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Research Article

Safety Assessment of Graphene Based Polyester Resin Composites

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ABSTRACT

The use, production and disposal of Engineering Nanomaterials (ENMs), including Graphene-Related Materials (GRMs), raise concerns and questions about possible adverse effects on human health and the environment, considering the lack of harmonized toxicological data on ENMs and the ability of these materials to be released to the air, soil or water compartment during common industrial processes and/or accidental events. Within this context, the potential release of graphene particles, their agglomerates and aggregates (NOAA) as a result of sanding of a battery of graphene based polyester resin composite samples intended to be used in the building was examined. The analysed samples were exposed to different weathering conditions to allow the evaluation of the influence of the weathering process on the morphology and size distribution of the particles released. Sanding studies were conducted in a tailored designed sanding bench connected to time and size resolving measurement devices. Particle size distributions and particle number concentration were assessed by an Optical Particle Counter (OPC) and a Condensation Particle Counter (CPC) respectively during the sanding operation. Scanning Electron Microscope/Energy Dispersive X-ray (SEM/EDX) analysis was performed to adequately characterize the morphology, size and chemical composition of released particles. A toxicity screening study of pristine and graphene-based nanocomposites released using the aquatic macro invertebrate *Daphnia magna* and relevant human cell-lines was conducted to support risk assessment and decision making. Results show a significant release of nano-scale materials during machining operations, including differences attributed to the % of graphene and weathering conditions. The cell lines testes demonstrated a higher effect in the human colon carcinoma cell line Caco2 than in the human fibroblasts (A549 cell line), which means that composites released to the environment can have an impact on human health and biota.

Keywords: Nanotechnology; Nanomaterial; Nanoparticle; Eco-toxicity; Risk assessment; Graphene

INTRODUCTION

Expectations in the use of Engineered Nanomaterials (ENMs) has exponentially grown over the last decade. However, there is a lack of information regarding the potential effects of EMMs on human health and the environment. These materials, understood as materials whose main constituents present one or more external dimensions in the size range from 1 nm to 100 nm, due to their unique physicochemical properties, enable the development of new products with extraordinary properties, including size, shape or surface area, can lead to adverse health effects. Graphene is a two-dimensional carbon allotrope nanomaterial. The many extraordinary properties of this material, such as mechanical stiffness, strength and elasticity as well as the high electrical and thermal conductivity, have produced considerable excitement since the initial discovery. Besides graphene and its derivatives are currently being explore for a multitude of applications as electronics, photo-catalysis, sensors, medicine, plastic or construction. Considering the large commercial interest in graphene products and their fast expansion,

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safe and sustainable development of GRMs in technologies and products required close attention to the potential impact of these materials on human health and the environment. Indeed, safety assessment is an integral part of the innovation process for the new GRMs products [1].

To date, material characterization is a key element of hazard assessment it has been reported, that graphene-based nanomaterials may exert different degrees of toxicity in animals or cell models by following with different administration routes and penetrating through physiological barriers. Some studies suggested that Graphene Oxide (GO) and reduced Graphene Oxide (rGO) might induce toxicity due to the generation of free oxygen radicals and oxidative stress. Besides, it has been reported that GO and RGO can induce significant toxicity to various cell lines, such as stem cells, germ cells, lung cells, skin cells, endothelial cells and macrophages. Similarly, Graphene Oxide (GO) related studies performed on the human alveolar adenocarcinoma cell line A549 showed a slight loss of cell viability.

Besides, it is known that pristine graphene induced some oxidative stress responses and cell death in GNP-2 cells, while particles resulting from the machining (*i.e.,* abrasion/sanding/drilling) of graphene related composites did not reveal significant cell damage in human THP-1 macrophages. Other studies suggest that CeO2-RGO nanocomposites did not generate significant effects even though pristine reduced GO has been reported to induce toxicity in A549 cells. Moreover, it is known that graphene could induce adverse effects at different levels in top predators and microorganisms at environmentally relevant concentrations. Toxicity to *Caenorhabditis elegans* has also been reported.

The release of graphene particles during the manufacture, use and end-of-life may reach the environment, including atmosphere, aquatic or terrestrial compartments, where the variety of target species and physical chemical factors creates a complex challenge. In fact, some studies have shown evidence on the potential effects of graphene related materials on aquatic and terrestrial organisms. In general, there is still large knowledge gaps with respect graphene's, environmental and toxicological effects and the (eco) toxicological information than can be extracted from the few developed studies, is not adequate to be applicable to real case scenarios in which consumers use graphene related polymer composites as components of final products.

