any organic surface coating (citrate, amine, etc.) contributes to the carbon detected from the CNT
Thermal Optical Transmittance (TOT) [16,23]	Sample on the filter won't be uniform, the split point of organic carbon/elemental carbon needs to be manually chosen instead of by optical information	Oxidation/degradation decreases the thermal stability and causes peak position shift; no issue with quantifying CNTs unless the sample has huge amount of organic carbon and the peak position of CNT after shifting is getting close to organic carbon	Having too much organic carbon may affect the thermal stability of CNT, and the signal from organic carbon will overlap that of elemental carbon; organic carbon should be resolved as much as possible
Isotopic labeling			
Carbon-13 Labelling[21,32,78]	Comprehensive oxidation of the CNTs required to prevent isotopic fractionation; agglomeration may affect thermal stability in closed-tube-combustion approaches, and efforts should be made to ensure complete combustion	Pre-analysis CNT oxidation may have slight impacts on CNT $\delta^{13}C$ signature (by virtue of reactive fractionation); these should be small depending on the extent of surface oxidation and/or if that process removes CNT-C from the CNT matrix	Wrapping with organic molecules will affect δ13C; the effect will depend on the δ13C of the molecule, and measures to separate the coating from the CNT are critical

Carbon-14 Labelling[15,26,30,99,124,152,173-181]	The impact of agglomeration would depend on the quantification procedure used; interference from self-quenching has been reported in some studies with agglomerates of CNTs, but this would not be expected for quantification using biological oxidation	This is not expected to impact this approach; carbon-14 analyses of released carbon dioxide has been used to quantify CNT degradation	This may impact measurements with liquid scintillation counting of dispersed CNTs but should not impact samples combusted using biological oxidation
Other radioactive isotopes[96-98]	This would not be expected to impact most isotopes unless self-quenching occurs	Oxidation or degradation may render this technique unusable if these processes lead to substantial separation of the radioactive isotope from the CNT	This would not be expected to impact most isotopes unless quenching occurs
Additional Techniques			
Analytical Ultracentrifugation (AUC)[60,165,182-184]	Aggregates rather than primary particle would be measured, data analysis potential decreased	Interpretation of results will become suspect due to differences in actual sample with respect to expected behavior	Variable effects, will likely bias size analysis
Gravimetric[185]	No impact	No impact unless there is complete degradation to CO_2	This can limit the accuracy of this approach since the concentration of these organic molecules will need to be assumed to be homogeneously distributed
Microwave Method[62,125,186,187]	These potential interferences have not been tested for this technique		
aF4-MALS[46]	Enhances material losses on the membrane and hinders accurate shape factor determination; thus agglomeration has to be avoided	Modifies CNT interactions with the membrane which can lead to higher or lower losses depending on carrier solution and membrane material	

Table S2: Potential Interferences for CNT Quantitation from Changes to CNTs for Selected Techniques

Abbreviations: Asymmetric flow field flow fractionation with multi-angle light scattering (aF4-MALS), analytical ultracentrifugation (AUC), black carbon (BC), carbon nanotube (CNT), chemothermal oxidation at 375 °C (CTO-375), multiwall carbon nanotube (MWCNT), near infrared fluorescence (NIRF), photoacoustic (PA), photothermal (PT), single particle inductively coupled plasma-mass spectrometry (spICP-MS), single-wall carbon nanotube (SWCNT), thermal gravimetric analysis (TGA), thermal gravimetric analysis-mass spectrometry (TGA-MS), total organic carbon (TOC), thermal optical transmittance (TOT).

