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Probabilistic approach for the risk assessment of nanomaterials: A case study for graphene nanoplatelets

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ABSTRACT

An experimental probabilistic approach for health risk assessment was applied for graphene nanoplatelets (GNPs). The hazard assessment indicated a low level of toxicity for the GNPs. The benchmark dose method, based on sub-chronic and chronic inhalation exposure studies, was used to quantify a guidance value (BMC_h) for occupational inhalation exposure to GNPs, expressed as a lognormal distribution with a geometric mean ± geometric standard deviation of 0.212 ± 7.79 mg/m^3 and 9.37 × 10^4 ± 7.6 particle/cm^3. Exposure scenarios (ES) were defined based on the scientific literature for large-scale production (ES1) and manufacturing (ES2) of GNPs; a third ES, concerning in-lab handling of GNPs (ES3) was based on results of experiments performed for this study. A probability distribution function was then assumed for each ES. The risk magnitude was calculated using a risk characterization ratio (RCR), defined as the ratio of the exposure distributions and the BMC_h distribution. All three ES resulted in RCR distributions ≥1 (i.e. risk present); however, none of the ES had a statistically significant level of risk at a 95% confidence interval. A sensitivity analysis indicated that ~75% of the variation in the RCR distributions was due to uncertainties in the BMC_h calculation.

1. Introduction

Engineered nanomaterials (NMs) are defined as man-made materials where at least 50% of the primary particles, aggregates, and/or agglomerates have one or more external dimension between 1 and 100 nm (nm) (Standardization and (ISO), 2008). Such materials possess unique physicochemical properties that make them suitable for use in various industrial sectors. Nevertheless, there is still limited knowledge about the impacts of NMs on human health and, consequently, health and safety policies related to NM exposure are in the initial phase of development. The possible health impacts of manufactured NMs need to be considered and, hence, tools for evaluating these factors, such as appropriate risk assessment (RA) procedures, need to be developed. In general, the methodology used in the RA process for NMs is similar to that already used for other types of chemicals (Leso et al., 2017). However, at a practical level, the RA process for the nanotechnology field is challenging. One of the major limitations lies in the very narrow amount of available information on NM hazard identification, dose-response relationships, and exposure evaluation. Thus, the commonly adopted deterministic RA methods do not explicitly state the certainty or likelihood of risk. This is because uncertainties in the assessment parameters (e.g., related to the lack of relevant toxicological data, known emissions across the life cycle, measured exposure, and appropriate models) are not considered in the assessment and therefore can't be expressed together with the results. A recent study proposed a probabilistic approach for performing human health RA in a case study of TiO_2, a widely used NM, for different occupational exposure scenarios (Tsang et al., 2017). This probabilistic approach allows (i) parameters in the RA process to be defined by distributions instead of single deterministic values with uncertainties that could undermine the value of the assessment, and (ii) estimation of uncertainties and variability in the RA process.

The objective of this study was to apply an experimental probabilistic approach, based on that proposed by Tsang et al. (2017), for performing human health RA for a case-study NM under selected occupational settings. The four steps in the RA process were followed, as defined by the United States National Academy of Sciences (National Research Council, 1983): hazard identification, dose-response assessment, exposure evaluation, and risk characterization. Different dosemetrics (mass concentration and number concentration) were considered (if possible) through all the phases of the RA.

The probabilistic RA approach was applied to graphene

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Abbreviations

95%CI	95% confidence interval
AIC	Akaike's information criterion
AM	arithmetic mean
BAL	broncho-alveolar lavage
BMC	benchmark concentration
BMC^h	benchmark concentration - human
BMCL	benchmark concentration (95% CI Lower Bound)
BMCU	benchmark concentration (95% CI Upper Bound)
BMD	benchmark dose
BMDL	benchmark dose (95% CI lower bound)
BMDU	benchmark dose (95% CI upper bound)
BMR	benchmark response
EF_{inter}	inter-species extrapolation factor
EF_{intra}	intra-species extrapolation factor
ES	exposure scenario
EXP	exposure data distribution
GFNs	graphene family nanomaterials
GM	geometric mean
GNPs	graphene nanoplatelets
GSH	glutathione
HBGV	health-based guidance value
LOAEL	lowest-observed adverse effect level
MN	manufactured (engineered) nanomaterial
NOAEL	no-observed-adverse-effect level
OEL	occupational exposure level
PMNs	polymorphonuclear cells
RA	risk assessment
RCR	risk characterization ratio
SD	standard deviation
TWA	time-weighted average
UF_i	uncertainty factor

nanoplatelets (GNPs); graphene is a single-atom thick, two-dimensional sheet of hexagonally arranged sp^2 carbon atoms that forms a platelet shape (Park et al., 2017; Tsai et al., 2017). Graphene has several related congeners, each with distinct properties, all of which are part of the graphene family nanomaterials (GFNs) (Bianco et al., 2013). GFNs are generally characterized by excellent mechanical properties, high elasticity, and high electric and thermal conductivity (Geim and Novoselov, 2007), making them suitable for various applications (Allen et al., 2010; De et al., 2011; Guo et al., 2010). Although several toxicity assessment studies have been conducted, there is a lack of information regarding potential health effects of GNPs in humans from inhalation exposure, which is the primary exposure pathway for engineered NMs in workplaces (Kim et al., 2016); the available data is not sufficient to make adequate conclusions about the potential hazards using a RA, or develop regulations. Comprehensive information regarding the general risks posed by GNPs is very limited (Kim et al., 2016; Schinwald et al., 2012).