It this sense, the evaluation of the potential release has gained importance on recent years, motivating the need to better understand the potential hazards posed by the production and use of polymer-based nanocomposites. A list of activities associated with a high potential for releasing particles has been identified in literature including diffusion, desorption or matrix degradation, this latter related with processes such as mechanical abrasion, thermal degradation, hydrolysis or UV exposure, which causes photodegradation. Mechanical abrasion plays a crucial role on industrial scenarios, where the nano-enabled products are subjected to machining operations, including cutting, grinding, shredding, sanding or drilling processes. The release of particles associated to these activities might be particles of matrix alone, free-standing particles, partially protruding or fully embedded particles in the polymer matrix or in limited cases, in the form of dissolved ionic forms of the added. Since the form and nature of the particles released may lead to different human/environmental effects, several studies have focused on identifying the nature and extent of nanoparticles and nanoparticle-containing fragments released from nano-enabled products because of weathering. Evaluations conducted to date do not indicate a high propensity for discreet nanomaterial release, but rather composite particles of matrix with partially or fully embedded nanomaterial. The few available studies reported a release of particles with an aerodynamic diameter smaller than 10 μm, which can penetrate to the alveolar region of the lung. Particles with a size ~200 nm has also been identified in sanding operations [2].

As mentioned above, the evaluation of the activities with a potential release of nanomaterials is important in order to establish control and safety measures to prevent the exposition from nanomaterial particles to workers, consumers and any person who may been in contact with these material during its life-cycle. The aim of this study is to present our first safety assessment of a pioneering graphene based polyester resin with usages on a wide range of applications, highlighting the building sector. Specifically, the assessment focuses on: 1) the characterization of aerosol particles released after mechanical abrasion on graphene-reinforced epoxy composites, (2) to quantify the amounts of protruding and free-standing graphene platelets in the abraded particles and (3) to assess the potential effects of the pristine and abraded graphene particles released on the aquatic micro invertebrate *Daphnia magna* and human cells lines. *Daphnia magma* was selected due to its importance as model organism in various biological disciplines, from aquatic ecology to biomedical sciences, while the cell lines A549 and Caco-2 were selected due to the relevance for improving current knowledge on the cellular response to foreign particles deposited in the lungs or in the gastrointestinal tract due to an accidental uptake by biota, which signify the acute inhalation and oral uptake toxicity *in vitro*.

MATERIALS AND METHODS

Materials

Graphene powder and related polyester resin was provided by Graphen glass. The material consisted of graphene particles of 10 μm-55 μm size formed by 2-6 graphene sheets with a BET surface >250 m^2/g. The properties of graphene are listed in Table 1.

The BET surface area was determined by nitrogen physisorption at 196°C (model AS1, Quantachrome, Boynton beach, FL, U.S.A.). The sample in the form of dried powder was first degassed for 24 h at 200°C.

Table 1: Properties of the graphene incorporated into the polymeric matrix.

Property	Description/value
Flake thickness (nm)	0.8-2 (2-6 mono-layers)
Particle size (μm)	10-55
Brunauer-Emmett-Teller (BET) surface area (m^2/g)	>250
Purity	>98%
Specific gravity (20°C)	1.18

Synthesis of graphene-polyester composite

Table 2 depicts the list of graphene-polyester composites samples produced and analysed under the scope of the present study. The composites were prepared by adding the required amount of graphene powder to the unsaturated polyester resin under mechanical agitation, followed by a ultrasonication and mechanical agitation in a temperature controlled bath.

Table 2: Properties curated graphene-based resins.

Property	Value (curated composite)
Young's modulus	3800 MPa
Fracture strain	2%
Tensile strength	60 Pa
Hardness (GYZJ 934-1)	40
Volatile content	41-45%
Water absorption (24 h/23°C)	15
Heat deflection temperature	70°C
Specific gravity (20°C)	1.182°C
Acid value	19-23 mg KOH/g

Physicochemical characterization of graphene based resins

The rheological properties of the polyester resin and the resulting composite were determined with a rheometer (TA instruments, AR2000) operated in cone and plate arrangements (stainless steel cone, with a 40 mm diameter and a 4° cone angle) at 25°C.

