

Exposure to airborne particles associated with the handling of graphene nanoplatelets

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KEY WORDS: Nanomaterial; graphene; occupational exposure

PAROLE CHIAVE: Nanomateriale; grafene; esposizione occupazionale

SUMMARY

Background: *It is recognized that engineering control measures are needed to reduce occupational exposure to engineered nanomaterials (NMs): of these, fume hoods are among the most widespread collective protection equipment used while handling NMs in occupational settings. It is known that in some circumstances, handling NMs in fume hoods can result in a significant release of NMs. Objective:* To assess the effectiveness of fume hoods in reducing exposure while handling graphene nanoplatelets and to define the conditions that result in a lower dispersion of particles and thus less operator exposure. **Methods:** *An experimental protocol was established to monitor the variations of airborne particle concentrations while handling graphene in fume hoods (transferring and pouring). The measurement locations were at the laboratory, inside the hood and at operator's breathing zone. Handling tasks were performed under different operating conditions: the variable factors included hood face velocity and sash height. Results:* Results of this study indicate that the handling of graphene nanoplatelets may pose a potential risk of contamination of the work environment and hence exposure of the involved operators, if adequate control measures are not taken. In fact, when inadequate or not sufficiently cautionary operational conditions were utilized, non-negligible increases in airborne graphene particle concentrations during the nanomaterial manipulation phases were observed. **Conclusions:** *Some operating conditions (e.g., face velocity, sash height) can be adjusted to avoid relevant personal exposure conditions and contamination of the work environment by NMs, thus ensuring safer conditions.*

RIASSUNTO

«**Esposizione a particelle aerodisperse associate alla manipolazione di nanoplatelets di grafene**». **Introduzione:** *Tra le misure utilizzate per ridurre l'esposizione professionale ai nanomateriali ingegnerizzati (NM) le cappe chimiche sono tra i dispositivi di protezione collettiva più utilizzati, soprattutto per i casi in cui è prevista la manipolazione di NM da parte di un operatore. Tuttavia, in alcune circostanze, la manipolazione di NM in cappe chimiche può comportare un rilascio di NM nell'ambiente di lavoro. Obiettivi:* Valutare l'efficacia delle cappe chimiche per la riduzione dell'esposizione associata alla manipolazione di un NM (nanoplatelets di grafene) e definire le condizioni che determinano una minore dispersione di NM e quindi una minore esposizione dell'operatore. **Metodi:** È stato

Pervenuto il 2.2.2018 - Revisione pervenuta il 4.5.2018 - Accettato il 4.6.2018

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Funding: Directa Plus S.p.A provided funding for this project to the Dipartimento di Scienza e Alta Tecnologia, Università degli Studi dell'Insubria. Any opinions, findings, and conclusions or recommendations expressed in this product are those of the authors and not of the sponsor.

definito un protocollo sperimentale per monitorare le variazioni delle concentrazioni di particelle di NM aerodisperse durante la manipolazione di nanoplatelets di grafene sotto cappa chimica. Le postazioni di misura sono state fissate in laboratorio, all'interno della cappa e nella zona respiratoria dell'operatore. La manipolazione del NM è stata eseguita in diverse condizioni operative, modificando la velocità di aspirazione e l'altezza del vetro di protezione della cappa.

Risultati: *I risultati di questo studio indicano che la manipolazione di nanoplatelets di grafene può rappresentare un potenziale rischio di contaminazione dell'ambiente di lavoro e quindi dell'esposizione degli operatori coinvolti, se non vengono adottate adeguate misure di controllo. Infatti, nella simulazione durante la quale sono state utilizzate condizioni operative inadeguate o non sufficientemente cautelative, è stato possibile osservare un aumento non trascurabile delle concentrazioni di particelle di grafene aerodisperse.* **Conclusioni:** *Alcune condizioni operative (velocità di aspirazione, altezza del vetro di protezione) possono essere regolate per evitare rilevanti condizioni di esposizione personale e la contaminazione dell'ambiente di lavoro, garantendo in tal modo condizioni di lavoro più sicure.*

INTRODUCTION

Engineered Nanomaterials (NMs) are materials containing nano-objects with at least one external dimensions in the size range of 1-100 nm (8). Due to their peculiar physico-chemical properties, several NMs are produced and used in many industrial sectors (13). Among these, the graphene family of nanomaterials (GFNs) has been introduced into several scientific and technological applications (9, 16). Graphene is a single-atom thick, two-dimensional sheet of hexagonally arranged carbon atoms and forms a platelet shape (22). This NM is characterized by excellent mechanical properties, high elasticity, electric and thermal conductivity (6). Given the potential occupational and public exposure to graphene due to the increase of GFNs applications, potential health impacts need to be assessed for GFNs applications (22). The toxicity of GFNs is still relatively underexplored: available results point to a variety of health effects that may be strictly related to some properties of graphene such as its shape, size, layer number, chemical groups, surface, stiffness, hydrophobicity, surface functionalization, and dosage (7, 10, 12, 15, 17, 18, 22). However, to date, limited data are available regarding occupational exposure assessment in the GFN production and application industry and thus further research is still required to come to conclusions regarding risk characterization for GFNs (and for NMs in general) (1). To date, for effective precautionary management of potential nanotechnology related risks, a “hierarchy