2. Methods

2.1. Hazard identification

A literature search was performed to identify recent studies (2010 to the present) from both peer-reviewed and grey literature, to identify relevant toxicity data for a RA of GNPs. Due to the differences in the toxicity of each class of GFNs, which could be dependent on different material characteristics (e.g., shape, size, purity, post-production processing steps, oxidative state, functional groups, dispersion state, synthesis methods) (Lalwani et al., 2016), this study focused on the results of in-vivo and in-vitro toxicology tests concerning GNPs and pristine graphene. When available, toxicological tests concerning subchronic and chronic inhalation exposures were prioritized, as these conditions were considered the most relevant potential exposure in the work environment.

2.2. Dose-response assessment

The benchmark dose (BMD) method was used to estimate a health-based guidance value (i.e., a threshold limit value for occupational exposure) (Davis et al., 2011; EFSA Scientific Committee, 2017). As previously proposed "benchmark concentration" (BMC) will be used instead of BMD, to emphasize that the adopted model refers to whole-body concentration data" (Tsang et al., 2017). Calculation of BMCs and the respective lower (BMCL) and upper bounds (BMCU) was undertaken using BMDS Wizard v. 1.10 (ICF et al., 2015) and BMD Software v2.7.0.4 (BMDS) (EPA). In addition, automated rules consistent with BMD modeling guidelines were applied (EPA, 2012b). BMDS Wizard recommended BMCs and associated BMCLs for the collected dose-response data based on the best-fit model selected according to decision logic determined prior to modeling and categorized the models as "Unusable", "Questionable", or "Viable". Only viable model outputs were considered in this study. Data sets consisted of continuous dose-response data, which guided the choice of BMRs and the types of models used to calculate BMDs. All models specified in the BMD modeling guidelines were used, if appropriate for the specific data type (EPA, 2012b). The BMR was defined as the change in the mean equal to one control standard deviation (SD) for continuous data. The viable models and associated BMCs (with corresponding BMCLs and BMCUs) for each dose-response set were selected according to criteria defined previously (Wignall et al., 2014). Since different dose-response data sets were considered, the lowest BMC and its associated BMCL were selected, regardless of the end-point/effect. The goal was not to find the single best fitting model, but rather to consider results from all valid applied models. The individual model results were combined by weighting, with higher weights for models that showed better fits. The model averaging approach was used to define an AIC-weighted average BMC and respective BMCL and BMCU values, considered as the lower and upper bounds of the BMC confidence interval, respectively.

Finally, a reference point (RP) also called the health-based guidance value (HBGV) was defined. The average BMC (and corresponding BMCL and BMCU values) calculated by BMD Software and BMDS Wizard based on the toxicological animal data defined from the hazard assessment are referred to as BMC_a, $BMCL_a$, and $BMCU_a$ (where "a" stands for "animal"). The lower bound ($BMCL_a$) was used as a starting point to calculate the potential RP as a precautionary approach. The $BMCL_a$ was extrapolated to a human effect threshold referred to as BMC_h (where "h" stands for "human"), which was considered equivalent to a HBGV or to an occupational exposure level (OEL), by applying extrapolation factors (EPA, 2012a), using Equation (1):

$$BMC_h = \frac{BMCL_a}{EF_{inter} * EF_{intra} * UF_i} \quad (1)$$

where EF_{inter} and EF_{intra} are inter- and intra-species extrapolation factors and UF_i contains other sources of uncertainty from the dose–response assessment. Based on the probabilistic approach defined by Tsang et al. (2017), lognormal distributions for these factors were defined, using a similar approach to that presented by Slob et al. (2014), and distributions for EF_{inter}, EF_{intra}, and UF_i were defined. The assumption is that these values would be log-normally distributed such that a value of 10 (as typically used in deterministic RA approaches) was one order of magnitude greater than the mean and occurred at the 99th percentile. A Microsoft Excel add-on software package (RiskAMP)

was used to supply probabilistic functions for stochastic functionality, along with a Monte Carlo simulation approach with Latin hypercube sampling (10000 iterations). A probability distribution function was assumed for each parameter.

2.3. Exposure assessment

A literature search was performed to identify recent studies (2015 to the present) from both peer-reviewed and grey literature regarding occupational exposure assessments for GNPs, in addition to the MARINA, NANEX, and NECID databases. Only studies with high-quality exposure data for occupational exposure during production or handling of GNPs were considered. The relevant studies were selected using the following inclusion and exclusion criteria. First, only data from 2015 to 2017 was included. Then, only studies conducted in industrial or laboratory facilities for a sufficient time period (i.e. > 1 h) were considered. Since exposure can be characterized by different dose metrics, no other restrictions for the measurement metric were applied. Finally, adequately descriptive statistics of the measurement data were required. For each exposure scenario (ES), distributions for exposure were then defined by means of Monte Carlo simulations using the same software and methods as that used for the Dose-Response Assessment described in the previous section. A probability distribution function was assumed for each ES or each activity described in the identified ES.