Morphology and thickness of the composite pieces were determined by cross-section and surface SEM through a Hitachi 4800 microscope. The FTIR spectra were evaluated on a Perkin Elmer 781 spectrophotometer on the powder samples from between 400 cm^{-1} to 4,000 cm^{-1}. Typical properties of the resin were as follow: Specific gravity measured at 25°C of 1, 10, viscosity at 25°C (Rheomat 37 sec^{-1}, 35 sec^{-1}) of 2, 0 to 2, 5 dPas, acid value of 19 mg KOH/g to 23 mg KOH/g and volatile content of 41 to 45. Table 3 depicts the main physicochemical properties of the curated graphene-based resins.

Table 3: Weathering conditions in test 1 and test 2.

Test	Hours	Temperature (°C) (Min/Av/Max)	HR (%) (Min/Av/Max)
1	481 h	24.6/62.62/82.9	5.5/10.21/43.4
2	537 h	18.5/58.23/84.9	7.0/18.81/72.3
Total	1018 h		

Weathering process

For the weathering process a UV chamber was used to simulate the environmental degradation conditions. The test was carried out in an accelerated weathering instrument purchased from Heraeus company (model suntest CPS+). A two-phases approach was conducted, including a first phase to replicate dry atmospheric conditions at 50°C, followed by an immersion process, where the temperature of the black body is automatically assigned as a function of the irradiance, to simulate wet atmospheric conditions. The testing programme applied included alternative cycles of wet and dry conditions of 500 hours during 20 days. The same process was repeated to reach a total weathering time of 1000 hours. Table 4 summarizes the weathering process conditions [3].

Table 4: Instrumentation used in the measurement campaign.

Sample ID	Function	Specifications
Condensation particle counter model 3007 (CPC) (TSI)	Quantify the particle number concentration	Size range: 0.01 to >1.0 μm
Optical Particle Sizer (OPS) model 3330 (TSI)	Classifies in concentration of number and particle size	Concentrations up to 105 particles/cm^3
Apex air sampling pump (Casella pump)	Obtain samples in filtering media to elucidate chemical nature by SEM	Size range: 0.3 μm-10 μm in up to 16 channels

Abrasion process and particle collection

In this study, a sanding beach including and a mechanical sander and tailored designed clamping system to guarantee and homogeneous sanding process to promote reproducibility was applied. Temperature (range 20°C-25°C) and humidity (range 40%-50% relative humidity) were monitored.

A Bosch sander (model Bosch PBS 75 AE) equipped with a sanding belt was used to simulate the sanding process on the surface of the graphene-based composites. A medium (G/K 120) grit-sized sanding paper from the brand Piranha (Black and Decker) was applied to the sanding machine.

The graphene based composite pieces were sanded at the low speed position of the sander during 20 seconds. The released particles from the abrasion/sanding area were collected in a stainless aluminum tray, that was placed under the sanding belt. Released particles were analyzed using a multimetric measurement approach.

Two instruments were used, including an Optical Particle Sizer (OPS) (model 3330, TSI), a Condensation Particle Counter (CPC) (model 3077, TSI). In parallel, the particles were collected on polycarbonate filters with a pore size of 0.2 μm mounted on 37 mm open-faced cassettes. Figure 1 shows pictures of the sanding bench.

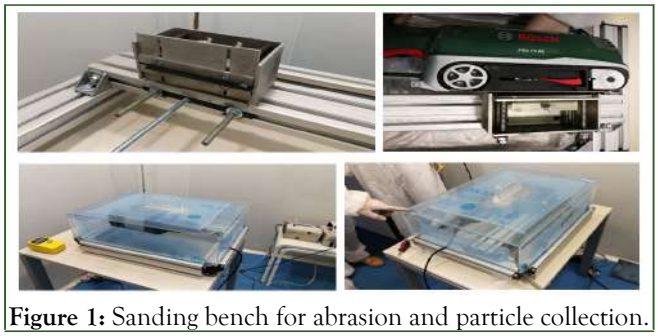

Figure 1: Sanding bench for abrasion and particle collection.

The morphology of pristine and the released particles from the composites was characterized using a HITACHI S-1800 Scanning Electron Microscope (SEM) equipped with EDX to analyze the elemental composition of the materials released. The background concentration, generated by the running abrasion module without sample contact but with operating the sanding machine, was investigated before the studies to characterize the level and nature of the background particle concentrations.

The inlets of the devices located at a height of 10 cm ± 0.2 cm and ~0.5 m from the particles source (sanding belt) using flexible Tygon® tubes attached to the inlets of the instruments. The main specifications of the suite of instruments used are listed in Table 5. The background concentration in the room was measured without sanding and with the sander turned off.

Table 5: Ecotoxicity measured as EC50 values at 24 and 48 h.