of controls” has been proposed (4). Following this hierarchy (which considers it as a priority to eliminate/to substitute the use of a hazardous substance and providing workers with adequate information and training), where it is conceivable that occupational exposure may occur, it is necessary to adopt engineering control measures to reduce exposure levels such as the application of local exhaust ventilation, pressure differentials, hoods and enclosed systems. In this regard, fume hoods are among the most widespread ventilation systems: airborne particles are captured and removed through the air flow when NMs are manipulated in the fume hood (20). However, previous evidence has outlined that the handling of NMs in laboratory fume hoods can result in a significant release of airborne particles into the laboratory environment and in the operator's breathing zone (20), while other studies have observed good containment effectiveness and lower particle emission rates (2, 21). Different behavior could be associated to different type of hoods and their design, as well as to different work practices adopted for the handling of NM. Then, the preliminary hypothesis of this study was that the handling of graphene nanoplatelets in fume hoods may cause exposure arising from the emission and dispersion of graphene particles, mainly in the form of airborne ultrafine particles (“UFP,” i.e., particles with diameter <100 nm), but also in the form of fine particles (“FP”, i.e., particles with diameter between 0.3 and 1 μm) or coarse particles (“CP”; i.e., particles with diameter >1 μm). The main objective of the study is

to assess the effectiveness of fume hoods in protecting against graphene NM exposure and to define the conditions that result in lower dispersion of particles in the chemical hood and in the laboratory environment and less exposure of the operators.

METHODS

Although different standard methods have been developed to evaluate hood performance and the potential for contaminant leakage, these methods typically rely on a tracer gas with no worker present, so their applicability to the actual handling of NMs in a fume hood is uncertain (20). Therefore, an experimental protocol was established based on previous studies concerning similar tests on handling NMs in fume hoods (2, 20, 21).

Material and hood

For this study, an extremely fine nanopowder of pristine graphene nanoplatelets was used (Directa Plus S.p.A., Lomazzo - Italy). The pristine graphene nanoplatelets have lateral dimensions of a few micrometers and thickness of a few nanometers; the nanoplatelets are weakly aggregated by van der Waals forces, exhibiting an apparent density of approximately 60 g/L. Particle handling was studied using a constant velocity hood (Typhoon Twin - model 2003HIC; Labosystem srl, Rovellasca - Italy) that uses a controller to vary the fan speed as the sash is moved to maintain a constant hood face velocity. It is important to note that the hood design is one of the most critical factors to affect the particle release.

NM handling and cleaning of the hood

NM handling was performed in each hood by transferring or pouring 100 g of the investigated material from beaker to beaker (3000 ml beakers were used). The transferring task was performed by using a spatula to transfer nanoplatelets from one beaker to another beaker, while for the pouring task, nanoplatelets were poured directly from one beaker into a second beaker at the center of the open top (the beakers were adjacent to each other at the open edge). After completion of the NM handling ac-

tivities, cleaning of the work surface inside the hood was performed with laboratory paper moistened with water (wet wiping). A standardized protocol, derived by previous studies, was used to perform the handling and cleaning tasks (2, 20, 21): all operations were performed sequentially, following a fixed schedule and at predetermined times: each single simulation / monitoring session had a total duration of 35 minutes (table 1). In addition to the operations described above, a 5-minute interval ("5-min lag") was provided at the end of each single operation to evaluate the possible re-suspension and/or deposition of FP, CP and UFP due to the performed activities. During these lag periods, the front glass of the hood and the suction front speed were left under the conditions provided for the test. For each test, prior to the beginning of nanomaterial manipulation, CP, FP and UFP background concentrations were measured at each monitoring site (i.e., natural and anthropogenic particles occurring in the workplace air considered in a non-perturbed condition).

Experimental conditions

NM handling simulations have been carried out under different operating conditions. Variables studied in this research include handling method, face velocity and sash position; the simulations and the contextual monitoring were carried out in 3 standardized operating conditions (for a total of 15 replicate simulations) as reported in table 2.

Since literature sources recommend that sash height should be kept as low as possible when manipulating NMs and to operate with a face velocity between 0.4 and 0.6 m/s (2, 20, 21), according to the initial hypothesis, the operating conditions defined for test #1 (sash height: 30 cm; face velocity: 0.50 ± 0.05 m/s) could be considered optimal conditions for NM manipulation in a chemical hood. Consequently, the operational conditions defined for Test #2 (sash height: 50 cm; face velocity: 0.40 ± 0.05 m/s) should be considered potentially less precautionary than those defined for Test #1. In contrast, the operating conditions defined for Test #3 (sash height: 30 cm; face velocity: 0.65 ± 0.05 m/s) could be considered potentially more precautionary than those defined for Test #1 (2, 20).

Table 1 - Time schedule for the task performed for each test

Timing (minutes)	Task	Description
0-5	Background concentrations	Determination of UFP, FP and CP concentrations prior to start of activity
5-10	Handling NM (Transferring)	Transferring of 100 g of NM from one beaker to another by spatula
10-15	5-min lag (Transferring)	Lag period - evaluation of the suspension and / or deposition of UFP, FP and CP associated with the previous operation
15-17	Handling NM (Pouring)	Transferring of 100 g of NM from one beaker to another
17-22	5-min lag (Pouring)	Lag period - evaluation of the suspension and / or deposition of UFP, FP and CP associated with the previous operation
22-25	Cleaning	Cleaning of the work surface inside the hood (wiping)
25-30	5-min Lag (Cleaning)	Lag period - evaluation of the suspension and / or deposition of UFP, FP and CP associated with the previous operation
30-35	Final concentrations	Determination of UFP, FP and CP concentrations at the end of the handling and cleaning activities

Table 2 - Summary of the operating conditions defined for each test

Test	N of replies	Sash position	Face velocity
#1	5	Low (30 cm)	Medium (0.50±0.05 m/s)
#2	5	High (50 cm)	Low (0.40±0.05 m/s)
#3	5	Low (30 cm)	High (0.65±0.05 m/s)

