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RESEARCH ARTICLE

Exposure monitoring of graphene nanoplatelets manufacturing workplaces

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Abstract

Graphenes have emerged as a highly promising, two-dimensional engineered nanomaterial that can possibly substitute carbon nanotubes. They are being explored in numerous R&D and industrial applications in laboratories across the globe, leading to possible human and environmental exposures to them. Yet, there are no published data on graphene exposures in occupational settings and no readily available methods for their detection and quantitation exist. This study investigates for the first time the potential exposure of workers and research personnel to graphenes in two research facilities and evaluates the status of the control measures. One facility manufactures graphene using graphite exfoliation and chemical vapor deposition (CVD), while the other facility grows graphene on a copper plate using CVD, which is then transferred to a polyethylene terephthalate (PET) sheet. Graphene exposures and process emissions were investigated for three tasks – CVD growth, exfoliation, and transfer – using a multi-metric approach, which utilizes several direct reading instruments, integrated sampling, and chemical and morphological analysis. Real-time instruments included a dust monitor, condensation particle counter (CPC), nanoparticle surface area monitor, scanning mobility particle sizer, and an aethalometer. Morphologically, graphenes and other nanostructures released from the work process were investigated using a transmission electron microscope (TEM). Graphenes were quantified in airborne respirable samples as elemental carbon via thermo-optical analysis. The mass concentrations of total suspended particulate at Workplaces A and B were very low, and elemental carbon concentrations were mostly below the detection limit, indicating very low exposure to graphene or any other particles. The real-time monitoring, especially the aethalometer, showed a good response to the released black carbon, providing a signature of the graphene released during the opening of the CVD reactor at Workplace A. The TEM observation of the samples obtained from Workplaces A and B showed graphene-like structures and aggregated/agglomerated carbon structures. Taken together, the current findings on common scenarios (exfoliation, CVD growth, and transfer), while not inclusive of all graphene manufacturing processes, indicate very minimal graphene or particle exposure at facilities manufacturing graphenes with good manufacturing practices.

Keywords

Exposure, graphene, monitoring, nanoplates, workplace

History

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Introduction

A recent analysis of the environmental and health literature database created by the International Council of Nanotechnology (ICON) has revealed that most nanomaterial safety research is hazard focused (83%), while only 16% deals with potential exposure (Froggett et al., 2014). Unlike the generation of hazard data for nanomaterials, which can be

conducted using animals, *in vitro* or *in silico*, the generation of exposure assessment data needs to be conducted in the workplace, indoors and outdoors, or under simulated conditions. Certain nanomaterial exposure studies also require consenting worker or consumer involvement, which only further restricts the exposure study. Despite the recent publication of nanomaterial hazard data dossiers by the OECD WPMN safety assessment sponsorship program, there is still a lack of exposure data on nanomaterials, especially to novel materials, technologies and products, along the life cycle of nano-enabled products, and end-of-life disposal and recycling. Thus, for a proper risk assessment of

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nanomaterials, exposure data need to be expanded to cover the full life cycle of the nanomaterial and their nano-enabled products.

Graphene, a two-dimensional engineered nanomaterial, is now being fabricated and used in many applications, such as electronics, biological engineering, filtration, lightweight and strong nanocomposite materials, and energy storage. Graphene is a recent addition to the family of carbon-based engineered nanomaterials (ENM), developed after the CNTs, and considered as a promising alternative to CNTs. Thus, many of the R&D facilities that previously worked on CNTs have now switched to graphene R&D to expand the commercialization of graphene. Graphene nanoplates have already been suggested to pose a risk to the lungs and pleural space due to their aerodynamic properties that allow them to be deposited beyond the ciliated airways after inhalation (Schinwald et al., 2012). Yet, while hazard data on graphene are slowly emerging, there are no data on graphene exposure in workplaces or research facilities. Several attempts have already been made to expose experimental animals to graphene for initial hazard assessment (Han et al., 2015; Ma-Hock et al., 2013; Shin et al., 2015). Yet, these initial graphene inhalation toxicity studies were conducted without considering the situation and characteristics of worker exposure, especially with regard to dosimetry, dose range, and aerosol size distribution. Furthermore, in realistic settings where other co-exposures to carbonaceous nanomaterials are present, identification and quantification of graphenes present unique methodological challenges.

The main objective of this study was to investigate workplace exposures to graphenes in real-world settings. Exposures to graphenes were assessed in two graphene-manufacturing research facilities utilizing several exposure metrics: mass concentration from gravimetric data, number concentration from different real-time instruments, elemental carbon analysis by thermal optical methods (for comparison with other carbon nanomaterials), and transmission electron microscopy to identify and characterize graphene nanoplate morphologies in the workplace ambient air.

Materials and methods

Sampling sites

The current study measured graphene airborne exposure concentrations at two research facilities manufacturing graphene.

Workplace A

The information related to each facility is shown in Table 1. Workplace A manufactures graphene on a small scale based on graphite exfoliation and chemical vapor deposition (CVD; Figure 1). First, graphite oxide is made by mild oxidation using a mixture of H₂SO₄ and KMnO₄, similar to the conventional modified Hummer's method (Gilje et al., 2007; Kovtyukhova et al., 1999). The next step is to add water and hydrogen peroxide followed by filtering and washing to form functionalized expanded graphite (FEG), followed by the intercalation of large organic

Table 1. Information on workplaces and processes investigated in this study.

Plant	Location	Manufactured materials	Process	Engineering controls	PPE in use	Size and dimension (L × W × H)
A (Lab)	Gwangju (1)	Graphene manufacturing and application	-Chemical synthesis (graphite exfoliation)-CVD	Inside fume hood	Working clothes, gloves, no respirator	7.5 m × 7.8 m × 3.6 m
B-1 (Research Institute)	Daejeon (1)	Graphene manufacturing	-CVD on copper plate	Clean room (class 10 000), local exhaust ventilation	Working clothes (dust-free garment) with gloves	5 m × 7 m × 4 m
B-2 (Research Institute)	Daejeon (2)	Graphene application	-Graphene layer transferring	Clean room (class 1 000), local exhaust ventilation	Working clothes (dust-free garment) with gloves	8 m × 12 m × 4 m