	% GR/trat.	EC_{50} mg/l-24 h	EC_{50} mg/l-48 h
N0206	Pure/pristine	318	146
M1 (B0)	0%/500 h	>100 (516)	>100 (304)
M2 (4)	50%/500 h	>100 (358)	>100 (221)
M6 (B0)	0%/1000 h	>100 (437)	>100 (251)

M7 (4)	50%/1000 h	>100 (298)	>100 (157)
M11 (4)	1%/0 h	>100 (381)	>100 (240)
M14 (1)	50%/0 h	>100 (216)	>100 (142)

Ecotoxicity

For ecotoxicity studies, the Daphtoxkit F™ bioassay (microbiotest, Ghent, Belgium) was used to estimate effect of pristine and abraded graphene particles to crustacean *D. magna*. Daphnia are planktonic crustaceans, characteristic of fresh water, with a size range between 0.5 mm-3 mm. The acute toxicity tests were performed according to the Toxkit® protocol. The Daphtoxkit™ F test is performed in accordance with test procedures of OECD Guideline 202 (OECD, 2004) and ISO 6341 (UNE-EN ISO 6341, 2012) [4].

The test is performed on neonates born from epiphyas (resistance eggs) of incuadas *Daphnia* 72-80 hours at controlled temperature of 20°C-22°C and light (6000 lux) in the OECD 202 medium previously aerated. The neonates placed in the trial therefore do not exceed 24 hours of life. *Daphnia* are fed spirulina during the two hours prior to the assay. It is carried out in conditions of darkness and temperature of 23°C.

To calculate median lethal concentration (LC50), 24 and 48-h EC50 values, as well as their associated 95% confidence intervals (95% CI) were calculated using the EPA-probit v1.5 program (USEPA).

A standard reference toxicity test with $K_2Cr_2O_7$ was run in parallel to each test series to verify the sensitivity of the *D. magna*.

Toxicity studies

Respiratory impact: Several assays were applied to the *in vitro* lung models to measure the levels of cytotoxicity (cell death or a reduction in cell viability), oxidative stress and inflammation.

The A549 cell line was selected as a representative cell of the alveolar epithelium of the gas-exchange region of the lung. A549 epithelial cells were grown in continuous culture in DMEM medium containing 10% FCS (life technologies), non-essential amino acids (diluted from 100x stock solution, sigma), sodium pyruvate (1 mM, life technologies), L-glutamine (diluted from 100x stock solution, life technologies), penicillin (100U, sigma) and streptomycin (100 µg/ml, sigma). This was designated complete medium. Cells were removed from culture by trypsinization and plated into 96-well plates at 2×10^5 cells/ml (100 µl/well). Plates were incubated for 24 hours at 370°C, wells washed with medium and treatments added to each well in a final volume of 100 µl in complete medium.

Oral route-ingestion: As in the previous case, several assays were applied to the *in vitro* Caco-2 cell model to measure the levels of cytotoxicity (cell death or a reduction in cell viability), oxidative stress and inflammation that occurred in response to exposure to pristine and abraded materials.

Caco-2 cell lines were cultivated for 10 days and then seeded at densities of 10^5 to 10^6 cells per ml (0.1 ml per well) in a 96-well plate by trypsinizing and centrifuging at 8.4 × g for 5 min. After a 24-h exposure, cell viability was measured by the conventional 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT; ATCC) reduction assay [5].

In both, fibroblasts and Caco-2, the ability of each material to generate Reactive Oxygen Species (ROS) was determined using the DCFH-DA assay. Dichlorodihydrofluorescin Diacetate (DCFH-DA) is able to cross cell membranes and enter cells where it is cleaved to form DCFH. The presence of ROS causes oxidization of DCFH into DCF which is fluorescent and can be evaluated to indicate a change in oxidative stress. ROS values are expressed as percent of fluorescence intensity relative to the control wells. Figure 2 shows a micrography of the cells lines used taken using an inverted microscope.