Real-time particle measurement

The measuring strategy consisted of: (i) determination of the “background” particle number concentrations (PNC); (ii) determination of the particle number concentration during the handling and cleaning activities by means of micro-environmental and personal measurements; (iii) distinction of operation-related GFN particles from background aerosols. Average airborne PNC was measured in each location before the handling of nanomaterial to obtain an average background number concentration, which is then subtracted from the measurements made during the subsequent activities; this approach basically assumes that the concentration determined in each location during no-activity periods is representative of the background concentration, and any increased concentrations during the work activity can be attributed to the NM process-

ing (11, 19). Once the background particle number concentrations have been determined, specific measurements are made simultaneously at different locations: GFN particle concentrations were estimated by comparing the measured concentrations with the background concentration, following different approaches for background distinction. However, note that these approaches are considered as a proxy for assessing NM airborne concentrations and exposure levels, despite there being several possible errors associated with use of count-difference methods for background distinction (11, 19). In this study, a precautionary and conservative approach was adopted, attributing the whole differential particle concentration as GFN particles (without any differentiation between incidental and process-related engineered nanoparticles).

Environmental measurements were taken at a laboratory room location (“laboratory”) and at the source location (“hood”); personal measurements were collected in the breathing zone of the operator who conducted the handling simulations (“operator”) (figure 1). Laboratory particle concentrations were measured 2 m in front of the hood, at the center of the room, and at the level of the respiratory tract (150 cm - standing adult person). Particle concentrations at the source were measured at the downstream side of the releasing source

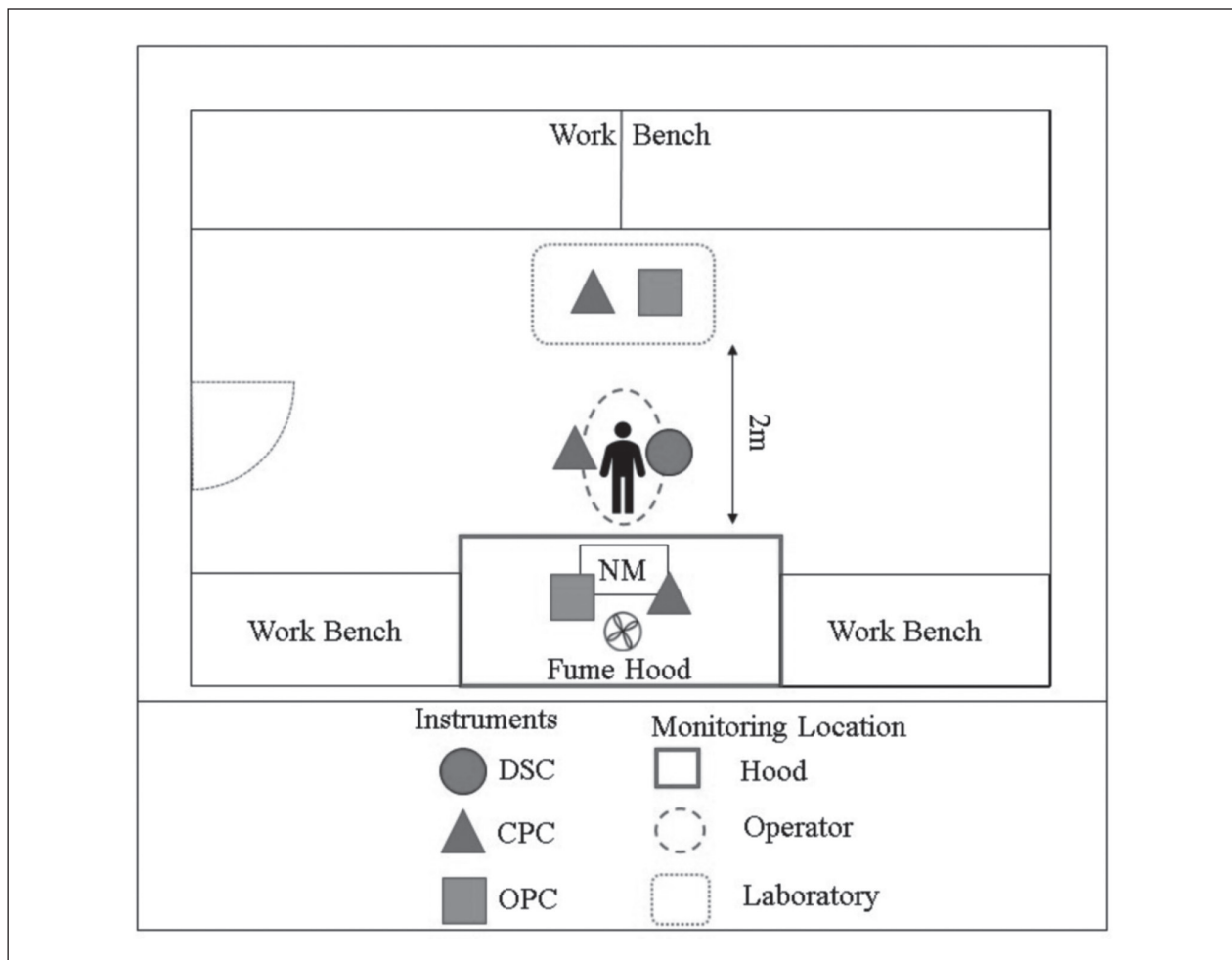


Figure 1 - Layout of the environment in the test environment, showing the “laboratory”, “hood” and personal (“operator”) monitoring locations

(instruments were placed at 15 cm vertically above the beaker at the downstream edge); NM handling tasks were performed in the hood at the center of the work surface. A time-activity diary was also used to separate the continuous data as a function of the different monitored tasks. All the instruments were used simultaneously for the entire length of the monitoring period.