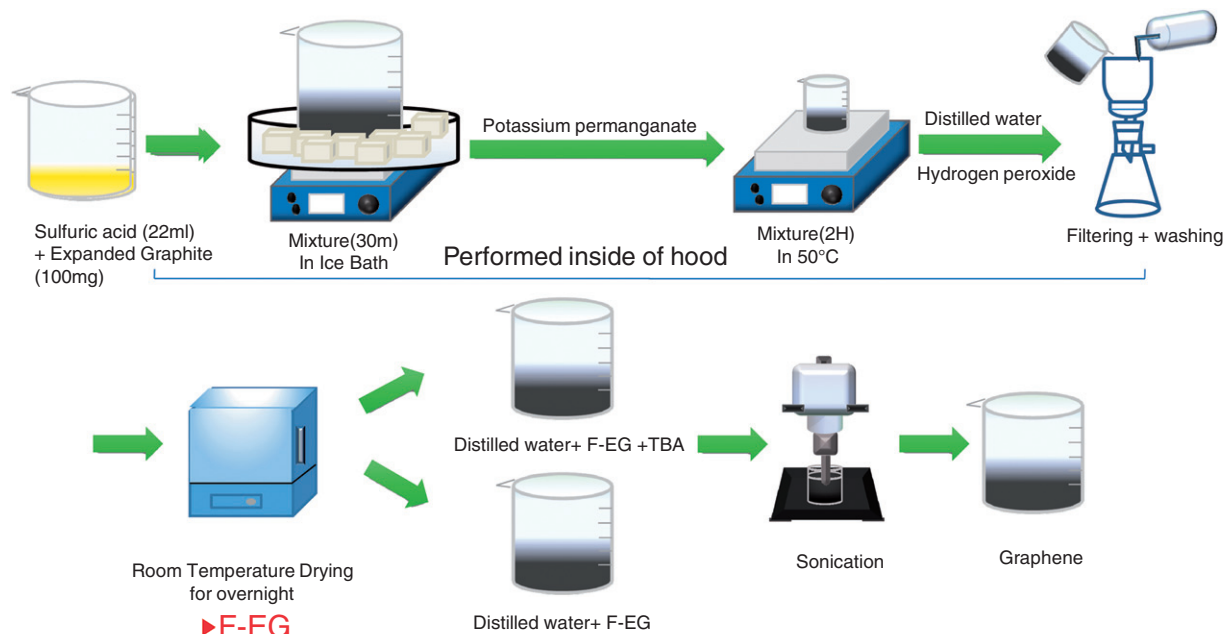


Figure 1. Graphite exfoliation process. FEG (functionalized expanded graphite); TBA (tetrabutylammonium).

tetrabutylammonium (TBA) ions into the layered graphite oxide. A colloidal suspension of graphene oxide is obtained via exfoliation of the TBA-intercalated graphite oxide using ultrasonication under 300 W for 30 min. Thermally reduced graphene oxide (rGO) is also obtained by annealing the graphene oxide at 900 °C for 2 h under an Ar atmosphere. For the graphene manufacturing process using CVD, the graphene is grown on a copper film. Before inserting the copper film into the furnace, it is cleaned by dipping it in acetone. A typical tube-furnace CVD system is used for the graphene growth. A polycrystalline Cu film is first annealed in an Ar/H₂ atmosphere at 1000 °C to increase the grain size and then exposed to a H₂/CH₄ gas mixture. During this step, the hydrocarbon decomposes and carbon atoms dissolve into a Cu crystal lattice to form a carbon solid solution. Finally, the samples are cooled in argon gas, and a graphene film is obtained on the Cu film.

Measurements were taken at Workplace A on two separate days: first day one, during the chemical synthesis of graphene (11:00–15:37) and CVD manufacturing (16:00–21:28; Supplementary Table S1), and on day two, during sonication (from 10:10 to 10:51; Supplementary Table S2). The sonication process was performed in a well enclosed sonicator.

Workplace B

Workplace B synthesized graphene using a CVD technique. All operations were conducted in a clean room class 10 000 (Workplace B-1 for CVD) and 1000 (Workplace B-2 for transfer) environment. A 35-μm-thick Cu foil without any pretreatment was loaded into a 4-inch quartz tube inside a furnace. A vacuum level of about 1 mTorr was maintained in the quartz tube, which was then heated to 1020 °C for 1 h with 300 sccm (standard cubic centimeter per min) of Ar and 100 sccm of H₂. The Cu foil is annealed under the same conditions for 45 min, and then 10 sccm of methane (CH₄)

gas is introduced for 35 min at 1020 °C. Thereafter, the furnace is cooled to 800 °C with 300 sccm of Ar and 30 sccm of H₂, and then rapidly cooled to room temperature. As a result, monolayer graphene films are synthesized on the Cu foil with good uniformity and a sheet resistance of around 300 ~400 Ohm/sq. The graphene-coated Cu foil is then laminated with a thermal release tape, and the Cu foil etched out using a 0.1-M ammonium persulfate solution including some chemical ingredients for doping and etching stability. After the etching process, the graphene on the thermal release tape is transferred to a polyethylene terephthalate (PET) substrate in a roll-to-roll manner at 190 °C, thereby eliminating the adhesion of the thermal release tape. The resulting monolayer graphene film on the PET substrate can be utilized as a transparent electrode for flexible displays and electronics. Measurements were also taken at Workplace B on two separate days: first day, at Workplace B-1 during the CVD synthesis of graphene on the copper plate (Supplementary Table 3A), and second day, at Workplace B-2 during the transfer of the synthesized graphene coating on the copper plate to the PET substrate (Supplementary Table 3B).

Personal and area sampling

The air samples were taken by drawing air through PVC (Polyvinyl chloride) filters in sampling cassettes (37 mm, 5 μm) obtained from SKC Inc. (Cat No. 225-5-37-P, Eighty Four, PA). The filter samples for the personal sampling were collected in the breathing zone using MSA (Escort Elf pump, Zefon International Inc., Ocala, FL) sampling pumps at a flow rate of 2.07–2.17 L/min and SKC (Leland Legacy pump, Eighty Four, PA) operated sampling pumps at a flow rate of 6.9–7.3 L/min when the work duration was short. Quartz filters (25-mm diameter quartz fiber filters, SKC Inc., Eighty Four, PA) were used to sample for elemental carbon (EC) concentration. The quartz filters were subsequently analyzed to determine the airborne mass concentration of elemental

carbon (EC). The filters were sent to KCOMWEL (Incheon, Korea) and analyzed according to NIOSH Manual of Analytical Methods (NMAM) Method 5040. The estimated LOD for elemental carbon was 0.3 µg per filter portion (NIOSH NMAM 5040). Sampling with personal samplers was performed during normal working hours. The personal samplers were attached to workers involved in the manufacturing of graphene. Area samples were also collected by placing the samplers 1–2 m away from the manufacturing devices, at suspected emission sources of graphenes, and at several representative locations throughout the workplace.