2.4. Risk characterization and uncertainty analysis

The potential risk magnitude was calculated using a risk characterization ratio (RCR), as defined by Equation (2), where EXP_i is the exposure value calculated from the exposure distributions defined for each scenario. A risky scenario (with exposure levels higher than BMC_h) was defined as RCR ≥ 1. The RCR distributions were calculated for each identified ES by sampling the EXP and BMC_h distributions over 10000 MC simulations per ES.

$$RCR = \frac{EXPi}{BMCh} \quad (2)$$

Uncertainty analysis was used to estimate the level of uncertainty in each step of the RA process, where possible sources of uncertainty were the use of (i) surrogate data (e.g. animal toxicology data); (ii) models (e.g. exposure estimates), and (ii) other assumptions (NM-specific sources of uncertainty, due to the lack of relevant data for toxicological profiles, known emissions, and measured exposure). The nominal range sensitivity analysis was selected to quantify the uncertainty; this is a local one-at-a-time (OAT) method where one input variable is modified at a time while all the others are kept constant. It is important to note that such methods cannot consider interactions between different input parameters; all options of the input parameters were considered equally likely. It is reasonable to assume that uncertainties in the analysis related to omitting interactions were much smaller than the other sources of uncertainties (e.g., using unknown probability distributions of the parameter options) (Riedmann et al., 2015). The nominal range sensitivity results were expressed as average percentage contributions to the uncertainty in the RCR calculation, considering each possible determinant (i.e. distributions of $BMCL_a$, EF_{inter}, EF_{intra}, UF_i, and exposure values).

3. Results and discussion

3.1. Hazard identification

Most of the data reported hereafter were derived from two recent review studies (Lalwani et al., 2016; Ou et al., 2016), which widely discussed the theme of toxicity of GFNs. Even though several studies conducted toxicity assessments, further research is required (Lalwani et al., 2016). Considering GNPs in particular, only few in vitro (Chowdhury et al., 2013; Li et al., 2012; Liao et al., 2011; Park et al., 2015; Sanchez et al., 2012; Zhang et al., 2010) and in vivo (Chowdhury et al., 2013; Duch et al., 2011; Kanakia et al., 2014, 2015; Kim et al., 2016; Park et al., 2015; Schinwald et al., 2012; Zanni et al., 2012; Roberts et al., 2016) studies were performed. Results from available studies outlined that (i) GNPs were found to deposit beyond the ciliated airways after inhalation (Schinwald et al., 2012), and (ii) acute inflammatory responses, cell inflammation, and frustrated macrophage phagocytosis were observed in mice (Schinwald et al., 2012). Results also suggested that (iii) graphene may not cause significant toxic responses at relatively high airborne concentrations, indicating a general low level of toxicity (Kim et al., 2016). Considering this, (iv) a NOAEL of ≥ 1.88 mg/m^3 (equivalent to 1.99×10^5 particle/cm^3) was defined, "based on no significant increases in inflammatory cells, inflammatory markers, or cytokines in the bronchoalveolar lavage fluid and lung tissues, lack of genotoxicity, and the overall negative findings from histopathological evaluation after the 1-day post-exposure and 28- and 90-days post-exposure" (Kim et al., 2016). Further details were reported in the supplementary material. This information, although not conclusive (containing a certain degree of uncertainty), was used in the following phases of the RA process for occupational exposure to GNPs.

3.2. Dose-response assessment

The benchmark dose method was used to quantify a health-based guidance value (which was considered equivalent to a OEL) for occupational inhalation exposure to GNPs, based on published inhalation exposure tests (Kim et al., 2016). The reference study, although classifiable as sub-acute test (and not as sub-chronic and chronic test) has been identified among those available as more representative of the investigated condition. On this basis, 15 valid mathematical models were fitted to the dose-response data based on mass concentration metrics (Table S6, supplementary material) and 16 valid mathematical models were fitted to the dose-response data based on number concentration metrics (Table S7, supplementary material). These models were then aggregated into an overall $BMCL_a$ distribution that was normally distributed ($p < 0.01$ using a Kolmogorov-Smirnov test) with a mean of 0.253 ± 0.065 mg/m^3 and $1.09 \times 10^5 \pm 8.11 \times 10^3$ particle/cm^3 to allow a stochastic BMC_h calculation (Table 1 summarizes the obtained data concerning BMC_a, $BMCL_a$, $BMCU_a$ and BMC_h). After applying EF_{inter}, EF_{intra}, and UF_i over 10000 Monte Carlo simulations, the results described the BMC_h (i) as a lognormal distribution with a GM of 0.212 mg/m^3 with a GSD of 7.796 mg/m^3 (mass concentration metric) and (ii) as a lognormal distribution with a GM of 9.37×10^4 particle/cm^3 with a GSD of 7.6 particle/cm^3 (number concentration metric). These BMCh values were considered as OEL values for the subsequent phase of risk characterization.