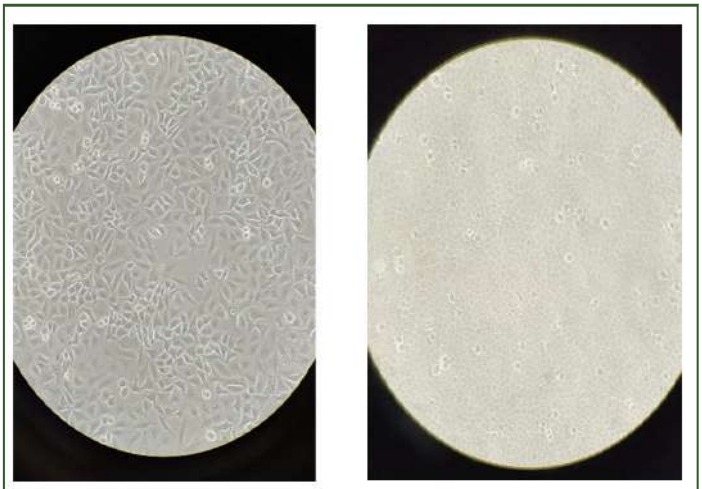

Figure 2: Right: A549 cell line micrograph and left: Caco-2 cell line.

RESULTS AND DISCUSSION

Abrasion test

Figure 3 shows the variations on the particle number concentration for the 14 samples processed during the operation. The minimum concentration reflects the background levels of particles before starting the sanding operation, reaching values below 6,000 particles/cm^3, as can be derived from the grey column depicted in Figure 3 [6].

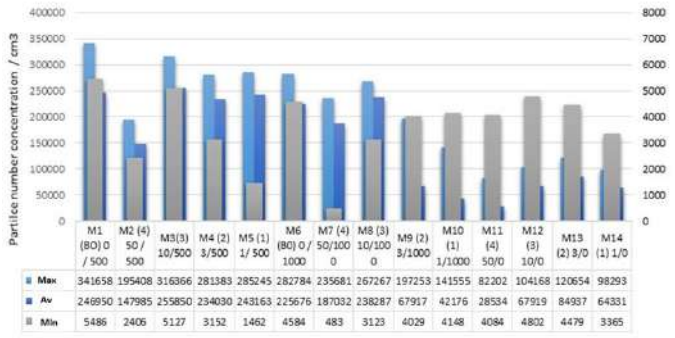

Figure 3: Concentration of particles measured with a CPC during the sanding activity. Maximun and average concentration values are referred to the Y axis.

A large increase immediately after the beginning of sanding operation was observed, with the highest peak values obtained reaching upto 350,000 particles/cm^3, which are about 50 times higher than the background levels.

The concentration of particles in the nano-range measured by the CPC revealed that weathered samples released a higher amount of sub-micron particles (ø<1 μm), especially significant for samples subjected to 500 hours aging process, where the maxim peaks were observed. Such issue denotes an alteration of the polymer surface, allowing a higher rate of particle release.

The differences between aging processes (1000 h *vs.* 500 h) are not significant, therefore it is estimated that the polymer's surface layer plays a relevant role in the exposure potential.

The levels of particles released for no-weathered samples was relatively low, with particles reaching a peak of 141,555 particles/cm^3 and an average particle level concentration of 60,000 particles/cm^3. This contrast with the high average levels found for weathered samples, with average levels of 220,000 particles/cm^3 and 135,000 particles/cm^3 for samples subjected to a weathering period of 500 and 1000 h respectively.

The analysis of the data measured by the particle sizer (TSI OPS 3300) showed different modes corresponding with particles with an average particle size of ~320 ± 2 nm, ~540 ± 2 nm and ~1150 ± 2 nm. The maximum peaks were observed for particles above 1 μm, being mainly due to number of particles embedded in the polymeric matrix, as can be derived from the SEM picture depicted in Figure 5 (Figure 4) [7].

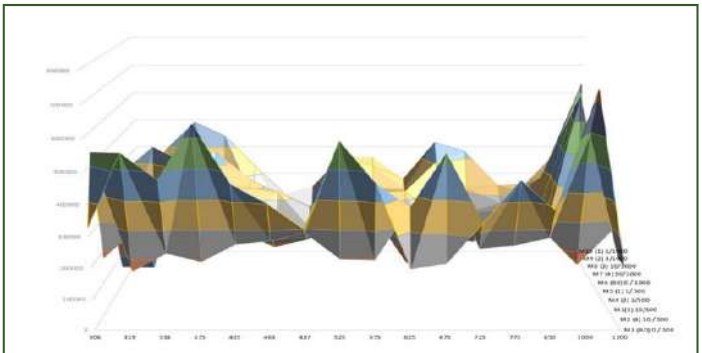

Figure 4: Size distribution of particles measured during the sanding process.