Elemental carbon sampling and analysis

Quartz filters (25-mm diameter quartz fiber filters, Whatman QMA, UK; 37-mm diameter quartz fiber filters, SKC Inc., Eighty Four, PA) were used to sample the elemental carbon (EC) concentration. The respirable elemental carbon was sampled using aluminum cyclone (cut-point 4 µm, SKC, Eighty Four, PA) with 2.5 liter/min. The quartz filters were subsequently analyzed to determine the airborne mass concentration of elemental carbon (EC). The filters were sent to KCOMWEL (Incheon, Korea) and analyzed according to NIOSH Manual of Analytical Methods (NMAM) Method 5040. The estimated LOD for the elemental carbon was 0.3 µg per filter portion (NIOSH NMAM 5040).

Real-time aerosol monitoring

A scanning mobility particle sizer (SMPS), combining a differential mobility analyzer (DMA, 4220, HCT Co., Ltd., Icheon, Korea) and condensation particle counter (CPC, 4312, HCT Co., Ltd., 0–10⁸ particles/cm³ detection range), was used to monitor the particle size distribution with an electrical mobility diameter ranging from 7.37 to 289.03 nm. Meanwhile, a dust monitor (Model 1.109, Grimm, Ainring, Germany) was used to observe the particle size distribution with a diameter ranging from 0.25 to 32 µm. The workplace air was sampled at a flow rate of 0.3 and 1.2 L/min for the SMPS and dust monitor, respectively. The SMPS scanned the particle sizes at a time resolution of 2.5 min (120 s for up-scan and 30 s for retrace), while the average time for the dust monitor was 1 min. Another CPC (3775, TSI Co Ltd., 1–10⁷ particles/cm³) was also used to monitor the number concentration. As a new metric, a nanoparticle aerosol monitor (NSAM, AEROTRAK™ 9000, TSI, Shoreview, MN) was used to measure the nanoparticle aerosol exposure–lung deposited surface area (aerosol concentration range: tracheobronchial region 1 to 2500 µm²/cc, alveolar region 1 to 10 000 µm²/cc). In addition, a portable aethalometer (Model AE 51; Magee Scientific, Berkeley CA) was utilized to measure the mass concentration of black carbon particles based on an optical absorption analysis. Here, the aethalometer sampled the total suspended particulate matter, and the air in the laboratories was sampled at a flow rate of 100 mL/min.

Transmission electron microscopy

A TEM equipped with an energy dispersive X-ray analyzer (TEM, H-7650, Hitachi, Japan) was used to measure the

nanoparticles based on NIOSH analytical method 7402 (NIOSH, 1994). The airborne nanoparticles were trapped in a TEM grid using a portable particle sampler (ESPnano Model 100, Spokane, WA; TEM, Partector, Naneos, Switzerland) containing a grid (Silicone monoxide substrates on 400 mesh nickel grid, Electron Microscopy Sciences, Hatfield, PA, USA, Cat No. SF 400-Ni; Formavar/carbon film grid: 10 nm ~10 µm range) with a flow rate of 0.1, 0.45 liter/min, and mini particle sampler (MPS, INERIS, France; Ecomesure, Saclay, France) containing a holey grid (Quantifoil R2/1 Holey carbon, Electron Microscopy Sciences, Hatfield, PA, USA, Cat No. Q225-CR-1; 3 mm diameter, 150 mesh Cu, range 1 nm to 1 µm, Okenshoji, Japan) support with a flow rate of 0.5 liter/min. The particles were measured at a magnification of 100 000, and the nanoparticles or graphene-like structures were analyzed using an energy dispersive X-ray spectrometer (EDS, TM200, Oxford, UK) at an accelerating voltage of 75 kV.

Ethics statement

The study protocol was approved by the authors' Institute Research Ethics Committee (Approval number: Hoseo University IRB 2015-014). All the subjects signed an informed consent form that conformed to the recommendations of the Committee.

Results

Workplace A

The work process at Workplace A on the first and second day is described in Supplements 1 and 2, and the sampling locations for the direct reading instruments and personal and area sampling are presented in Figure 2. The particle number concentrations ranged from 62 to 16 804 particles/cm³ when measured using the CPC, and from 2342.26 to 23 977.49 particle/cm³ when measured using the SMPS. The black carbon particles ranged from 71 to 3651 particles/cm³ when measured using the aethalometer (Figure 3).

The sonication of the graphene, which was expected to release many particles, did not actually release particles due to the encapsulated sonication process. The total and respirable particle concentrations measured by area sampling are described in Table 2. The particle mass concentrations in terms of the 8-h TWA ranged from 0.0286 to 0.0480 mg/m³ on the first day, and were 0.00058 mg/m³ on the second day (Table 2). The average TSP concentrations at Workplace A are presented in Table 3. No distinct manufacturing process was identified by the mass sampling as releasing graphene particles at Workplace A. The task performed in the areas, Hood 1 (wet mixing and reaction), Hood 2 (wet filtration), CVD table (synthesis), and sonication table (sonication) did not result distinctive particle release due to wet procedure and encapsulated sonication. To specify the graphene exposure using the elemental carbon concentration, respirable elemental carbons were sampled onto quartz filters using a cyclone. However, most of the elemental carbon concentrations were below the detection limit and lower than background levels (Table 4). EC concentrations higher than the background were detected at 1 m away from CVD and inside of sonicator in the

Figure 2. Sampling locations for direct reading instruments and personal and area samplings in the Workplace A. T, TSP; EC, elemental carbon; R, respirable.

