Review; Risk Assessment of Aerosolized SWCNTs, MWCNTs, Fullerenes and Carbon Black[†]

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Abstract

In this paper we review the risk assessment of carbonaceous nanomaterials, such as single-wall carbon nanotubes (SWCNTs), multi-walled carbon nanotubes (MWCNTs), fullerenes and carbon black, and summarize elemental carbon (EC) analyses for the determination of the those nanomaterials, focusing on the inhalation exposure of airborne nanomaterials. In the reports of hazard assessment, the proposed OELs (Occupational Exposure Limits) of MWCNTs and SWCNTs ranged from 1 to 50 $\mu\text{g}/\text{m}^3$. The fullerenes and carbon black seem to be less toxic than the CNTs. In the reports of exposure assessment, the aerosol concentrations of MWCNTs and SWCNTs in work environments were from less than 0.1 to more than 100 $\mu\text{g}/\text{m}^3$. The expected minimum concentration of airborne MWCNTs in the EC analyses was around 1 $\mu\text{g}/\text{m}^3$, but the concentrations of EC in ambient particulate matters (APM) were more than 1 $\mu\text{g}/\text{m}^3$ in urban environments. The EC analysis introduced in this paper is a convenient method to quantify the carbonaceous nanomaterials in the samples, but size-classification of aerosol samples by cascade impactor and observation using electron microscopes are needed to confirm the characteristics of the nanomaterials.

Keywords: nanomaterials, SWCNTs, MWCNTs, fullerenes, carbon black, thermal carbon analysis

1. Introduction

Ten years ago, Maynard (2007) wrote “Nanotechnology is clearly a concept whose time has come. Yet it is now being promoted in the scientific and popular press as a major technological breakthrough, heralding the next industrial revolution.” He also foresaw, “At the same time, there are increasing concerns that new nanotechnologies will bring about new risks to human health and the environment.” That is, nanotechnology will have both benefits and risks. How can we avoid the risks and obtain the benefits? In this paper, we review the risk-management of carbonaceous nanomaterials, as shown in **Fig. 1**.

Carbon black (CB), a representative carbonaceous nanomaterial, has a long history and is mass-produced worldwide. Primary CB particles are produced during partial combustion or thermal decomposition of hydrocarbons by gas-to-particle conversion. The sizes of primary

particles range from 10 nm to 100 nm. A few to many tens of primary particles immediately form highly branched chains of primary particles called aggregates. CB is added to rubber to reinforce final products and is also used as a pigment for printing ink, paints and toners. Other carbonaceous nanomaterials, such as fullerenes and carbon nanotubes, have been developed recently and supplied to markets.

Kroto et al. (1985) determined the atomic structure of C_{60} fullerene having the same geometry as soccer ball, and since then there have been many studies about fullerene derivatives in the fields of chemistry and pharmacology. C_{60} fullerene is soluble in organic solvents, such as toluene and tetrahydrofuran, but insoluble in water. Purified C_{60} fullerene forms a dense molecular crystal structure (brown powder), not a random-shaped agglomerate of the single molecule of C_{60} fullerene. A sensational study on nano-risk reported that C_{60} fullerenes induced oxidative stress in the brain of juvenile largemouth bass, but later the authors admitted that the results were mainly affected by the tetrahydrofuran used as a dispersant of C_{60} fullerene (Oberdörster, 2004; Zhu et al., 2006).

Carbon nanotubes (CNTs) possess unique properties and have been the focus of extensive research originated by Japanese scientists (Endo, 1988; Iijima, 1991) during the last two decades. CNTs are fiber-shaped substances

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Fig. 1 Carbonaceous nanomaterial in this review.

that consist of graphite hexagonal-mesh planes (graphene sheets) that present as a single-layer or in multiple layers. Tubes with single-wall structures are called single-wall carbon nanotubes (SWCNTs), and those with multi-wall structures are called multi-walled carbon nanotubes (MWCNTs). The physical properties of SWCNTs and MWCNTs, including high tensile strength and conductivity, make them increasingly desirable for manufacturing and medical applications (Maynard, 2007). Carbon nanofiber (CNF) is also used as a general term of the products, such as VGCNF (Vapor Grown Carbon Nanofiber). Carbon nanofibers with graphene layers wrapped into perfect cylinders are called CNTs.