Numerical concentrations of airborne UFP were measured using a miniature diffusion size classifier (DSC) and condensation particle counters (CPC). The DSC used for this study (DiSCmini, Matter Aerosol AG, Wohlen AG, Swiss) measures the particle number concentrations (range: 10^3 - 10^6 particle/ cm^3) and particles' average diameter in the size range

of approximately 10-700 nm (5). The DSC also estimates with reasonable accuracy the lung-deposited surface area concentration (LDSA), defined as the particle surface area concentration per unit volume of air, weighted by the deposition probability in the lung (5). Portable CPCs (P-Trak Ultrafine Particle Counter model 8525; TSI Inc., Shoreview, MN, USA) were also used in this study to quantify the airborne UFP number concentration (size range: 0.02 to 1 μm). Finally, numeric airborne concentrations FP and CP were also measured using optical particle counters (“OPC,” mod. Handheld 3016, Lighthouse Worldwide Solutions, Fremont, CA, USA), which can provide real-time measurement of particles with optical diameter for six different

dimensional fractions in the 0.3-10 μm range. It should be noted that for this study the UFP fraction includes particle with dimension >100 nm, although their number concentration is negligible with respect to those in the 0 - 100 nm interval. Further, results obtained with the OPC were referred to two cumulative dimensional fractions: fine particles (particle between 0.3 μm and 1 μm) and coarse particles (particles >1 μm). Data were measured with a 1-s (CPC, DSC) or 10-s frequency (OPC). Table 3 summarizes the monitoring design and strategy. It should be noted that the combined characterization of CP, FP and UFP can provide information on the nature and magnitude of potential contamination of the environment and of the professional exposure to nanomaterials. In fact, this experimental design provides complete aerosol evaluation and allows the presence of sources of nanomaterials and aggregates of nanomaterials (also of micrometric size) to be identified with reasonable certainty (3, 14).

RESULTS

The variations of airborne UFP, FP and CP concentrations from background concentrations are reported in table 4 and table 5. The background number concentrations defined for each location were subtracted from the measurements made during handling of NMs; thus, results from this type of measurement should be interpreted as absolute variations in the concentrations of airborne NM particles with respect to the background values. By comparing the absolute variation to the background value, it is also possible to determine the relative variation (%).

Operating conditions - Test #1

Results for Test #1 outlined a general reduction of mean UFP concentrations (mean \pm standard deviation (particle/cm³); mean \pm standard deviation (%) when compared to the background value measured at the three monitored stations: hood (-556 \pm 1155 particle/cm³; -11.4 \pm 18%), operator (-459 \pm 1764 particle/cm³, -1.4 \pm 23%) and laboratory (-362 \pm 1882 particle/cm³; -0.7 \pm 27%). However, no relevant variations of the mean diameter or LDSA were observed in association with the reduction of PNC. By analyzing the variations in UFP concentrations recorded for the various tasks performed during Test #1 (table 4), it should be noted that only the transferring task involved a slight increase in the concentrations measured for the operator (52 particle/cm³, 1.9%) and laboratory (26 particle/cm³, 0.3%). However, this increase is followed by a sharp decrease in concentrations during the next observation period (5 min lag - transferring). After cleaning the hood's work surface UFP concentrations decrease again at the laboratory site and for operator exposure. On the other hand, UFP concentrations showed a regular and stable reduction in the "hood" site, contributing to the hypothesis that the operational conditions for Test #1 are adequate to ensure efficient removal from the hood compartment of UFP, which can eventually be re-suspended during the handling of the NM. It should be noted that overall, at the end of the test, UFP concentrations at the three measurement sites are consistently lower than the background concentrations. Concerning FP and CP concentrations, different behavior could be observed (table 5). Although the results outlined overall re-

Table 3 - Monitoring strategy and instruments used for measurements

Monitored task	Particles fraction	Location		
		Laboratory	Hood	Personal
Background Concentrations (site-specific)	UFP	CPC (PNC)	DSC (PNC, mean diameter, LDSA)	
	FP, CP	OPC (PNC, size-resolved)		
Handling and cleaning operations	UFP	CPC (PNC)	DSC (PNC, mean diameter, LDSA)	
	FP, CP	OPC (PNC, size-resolved)		

Table 4. Absolute and relative (%) average variation of UFP concentrations from the background value. Results are calculated on 5 replicates of each test