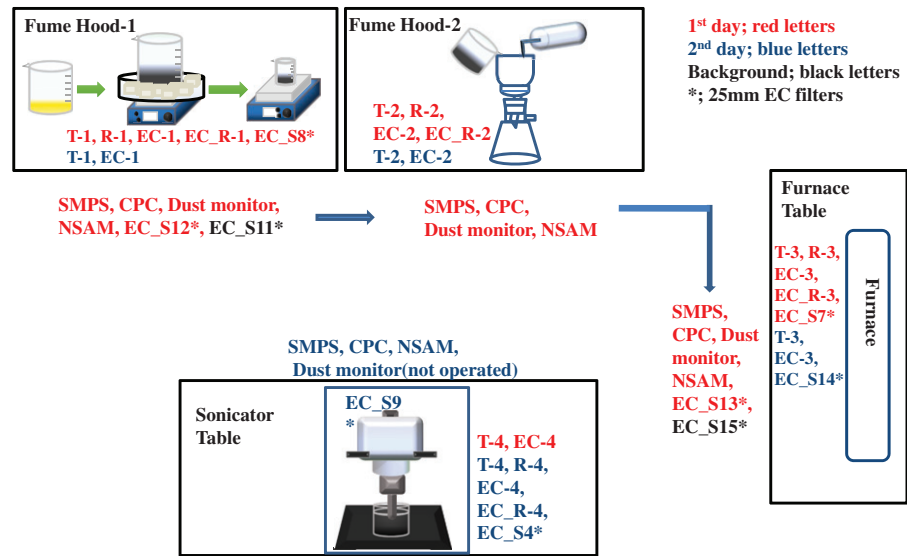


Figure 3. Particle number concentrations during graphene manufacturing at Workplace A. A. First day, B. Second day.

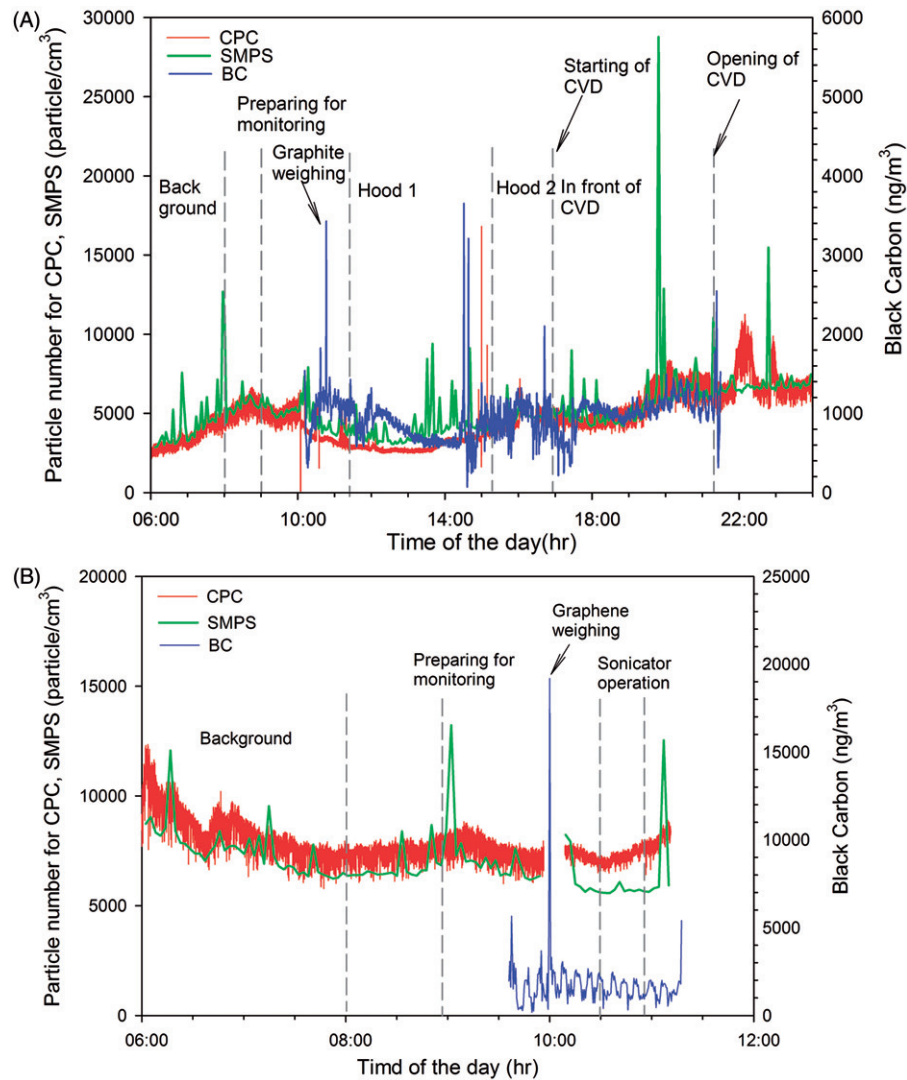


Table 2. Airborne mass concentrations (8-h TWA) in total and respirable samples from Workplace A.

Task	Location	Sampling site	First day					Second day				
			Filter weight (mg)	Flow rate (L/min)	Sampling time (min)	Mass concentration (mg/m ³)	8hr TWA (mg/m ³)	Filter weight (mg)	Flow rate (L/min)	Sampling time (min)	Mass concentration (mg/m ³)	8hr TWA (mg/m ³)
Mixing and reaction in solution	Inside hood 1	Total dust (T-1)	0.0413	1.9885	390	0.05330	0.04330	0.0007	2.3820	31	0.00903	0.00058
		Respirable dust (R-1)	0.0353	2.4935	390	0.03633	0.02952	NM			NM	
Filtering of solution	Inside hood 2	Total dust (T-2)	0.0340	1.9610	388	0.04469	0.03612	ND	2.5320	31	ND	ND
		Respirable dust (R-2)	0.0330	2.4035	388	0.03539	0.02860	NM			NM	
Graphene synthesis	CVD table	Total dust (T-3)	0.2947	4.7290	631	0.03654	0.04803	ND	2.5155	97	ND	ND
		Respirable dust (R-3)	0.0517	2.5080	633	0.03254	0.04292	NM			NM	
Sonication	Sonicator table	Total dust (T-4)	0.0700	4.8255	397	0.03654	0.03022	ND	2.5320	31	ND	ND
		Respirable dust (R-4)	NM			NM		ND	2.4780	31	ND	ND

NM, not measured; ND, not detectable.

second day measurement when 25 quartz filters were used. A further TEM analysis of the air samples taken from Workplace A revealed graphene-like structures, as well as some aggregate/agglomerate carbon structures (Figure 4). An EDS analysis of sampled structures indicated that the major components were carbon (Figure 4G and H) and some contamination of Si.