Hazard assessment and exposure assessment are essential elements in the risk-assessment and risk-management of nanomaterial production. Occupational exposure limits (OELs), key issues in hazard assessment, are determined by toxicological studies using laboratory animals, such as rats and mice, if epidemiological data are not available especially for nanomaterials. The Japan Society for Occupational Health (JSOH, 2015) defined “Occupational Exposure Limit-Mean (OEL-M) for mean concentration of a chemical substance is defined as the reference value to the mean exposure concentration at or below which adverse health effects caused by the substance do not appear in most workers working for 8 hours a day, 40 hours a week under a moderate work-load”. The mean exposure concentration can be determined as the 8-hour time-weighted average (TWA) concentration of target materials.

For exposure assessment, we need analytical techniques for monitoring the concentrations of target nanomaterials in the ambient contaminants. Frequently, we have not suitable OELs or the monitoring techniques ranged from 1/10 level of OELs for the nanomaterials.

2. Health effects of carbonaceous nanomaterials

2.1 Carbon black

CB was the most examined nanomaterial in the past. Several studies have shown that inhaled CB particles induce lung tumors in rats when they are administered at doses that cause particle overload in the lungs, as well as chronic inflammation and epithelial hyperplasia. The In-

ternational Agency for Research on Cancer (IARC) has classified CB as possibly carcinogenic to humans (Group 2B; IARC, 1996; 2010). Elder et al. (2005) reported that this evaluation was based on inadequate evidence in humans, but sufficient evidence in experimental animals. In the case of human epidemiological evidence, U.S. studies on cancer in carbon black workers showed no excess of lung cancer. Rats, mice, and hamsters were exposed for 13 weeks to inhaled CB, and, based on the results, a sub-chronic No-observable-adverse-effect-level (NOAEL) of 1 mg/m³ respirable CB (Printex 90) can be assigned to female rats, mice, and hamsters.

Sager et al. (2009) reported that pulmonary responses to the instillation of ultrafine CB (Printex 90) to rats were comparable to equivalent particle surface area doses of ultrafine titanium dioxide, concluding that ultrafine titanium dioxide appears to be more bioactive than ultrafine CB on an equivalent surface area of particles on the delivered basis.

A short-term (5 consecutive days) inhalation study using rats (Ma Hock et al., 2013) was conducted to compare the effects of different carbon-based materials with different structures. No relevant toxicity was observed for CB (larger size than Printex 90) of 10 mg/m³, but MWCNT of 0.5 mg/m³ induced lung toxicity.

At present, the Japan Society of Occupational Health recommends that the OEL of CB should be 1mg/m³ as the respirable fraction (JSOH, 2015).

2.2 Fullerene

The first assessment of the toxicity resulting from inhalation exposures to C₆₀ fullerene nanoparticles and microparticles found minimal changes in the toxicological endpoints examined (Baker et al., 2008).

In a project (P06041, Risk assessment of manufactured nanomaterials) of Japanese New Energy and Industrial Technology Development Organization (NEDO), intratracheal instillation (IT) and inhalation (IH) studies on rats exposed to C₆₀ suggested that well-dispersed fullerenes do not have a strong potential of neutrophil inflammation (Morimoto et al., 2010), and the pulmonary inflammation pattern after exposure to C₆₀ was slight and transient in the pathological features of rat lungs. (Ogami et al., 2011) The half-life of intratracheally instilled C₆₀ (0.1 to 1 mg/rat) was 15–28 days; a short period compared with other nanomaterials (Shinohara et al., 2010). An OEL of 0.39 mg/m³ was adopted for fullerenes in a report by the NEDO project (Nakanishi, 2011).

2.3 Carbon nanotubes

The continuing increase in CNT production will lead to heightened risks of occupational exposure, raising con-

cern that CNT exposure via inhalation, ingestion or dermal contact will lead to harmful effects (Donaldson et al., 2006). Many in vivo experimental studies have been conducted to assess the potential effects of MWCNTs.