Table 1
BMC_a, $BMCL_a$, $BMCU_a$ (mean ± SD) and BMC_h (GM ± GSD) calculated for graphene nanoplatelets and expressed as mass [mg/m^3] and number concentrations [particle/cm^3].

Metric	BMC_a	$BMCL_a$	$BMCU_a$	BMC_h
	Mean (± SD)			GM (± GSD)
Mass concentration [mg/m^3]	1.698 (± 0.438)	**0.253 (± 0.065)**	6.721 (± 1.791)	0.212 (± 7.696)
Number concentrations [particle/cm^3]	4.17×10^5 (± 3.68×10^4)	**1.09×10^5 (± 8.11×10^3)**	6.70×10^{13} (± 9.47×10^{12})	9.37×10^4 (± 7.655)

3.3. Exposure assessment

After selection of data using the inclusion/exclusion criteria, two studies were found to be suitable for the present analysis. The first study presented a multi-metric occupational exposure assessment of GNPs for workers engaged in large-scale production of graphene (Spinazzè et al., 2016), referred to here as ES1. The second study investigated the exposure of workers and research personnel to graphene in two research facilities (Lee et al., 2016) and is referred to here as ES2. As mentioned previously, the MARINA, NANEX, and NECID databases were also consulted to provide an overview of studies that measured workers exposure while handling GNPs. Data concerning the handling of graphene or GNPs was located only in the MARINA database. Among 16 possible ES, only 2 contained measurements performed during the 2015–2016 period; however, these data could not be included in this study due to some lack of information, that did not allow to obtain a correct stochastic simulation of the exposure distribution. Considering that limited data were available regarding occupational exposure associated with the handling of GNPs lab-scale measurement of such exposure was performed (ES3) (Spinazzè et al., in preparation). Further details on the selection and characterization of the ESs were reported in the supplementary material.

Hence, three ES were defined based on data taken from the scientific literature and based on results of an experimental study. The obtained data described large-scale production of GNPs (ES1) (Spinazzè et al., 2016), manufacturing of GNPs (ES2) (Lee et al., 2016), and in-lab handling of GNPs (ES3) (Spinazzè et al., 2018). Each of these ES were subdivided into different exposure profiles if relevant differences were defined considering exposed worker profiles or workplace descriptions. Table 2 summarizes the obtained data concerning exposure to GNPs, expressed as mass [mg/m^3] and/or number concentrations [particle/cm^3] for the three selected ES studied here. The results are presented as a geometric mean (GM) and geometric standard deviation (GSD). Based on these data, for each ES, distributions for exposure were then defined using Monte Carlo simulations: a Microsoft Excel add-on software package (RiskAMP) was used to supply the probabilistic functions for the stochastic functionality; a Monte Carlo simulation approach with Latin hypercube sampling (10000 iterations) was then used. A probability lognormal distribution function (checked with the Kolmogorov-Smirnov test on log-transformed data; $p < 0.01$) was assumed for each ES or exposure profile. The results of the simulations are shown in Table 4 (Table 3).

Considering mass concentration, the geometric means for exposure ranged from 3.8×10^{-4} mg/m^3 (ES1, Profile 1) to 2.92×10^{-1} mg/m^3 (ES2, Profile 4) (Table 4). Considering number concentrations, the geometric means for exposure ranged from 2.40×10^2 particle/cm^3 (ES3, Profile 6) to 6.44×10^3 particle/cm^3 (ES1, Profile 2). It is necessary to observe that for ES2, particle mass concentrations presented in Table 2 couldn't be totally referred as GNPs: In fact, the elemental carbon concentrations measured on gravimetric samples were between values below detection limit and to 0.26 μg/m^3, meaning GNPs should be lower than the gravimetric results of one order of magnitude or more. This suggests that the workplaces were strongly influenced by the presence of other particles than GNPs. However, gravimetric mass was adopted as opposed to elemental mass (which would be more specific), to represent the exposure in this risk assessment study. This choice was made both for continuity with ES1 (in which elemental carbon concentrations were not determined) and to ensure a precautionary approach (therefore overestimating the exposure to GNPs in ES2).

3.4. Risk characterization and uncertainty analysis

Table 4 shows the results of the risk characterization for the three exposure scenarios. ES1, ES2, and ES3 all resulted in RCR distributions with RCR ≥1 (i.e. risk present); however, none of the values showed a statistically significant level of risk at the 95% CI. Nearly 95–99% of the exposures sampled using the Monte Carlo simulation were < 1 (i.e. in the worst case only 6% of the estimated RCR indicated a possible risk to the exposed workers; ES1-P2 mass metric). In accordance with the lower exposure concentrations in ES1-P1 (mass metric) and ES3-P6, the probability of RCR values ≥ 1 were lower than those for other ES and very close to 0%, while other exposure profiles had a higher, although very small, risk for the exposures estimated in those ES. Only ES1-P2 and ES2-P4 resulted in a probability > 5% of obtaining RCR ≥1, though no ES resulted in a significant RCR ≥1 at a 95% CI.