Workplace B

The work process at Workplace B on the first and second day is described in detail in Supplementary Table 3, and the sampling locations for the direct reading instruments and personal and area sampling are presented in Figure 5. The particle number concentrations ranged from 634 to 33 974 particles/cm³ when measured using the CPC, and from 0.05 to 2.26 particle/cm³ when measured using the dust monitor. The nanoparticle surface area monitor (NSAM) results ranged from 4.84 to 38.54 µm²/cm³ (Figure 6). An increased particle number was noted at 16:00 at Workplace B-1 when the graphene synthesized on the copper plates was taken out (Figure 6A). However, the transfer of the copper plates to the PET sheets and the cutting lasted less than 10 min. Meanwhile, at Workplace B-2, while the CPC and dust monitor recorded an increased particle number concentration, no distinct increase in the particle number was detected (Figure 6B). The total and respirable particle concentrations measured by area sampling are described in Table 5. The particle mass concentrations in terms of the 8-h TWA were negligible at both Workplaces B-1 and B-2 (Table 5) because of good practice in the clean room environment. The respirable elemental carbon concentrations, indicating possible graphene exposure, were all below the detection limit (Table 6). A further TEM analysis of the air samples taken from Workplace B showed graphene-like structures, as well as some aggregate/agglomerate carbon structures (Figure 7).

Discussion

This study attempted to monitor graphene exposure in R&D graphene manufacturing facilities. Unlike other carbon nanomaterials, such as CNTs and CNFs, which have been the focus of various exposure assessment (Dahm et al., 2012, 2013; Han et al., 2008; Lee et al., 2010, 2015a) and health surveillance (Lee et al., 2015b; Liao et al., 2013; Liou et al., 2012) studies, there has been no previous graphene exposure study. While graphenes and CNTs share similar manufacturing methods, such as CVD, graphenes can also be manufactured by graphite exfoliation in which graphene oxide sheets are reduced to graphite. Furthermore, the CVD applied in graphene manufacturing uses a copper plate to make a single layer of graphene, while the CVD in CNT manufacturing uses fine metal particles as catalysts to grow carbon nanotube structures. Of note, no definitive method has yet been identified for graphene exposure monitoring and quantification.

The current exposure assessment of three graphene manufacturing processes – exfoliation, CVD, and transferring to a PET sheet – while not inclusive of all graphene manufacturing processes and activities, represents the

Table 3. Average TSP concentrations at Workplace A.

	Range (mg/m ³)	Mean (mg/m ³)	SD	GM (mg/m ³)	GSD
Total	ND-0.05330	0.03359	0.016	0.02917	1.917
Respirable	ND-0.03633	0.03475	0.002	0.03472	1.059

Table 4. EC concentration in the Workplace A.

Process	Sampling site	First day		Second day	
		Sampling volume (L)	EC (µg/m ³)	Sampling volume (L)	EC (µg/m ³)
Personal	Respirable dust	1029.0	<DL	72.7	<DL
Mixing and reaction in solution	Total dust (EC-1)	2881.1	<DL	77.8	<DL
	Respirable dust (EC_R-1)	957.6	<DL		
	Inside of hood (EC_S8*)	3465	0.26		
	50 cm in front of hood (EC_S12*)	2661	0.40		
Filtering of solution	Total dust (EC-2)	1879.6	<DL	60.7	<DL
	Respirable dust (EC_R-2)	936.0	<DL		
Graphene synthesis	Total dust (EC-3)	4530.5	<DL	235.7	<DL
	Respirable dust (EC_R-3)	1577.8	<DL		
	1 m away from CVD (EC_S13*, EC_S14*)	2652	0.47	390	1.15
	Just about CVD (collection of graphenes from CVD, EC_S7*)	63	<DL		
Sonication	Total dust (EC-4)	1758.8	<DL		
	Respirable dust (EC_R-4)			73.2	<DL
	Inside of sonicator (EC_S9*)			140	0.91
	Outside of sonicator (EC_S4*)			384	<DL
Background	50 cm in front of hood (EC_S11*)	1440	0.27		
	1 m away from CVD (EC_S15*)	1440	0.46		

<DL, less than indicated detecting limit (DL, 0.3 µg/filter); EC, elemental carbon; R, respirable.

*Indicates use of 25 mm filter. The sampling sites correspond with Figure 2.