In the NEDO project, MWCNTs and SWCNTs were evaluated in IH tests and/or IT tests using rats (Nakanishi, 2011), and the biological results from the project were published by Kobayashi et al. (2010) and Morimoto et al. (2012). In one specific result of the project, Lee et al. (2013) analyzed the data of rat lungs administered two samples of MWCNTs (Nikkiso) with different average lengths derived from one bulk sample (44 nm in diameter) by IT and found that, based on histopathological changes, the pulmonary surfactants and inflammation scores of the lungs were significantly higher by MWCNT-Long (3.4 μm in mean length) than by MWCNT-Short (0.94 μm in mean length) exposure. Nakanishi et al. (2015) analyzed the relationship between the specific surface area of CNTs and the rate of increase of Polymorphonuclea leucocytes (PMNs) in Bronchoalveolar-lavage-fluid (BALF) and reported that the larger the specific surface area, the larger the rate of increase. The authors concluded that there was only a common relationship between the specific surface area and the increased rate of PMNs, regardless of SWCNTs or MWCNTs. The surface energy or surface activity of the CNTs was assumed to be the real reason for this difference, and the surface area reflects this indirectly. Generally, a larger specific surface area of CNTs means a smaller fiber diameter. An OEL of 0.03 mg/m^3 was adopted for SWCNTs, DWCNTs, and MWCNTs as a common criterion in a report by the NEDO project (Nakanishi, 2011), based on the integration of IT and IH tests. Considering the uncertainties in the data processing, they proposed limiting the period of application of the OEL to 15 years on the assumption that the values will be reviewed whenever new data are obtained.

One of the earliest OELs for CNTs was proposed by the British Standards Institution (2007); they proposed a “benchmark exposure limit” of 0.1 fiber/ cm^3 . Other OELs, however, were based on mass concentrations of CNT particles as a metric.

The U.S. NIOSH reviewed animal and other toxicological data relevant to assessing the potential non-malignant adverse respiratory effects of CNTs and CNFs, provided a quantitative risk assessment based on animal dose-response data, and proposed a recommended exposure limit (REL) of 0.001 mg/m^3 (1 $\mu\text{g}/\text{m}^3$) for elemental carbon (EC) as a respirable mass 8-hour time-weighted average (TWA) concentration in the NIOSH Current Intelligence Bulletin 65 (2013). Irrespective of many reports on MWCNT toxicity studies from the US NIOSH, the recommended OEL for CNTs was based on the limit of quantification of Method 5040 (NIOSH, 2003), a thermal-optical carbon analysis method for determining airborne exposure to re-

spirable CNTs.

Pauluhn (2010) selected 0.1 mg/m^3 as the NOAEL in a rat 13-week inhalation study to derive a human-equivalent concentration, and proposed 0.05 mg/m^3 (8-h TWA) as the OEL for a specific MWCNT (Baytubes).

Ma-Hock et al. (2009) conducted a MWCNT inhalation test in which rats were head-nose exposed for 13 weeks to concentrations of 0.1, 0.5, or 2.5 mg/m^3 . At 0.1 mg/m^3 , there was still minimal granulomatous inflammation in the lung and in lung-associated lymph nodes, while there was no substance-related systemic toxicity at 2.5 mg/m^3 . They did not select a NOAEL for MWCNT (Nanocyl NC 7000) from their study.

The Japan Bioassay Research Center conducted a rat 2-year inhalation study of MWCNT (MWNT-7) at concentrations of 0.02, 0.2, and 2 mg/m^3 . Adenoma and adenocarcinoma in rat lungs were observed dose-dependently in male rats. Inflammatory responses were observed from 0.02 mg/m^3 (Kasai et al., 2016). Before publication of the study, IARC (Kuempel et al., 2017) classified MWCNT-7 as possibly carcinogenic to humans (Group 2B); and SWCNTs and MWCNTs excluding MWCNT-7 were categorized as not classifiable as to their carcinogenicity to humans (Group 3). These categories of CNTs may be changed in the future along with the progress of toxicological studies.