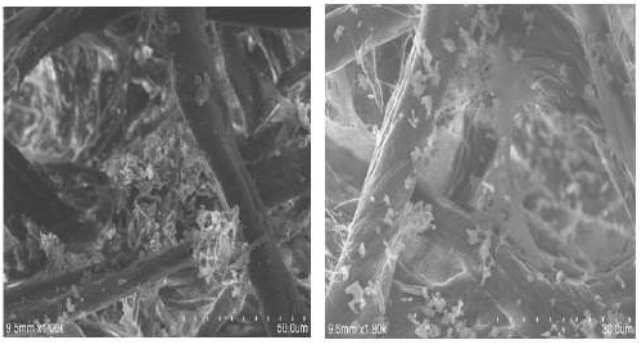

Figure 5: Detail of SEM images including particles collected in filters during the abrasion process.

The SEM images, which are shown in Figure 5, show only wear particles from the resin material. Therefore, it can be assumed that the abraded resin is the main source of the particles released. No free graphene particles were collected.

SEM/EDX analysis of sander emission also revealed a reasonable amount of carbon agglomerates ranging from 0.1 μm to 20 μm. As can be derived from the SEM/EDX analysis, graphene nanoparticles were embedded in the matrix or attached to the surface of these particles indicating the importance of measuring micrometric-sized particles in addition to nanosized particles. Figure 6 shows a high number of particles collected into the filters.

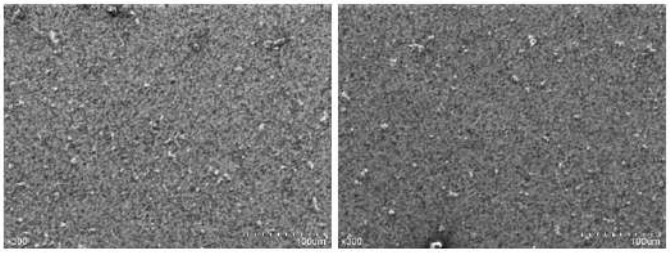

Figure 6: Detail of a polycarbonate filter covered by particulate matter immitted during the abrasion process.

Figure 7 includes an EDX spectrum identifying the elements that are represented in the filtration media.

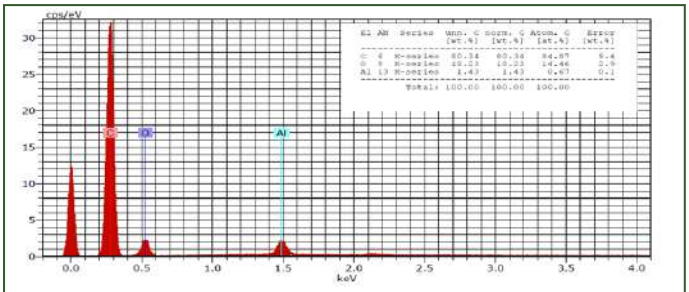

Figure 7: SEM/EDX analysis of particles collected in filters during the abrasion process.

Ecotoxicity analysis

The results on the aquatic toxicity of graphene showed that the immobility and mortality of the microcrustacean *Daphnia magna* was not affected, indicating a low acute toxicity in micro-invertebrates. Table 6 depicts Effect Concentration levels (EC) for the samples analysed [8].

Table 6: Toxicity measured as EC50 values at 24 and 48 h.

	Value	Low limit	Upper limit
EC50 A549	205 ppm	140.7	376.5
EC50 Caco2	68 ppm	36.1	1245.0

Toxicity analysis

The cell viability showed a dose-dependent pattern for pristine graphene to A549 cells in the concentration range of 12, 5 to 200 ppm, with a first effect at a concentration higher than 25 ppm (citoxicity above 80%), which indicates a low toxicity potential of graphene in this cell free system. This dose dependent effect of the particles can be found in Figure 8, where the effect of the particles over time at different doses is depicted. For example, for 50 ppm of graphene, the cell viability 71% after 24 h of exposure.

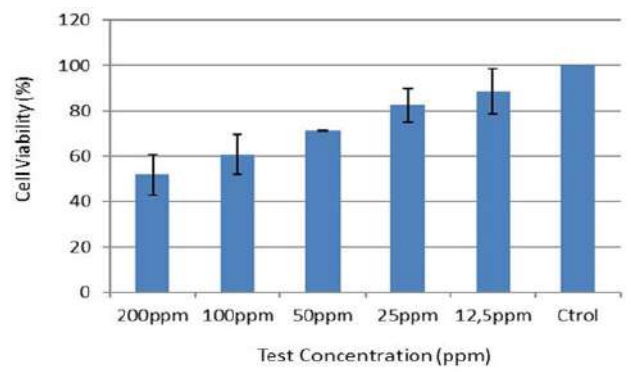

Figure 8: Effect of graphene on the generation of cytotoxic effects in human skin fibroblast cells.