Test	PNC - Hood (particle/cm ³)	PNC - Operator (particle/cm ³)	Mean Diameter - Operator (nm)	LDSA - Operator (µm ² /cm ³)	PNC - Laboratory (particle/cm ³)
#1 Background (mean)	6785	5497	93.8	26.87	6446
Transferring	-190 (-7.2)	52 (1.9)	0.3 (0.4)	0.28 (1.0)	26 (0.3)
5-min lag (Transferring)	-397 (-8.1)	-231 (-0.1)	0.1 (0.01)	0.13 (0.6)	-387 (-4.2)
Pouring	-580 (-9.9)	-515 (-3.4)	-0.2 (-0.4)	-0.17 (-0.4)	-440 (-3.3)
5-min lag (Pouring)	-641 (-13.4)	-651 (-5.1)	-0.1 (-0.4)	-0.11 (-0.1)	-735 (-7.4)
Cleaning	-776 (-21.7)	-834 (-7.8)	-0.5 (-0.9)	-0.54 (-1.8)	-753 (-6.3)
5-min lag (Cleaning)	-771 (-21.3)	-710 (-2.8)	-0.6 (-1.2)	-0.63 (-1.9)	-133 (7.7)
Final concentration	-782 (-10.1)	-509 (3.8)	-0.5 (-1.1)	-0.51 (-1.3)	-314 (4.7)
Mean variation	-556±1155	-459±1764	-0.2±1.4	-0.21±1.45	-362±1882
±SD (%)	(-11.4±18)	(-1.4±23)	(-0.5±2.2)	(-0.5±4.8)	(-0.7±27)
#2 Background (mean)	5748	4228	102.1	24.48	5227
Transferring	-36 (-0.4)	17 (0.9)	-0.5 (-0.5)	-0.46 (-1.7)	-2 (0.1)
5-min lag (Transferring)	-120 (-1.8)	27 (0.8)	-0.4 (-0.4)	-0.40 (-1.9)	-11 (0.03)
Pouring	-67 (-0.7)	65 (2.4)	-0.6 (-0.6)	-0.57 (-2.5)	129 (3.1)
5-min lag (Pouring)	-41 (-0.1)	190 (6.0)	-0.2 (-0.3)	-0.22 (-0.9)	142 (3.3)
Cleaning	-45 (-0.3)	197 (5.5)	-0.04 (-0.1)	-0.04 (-0.1)	238 (5.0)
5-min lag (Cleaning)	-87 (-1.2)	327 (8.7)	0.3 (0.3)	0.31 (1.2)	140 (3.3)
Final concentration	-122 (-1.99)	290 (7.7)	0.1 (0.2)	0.13 (0.6)	26 (1.2)
Mean variation	-74±309	166±389	-0.1±1.0	-0.15±1.0	81±310
±SD (%)	(-0.9±5.8)	(4.8±10.4)	(-0.2±1.0)	(-0.6±4.2)	(2.0±6.1)
#3 Background (mean)	4818	3828	95.7	20.69	4627
Transferring	-43 (-1.0)	-17 (-0.8)	-0.2 (-0.2)	-0.22 (-1.2)	-45 (-0.8)
5-min lag (Transferring)	-97 (-1.8)	-121 (-2.7)	-0.3 (-0.3)	-0.26 (-1.3)	-30 (-0.4)
Pouring	-127 (-2.1)	-172 (-3.5)	-0.2 (-0.2)	-0.22 (-0.9)	-73 (-1.0)
5-min lag (Pouring)	-191 (-3.0)	-226 (-4.3)	-0.4 (-0.5)	-0.44 (-1.8)	-124 (-1.9)
Cleaning	-304 (-5.3)	-335 (-7.2)	-0.7 (-0.7)	-0.66 (-2.8)	-196 (-3.3)
5-min lag (Cleaning)	-278 (-4.6)	-332 (6.9)	-0.6 (-0.6)	-0.57 (-2.3)	-172 (-2.8)
Final concentration	-341 (-5.9)	-290 (-5.5)	-0.7 (-0.7)	-0.70 (-2.9)	-89 (-0.8)
Mean variation	-200±443	-209±549	-0.4±1.2	-0.44±1.20	-101±418
±SD (%)	(-3.4±8.6)	(-4.3±13.7)	(0.5±1.3)	(-1.9±5.9)	(-1.5±8.9)

ductions of FP concentrations in personal exposure (-1.9 ± 5.7 particle/cm³; $-5.5\pm 12.3\%$) and in the laboratory site (-1.0 ± 3.9 particle/cm³, $-3.3\pm 9.6\%$), increases in CP concentrations were observed for these same two positions (operator: 0.07 ± 1.13 particle/cm³; $35\pm 56\%$; laboratory: 0.05 ± 0.06 particle/cm³; $33\pm 40\%$), as was an increase in airborne particles concentrations at the hood monitoring site (FP: 1.5 ± 11 particle/cm³; $8.5\pm 41.3\%$; CP: $0.86\pm 3.03\%$ particle/cm³; $188\pm 523\%$). This increase can be attributable to transferring and pouring activities, which involve a relevant increase in fine particulate

concentrations and especially of coarse particulate matter. However, this increase in concentrations is efficiently offset by the “lag” period provided after the NM handling and subsequently by the cleaning of the work surface; at the end of the test these two procedures contributed to the attainment of UFP, FP and CP concentrations that were lower or comparable to the background values. Therefore, although not-negligible increases in UFP, FP and CP concentrations were observed during the NM handling tasks, the operating conditions for Test #1 are adequate to ensure efficient removal of particles

Table 5 - Absolute and relative (%) average variation of fine particulate (< 1 µm) and coarse particulate (> 1 µm) concentrations from the background value. Results are calculated on 5 replicates of each test

