



Development of a conceptual framework for evaluation of nanomaterials release from nanocomposites: Environmental and toxicological implications



James Ging^a, Raul Tejerina-Anton^a, Girish Ramakrishnan^a, Mark Nielsen^b, Kyle Murphy^b, Justin M. Gorham^c, Tinh Nguyen^c, Alexander Orlov^{a,*}

^a Materials Science and Engineering, Stony Brook University, Stony Brook, NY, United States

^b University of Dayton, Dayton, OH, United States

^c National Institute of Standards and Technology, Gaithersburg, MD, United States

HIGHLIGHTS

- The UV-induced degradation of multiple carbon nanotube-epoxy composites is studied.
- The toxicology of these materials is explored with a *Drosophila* model.
- A life cycle analysis of carbon nanotube release from composites is proposed.

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ABSTRACT

Despite the fact that nanomaterials are considered potentially hazardous in a freely dispersed form, they are often considered safe when encapsulated into a polymer matrix. However, systematic research to confirm the abovementioned paradigm is lacking. Our data indicates that there are possible mechanisms of nanomaterial release from nanocomposites due to exposure to environmental conditions, especially UV radiation. The degradation of the polymer matrix and potential release of nanomaterials depend on the nature of the nanofillers and the polymer matrix, as well as on the nature of environmental exposure, such as the combination of UV, moisture, mechanical stress and other factors. To the best of our knowledge there is no systematic study that addresses all these effects. We present here an initial study of the stability of nanocomposites exposed to environmental conditions, where carbon nanotube (CNT) containing polymer composites were evaluated with various spectroscopic and microscopic techniques. This work discusses various degradation mechanisms of CNT polymer nanocomposites, including such factors as UV, moisture and mechanical damage. An *in vivo* ingestion study with *Drosophila* showed reduced survivorship at each dose tested with free amine-functionalized CNTs, while there was no toxicity when these CNTs were embedded in epoxy. In addition to developing new paradigms in terms of safety of nanocomposites, the outcomes of this research can lead to recommendations on safer design strategies for the next generation of CNT-containing products.

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1. Introduction

There is growing interest in the development of new organic polymer composites. Traditional composites have been used extensively in civil infrastructure applications; their use continues to grow in new and existing structures *via* retrofits, rehabilitations, and repairs. They are also used in consumer products, and in the automotive and

aerospace industries. One of the most promising trends in composites is the use of nanomaterials to revolutionize properties of existing composites. Polymer nanocomposites are multicomponent systems where polymers are combined with nanomaterials, often called nanofillers. Nanofillers, even at small concentrations, can dramatically enhance material properties, such as scratch resistance, elasticity, conductivity, etc. These new materials are already being used in the place of conventional composite materials.

Among many different types of polymers used in nanocomposites, epoxy polymers are standard materials used for aerospace, infrastructure, and consumer products owing to their excellent mechanical properties (specific strength, stiffness), good chemical resistance, and resistance to hydrolytic degradation (Spitalsky et al., 2010). These

* Corresponding author at: Materials Science & Engineering, Room 314 Old Engineering, Stony Brook University, Stony Brook, NY 11794-2275, United States. Tel.: +1 631 632 9978, +1 631 356 4675.

E-mail address: alexander.orlov@stonybrook.edu (A. Orlov).

properties can be increased even further through the addition of nanofillers such as carbon nanotubes (CNTs). Given the CNTs' high tensile modulus, strengths, low mass density, and large aspect ratio, their addition to polymers can improve the tensile stress, Young modulus, fracture toughness and storage modulus of the polymer matrix. Among other industrially relevant properties of nanocomposites attributed to CNTs are reduced gas and liquid permeability, increased thermal stability, lower flammability, low percolation threshold for electrical conductivity and higher wear resistance (Schlagenhauf et al., 2012) (Wohlleben et al., 2013a,b). CNT composites have already been used in products including sports bicycles, ship hull antifouling treatment, transistors, and even on the Juno spacecraft (De Volder et al., 2013).