In ES 1, Profile 1 (Engineer), Profile 2 (Operator - graphite expansion), and Profile 3 (Operator - Drying) resulted in RCR distributions ≥ 1 (i.e. risk present) with a percentage between 0.04% and 1.7% (when EXP and BMCh were expressed as mass concentrations) or between 0.5% and 5.7% (when EXP and BMCh were expressed as number concentrations). It should be noted that none of the three exposure profiles had a statistically significant level of risk measured at a 95%CI. Therefore, ~94–99.5% of the estimated exposures were at RCR < 1 (i.e. only 0.04–5.7% of the results resulted in a risk to the exposed population). Interestingly, in the study used as a data source for this scenario (Spinazzè et al., 2016), the authors concluded their evaluation by claiming that: (i) workers in ES1 were exposed to mean levels of GNPs lower than the adopted reference value (8-hr time-weighted average (TWA) = 40,000 particle/cm^3); (ii) the possibility of significant exposure by transient high GPN particle peaks was not likely, and (iii) workers who are directly involved in a specific task (material sampling for quality control) have higher potential for occupational exposure. These findings were further corroborated by quantitatively defining the probability of exceeding a recommended limit value, calculated specifically for the NM being assessed. Further, the three exposure profiles defined for ES 1 were hierarchically (and quantitatively) classified in terms of the risk probability, showing a higher level of risk (i.e. higher probability of RCR ≥1) for Profile 2 (Operator - graphite expansion) than for Profile 3 (Operator - drying) and Profile 1

Table 2
Exposure to graphene nanoplatelets expressed as mass [mg/m^3] and/or number concentrations [particle/cm^3] for the three selected exposure scenarios (ES). The data are presented as geometric mean (GM) and geometric standard deviation (GSD).

ES [Source]	Worker Profile	GM	GSD
	Metric		
ES1 (Spinazzè et al., 2016)	**Profile 1 (P1) - Engineer**		
	Number concentration [particle/cm^3]	3.32×10^3	1.95×10^2
	Mass concentration [mg/m^3]	3.80×10^{-4}	1.00×10^{-6}
	Profile 2 (P2) - Operator (Graphite Expansion)		
	Number concentration [particle/cm^3]	6.44×10^3	3.78×10^3
	Mass concentration [mg/m^3]	3.86×10^{-3}	8.50×10^{-5}
	Profile 3 (P3) - Operator (Drying)		
	Number concentration [particle/cm^3]	9.09×10^2	5.36×10^2
	Mass concentration [mg/m^3]	3.68×10^{-3}	7.80×10^{-5}
ES2 (Lee et al., 2016)	**Workplace**	**GM**	**GSD**
	Metric		
	Profile 1 (P4) - Workplace A		
	Mass concentration [mg/m^3] - Total suspended particles	2.92×10^{-2}	1.92
	Mass concentration [mg/m^3] - Respirable particle	3.47×10^{-2}	1.06
	Profile 2 (P5) - Workplace B		
	Mass concentration [mg/m^3] - Total suspended particles	3.30×10^{-3}	1.30
ES3 (Spinazzè et al., 2018)	**Worker Profile**	**GM**	**GSD**
	Metric		
	Profile 1 (P6) - Handling GNPs		
	Number concentration [particle/cm^3]	2.40×10^2	3.10

Table 3
Descriptive statistics for results of the 10000 Monte Carlo simulations used to estimate the distribution of exposure values for ES1, ES2, and ES3. Exposure to graphene nanoplatelets was expressed as mass [mg/m^3] and/or number concentrations [particle/cm^3] for the three selected ES. The data are presented as geometric mean (GM), geometric standard deviation (GSD), minimum and maximum values (min, max), and 5th and 95th percentiles.

ES	Worker Profile	GM	GSD	min	5th percentile	95th percentile	max
	Metric						
1	**Profile 1 (P1) - Engineer**						
	[*particle/cm^3*]	3.32×10^3	1.95×10^2	2.64×10^3	3.01×10^3	3.07×10^3	4.23×10^3
	[*mg/m^3*]	3.80×10^{-4}	1.00	3.76×10^{-4}	3.78×10^{-4}	3.79×10^{-4}	3.84×10^{-4}
	Profile 2 (P2) - Operator (Graphite Expansion)						
	[*particle/cm^3*]	6.47×10^3	5.57×10^3	6.19×10^3	2.30×10^3	2.74×10^3	4.15×10^4
	[*mg/m^3*]	3.86×10^{-3}	1.00	3.53×10^{-3}	3.72×10^{-3}	3.75×10^{-3}	4.18×10^{-3}
	Profile 3 (P3) - Operator (Drying)						
	[*particle/cm^3*]	9.04×10^2	7.79×10^2	9.51×10^1	3.17×10^2	3.79×10^2	7.44×10^3
	[*mg/m^3*]	3.68×10^{-3}	1.00	3.40×10^{-3}	3.55×10^{-3}	3.58×10^{-3}	4.04×10^{-3}
2	**Workplace - Profile**	**GM**	**GSD**	**min**	**5th percentile**	**95th percentile**	**max**
	Metric						
	Profile 4 (P4) - Workplace A						
	[*mg/m^3*] - *TSP*	2.92×10^{-2}	1.92	$7{,}73 \times 10^{-9}$	$3{,}4 \times 10^{-6}$	$9{,}57 \times 10^{-2}$	$1{,}21 \times 10^1$
	[*mg/m^3*] - *Resp. particle*	3.23×10^{-2}	1.06	4.3×10^{-8}	1.48×10^{-5}	3.72×10^{-5}	3.42×10^1
	Profile 5 (P5) - Workplace B						
	[*mg/m^3*] - *TSP*	3.22×10^{-3}	1.30	1.88×10^{-11}	2.99×10^{-8}	8.83×10^{-8}	1.14×10^1
3	**Worker Profile**	**GM**	**GSD**	**min**	**5th percentile**	**95th percentile**	**max**
	Metric						
	Profile 6 (P6) - Handling GNPs						
	[*particle/cm^3*]	2.40×10^2	2.40×10^2	2.27×10^2	2.35×10^2	2.36×10^2	2.53×10^2