3. Workplace handling of carbonaceous nanomaterials

Aerosol concentrations of carbonaceous nanomaterials in work environments are usually measured by *online* instruments, such as a scanning mobility particle sizer (SMPS), and *offline* measurement, such as Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM) and carbon analysis. *Online* measurement of particle size, number, and surface area is convenient, but the instruments used for these measurements respond to all particles, not just carbonaceous nanomaterials, and do not show information on shape/morphology and chemical composition.

Carbon is a common element on the earth and it is not easy to distinguish the sources of EC in ambient particles, such as carbonaceous nanomaterials and ambient particulate matter (APM) (see Fig. 2), but it has the merit that carbon analysis for the mass concentration of EC is a relatively sensitive and quick procedure from sampling to analysis.

Kuhlbusch et al. (2006) studied the particle characteristics in reactor and pelletizing areas of CB production. Filters that collected PM_{2.5} and PM₁₀ at worksites and outdoor sites within the same production facilities were analyzed for EC and organic carbon (OC) to assess and

Fig. 2 Many sources of elemental carbon in work environment atmosphere.

quantify the magnitude and proportion of the carbon fractions in the total particulate matter in the work areas. The particle number and mass concentrations in the reactor and pelletizing areas of the CB production plants appeared to fall within the range of ambient air values, but the pelletizing areas for CB showed high ratios of EC to PM10: 60–74 %, compared with 9–16 % for APM.

Fujitani et al. (2008) reported on a work environment in a C₆₀ production facility in Japan. They used SMPS and optical particle counter (OPC) for *online* measurement, and filter sampling and SEM observation for *offline* analysis. The particle volume concentration in the coarse size range (> 2 µm) was higher during the removal of fullerenes from the storage tank and/or weighing. The presence of fullerenes in the filter samples was confirmed by SEM observation of the morphology of microparticles collected during the bagging operation.

Takaya et al. (2010) carried out aerosol monitoring in two types of packing facilities of MWCNTs, where one of the packing facilities was manually operated and the other was automated, and found that the personal exposures were 2.39/0.39 (total/respirable) mg/m³ and 0.29/0.08 (total/respirable) mg/m³, respectively. The task, however, was related to nanoscale particle release and was not observed by real-time aerosol monitors. In further research by Takaya et al. (2012), micron-size particles containing MWCNTs were released into the air in a weaving process of MWCNT-coated yarn. The mass concentrations of total/respirable dust collected by personal sampling were 0.159/0.093 mg/m³ (total/respirable). The concentrations of EC in the respirable mass ranged from 1 to 5 µg C/m³.

Hedmer et al. (2014) quantified the occupational exposures and emissions during arc discharge production, purification, and functionalization of MWCNTs in a laboratory-scale worksite. In the personal exposure measurements, respirable dust ranged between detection limit and 93 µg/m³, and EC ranged between detection limit and 7.4 µg C/m³.

Dahm et al. (2015) assessed exposures to CNT (13 sites) and CNF (1 site). Personal breathing zone (PBZ) and area samples were collected for both the inhalable

and respirable mass concentration of EC, using the NIOSH Method 5040. The respirable EC PBZ concentrations ranged from 0.02 to 2.94 µg/m³, with a geometric mean of 0.34 µg/m³ and an 8-h TWA of 0.16 µg/m³.

Kuijpers et al. (2016) assessed personal exposure to MWCNTs during the synthesis and handling of MWCNTs in a commercial production facility and linked these exposure levels to specific activities. The exposure levels of the MWCNTs observed in the production area during the full scale synthesis of MWCNTs (*N* = 23) were comparable to levels observed during further handling of MWCNTs (*N* = 19); 41 µg/m³ and 43 µg/m³, respectively. Carbon analysis has also been used to determine the mass concentration of SWCNT aerosols generated (Maynard et al., 2004), the concentration of MWCNT aerosols in a laboratory (Han et al., 2008), and the concentration of carbon nanofibers and MWCNTs in factories (Methner et al., 2010; Birch et al., 2002).