Concerning the Caco-2 cell line, the cell viability showed alsodose-dependent pattern for pristine graphene in the concentration range of 6, 25 to 100 ppm, with a first effect at a concentration higher than 12, 5 ppm (citoxicity above 60%), which indicates a low to moderate toxicity potential of graphene in this cell free system. This dose dependent effect of the particles can be found in Figure 9, where the effect of the particles over time at different doses is depicted. For example, for 100 ppm of graphene, the cell viability calculated was 46%, 33% after 24 h of exposure. That means that the cytotoxic potential for the oral route is higher than the one observed for the A549 cell line [9].

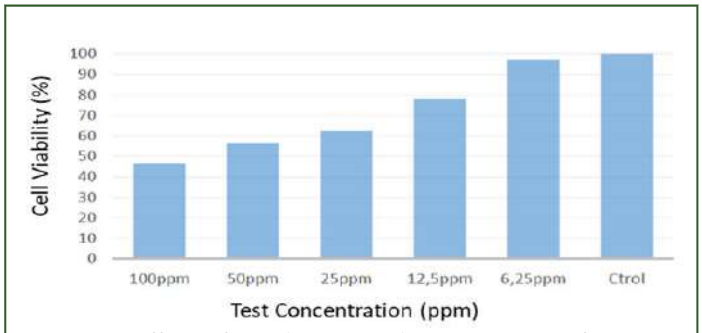

Figure 9: Effect of graphene on the generation of cytotoxic effects in the colon adenocarcinoma cell line.

Table 7 shows the effect concentration values calculated, including lower and upper limits. The dose range chosen is expansive in order to include relevant exposure concentrations (lower range) but also higher concentrations to allow EC50 values to be calculated for comparison and ranking purposes. On the basis of the data retrieved using A549 and Caco-2 cells, the toxicity for graphene was estimated to be 205 ppm and 68 ppm for the inhalation and oral route respectively, which means that the oral route is of prime importance.

Table 7: Toxicity results for samples analysed.

	%GR/Trat.	A549		Caco-2	
		EC_{50} mg/l-24 h	ROS	EC_{50} mg/l-24 h	ROS
N0206	Pure/pristine	205	+C1	68	+C2
M14 (1)	50%/0 h	68	+C1	>100	+C2

Figure 10 shows the effects of the graphene related composites on the A549 cell. Our results suggest that cell viability was not decreased by the pristine composite and the graphene based composite sample, being according to other recent reports that showed non-toxicological effects of graphene related composites. The EC50 values were above 100 ppm, which means that the samples tested can be considered non-toxic. These results also suggest that the graphene based composite induced much less response in A549 cells that those of the graphene nanosheets.

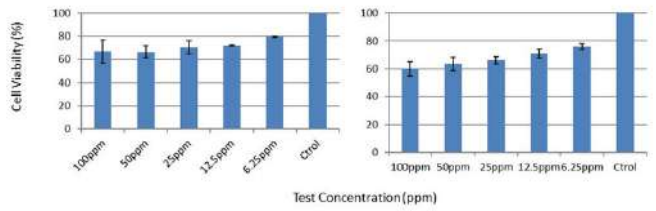

Figure 10: Effect of graphene related composites on the generation of cytotoxic effects in human skin fibroblast cells. Right: Pristine nanocomposite. Left: Graphene based composite sample (M14: 50% of graphene).

Similarly, Figure 11 shows the effects of the graphene related composites on the Caco2 cell. A similar behavior to the one observed for A549 cells can be identified. Notwithstanding, a higher level of decrease was observed for the Caco2 cell line, which means. The EC50 values were above 100 ppm, which means that the samples tested can be considered non-toxic. These results also suggest that the grapheme based composite induced much less response in A549 cells that those of the graphene nanosheets.

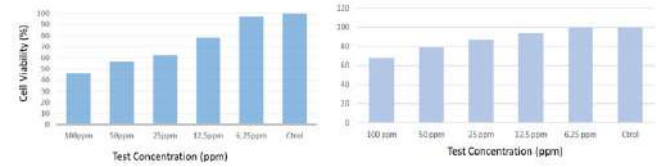

Figure 11: Effect of graphene related composites on the generation of cytotoxic effects in Caco-2 cells. Right: Pristine nanocomposite. Left: Graphene based composite sample (M14: 50% of graphene).