Method	Relevant reference materials or standard methods	Key steps in instrument calibration	Challenges to standardization (i.e., traceability to the SI)
Spectroscopic			
Absorbance	Informational values RM8281, ISO/TS 10868:2011	Calibration of wavelength and intensity performed at 100 % transmittance. Usable wavelength range should be established by testing the absorbance of a blank sample and considering regions of high absorbance, scattering interference and Beer's law considerations.	Chemical environment can affect intensity and peak positions. Absorbance of water in NIR wavelengths for cell path lengths > 1mm.
Near infrared fluorescence	Informational values RM8281, ISO/TS 10867:2010	Traceable lamp detector train calibration	Chemical environment can affect intensity and peak positions, in-filter effects
Photoacoustic and photothermal	CNTs with well characterized absorbance of narrow size distribution in a pure solvent could be used for calibration	A standard sample with material similar to the sample CNTs (that absorbs the same wavelength) can be used to tune the setup. Laser power, sensitivity, and time constant can be adjusted for the sample as needed.	No standards published or referenced to date. Short shelf life for samples if in situ CNTs degrade or change over time. Difficult traceability to SI.
Raman	Frequency (x-axis) calibration standards ASTM E1840, Intensity (Y-axis) E2911, E2529, NIST SRM series 224X	Choose the appropriate standard for frequency and intensity depending on the excitation wavelength. Alternatively, a series of standard solutions (dilution series) of the pure analyte in combination with the internal standard can be used	
Spectrometric			
Inorganic Element Analysis	Dissolved standards of the monitored elements are required to determine the instrument sensitivity for the elemental quantification. Potential influences from residual carbon content and dissolved solids can be accounted for by suitable calibration techniques, including isotope dilution, matrix matched standards and the method of additions. SRM 2483 (single-wall carbon nanotubes (raw soot)) could be used to test instrument performance	ISO/TS 13278:2011E, This Technical Specification provides reference standard methods for the determination of elemental impurities in CNTs using ICP-MS. Results traceable to the SI can be readily achieved using traceable high-purity calibration standards. Calibration is performed with solutions having known concentrations of the metallic analytes of interest and matrix-matched to the composition of the prepared samples.	Lack of control environmental and biological matrices. Guarantee that sample digestion is quantitative prior to elemental analysis.

Single particle inductively coupled plasma-mass spectrometry (spICP-MS)	Reference Materials: Single element standard solutions available from numerous reference materials producers; NIST RM 8013 Gold Nanoparticle, Nominal 60 nm Diameter; Standards: ISO TS13278 Determination of metal impurities in samples of carbon nanotubes using inductively coupled plasma mass spectrometry	Calibration of ICP-MS instrument sensitivity is performed with solutions having known concentrations of the metallic analytes of interest and matrix-matched to the composition of the prepared samples. Calibration of sample transport efficiency is performed using metallic nanoparticles having known size (metal does not need to be the same as the trace metal analytes). Sample transport efficiency may also be calibrated using the waste collection method, but this method is generally less reliable.	Reference CNT samples with homogeneous size and controlled metal impurities contents are required to address the standardization.
Microscopic			
Atomic Force Microscopy	ASTM E2859-11 Standard guide for size measurement of nanoparticles Using atomic force microscopy, NIST RM8281, NRC Canada SWCNT-1	In-plane resolution, i.e. distance/pixel should be selected to enable identification of smallest expected particles of interest.	Surface roughness of deposition substrates varies significantly with preparation methodology. Polydisperse samples may require measurements at multiple resolutions to identify small particles, and to locate larger particles.
Hyperspectral Imaging		A representative spectral library is generated from the parent material. The spectral library is then used to detect the same material in a sample (e.g. cell) using a mapping algorithm	Unspecific absorption in the VNIR spectral range
Scanning Electron Microscopy	ISO/TS 10798:2011 Nanotechnologies -- Charaterization of single-wall carbon nanotubes using scanning electron microscopy and energy dispersive X-ray spectrometry analysis	Reference materials (regarding CNTs number concentrations) must be use to evaluate instrumental losses during sample preparation	Reference CNT suspensions with certified number concentrations must be developed; the shelf life of these suspensions maybe limited due to CNT agglomeration
Transmission Electron Microscopy	ISO/TS 10797:2012 Nanotechnologies -- Characterization of single-wall carbon nanotubes using transmission electron microscopy	Reference materials (regarding CNTs number concentrations) must be use to evaluate instrumental losses during sample preparation	Reference CNT suspensions with certified number concentrations mus be developed. The shelf life of these suspensions maybe limited due to CNT aggregation.
Thermal			
CTO-375			
Thermal Gravimetric Analysis	ISO/TS 11308:2011 Nanotechnologies -- Characterization of single-wall carbon nanotubes using thermogravimetric analysis	Temperature and mass calibration required	No reference materials for temperature calibration; traceable mass standards available
Thermal Gravimetric Analysis-Mass Spectrometry		Temperature and mass calibration required. MS peak identification database needed.	No reference materials for temperature calibration; traceable mass standards available
Total Organic Carbon Analysis			
Thermal Optical Transmittance	NIOSH, Elemental Carbon (Diesel Particulate): Method 5040. In NIOSH, Manual of Analytical Methods, 4th ed.; 2003.	Sucrose solution and methane gas carbon standandards are often used for mass calibration	Each CNT has slightly different peak position depending on defect, purity, functional group etc.; also it differs by the temperature program