The chemical, physical, and structural properties of the polymer/nanofiller–interface all play a vital role in nanocomposite degradation. Interfacial adhesion of CNTs to the polymer can be enhanced by chemical modification of the CNTs (*i.e.*, functionalization), or by using surfactants to moderate the physical interactions between the carbon nanofillers and matrix. There are certain advantages of this latter method, given its simplicity and lower cost compared to chemical functionalization (Fiedler et al., 2006).

Despite promising developments in nanocomposites, several important questions related to their safety for humans and the environment have not been properly addressed. For example, it is known that CNTs provide vehicles for drug delivery, as they are able to cross cell membranes carrying a variety of biologically functionalized amendments (Kostarelos et al., 2007). *In vitro* studies give mixed results, some find no toxicity (Cherukuri et al., 2004), or toxicity only in modified CNTs (Kam et al., 2004), possibly because they more readily cross cell membranes. *In vivo* toxicological studies under various delivery methods revealed that longer CNTs are more toxic (Poland et al., 2008); their needle-like fiber shape has similarities to that of asbestos. Inhalation studies in mice showed the lung has difficulty clearing CNTs. Longer multi-walled CNTs (MWCNTs) (higher aspect ratio) induced inflammation, nodular lesions (granulomas) and scarring in the mesothelial lining, symptoms which mirror asbestos inhalation phenotypes (Nagai et al., 2011; Ryman-Rasmussen et al., 2009). Shorter or tangled nanotubes, and carbon black controls, had much less of an effect. Inhalation studies in mouse mesothelial lining also resulted in granuloma formation (Poland et al., 2008). These CNTs were found three months following intravenous injection, and resulted in low levels of oxidative stress (Yang et al., 2008). Minimal bioaccumulation and toxicity were generally reported for unmodified MWCNTs in environmental studies (Li et al., 2013; Shrestha et al., 2013). Research still needs to be done to verify if this finding for a broader range of CNT functionalizations and types (*i.e.* single-walled and double-walled).

Encapsulation into a polymer matrix is thought to negate these aspects of CNTs' toxicity, but with little experimental support. The most probable source of CNT nanocomposite toxicity is in their potential to generate degradation products in an environmental setting *via* weathering. Polymer photodegradation by UV light can expose CNT ends at the nanocomposite surface, or potentially release free CNTs. Epoxy-CNT composites showed significantly less photodegradation than equivalent SiO₂ nanocomposite (Nguyen et al., 2011). *In vivo* toxicity studies have not yet been performed on nanocomposites or nanocomposite byproducts during manufacturing or environmental weathering. These are needed, as *in vitro* nanoparticle toxicology does not necessarily predict *in vivo* toxicology (Posgai et al., 2011).

An important question to address is whether there are realistic scenarios of CNT release from the polymer nanocomposites which could cause unwanted human and/or environmental toxicity. It is conceivable that such release can be caused by mechanical damage, chemical degradation under UV light and moisture, and biodegradation. This paper addresses some of the gaps in current knowledge in terms of clarifying the release pathways and the most likely release scenarios.

Current research on the environmental stability of nanocomposites has focused primarily on short-term stability and performance, whereas

the longer-term issues have not been properly addressed. This knowledge gap has the potential to hinder both applications and acceptance of these advanced composites in various industries. It is known that polymer matrices can undergo degradation when exposed to various environmental conditions during production, use and disposal. Therefore, it is critical to scrutinize the behavior of nanocomposites under relevant environmental conditions at all stages of the material's life cycle. Ideally, this approach should be applied to all manufacturing processes that use nanomaterials. Undertaking a comprehensive risk analysis of the environmental and health impacts of nanocomposites is still a considerable challenge (Petersen et al., 2011a). Moreover, it is also important to consider other mechanisms related to end-of-life stage of these materials, including incineration. (Petersen et al., 2011b). Fig. 1 shows a conceptual scheme of Life Cycle (LC) and potential mechanisms of release of nanomaterials from the composites. More specifically, it considers several stages of the LC, including the following:

1.1. Manufacturing stage

This stage includes handling of nanomaterials, which are present either in dry or already liquid-dispersed form. Due to the fact that this step involves raw nanofiller is conceivable that the highest risk of freely dispersed CNT release would occur during this stage. There are several other manufacturing steps beyond raw nanomaterial handling which could lead to exposure. These include nanofiller encapsulation into composites, machining (such as cutting, drilling and sanding), and assembly of nanomaterials into the final products. There are, however, several methods of nanomaterials containment, which, coupled with personal protection, can mitigate the exposure risks, especially those resulting from inhalation exposure (Kohler et al., 2008; Schlagenhauf et al., 2012; Bello et al., 2009).