(Engineer) when considering the mass metric. When considering the numeric metric instead, the profile 1 shows a risk level higher than the profile 3, while the profile 2 still resulted the one with the higher probability of RCR > 1.

In ES 2, both Profile 4 (Workplace A) and Profile 5 (Workplace B) resulted in RCR distributions ≥ 1 (i.e. risk present) with a percentage between 0.8% and 6.1%, while no exposure profile had a statistically significant level of risk measured at the 95%CI. In general, ~94–99% of the estimated exposures were at RCR < 1 (i.e. only 0.04–5.7% of the results resulted in a risk to the exposed population). Note that the study used as a data source for this scenario (Lee et al., 2016) claimed that results obtained from environmental monitoring (not including all graphene manufacturing processes) indicated very minimal graphene exposure at facilities manufacturing graphene with good practices. However, our results showed a not negligible (although not statistically significant) probability of exceeding the recommended limit specifically for the ES2-P4 case.

In ES 3, Profile 6 (handling of GNPs) resulted in RCR distributions ≥ 1 (i.e. risk present) with a percentage of 0.02%; this exposure profile was not statistically significant at a 95%CI. Thus, ~99% of the estimated exposures were at RCR < 1 (i.e. only 0.02% of the results resulted in a risk to the exposed population). The reference study for ES3 (Spinazzè et al., 2018), which followed a deterministic approach, claimed that the concentration levels observed for this lab-scale simulation were particularly small; personal monitoring experiments showed exposure concentrations between 3649 and 5103 particle/cm^3, with a maximum value of 12190 particle/cm^3. Hence, the total average particle concentrations (including background aerosols) measured under these operating conditions were of the order of 1/10 of the threshold values used in that study (40000 particle/cm^3). The probabilistic approach used here, although defining a scenario without

Table 5
Results of the sensitivity analysis for the model used for RCR estimations, presented as averaged percent contribution to the uncertainty.

Metric used in RCR estimates	Variable average contribution to the uncertainty (%)				
	BMCLa	EFinter	EFintra	Ufi	EXP_i
Mass concentration ESs	0.4	23.1	22.5	22.9	31.0
Number concentration ESs	0.1	33.5	32.6	33.3	0.5
Overall RCR estimations	0.3	26.4	25.6	26.2	21.4

Table 4
Results of the 10000 Monte Carlo simulations used to estimate the RCR distributions for ES1, ES2, and ES3. RCR distributions were calculated considering number concentrations [particle/cm^3] and/or mass concentrations [mg/m^3] as metrics for exposure and BMCh distributions. The data are presented as geometric mean (GM), geometric standard deviation (GSD), minimum and maximum values (min, max), and lower and upper limits of the confidence interval of the mean (95% CI).

ES-Profile *(Metric)*	GM	GSD	min	max	Lower 95%CI	Upper 95%CI	RCR ≥ 1 (95%CI)	Probability (%) RCR ≥ 1
ES1 - P1 *(Number)*	2.58×10^{-2}	6.65	2.15×10^{-5}	26.0	2.49×10^{-2}	2.68×10^{-2}	No	2.7
ES1-P1 *(Mass)*	1.47×10^{-3}	7.24	1.11×10^{-6}	1.9	1.42×10^{-3}	1.53×10^{-3}	No	0.04
ES1-P2 *(Number)*	4.37×10^{-2}	7.17	1.99×10^{-5}	60.3	4.21×10^{-2}	4.54×10^{-2}	No	5.7
ES1-P2 *(Mass)*	1.50×10^{-2}	7.24	1.06×10^{-5}	18.8	1.44×10^{-2}	1.56×10^{-2}	No	1.7
ES1-P3 *(Number)*	6.05×10^{-3}	7.14	4.08×10^{-6}	7.2	5.82×10^{-3}	6.29×10^{-3}	No	0.5
ES1-P3 *(Mass)*	1.43×10^{-2}	7.24	1.05×10^{-5}	18.4	1.37×10^{-2}	1.48×10^{-2}	No	1.6
ES2 - P4 *(TSP - mass)*	7.46×10^{-3}	19.0	7.71×10^{-8}	1452	7.04×10^{-3}	7.90×10^{-3}	No	4.6
ES2-P4 *(Resp.- mass)*	1.43×10^{-2}	20.0	1.77×10^{-7}	2052	9.10×10^{-3}	1.02×10^{-2}	No	6.1
ES2-P5 *(TSP - mass)*	3.26×10^{-4}	28.6	8.17×10^{-10}	62.2	3.05×10^{-4}	3.48×10^{-4}	No	0.8
ES3 - P6 *(Number)*	1.87×10^{-3}	6.64	1.52×10^{-6}	1.9	1.80×10^{-3}	1.94×10^{-3}	No	0.0

significant risks, allows quantification of the likelihood of the risk.