Test	FP - Hood	CP - Hood	FP - Operator	CP - Operator	FP - Laboratory	CP - Laboratory
#1 Background (mean)	32.7	0.91	43.9	0.24	39.0	0.15
Transferring	0.8 (4.6)	3.72 (775)	1.9 (3.6)	0.09 (41)	1.4 (2.9)	0.06 (39)
5-min lag (Transferring)	2.8 (4.6)	0.11 (36)	-1.0 (-3.5)	0.05 (22)	-0.2 (-1.2)	0.04 (26)
Pouring	3.6 (12.7)	3.61 (525)	1.7 (2.3)	0.12 (57)	0.3 (0.1)	0.05 (39)
5-min lag (Pouring)	2.7 (14.9)	0.29 (71)	-0.8 (-3.7)	0.07 (34)	-0.5 (-2.1)	0.05 (33)
Cleaning	1.9 (12.3)	0.32 (72)	-2.5 (-6.8)	0.12 (57)	-1.0 (-3.3)	0.06 (39)
5-min lag (Cleaning)	0.6 (10.3)	-0.16 (17)	-4.0 (-11)	0.10 (47)	-2.6 (-7.3)	0.05 (35)
Final concentration	-0.3 (6.2)	-0.34 (-14)	-6.7 (-16)	0.005 (7.1)	-4.0 (-10)	0.04 (26)
Mean variation	1.5±11	0.86±3.03	-1.9±5.7	0.07±1.13	-1.0±3.9	0.05±0.06
±SD (%)	(8.5±41.3)	(188±523)	(-5.5±12.3)	(35±56)	(-3.3±9.6)	(33±40)
#2 Background (mean)	38.4	0.58	47.9	0.27	43.0	0.19
Transferring	-1.6 (-4.1)	3.39 (679)	-1.0 (-2.1)	0.01 (5.1)	-0.4 (-1.0)	-0.01(-3.5)
5-min lag (Transferring)	-1.5 (-3.8)	-0.01 (0.4)	-1.7 (-3.4)	-0.02 (-5.4)	-1.1 (-2.8)	-0.01(-4.0)
Pouring	-1.9 (-5.0)	1.04 (173)	-1.7 (-3.4)	-0.02 (-5.9)	-1.4 (-3.5)	-0.01 (-2.4)
5-min lag (Pouring)	-0.6 (-1.5)	0.01 (3.7)	-1.5 (-2.8)	-0.02 (-2.5)	-1.1 (-2.8)	-0.01 (-3.7)
Cleaning	0.3 (0.8)	0.08 (19)	1.2 (2.3)	0.00 (5.9)	-0.3 (-0.9)	-0.01 (-4.6)
5-min lag (Cleaning)	-0.4 (0.8)	0.04 (9.1)	-0.4 (-0.8)	-0.01(1.7)	-0.7 (-1.7)	0.01 (4.5)
Final concentration	-0.5 (-0.8)	-0.02 (-2.7)	-2.3 (-4.4)	0.003 (2.6)	-1.2 (-2.6)	0.01 (4.2)
Mean variation	-0.8±3.7	0.64±2.19	-1.2±3.0	-0.01±0.09	-0.9±2.2	-0.004± 0.05
±SD (%)	(-2.1±9.9)	(128±472)	(-2.3±6.1)	(-0.2±32)	(-2.1±5.3)	(-1.1±24)
#3 Background (mean)	44.4	0.59	54.2	0.27	50.2	0.16
Transferring	-1.9 (-4.1)	3.92 (838)	-1.0 (-1.2)	-0.04 (-10)	-1.4 (-2.2)	-0.01 (-6.7)
5-min lag (Transferring)	-1.8 (-3.4)	0.11 (25)	-1.8 (-2.2)	-0.06 (-17)	-2.0 (-2.6)	-0.02 (-11)
Pouring	-2.6 (-4.6)	1.08 (197)	-2.2 (-2.7)	-0.03 (-7.7)	-2.7 (-3.7)	-0.02 (-7.8)
5-min lag (Pouring)	-2.5 (-3.9)	-0.04 (-1.5)	-3.3 (-4.2)	-0.06 (-18)	-3.4 (-4.6)	-0.02(-9.0)
Cleaning	-2.4 (-3.6)	0.02 (11)	-3.2 (-3.6)	-0.04 (-14)	-4.1 (-5.6)	-0.02 (-12)
5-min lag (Cleaning)	-2.5 (-3.3)	-0.08 (-6.7)	-3.3 (-3.2)	-0.05 (-13)	-4.1 (-5.4)	-0.01 (-6.4)
Final concentration	-2.9 (-3.4)	-0.17 (-24)	-3.4 (-2.7)	-0.08 (-24)	-4.5 (-5.6)	-0.02 (-11)
Mean variation	-2.3±4.0	0.69±2.51	-2.6±5.8	-0.05±0.09	-3.1±5.5	-0.02±0.04
±SD (%)	(-3.7±8.5)	(152±582)	(-2.8±8.3)	(-15.4±28)	(-4.2±8.8)	(-9.1±22)

possibly raised during the handling of the NM, especially if appropriate compensation times are set after these operations.

Operating conditions - Test #2

In contrast to the above, for Test #2 an increase in mean UFP concentration compared to the background value was observed for the operator exposure (166±389 particle/cm³, 4.8±10.4%) and at the laboratory site (81±310 particle/cm³, 2.0±6.1%), while

a simultaneous decrease in UFP concentration at the hood position was observed (-74±309 particle/cm³; -0.9±5.8%) (table 4). No relevant variations of the mean diameter or LDSA of the particles were observed. Interestingly, UFP concentrations at the hood position never showed an increase over the background value, thus corroborating the hypothesis that the operating conditions for Test #2 are still adequate to ensure efficient removal of UFP possibly raised during the handling of the NM. However, for the operator exposure and at the laboratory site, aver-