There have been other studies to determine the number concentration of CNTs as asbestos fibers. We think that morphological observation by SEM and/or TEM can yield important data to confirm the presence of CNTs, but that the counting of CNT fibers is not a good way to determine the exposure assessment because the toxicological studies mentioned above were based on mass concentration and did not observe any clear toxicological similarity between CNTs and asbestos fibers. In addition, most CNT particles observed in work environments are large (micron size) agglomerates, which should be omitted according to the rules of asbestos counting.

4. Analysis of airborne carbonaceous nanomaterials

In the case of carbonaceous nanomaterials, carbon analysis has been used to determine the aerosol concentrations mentioned above. The U.S. NIOSH recommended applying Method 5040, a thermal-optical carbon analysis method, to determine airborne exposure to respirable CNT (Birch and Cary, 1996). Method 5040 was originally developed to measure EC concentration to estimate diesel particulates in work-environments. A similar carbon analysis method that determines the EC concentration in APM was also published by Chow et al. (1993). Both methods were originally intended to determine the EC mass generated by combustion processes and focused on the separation of organic carbon and elemental carbon in the particulates. The concentrations of EC in APM measured in urban areas of Japan were usually more than 1 µg/m³ (Ono-Ogasawara et al., 2009; Kim et al., 2011; Kudo et al., 2011), which value is similar to the level proposed by the US NIOSH as the OEL for CNT: 1 µg/m³ as EC.

In our pilot study, we obtained samples from quartz fi-

ber filters at a factory for CNT production. The color of the obtained samples was almost black, and the automatic function of thermal optical split of OC and EC by the carbon analyzer did not work. The samples were also resistant to high temperatures (850 degree C) and remained after the carbon analysis because the CNT had a graphite structure and annealed in the inert gas atmosphere. We decided to stop using the Method 5040, based on the optical split of OC and EC, and adopted a modified IMPROVE protocol by Chow et al. (1993). We used the modified IMPROVE protocol at each temperature stage, defined as OC1–OC4 and EC1–EC3, as shown in **Table 1** (Ono-Ogasawara and Myojo, 2013). This protocol was adopted for the analysis of CNTs in the reports by Takaya et al. (2010, 2012).

Airborne particle samples on the quartz fiber filters in the impaction stages of a Sioutas cascade impactor (SCI; SKC Inc., Eighty Four, PA, USA) were observed by SEM. **Fig. 3** shows particles collected on the filter fibers of the stage ($> 2.5 \mu\text{m}$). The fine and tangled fibers in the debris must be carbon nanotubes. We assumed that CNTs, in particular MWCNTs, decomposed at a higher temperature than APM and behaved as micron-size agglomerates irrespective of the nano-size diameter. In our method, at first, size-segregated particles are collected using an impactor, and then the EC concentrations of the collected particles of each size fraction are measured by carbon analysis without optical correction. The presence of MWCNTs is evaluated using factors of both size and the oxidation temperature in the measured data. Airborne particles were collected using a SCI, and the EC in the particles was analyzed using a carbon monitor. A schematic diagram of this procedure is shown in **Fig. 4** (Ono-Ogasawara and Myojo, 2011). The SCI is designed to collect size-segregated particles in six stages of $> 6.6 \mu\text{m}$, $> 2.5 \mu\text{m}$, $> 1.0 \mu\text{m}$, $> 0.5 \mu\text{m}$, $> 0.25 \mu\text{m}$, and smaller than $0.25 \mu\text{m}$ at a flow rate of 9 L/min. The middle four stages are called stages A to D, and the final stage is a back-up filter. A quartz fiber filter (2500QAT-UP, PALL, Port Washington, NY, USA) was used to collect particles, as shown in **Fig. 4**.

Typical cases of simulated MWCNT aerosols and APM are shown in **Fig. 5**. The EC of the MWCNTs is localized at stage A as EC3, and the EC of APM is localized at backup filter F as EC2. We analyzed several samples from the maintenance work of instruments for heat treatment of MWCNTs: automated packing during and not during work; and outside of the factory at 5 m from the air inlet to the factory. These real airborne particle samples at CNT production facilities showed tendencies somewhere between the MW1 and APM shown in **Fig. 5**. The details were shown in our previous paper (Ono-Ogasawara and Myojo, 2013).