Finally, Table 5 summarizes the results of the nominal range sensitivity analyses (SA), expressed as an average percent contribution to the uncertainty in the RCR estimates, considering each possible determinant (i.e. distributions of $BMCL_a$, EF_{inter}, EF_{intra}, UF_i, and exposure values). Two different nominal SA were performed, to evaluate both the mass concentration and number concentration metric. For all ES, ~75% of the variation in the RCR distributions was influenced by uncertainties in EF_{inter}, EF_{intra}, and UF_i, while the remaining uncertainty was mainly from the exposure estimations. These results are consistent with the analysis of Tsang et al. (2017).

3.5. Risk management implications

To date, for effective precautionary management of potential NM-related risks, a hierarchy of controls has been proposed (Engeman et al., 2013), which largely adhere to the classic approach of risk management. This hierarchy prioritizes the elimination or substitution of a hazardous substance and provides workers with adequate information and training. When occupational exposure is expected to occur, it is necessary to adopt engineering controls to reduce exposure levels, such as the use of local exhaust ventilation, pressure differentials, hoods, and enclosed systems (Schulte et al., 2013; Heitbrink et al., 2015). Risk management procedures were already defined in the ES1 and ES2 facilities; nevertheless, the use of a probabilistic approach for risk assessment indicated the presence of a certain level of risk (although not statistically significant) in those scenarios, which could be reduced by implementing risk management procedures. A typical hierarchical risk management approach based on organizational procedures (i.e., warning, trainings, and specific operating procedures), engineering controls (e.g., enclosing activities with high exposure potential and using fixed containment booths) and personal protective equipment (e.g., filtering half-face masks with FFP2- or FFP3-class filters) was implemented at the ES1 company, consistent with a precautionary approach, to control the exposure to GNPs associated with the production process. Overall, this approach appeared to ensure an adequate level of control, since the probability of having a RCR ≥ 1 was 5.7% in the worst-case profile. A technical report performed an evaluation of engineering controls for manufacturing and handling GNPs in the workplace and concluded that some specific tasks in the production process were sources of NP release into the workplace (Lo et al., 2011); the same report also stated that appropriate engineering controls could help mitigate exposure to NMs in production areas. Similarly, in ES1, specific work tasks showed the potential to cause serious contamination within the workplace; however, the development of appropriate procedures for workplace and worker monitoring, improved removal and containment systems, education and training of workers, and periodic exposure assessments, were all helpful in preventing workplace contamination and reducing workers' exposure. The implementation of up-to-date control strategies (e.g., improvement of the existing local exhaust ventilation systems with mobile HEPA-filtered inlets near potential sources of GNPs) was recommended. This was expected to contribute to lowering occupational exposure, workplace contamination levels, and high-level transient exposure peaks, further reducing the risk level for ES1.

ES2 indicated potential exposure of workers and research personnel to GNPS in two research facilities: a laboratory that manufactures graphene on a small-scale using graphite exfoliation and chemical vapor deposition (workplace A) and a research institute that grows graphene on a copper plate using chemical vapor deposition, which is then transferred to a polyethylene terephthalate (PET) sheet (workplace B). In workplace A the production processes were performed inside a fume hood, while in workplace B the work activities were performed in a clean room (class 10000) with local exhaust ventilation, while the subsequent graphene application was performed in a class 1000 clean room with local exhaust ventilation. Workers involved in the manufacturing processes both in workplace A and B wore personal protective equipment (working clothes and gloves, but not respirator). It should be noted that in ES 2, Profile 4 (workplace A) resulted in RCR distributions ≥ 1 (i.e., risk present) with a higher percentage than workplace B. This could be due to the different engineering controls used. In fact, although fume hoods are among the most widespread control systems, previous studies showed that the handling of NMs in fume hoods can result in the significant release of airborne particles into the laboratory environment and within the operator's breathing zone (Tsai et al., 2009), while other studies observed good containment effectiveness and lower particle emission rates (Dunn et al., 2014; Fonseca et al., 2018; Plitzko, 2009; Tsai et al., 2010). Specific discussion of these factors regarding the handling of GNPs was included in the ES3 study. Further information is required to clarify this point; the different RCR distributions for the two workplaces could be due to several other aspects. The implementation of up-to-date control strategies could further reduce the risk level for ES2.

ES3 involved lab-scale experiments designed for this study that measured the exposure associated with handling GNPs under chemical fume hoods. ES3 resulted in RCR distributions ≥ 1 (i.e., risk present) with a percentage of 0.02%; the exposure profile was not statistically significant at a 95%CI. Hence, although the handling of GNPs in fume hoods may result in exposure due to emission and dispersion of graphene particles, the operating conditions can be adjusted to avoid significant personal exposure and contamination of the work environment, ensuring safer conditions.