Isotopic labeling			
Carbon-13 Labelling	Standards include calcium carbonate (commonly used Vienna PeeDee Belemnite and NBS ^{13}C standard), barium carbonate	Ensuring accuracy of standards is key to reliable measurements. Also, running standards throughout sample analysis is required to understand measurement drift.	Samples containing sulfate cause contamination in the final product. Small samples may not release enough gas for the analysis. Nanotubes will differ in their 13C ratios based on original source of C.
Carbon-14 Labelling	Standards include NIST 4222C (carbon-14 hexadecane radioactivity standard solution)	Calibration depends on the method (liquid scintillation counting, autoradiography, biological oxidation) used to quantify the radioactivity. For all methods, it may be necessary to calibrate the instrument using other radioactive carbon-14 chemicals or elements.	
Other isotopes	Multiple radioactivity standards are available from NIST (e.g., 4915F cobalt-60 radioactivity standard solution) and from other organizations	Calibration depends on the instrument used to measure the radioactivity	One principal challenge is the stability of the radioactive isotope onto the carbon nanotube.
Additional techniques			
Analytical Ultracentrifugation	SRM under development for ensuring radial measurement precision; sedimentation of Bovine Serum Albumin (BSA) frequently used as an unofficial standard.	External evaluation of temperature calibration and bulk solution viscosity and density properties are critical for correct measurements.	Requires unique absorbance or refractive index signals from solute differentiable from media.
Gravimetric	A broad range of mass RMs and protocols are available for gravimetric measurements	Balances can be calibrated using device-specific procedures, reference masses are readily available	Works only for a limited number of conditions and matrices
Microwave Method	CNT material used in the exposure experiment, (reference) control material (e.g., CNT-free biological tissue such as NIST SRM 1573 Tomato leaves)	The very same CNT material that is to be quantified must be used to calibrate the instrument; a calibration curve is generated using the thermal response as a function of known CNT amounts spiked into tissue samples	A main limitation to standardization is that the instrument used to make these measurements is not readily available
Field flow fraction/asymmetric flow field flow fraction/asymmetric flow field flow fraction-multi-angle light scattering	Certified polystyrene (PS) beads (Single or mix, available from NIST or other sources). Bovine serum albumin (BSA). Any other certified particle standard (e.g., Au, Ag, and SiO2) that can be dispersed in the carrier solution	PS beads dispersed in the used carrier solution are used for retention time calibration (hydrodynamic diameter). An isotropic scatterer is used for normalization of the MALS detector angles (e.g. 20nm PS beads). BSA is used for molecular weight calibration of the MALS detector	Reference CNT samples with homogeneous size and controlled particle impurities (e.g. soot) would be required for aF4-MALS quality assurance. Changes in the chemical environment of the CNTs as well as changes of the CNTs themselves (e.g., surface functionalizations, length distributions) can affect retention time in aF4; standardized methods must include extensive methodological details to ensure reproducibility

Table S3: Standards and calibration of selected carbon nanotube quantification techniques

Material	NIST SRM 2483 Single-wall carbon nanotube soot	NIST RM8281 Single-wall carbon nanotubes (dispersed, three length-resolved populations)	NRC Canada: SWCNT-1 Single-wall carbon nanotube certified reference material	JRC Multiwall carbon nanotube representative test materials
Reference Material (Y/N)	Yes	Yes	Yes	No
Are certified, reference, or information values provided for these characteristics?				
AFM imaging	No	Information values (length distributions)	Information values	No
Elemental composition	Certified, reference, and information values	No	Certified and reference values	Yes
NIR fluorescence spectra	No	Information values	Information values	No
Raman ratio	Reference values	Reference values	Reference values	Yes
Raman spectra	Information values	Information values	Reference values	Yes
SEM imaging	Information values	No	Information values	No
Specific surface area	No	No	Reference values	Yes
TEM imaging	Information values	Information values	Information values	Yes
Thermogravimetric analysis (residual mass and oxidation temperature)	Reference values	No	Reference values	Yes
UV-vis-NIR absorbance spectra	Information values	Information values	Information values	No
X-ray diffraction	No	No	Reference values	Yes

Table S4: Characterization of carbon nanotube reference materials, standard reference materials, and representative test material.