Based on our data, a schematic diagram of the size and

Table 1 IMPROVE protocol for carbon aerosol analyzer.

	Time* (s)	Temperature (°C)	Atmospheric gas
OC1	180	120	He
OC2	180	250	He
OC3	300	450	He
OC4	300	550	He
EC1	360	550	2% O ₂ /He
EC2	600	700	2% O ₂ /He
EC3	360	920	2% O ₂ /He

*: Time of each step was adjusted for non-MWCNT samples. Inset shows a thermogram of an aerosol sample (Ono-Ogasawara and Myojo, 2013).

Fig. 3 Scanning electron microscopy of MWCNTs suspended in a workplace air. The bold fibers in the image are fibers of quartz filter. The white bar in the photo shows 10 μm .

EC amounts that evolved at different oven temperatures could be depicted as shown in **Fig. 6** (Ono-Ogasawara and Myojo, 2013). Fullerene (C₆₀) decomposed easily at a low temperature (Myojo et al., 2011). SWCNTs and thin MWCNTs also oxidized at 700 degree C, and the main proportion was EC2. The MWCNTs oxidized as EC2, as indicated by the presence of thinner MWCNTs, then EC3 must be an indication of the presence of thick MWCNTs. Well-produced carbon black (CB) is stable at high temperatures, and EC3 was a main constituent. **Fig. 6** shows

Fig. 4 Schematic diagram of sampling and analysis of ambient MWCNT particles.

* Determined by measured data (Ono-Ogasawara and Myojo, 2011). Nominally 75 % (= 15 mm/20 mm).

Fig. 5 Typical size effects on EC2 and EC3 of carbon analysis for typical samples of APM and MW1 (a sample of MWCNTs). Agglomerates of MWCNTs were detected at large fractions ($> 2.5 \mu\text{m}$ and EC3). EC in a sample of ambient particulate matters were detected at small fractions ($< 0.25 \mu\text{m}$ and EC2). Real samples were mixtures of the both graphs. (Ono-Ogasawara and Myojo, 2013)

a general tendency of the appearance of different types of carbon from carbonaceous nanomaterials in a carbon analysis. This tendency depends not only on the graphitization but also on the impurities in each sample, such as catalytic metals. To apply this method to the workplace, it is important to know the properties of the target materials used in each workplace. For measurements using a carbon monitor, the limit of quantitation is around $1 \mu\text{g EC}$ on a punched filter. This means stages A to D need to be more than $1.5 \mu\text{g EC}$ at each stage. The quantitation limit for the backup filter is expected to be $5.3 \mu\text{g}$ on the filter, and the expected minimum concentration of 2 hours sampling by SCI should be $1 \mu\text{g EC}/\text{m}^3$.

5. Conclusion

The lifecycle of carbonaceous nanomaterials can be categorized into the following six stages: manufacturing or synthesizing; manufacturing of interim products (master batches and dispersed solutions); manufacturing of products (composite, papers or fibers); processing of the products (cutting or sanding); use of the products; and disposal/recycling (Ono-Ogasawara et al. 2015). In these phases of the life cycle, the isolated nanoparticles or nanofibers discussed above may exist only in the first two phases and the last phase. The binding strength between nanomaterials and base materials of the composite ranges from weak surface adhesion to chemical bonding. The

Fig. 6 Estimated 2 dimensional map of size and EC1 to EC3 by our carbon analysis for MWCNT and other carbonaceous materials. CB: carbon black, APM: ambient particulate matter, d_i : diameter of MWCNTs. A to D and F are samples on the stages of SCI. (Ono-Ogasawara and Myojo, 2013)

exposure assessment and risk assessment of nanomaterials should be anticipated at all these stages. The thermal carbon analysis method with size-segregation of airborne particles is applicable to the measurement of exposure to carbonaceous nanomaterials at any stage of the life cycle. Further validation of the method and examples of its application are needed, however, in order to reach consensus regarding exposure measurements of carbonaceous nanomaterials.

This review is not a systematic review and mainly focused on reports on the risk assessment of carbonaceous nanomaterials as conducted by the authors and their co-workers or related work by others.

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