3.6. Limitations and strengths

Some assumptions and limitations must be considered in the interpretation of the results of this study as the study design limits the general applicability of the findings. Major limitations of the present study are summarized hereafter: (i) first, it should be considered that NMs have a wide range of chemical and physical properties (e.g., graphene type, size, morphology, etc.)., which poses immediate challenges for the RA due to its impact on toxicological effects and exposure potentials. Although this study focused on a specific NM, to perform case-specific RA, a certain level of uncertainty could be included in the study. In this regard, for example, it is likely that differences can be observed for the same ES by comparing exposure levels expressed as mass or number metric; this could lead, for the same scenario, in defining different probabilities of risk, by comparing the exposure with the respective metric-specific thresholds. Also for this reason, it is important, when possible, to perform a multi-metric risk assessment. (ii) The simplifications used in parametric distribution simulations inevitably introduce uncertainties into the model dose-response, exposure assessment, and risk characterization phases. The model uncertainties depend on selection of the distributions, definition of the exposure scenario and profiles, modeled activities, selection of averaging times and number of iterations, and generation of the random numbers. Hence, an SA was performed to quantify the impact of this uncertainty on the overall results. (iii) Having regard to the limited number of available studies, the selection of sources used for the hazard assessment phase could have introduced bias in the risk assessment process; however, considering that the available sources are still not sufficient to reach conclusions about the potential hazards connected with risk assessment and regulation of GPNs, results from available studies were carefully considered, with a precautionary approach. (iv) In the dose-response assessment, the BMR calculated from a change in the mean equal to one control SD was used as it is the standard reporting level for each dose-response type and does not necessarily represent equivalent values. However, Crump (1995) found that using a 1 control-group SD change for the continuous end-point results in an overestimated risk of approximately 10% for the proportion of individuals $< 2^{nd}$ percentile or $> 98^{th}$ percentile of controls for normally distributed effects. It should be noted that the EPA Benchmark Dose Technical Guidance

(EPA, 2012b) recommends always reporting the estimated BMD associated with the BMR in terms of a difference in means equal to 1 SD. However, one of the weaknesses of this BMR definition is that the associated BMD then depends on study-specific factors. Another limitation of using the 1 SD metric is that the estimate of the associated BMD cannot be translated into an equipotent dose in populations with larger within-group variation. The adopted precautionary (i.e., use of $BMCL_a$ for BMC_h) and probabilistic approach is expected to contribute to reducing the effect of this limitation. Finally, (v) the study design implies some limitations in the general applicability of the findings. Exposure data for ES1 and ES2 were taken from published studies after a bibliographic search and the application of inclusion and exclusion criteria, with the aim of obtaining high-quality exposure assessment data. Some relevant scenario-specific variables may not have been considered (e.g., use of personal protective equipment, measurement locations, etc.), for the purposes of the applicability of the probabilistic model. Further, some other valuable studies could have been excluded from this study due to the strict selection criteria. Exposure data for ES3 were derived from an in-lab simulation performed especially for this study; measurements were taken within a specific occupational setting, according to a systematic and technical protocol which included some intrinsic limitations in accuracy.

Despite these limitations, this study has several strengths, as it provides important insights into the RA procedure for engineered NMs in occupational settings and validates the use of a probabilistic approach to human health RAs for emerging technologies, such as NMs. In summary, (i) this is the first study to estimate a threshold limit value (BMC_h) for occupational exposure to graphene nanoplatelets expressed both in number and mass concentrations. In addition, (ii) the probabilistic approach allowed the introduction of randomness derived from the uncertainty or variability in the source data into the dose-response assessment, exposure assessment, and risk characterization stages. Finally, (iii) the probabilistic approach allowed the quantification of the likelihood of a potential risk, while quantitatively including the randomness in the RA parameters (i.e., toxicity and exposure data) and explicitly reporting their influence on risk characterization. The results obtained in this study will have to be updated in the future, according to the evolution of knowledge on hazard identification (e.g. if new data from sub-chronic and chronic toxicity studies will be available) and exposure assessment of GNPs and NMs. However, at the current state, this study could represent an advancement of the knowledge about the risk related to occupational exposure to GNPs.

4. Conclusions

The use of a probabilistic approach in a RA of human health was applied for GNPs under three occupational ES. Stochastic simulations were used to perform the dose-response assessment, exposure assessment, and risk characterization phases, allowing the introduction of sources of uncertainty, whose impact on the RA procedure was subsequently quantified by means of a sensitivity analysis (about 75% of the variation in the RCR distributions was due to uncertainties in the BMC_h calculation). The adopted probabilistic approach allowed to estimate a specific threshold limit value for occupational exposure and to quantitatively define the probability of a risk condition occurring for each investigated ES. All the considered ES resulted in RCR distributions ≥ 1 (i.e. risk present); but, none of these had a statistically significant level of risk. On this basis, ES-specific risk management measures were evaluated.

Conflicts of interest

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ijheh.2018.08.011.

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