age concentration values are constantly higher than the background values, and they showed a moderate but steady increase between the transferring and cleaning tasks. Only after cleaning the hood work surface did the UFP concentrations measured at the laboratory site return to values comparable to background concentrations (i.e., resulting in an average increase of 26 particles/cm³, which is equivalent to an average increase of 1.2%), while personal exposure resulted in an increase of approximately 300 particle/cm³ (equivalent to an 8% increase) with respect to background concentrations. Regarding FP and CP concentrations (table 5), an overall reduction of FP concentration was established for personal exposure (-1.2±3.0 particle/cm³; -2.3±6.1%) and for the laboratory site (-0.9±2.2 particle/cm³; -2.1±5.3%). For these two same sites, a reduction of CP concentrations (personal: -0.01±0.09 particle/cm³; -0.2±32%; laboratory: -0.004±0.05 particle/cm³; -1.1±24%) was also observed. At the hood site a reduction of average FP concentrations (-0.8±3.7 particle/cm³; -2.1±9.9%) and a simultaneous increase in average CP concentrations (0,64±2.19 particle/cm³, 128±472%) were observed. The increase of CP could be attributable to NM handling operations (transferring and pouring). However, even in this case, the increase in concentrations is efficiently offset during the lag period expected after the material handling and, subsequently, with the cleaning operations of the hood's work surface. At the end of the test particles concentrations were lower than (or comparable to) the background values. Thus, considering results obtained during NM handling performed under Test #2 operating conditions, the potential for dispersion of NMs in the hood compartment and in the laboratory environment, as well as potential exposure of the operator involved in the manipulation of nanomaterials, cannot be excluded.

Operating conditions - Test #3

Analogously to what was observed for Test #1, measurements for Test #3 generally showed a reduction with respect to background for UFP concentrations at the three monitored stations: hood (-200±443 particle/cm³; -3.4±8.6%), operator (-209±549 particle/cm³, -4.3±13.7%) and laborato-

ry (-101±418 particle/cm³; -1.5±8.9%). In association with this reduction of PNC, a non-negligible variation of the particle LDSA (-0.44±1.20 μm²/cm³; -1.9±5.9%) was also observed. By analyzing the variations in UFP concentration observed for the various tasks of Test #3 (Table 4), none of the operations performed resulted in an increment of UFP concentration in any of the investigated locations; conversely, the reduction of UFP concentrations appears to be characterized by a steady and constant trend; a slight increase in the concentrations at the laboratory and hood site was observed because of the cleaning activity. This last was not enough to determine an exceedance of the initial background value anyway. Overall, at the end of the test, the UFP concentrations at the three measurement sites were lower than the background values. This evidence corroborates the hypothesis that the conditions for Test #3 are adequate to ensure efficient removal of UFP during the handling of NM. A reduction of FP concentrations was also determined for personal exposure (-2.6±5.8 particle/cm³; -2.8±8.3%) and the laboratory site (-3.1 ± 5.5 particle/cm³; -4.2±8.8%). At the same positions, a reduction of CP concentrations was also observed (operator: -0.05±0.09 particle/cm³, -15.4±28%, laboratory: -0.02±0.04 particle/cm³; -9.1±22%). In the hood site, a reduction of average FP concentration (-2.3±4.0 particle/cm³; -3.7±8.5%) and a simultaneous increase in CP concentration (069±2.51 particle/cm³, 152±582%) were also observed. As discussed above, this increase is mainly attributable to transferring and pouring activities; however, even in this case, the increase in concentrations is efficiently offset during the lag period expected after the NM handling and then with the cleaning of the work surface. At the end of the test, FP and CP concentrations were lower than the background values. In summary, although non-negligible increments of particles concentrations have been observed during the NM handling tasks, the above operating conditions could be considered adequate for NM handling in a chemical hood; further, Test #3 conditions appeared to ensure efficient removal of particles eventually re-suspended because of NM handling, especially if adequate compensation times are provided after such activities.

DISCUSSION

In the present study, the intensity of UFP, FP and CP contamination (and therefore of the potential exposure) resulting from handling of NM in a fume hood depends on several factors, primarily the face velocity and the sash height of the hood. The performed tests were characterized by distinct values for these two variables with the intention of simulating three scenarios characterized by operational conditions considered optimal (Test #1), potentially worse (Test #2) or better (i.e., more precautionary) (Test #3) for handling the studied NM.

In this regard, although the concentration levels observed for this study are of particularly modest entities, results showed that Test #2 represents a non-precautionary operating condition for which it is not possible to exclude the potential contamination of work environments by UFP, FP and CP generated by the handling of NM. This statement is based mainly on the observed increase in UFP concentrations in the laboratory and at the level of personal exposure, a behavior in contrast to what was observed during the other simulations. On the other hand, Test #1 showed in general the containment of potential UFP, FP and CP contamination generated by the handling of the NM, but it also showed non-negligible increases in UFP concentrations (i.e., during the transferring task) and FP. These increments suggest that it would be preferable to adopt more precautionary conditions such as those defined in Test #3. In this latter simulation, increases of UFP and FP concentrations were not observed at any of the three monitoring stations. Concerning CP, non-negligible concentration increases were observed during transferring and pouring operations at the hood position. This increment is effectively compensated for by the lag periods that were defined in the time schedule after the material handling and cleaning activities: this practice contributed to the achievement of final CP concentrations that were lower than the background values. This pattern occurs likewise for all the performed tests regardless of the adopted operating conditions. In this regard, it is possible to state that overall, all tested operating conditions can be considered adequate to ensure efficient removal of the coarse frac-

tion of airborne particles from the hood compartment during the handling of the NM if appropriate lag periods are respected after such operations. As mentioned, even the cleaning operations of the hood's work surface contributed to the reduction of airborne concentrations of UFP, FP and CP. In this study, wet wiping was adopted: equivalent systems or more efficient ones (e.g., high efficiency particulate air filter (HEPA) - filtered vacuum cleaners) are equally valid for this purpose.