Depending on the type of CNT (SWCNT or MWCNT), surface functionality, and presence or absence of catalyst residue, unencapsulated CNTs pose different toxicity. There are already numerous studies of this scenario (Ahamed et al., 2010; Fenoglio et al., 2012; Sharifi et al., 2012), although reproducibility and consistency of this data is an issue. Although chronic respiratory exposure to catalyst metals such as cobalt, a common nanotube contaminant, can cause bronchial asthma (Swenn et al., 1993), the extremely small percent composition of catalyst impurities after processing (generally <0.2 at.%), would most likely cause their health effects to be greatly outweighed by those of the CNTs.

1.2. Use

This stage poses the most significant risk to consumers. Repairs and certain patterns of use might lead to mechanical abrasion and to various types of materials failure originating from mechanical stress (Bello et al., 2009). Given that consumers do not usually practice nanomaterials containment procedures available at the manufacturing stage, there is a vital need to ensure that normal use of nanocomposites does not pose any risk greater than those inherent with the polymer substrate.

Normal use of nanocomposites might entail mechanical, physical and chemical factors leading to composite degradation. For example, environmental conditions might result in combined or individual exposure to UV light, humidity, temperature, chemical and biological factors. Linking nanomaterials release to physicochemical properties of nanocomposites and the environment has only been addressed sparsely in the current literature (Wohlleben et al., 2013a,b). Wohlleben *et al.* examined the combined effects of mechanical stress *via* shaking and ultrasonication with UV light exposure, but the particulate detection methodology could not discern between exposed CNTs and other particulates smaller than 150 nm.

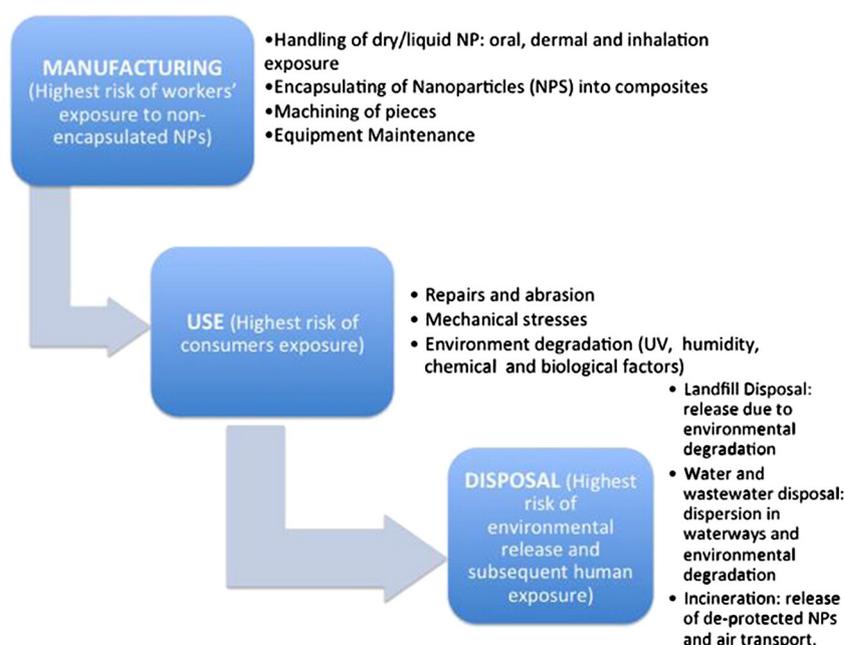


Fig. 1. Conceptual scheme of the life-cycle of CNT nanocomposites and potential associated environmental/health risks.