Regarding the ROS generation, both cells show a base production, identified in Figure 12 as negative control C. In the case of the A549 cell line, a concentration of up to 20 ppm in the media generates a significant response in the cell, however tested concentrations of 10 ppm and 5 ppm did not cause an increase above the C response. The Caco-2 cell line was more sensitive to graphene, with a high increase on the ROS generation at 10 ppm, where the A549 did not show any adverse response [10].

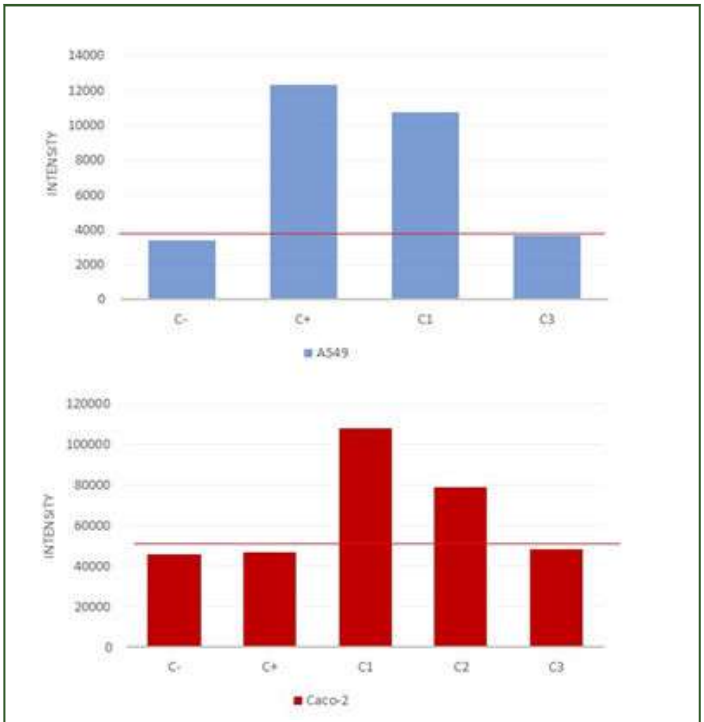

Figure 12: ROS generation in the cell lines A549 and Caco-2 after an exposure period of 24 h. Concentrations: C1:20 ppm/C2: 10 ppm/C3: 5 ppm.

CONCLUSION

With the present work we have attempted to give a study of the current level of human and environmental hazard assessment of GBMs and to emphasize the importance of understanding the structure and the activity relationships that underlie the potential toxicity of these materials.

The safe assessment for resins reinforced with graphene derivatives is essential because the huge applications of these composites as aircraft, wind turbines, bridges, ships, cars and sports equipment to name a few. In addition, the toxicological and release potential of graphene and graphene-based resins was investigated considering inhalation as main route of exposure for risk assessment purposes.

Furthermore, whereas real time measurements demonstrated a high release of particles below 1 μm. However, these particles, according with the SEM images, are rather made up from matrix materials, which contains the embedded particles. It was clearly visible that a higher concentration level was obtained for weathered samples, with suggest that a significant impact of the weathering process on the release of nano and micro-sized particles. Particle size distribution, did not show differences in the size of the particles released, suggesting that the size of the particles could be related with the specifications of the sanding device applied.

Our data suggest that graphene related composites can be considered a safe material considering the inhalatory route. The toxicity studies concluded also that the grapheme and graphene resins have a low toxicity profile. However, more investigation is needed to specify sub-lethal effects, including ROS and inflammation. Nevertheless, it is hoped that as this framework is populated with additional studies, ideally using GMRs that have undergone rigorous characterization, the structure-activity relationship of these materials. Indeed, it is also important to move from a descriptive to a predictive toxicological model in order to be able to use these promising GRMs to the multiple applications in a safety context for the human and the environment.

AUTHOR CONTRIBUTIONS

Conceptualization, C.F.L methodology, C.F.L and F.A.M.; validation, J.H.A, C.F.L. and F.A.M.; investigation, J.H.A, C.F.L. and F.A.M.; resources, C.F.L. and F.A.M.; writing-original draft preparation, F.A.M and C.F.L. All authors have read and agreed to the published version of the manuscript.

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CONFLICTS OF INTEREST

Francisco Aznar Molla, Carlos Fito-Lopez, Jose Antonio Heredia Alvaro and Inmaculada Colmenar-Gonzalez declare that we have no conflict of interest.

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