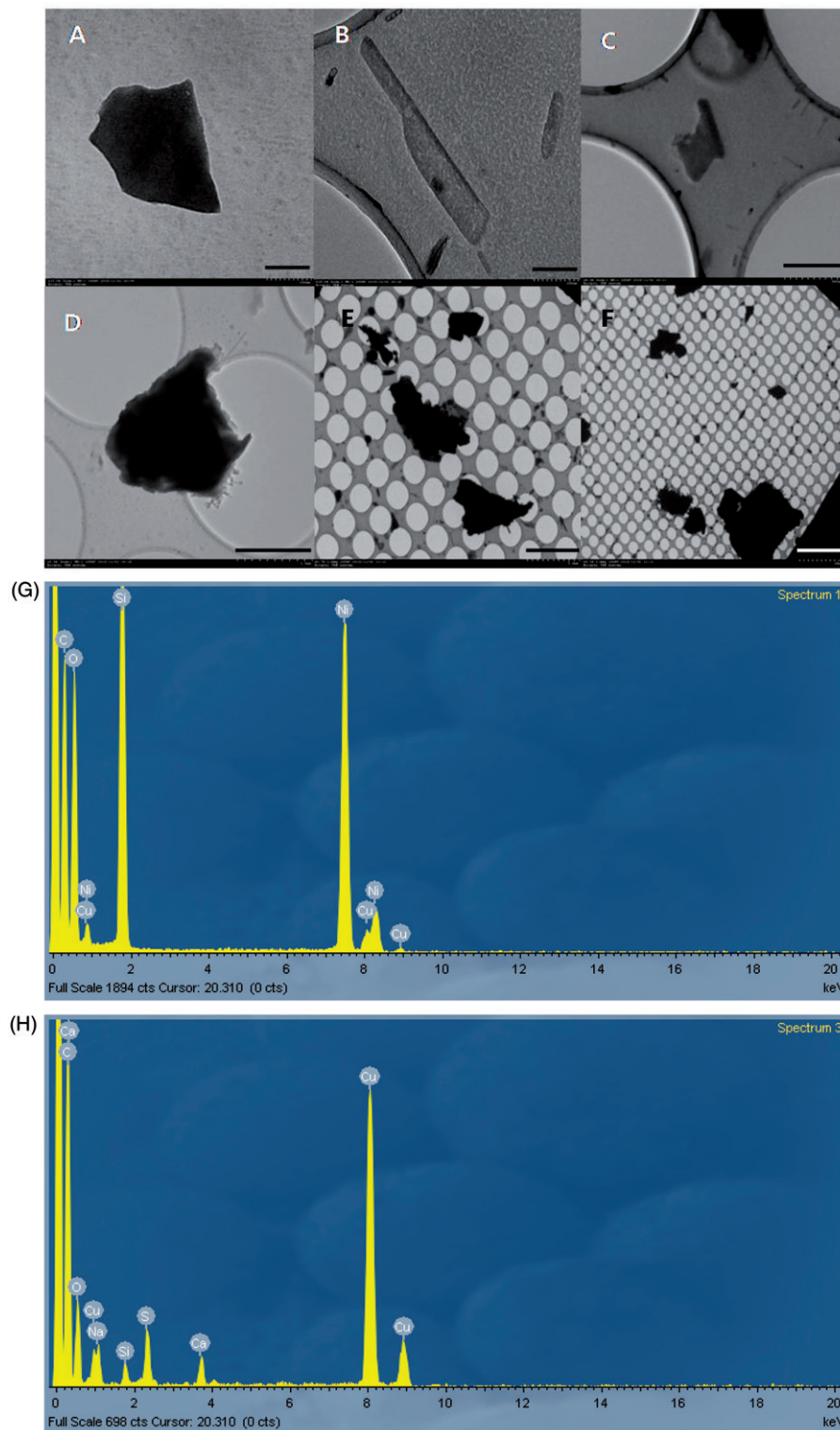
graphene manufacturing processes currently used in R&D facilities and some pilot-scale industries. The present results showed minimum release of graphenes or other particles during manufacturing based on real-time aerosol monitoring, and negligible exposure to graphenes based on personal and area sampling for the TSP and EC. For real-time monitoring, several of the direct reading instruments used in this study, including the CPC which was effective at monitoring particle number concentration for graphene inhalation exposure study (Kim et al., 2016), dust monitor, NSAM, and SMPS, were not found to be effective in monitoring particle or graphene release in these workplaces. This was due to the low particle release during the graphene manufacturing process, clean environmental conditions, especially at Workplace B, and two-dimensional material characteristics in contrast to three-dimensional particles. Another CNT measurement study using a real-time monitor previously suggested that the measurement of one-dimensional nanomaterials, such as CNTs, using a SMPS is unable to accurately represent CNTs due to the arc charge caused by the charged CNTs in the DMA (Ku et al., 2007). Notwithstanding, the aethalometer detected a possible particle release during the graphene manufacturing, particularly during the weighing process and opening of the CVD due to black carbon-like material release. These process points have also been previously identified as emission points of nanomaterials during CNT manufacturing (Lee et al., 2010). In the case of MWCNTs, an aethalometer was effective in identifying an increased black carbon concentration when the blending equipment was opened (Han et al., 2008). Thus, an aethalometer would seem to be effective for monitoring exposure to graphenes or black

carbon particles, although this may be worth investigating further in other graphene workplaces.

The respirable mass concentration of elemental carbon has already been suggested as the recommended exposure level for CNTs and CNFs (NIOSH, 2013). In a 28-day inhalation toxicity study of graphenes, the elemental carbon concentration corresponded well with the mass concentration of graphenes (Kim et al., 2016). Therefore, this study measured the respirable elemental carbon concentration, and most of the samples were found to be lower than the detection limit (0.3 µg/filter). Only few samples collected by 25-mm quartz filter showed some detectable levels of elemental carbon. Although sensitivity of 25-mm filter over 37-mm filter has been reported for asbestos sampling (WHO, 1997), sampling efficiency of elemental carbon between 25-mm and 37-mm quartz filter needs to be studied further. Consequently, the results indicated that while carbonaceous materials were released during manufacturing, the concentration of graphene was very low, but this is largely due to clean room, hoods, and small-scale processes with good manufacturing practices.

Particle shape characterization is another important step in assessing nanomaterial exposure, as it provides information on the particle size distribution, particle aggregation/agglomeration, and possible lung deposition. Such particle shape information can also be used to design aerosol generation for inhalation toxicity studies. For example, several inhalation studies of MWCNTs (Ahn et al., 2011; Kasai et al., 2014; Kim et al., 2012; McKinney et al., 2009) were based on an exposure study that revealed the shape of airborne MWCNTs in the workplace (Han et al., 2008). All these aerosol

Figure 4. Graphene-like structures from samples obtained from Workplace A by TEM. Graphene sheet-like structure (A&B) bar in A & B indicate 200 nm and C indicates 500 nm, Aggregated/agglomerated carbon structures (D, E, F). Bars in D, E, and F indicate 1 μm , 5 μm , and 10 μm , respectively. G, EDS analysis of image A on nickel grid; H, EDS analysis of image C on copper grid.



generators tried to simulate and generate dispersed MWCNTs found in MWCNT manufacturing workplace air. Therefore, the present study also identified the shapes/morphologies of airborne graphene in the workplace, which is useful in facilitating generation of more realistic aerosol distributions for inhalation toxicity studies, which can be summarized as individual graphene platelets and their agglomerates in workplace air. Thus dispersed graphene platelets with some agglomerates with varying sizes and thickness, depending on

graphene manufacturing purpose, would be desired for inhalation studies.

From the current study, several graphene exposure strategies can be suggested. For real-time release monitoring, the use of an aethalometer in combination with other direct-reading instruments can be useful to identify emission sources irrespective of composition or morphology of nanoparticles. TEM identification of structural features of airborne nanoparticles is essential for identifying/confirming the presence

Figure 5. Sampling locations for direct reading instruments and personal and area sampling at Workplace B-1 and B-2. EC, elemental carbon; R, respirable, T, TSP.