1.3. Disposal

This stage of the life cycle has several potential release pathways of NPs. The disposal stage includes landfill disposal, water and wastewater disposal, and incineration. Similarly to environmental factors encountered at the use stage of LC, nanocomposites disposed in landfills will potentially be exposed to heat, moisture, liquids of variable pH and ionic strengths, microorganisms and other physicochemical factors. If done correctly, incineration could potentially combust nanomaterials such as CNTs, while particulate matter control procedures could potentially retain a significant fraction of inorganic nanomaterials. Similarly, nanomaterials disposed in wastewater might be retained at various stages of water treatment; however, the efficiency of removal may vary from facility to facility, and may be dependent on the type of nanomaterials. Overall, there are many uncertainties related to each of the disposal methods, and more studies are needed to determine the efficiencies of the existing methods while developing new methods geared towards nanomaterials removal.

It is important to note that the published literature focuses primarily on manufacturing-stage composite degradation, such as potential dust release during sawing, drilling and sanding operations (Goehler et al., 2010; Reijnders, 2009). In one such study, Goehler et al. found that sanding of acrylate and polyurethane coatings containing inorganic nanoparticles did not result in a significant release of nanomaterial from the matrix (Goehler et al., 2010). Additionally, Ren et al. studied the mechanical degradation of nanocomposites such as cyclic fatigue behavior of SWCNT-epoxy composites. The results of this study showed several areas where CNTs were partially pulled out of the matrix due to composite deformation, with CNTs bridging surface cracks (Ren et al., 2003).

Even though the studies mentioned above found a very limited release of nanomaterials (Cena and Peters, 2011; Nowack et al., 2012), several other mechanisms of composite degradation, such as UV exposure, must be comprehensively evaluated under environmentally relevant conditions such as high light intensity, temperature cycles and humidity (Cena and Peters, 2011; Wohlleben et al., 2011). Moreover, comprehensive studies are needed to link the UV-induced release of nanomaterials (and their physicochemical properties) to their potential toxicity. It is important to note that exposure of composites to combined UV radiation and condensation (moisture) can significantly accelerate

nanocomposite degradation, resulting in a deterioration of mechanical properties, changes in chemical structure, and significant mass loss (Chang and Chow, 2010; Kumar et al., 2002; Nakamura et al., 2006; Singh et al., 2010; Zhao et al., 2009). These combined conditions are strongly correlated to outdoor weathering, where materials are exposed to sunlight, moisture, and temperature extremes (Petersen et al., 2011a,b).

The very limited literature on combined moisture and UV exposure provides only partial answers. A study done by Wohlleben et al. (2013a), which included toxicity evaluation, explored two separate degradation scenarios of polyurethane composites: accelerated weathering and mechanical degradation. The authors did not find a notable release of CNTs from polymer matrix, and therefore the toxic effects were not observed. The study noted accelerating effects of a combination of relative humidity and UV exposure, as well as an appearance of freestanding CNTs when the polymer matrix was substantially photodegraded. Although the authors suggested that the CNTs tend to stay on the surface, a lack of methodology to assess such release, as well as uncertainties related to the relevance of experimental parameters to environmental conditions render this suggestion inconclusive.

The authors have also noted that the absence of CNT release in one polymer does not necessarily indicate that absence in other polymers (Wohlleben et al., 2013a). This conclusion highlights the need to perform composite degradation studies for various types of nanofiller-matrix composites under a variety of simulated weathering scenarios. For example, the above-mentioned polyurethane study cannot be extended to epoxy composites, given the differences in chemical composition, rheological behavior and mechanical properties of these two composites, which will result in remarkably different release scenarios.