It is important to note that some limitations in the study design and methods could have had an impact on the results, including possible errors associated with the use of the count-difference method to estimate particle number concentrations of GFN particles and direct-reading instrument sensitivity. Further, data were derived from an in-lab simulation: measurements were taken within a specific setting, according to a systematic and technical protocol which comprises some intrinsic limitations in accuracy. However, the distinction of GFN particles from background aerosols was performed with a conservative approach, and in conclusion, this approach was likely to result in an overestimation of contamination and exposure, as discussed previously (19). Further, although the basic concepts of adopted approach were based on those proposed by previous authors (2, 20, 21) and then applied for a case-study NM under selected occupational settings, some further issue should be considered for a complete evaluation. Among these, specific hood's features (i.e. hood design, exhaust flow, interior equipment loading, etc.), as well as other boundary conditions (i.e. location of laboratory air supply, proximity to doors, etc.) could have an impact on the results and should be considered (2, 20, 21). More in general, a further study would also be useful in reducing the uncertainty associated with the measurements, whose variability appears to be high and possibly in confirming the conclusions obtained with this study. However, despite some limitations, this study allows a first characterization of the relationship between basic fume hood settings (i.e. sash height, face velocity) and release of graphene nanoplatelets during handling tasks and therefore allows to define appropriate procedures for the safe handling of graphene nanoplatelets.

In summary, this work discussed a case study meant to provide data on the performance of laboratory fume hoods when handling NMs for risk assessment purposes. Results indicate that the handling of NMs may pose a potential risk of contamination of the work environment and hence exposure of the involved operators. However, some operating conditions can be adjusted to avoid relevant personal exposure conditions and contamination of the work environment by the NMs themselves, to ensure safer conditions. For this reason, some good-laboratory procedures are reported below to be considered with the purpose of defining appropriate procedures for the handling of NMs in a chemical hood; these were derived from indications in scientific literature and international guidelines (2, 13, 20, 21). The height of the sash must be kept at the lowest possible level (in the case: 30 cm) while maintaining a proper frontal velocity, as reported in good technical practices. The face velocity of the chemical hood should be set between 0.4 and 0.6 m/s; lower or higher (> 0.8 m/s) frontal velocities can result in dispersion of the NM. Constant volume hoods represent an adequate choice for this type of application. Handling of NMs in fume hoods should be carried out by preferring manipulation techniques that involve the least possible material dispersion and avoid inducing strong turbulence. These indications were integrated considering the evidence obtained during this study: (i) The simulation carried out with a frontal velocity of 0.50 ± 0.05 m/s showed consistent results with existing indication, i.e., these conditions assured adequate containment of the potential UFP, FP and CP contamination generated by the manipulation of the nanomaterial. More cautionary conditions can also be considered: lower dispersion of particles was observed for a velocity of 0.65 ± 0.05 m/s. (ii) Provide a lag period at the end of NM handling, before proceeding with other activities, to allow the concentration of airborne particles to be reduced in the hood compartment. In this study, a 5-minute lag was sufficient to compensate for increases in particulate concentrations that may have been re-suspended during NM handling. (iii) Cleaning the hood's work surface after any NM handling activity. In this study, wet wiping proved to be an efficient technique; methods equivalent or

more efficient (e.g., cleaning with an absolute filter extractor) are equally valid for the reduction of re-suspended UFP, FP and CP concentrations and for containment of the potential contamination. (iv) Given the uncertainty related to the toxic potential of GFNs and NMs in general, it is advisable to ensure the protection of workers engaged in the handling and cleaning processes with adequate personal protective equipment (e.g., filtering half-facemask with (FFP2- or FFP3-class filter), nitrile gloves and protective glasses).

CONCLUSIONS

At first, it should be noted that the concentration levels observed for this study are particularly modest, so the occupational exposure associated with the handling of the nanomaterials under the investigated conditions may be considered not relevant. Anyhow, results of this study indicate that the handling of graphene nanoplatelets may pose a potential risk of contamination of the work environment - and hence exposure of the involved operators, if adequate control measures are not taken. In fact, if inadequate or not sufficiently cautionary operational conditions are adopted non-negligible increases in UFP, FP, and CP concentrations during the nanomaterial manipulation phases are observed. Similarly, evidences also outlined that the adoption of appropriate operating procedures avoids relevant personal exposure conditions and contamination of the work environment by the NM itself, thereby ensuring safer conditions. Results also indicated that some operating conditions (e.g., face velocity, sash height) can be adjusted to avoid relevant personal exposure conditions and contamination of the work environment by the NM itself. In this regard, some indications were reported in this study to be considered with the purpose of defining appropriate procedures for the handling of NMs in a chemical hood. These were derived from scientific literature and international guidelines and could be easily applied in any NM-related laboratory scenario to ensure safer conditions. Among the other indications, a particularly important suggestion is to provide a lag period at the end of NM handling, before proceeding with other activities, to allow the concentration of air-

borne particles to be reduced in the hood compartment. In this study, a 5-minute lag was sufficient to compensate for increases in particulate concentrations that may have been re-suspended during NM handling.

NO POTENTIAL CONFLICT OF INTEREST RELEVANT TO THIS ARTICLE WAS REPORTED BY THE AUTHORS

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ACKNOWLEDGMENTS: *The authors gratefully acknowledge Directa Plus S.p.A, and in particular Giulio Cesareo (CEO), Valerio Giughiano (Project Manager and Project Coordinator), Carolina Volontè (Quality Control) and Laura Giorgia Rizzi (CTO - Chief Technical Officer) for participating in this research project and for the permission to publish; the authors also acknowledge Directa Plus' administrative and operation personnel for their kind and helpful collaboration.*