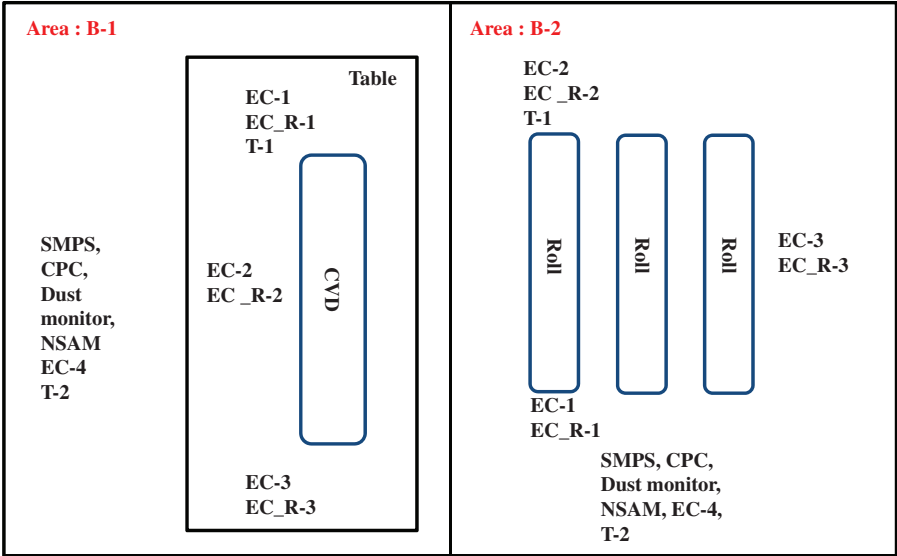


Figure 6. Particle number concentrations during graphene manufacturing at Workplace B. A. Workplace B-1, B. Workplace B-2.

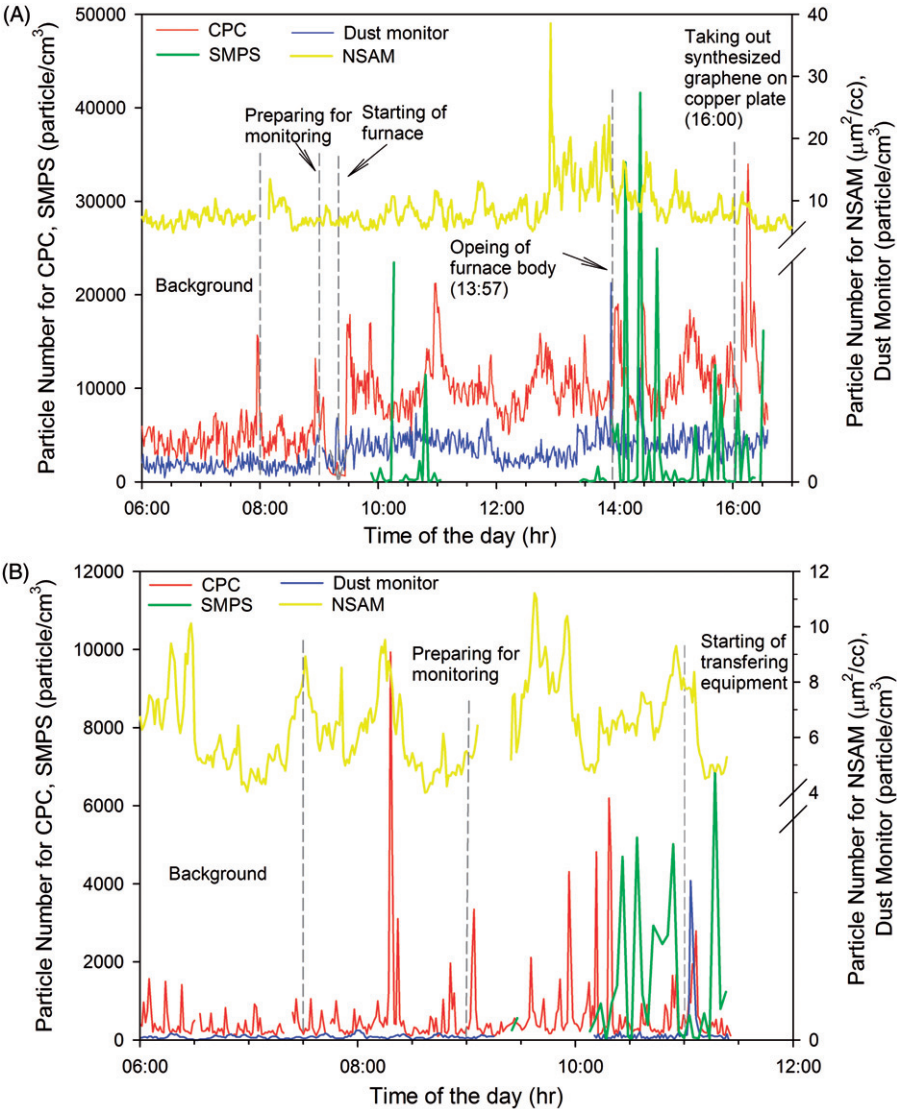


Table 5. TSP mass concentrations (8-h TWA) at Workplace B. The sampling sites correspond with Figure 5.

Process	Sampling sites	Filter weight (mg)	Flow rate (L/min)	Sampling time (min)	Mass concentration (mg/m ³)	8-h TWA (mg/m ³)
A. B-1						
CVD process	Left side of CVD door	ND	2.518	427	ND	ND
	In front of CVD	0.00367	2.5042	426	0.00344	0.00305
B. B-2						
Transfer process	Left side of roll	0.00333	2.609	91	0.01404	0.00266
	Right side of roll	0.00533	2.505	86	0.02476	0.00444

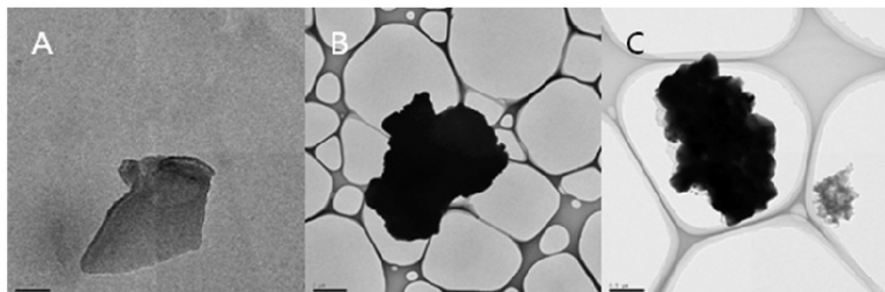
Table 6. EC concentrations at Workplace B-1 and B-2. The sampling sites correspond with Figure 5.

	First-day Workplace (B-1)		Second-day Workplace (B-2)	
	Sampling volume (L)	EC (μg/m ³)	Sampling volume (L)	EC (μg/m ³)
EC-1	2249.525	<DL	473.616	<DL
EC_R-1	1073.4348	<DL	215.6665	<DL
EC-2	2258.865	<DL	476.7285	<DL
EC_R-2	1060.2501	<DL	217.3952	<DL
EC-3	2291.296	<DL	459.326	<DL
EC_R-3	1032.1871	<DL	213.356	<DL
EC-4	2175.12	<DL	447.093	<DL
EC_R-4	1058.3195	<DL	205.6159	<DL

<DL, less than indicated reporting limit (DL).
Elemental carbon (EC, 0.3 μg/filter).