Under certain exposure conditions, it is feasible that CNTs will remain on the composite surface even after degradation. When Nguyen et al. considered an accelerated scenario of UV- and moisture-induced degradation of bisphenol-A epoxy composites, they found significant accumulation of CNTs on the surface, but no detectable release of CNTs after nine months of exposure (Nguyen et al., 2011). Moreover, the authors found that composites' degradation slowed down after the layer of accumulated CNTs began to act as a screen against the radiation, preventing it from reaching the polymer underneath. This could also explain the increase in resistance to weathering described by other researchers (Asmatulu et al., 2011; Loos et al., 2012). The hypothesis

that CNTs have screening effects is also supported in studies of composites involving similar nanofillers. For example, graphene, a material similar to CNTs in its chemical composition, was also found to accumulate on the composite surface upon exposure of the nanocomposite to UV radiation (Bernard et al., 2011). However, the release of graphene was not detected. Given the challenges inherent with detecting released nanosize particles, it is quite possible that the problem is with detection rather than with the absence of released nanomaterials which lead to the results described above.

Linking the most probable release scenarios with changing physico-chemical properties of nanocomposites when subjected to the variables present in a given point in their lifecycle is a complex but important challenge. This is made even more complex because it is important to consider the toxicological implications of such a potential nanomaterial release. Addressing this challenge at every stage of the composites' life cycle would not only improve occupational safety, but also would protect consumers and environment.

2. Experimental methods¹

2.1. Materials

Three types of samples were prepared: neat epoxy without nanofiller, epoxy with 1.0% mass fraction unfunctionalized MWCNTs, and epoxy with 1.0% mass fraction amino-functionalized multi-walled CNTs. Hereafter, unfunctionalized MWCNTs and amino-functionalized MWCNTs are designated as plain CNTs and amino-CNTs respectively. The epoxy matrix consisted of diglycidyl ether of bisphenol F (DGEBF)-based resin cross-linked with an aliphatic amine curing agent (hardener) in a stoichiometric ratio of 7:3 by mass. Amino-CNTs (SKU-030114) and plain CNTs (SKU-030111) were obtained commercially (Cheap Tubes Inc.). They were produced by the catalytic chemical vapor deposition (CCVD) process, and subsequently treated with a plasma purification process to remove residual catalyst. The surface area and surface atomic percentages (Table 1) of these nanotubes were measured by N₂ adsorption and X-ray photoelectron spectroscopy (XPS), respectively. XPS was also employed to identify the catalyst residues. Only trace amounts (<2 at.%) of the remaining Co catalyst were detected by XPS. The concentration of this residue was low enough that it was difficult to quantify accurately.

In our experiments, two different types of MWCNTs were used (plain and amino-functionalized MWCNTs). The rationale for the selection of these types of CNTs is threefold. First, they are among the most widely used on a commercial scale. Second, they are some of the most well described CNTs in literature. Thirdly, the amino groups in amino-functionalized MWCNTs can bond covalently with the epoxy resin (Fiedler et al., 2006), leading to mechanically stronger and more environmentally stable composites as compared to plain CNT composites. The rationale for selecting DGEBF instead of the more common diglycidyl ether of bisphenol A (DGEBA) is related to recent efforts to replace DGEBA in various consumer products with safer alternatives.

2.2. Nanocomposite preparation

Unless specifically mentioned, all samples were prepared with identical methodology as follows. The critical step of dispersion of CNTs in the epoxy matrix was performed using an established method adopted by several groups (Ahamed et al., 2010; Kim et al., 2006). This method, usually called solution-mixing, involves several stages, including bath sonication of CNTs; the sonication took place in two one-hour

Table 1

Information concerning CNT controls, undamaged epoxy and the UV-irradiated epoxy.

Sample	C%	St. Dev	O%	St. Dev	N%	St. Dev	Catalyst residue present?	CNT surface area (m ² /g)
Plain CNT	88.4	3.5	7.8	1.1	0.2	0	Yes, Cobalt	209.112
Amino-CNT	92.1	0.5	5.7	0.2	1.1	0.3	Yes, Cobalt	282.961
Native epoxy	76.7	0.4	20.7	0.2	2.6	0.1	N/A	N/A
UV epoxy	63.5	0.5	28.6	0.4	7.8	0.2	N/A	N/A

Percent surface concentrations of the C, O and N for these samples is noted as well as the presence and identity of any residual catalyst and nanofiller surface area. Each set of percentages are derived from the average of 3 unique measurements ± 1 standard deviation.

sonication stages using an ice water-cooled Branson 2510 45 kHz bath sonicator. The sonicator had a power of 60 W \pm 5 W, calculated using NIST's published recommendations for ultrasonic dispersion of nanoparticles (Taurozzi et al., 2011). 0.5 g of CNTs were placed in 100 g ethanol and sonicated for 1 h. After pouring this CNT suspension into a beaker containing 34.5 g epoxy resin, the CNTs-epoxy-ethanol mixture was sonicated for another hour. It should be noted that the sonication process could cause CNT breakage, thus modifying the aspect ratio and possibly, their toxicity.