Figure 7. Graphene-like structures in samples obtained from Workplace B based on TEM.

A. Graphene sheet-like structure at Workplace B-1, bar indicates 100 nm, B. aggregated/agglomerated carbon structures at Workplace B-1, bar indicates 2 μm, and C. aggregated/agglomerated carbon structures at Workplace B-2, bar indicates 0.5 μm.



of graphene-like structures in the manufacturing workplace air. Elemental carbon concentration measurements are recommended to evaluate the intensity of graphene exposure during the manufacturing process. This approach is not that different from monitoring strategies for other ENM, especially the carbon-based ones such as CNTs and requires measuring multiple exposure metrics. Additional exposure assessment studies such as monitoring of other work processes in the manufacturing of graphenes, graphene handling, including packaging, and graphene fabrications for various purposes, are still needed for further characterization of graphene exposure in the workplace. In addition, inhalation chambers and simulation studies to characterize graphenes in terms of number, surface area, and mass using direct-reading instruments or filter would be helpful.

Declaration of interest

The authors have no conflicting interest and nothing to disclose.

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References

- Ahn KH, Kim SM, Yu IJ. (2011). Multi-walled carbon nanotube (MWCNT) dispersion and aerosolization with hot water atomization without addition of any surfactant. *Safe Health Work* 2:34–8.
- Dahm MM, Evans DE, Schubauer-Berigan MK, et al. (2012). Occupational exposure assessment in carbon nanotube and nanofiber primary and secondary manufacturers. *Ann Occup Hyg* 56:542–56.
- Dahm MM, Evans DE, Schubauer-Berigan MK, et al. (2013). Occupational exposure assessment in carbon nanotube and nanofiber primary and secondary manufacturers: mobile direct-reading sampling. *Ann Occup Hyg* 57:328–44.

- Froggett SJ, Clancy SF, Boverhof DR, Canady RA. (2014). A review and perspective of existing research on the release of nanomaterials from solid nanocomposites. *Part Fibre Toxicol* 11:17.
- Gilje S, Han S, Wang M, et al. (2007). A chemical route to graphene for device applications. *Nano Lett* 7:3394–8.
- Han JH, Lee EJ, Lee JH, et al. (2008). Monitoring multiwalled carbon nanotube exposure in carbon nanotube research facility. *Inhal Toxicol* 20:741–9.
- Han SG, Kim JK, Shin JH, et al. (2015). Pulmonary responses of Sprague-Dawley rats in single inhalation exposure to graphene oxide nanomaterials. *Biomed Res Int* 2015:376756.
- Kasai T, Gotoh K, Nishizawa T, et al. (2014). Development of a new multi-walled carbon nanotube (MWCNT) aerosol generation and exposure system and confirmation of suitability for conducting a single-exposure inhalation study of MWCNT in rats. *Nanotoxicology* 8:169–78.
- Kim JS, Sung JH, Song KS, et al. (2012). Persistent DNA damage measured by Comet assay of Sprague-Dawley rat lung cells after five days of inhalation exposure and 1 month post-exposure to dispersed multi-wall carbon nanotubes (MWCNTs) generated by new MWCNT aerosol generation system. *Toxicol Sci* 128:439–48.
- Kim JK, Shin JH, Lee JS, et al. (2016). 28-Day inhalation toxicity of graphene nanoplates in Sprague-Dawley rats. *Nanotoxicology*. [Epub ahead of print].
- Kovtyukhova NI, Ollivier PJ, Martin BR, et al. (1999). Layer-by-layer assembly of ultrathin composite films from micron-sized graphite oxide sheets and polycations. *Chem Mater* 11:771–8.
- Ku BK, Maynard AD, Baron PA, Deye GJ. (2007). Observation and measurement of anomalous responses in a differential mobility analyzer caused by ultrafine fibrous carbon aerosols. *J Electrostat* 65:542–8.
- Lee JH, Lee SB, Bae GN, et al. (2010). Exposure assessment of carbon nanotube manufacturing workplaces. *Inhal Toxicol* 22:369–81.
- Lee JH, Ahn KH, Kim SM, et al. (2015a). Three-day continuous exposure monitoring of CNT manufacturing workplaces. *Biomed Res Int* 2015:237140.
- Lee JS, Choi YC, Shin JH, et al. (2015b). Health surveillance study of workers who manufacture multi-walled carbon nanotubes. *Nanotoxicology* 9:802–11.
- Liao HY, Chung YT, Lai CH, et al. (2013). Six-month follow-up study of health markers of nanomaterials among workers handling engineered nanomaterials. *Nanotoxicology* 1:100–10.
- Liou SH, Tsou TC, Wang SL, et al. (2012). Epidemiological study of health hazards among workers handling engineered nanomaterials. *J Nanopart Res* 14:878–82.
- Ma-Hock L, Strauss V, Treumann S, et al. (2013). Comparative inhalation toxicity of multi-wall carbon nanotubes, graphene, graphite nanoplatelets and low surface carbon black. *Part Fibre Toxicol* 10:23.
- McKinney W, Chen B, Frazer D. (2009). Computer controlled multi-walled carbon nanotube inhalation exposure system. *Inhal Toxicol* 21:1053–61.
- NIOSH (National Institute of Occupational Safety and Health). (1994). Manual of analytical methods (NMAM) method 7300: asbestos by TEM. Cincinnati, OH: NIOSH.
- NIOSH (National Institute of Occupational Safety and Health). (2003). Manual of analytical methods (NMAM) method 5040: elemental carbon. Cincinnati, OH: NIOSH.
- NIOSH (National Institute of Occupational Safety and Health). (2013). Current intelligence bulletin 65: occupational exposure to carbon nanotubes and nanofibers. Cincinnati, OH: NIOSH.
- Schinwald A, Murphy FA, Jones A, et al. (2012). Graphene-based nanoplatelets: a new risk to the respiratory system as a consequence of their unusual aerodynamic properties. *ACS Nano* 6:736–46.
- Shin JH, Han SG, Kim JK, et al. (2015). 5-Day repeated inhalation and 28-day post-exposure study of graphene. *Nanotoxicology* 9:1023–31.
- WHO (World Health Organization). (1997). Determination of airborne fibre number concentrations: a recommended method, by phase-contrast optical microscopy (membrane filter method). Geneva: WHO.

Supplementary material available online