All three solutions (neat epoxy, bare- and amino-CNT-containing) were finally mixed with the amine curing agent at appropriate amounts using a mechanical mixer. After an additional 15 min of sonication, the CNT-epoxy-curing agent mixture was poured onto separate polyethylene terephthalate (PET) sheets and drawn into 400 μ m thick films using a thin-film applicator. These films were then left to pre-cure at ambient conditions for 48 h and subsequently post-cured in an oven at 120 °C for 20 min.

2.3. Exposures to environmental conditions

After curing and storing at ambient conditions (24 °C and ~50% relative humidity) for one week, the nanocomposite films were cut into approximately 2.54 cm diameter circles, which were then placed into the Simulated Photodegradation via High Energy Radiant Exposure (SPHERE) accelerated environmental chamber (Chin et al., 2004) at the National Institute of Standards and Technology (NIST). This UV chamber produces a highly uniform UV flux of approximately 480 W/m² in the 295 to 400 nm wavelength range; approximately 22 suns worth of ultraviolet radiation. In this setup, the temperature and humidity were tightly controlled and kept at constant 30 °C and 75% relative humidity.

In addition to this accelerated UV exposure, a supplemental degradation experiment was conducted by subjecting samples to alternating UV exposure and high relative humidity in a QUV weathering system, as sold by Q-Lab corporation. The main purpose of this experiment, which was conducted at Stony Brook University, was to obtain degraded samples for toxicity study. This setup subjects samples to UVA radiation (340 nm wavelength) exposure at 0.68 W/m² irradiance and 40 °C, which matches typical maximum irradiance at noon for Floridian summer sunlight, followed by sequential exposure to water vapor condensation (100% relative humidity) on a 3 h cycle at 40 °C. The condensation simulates dew and rainfall and serves a dual purpose: uptake of moisture into the nanocomposite as well as washing the nanocomposite surface. This cyclic exposure provides 12 h of both UV radiation and condensation within a 24 h period.

2.4. Characterization of nanocomposite degradation

Samples exposed in the NIST SPHERE were periodically removed for attenuated total reflection-Fourier transform infrared (ATR-FTIR) analysis to monitor chemical change over time. After 1380 h of exposure, a subset of these samples was removed from the SPHERE chamber for XPS analysis and cross-sectional morphological imaging.

¹ Certain trade names and company products are mentioned in the text or identified in illustrations in order to specify adequately the experimental procedure and equipment used. In no case does such identification imply recommendation or endorsement by National Institute of Standards and Technology, nor does it imply that the products are necessarily the best available for the purpose.

ATR-FTIR spectroscopy was performed using a Nicolet Nexus 670x spectrometer equipped with a liquid nitrogen-cooled mercury cadmium telluride (MCT) detector. All spectra were recorded at an incident angle of 45°, a resolution of 4 cm⁻¹, a ZnSe prism, and dried air as the purge gas. All FTIR results were the average of at least three locations on three specimens. Surface and cross-sectional morphological changes due to environmental exposures were recorded using a Zeiss Ultra-60 field emission scanning electron microscope (FE-SEM).

XPS spectra were taken on a Kratos Axis Ultra DLD spectrophotometer equipped with monochromatic Al K α radiation ($h\nu = 1486.6$ eV) under ultra-high vacuum (UHV) conditions ($P_{\text{base}} \approx 7 \times 10^{-8}$ Pa). Due to the insulating nature of the CNT-epoxy nanocomposites, spectra were acquired with and without a charge neutralizer running to obtain quantitative information of the epoxy matrix and the surface enhancement of CNTs, respectively. The charge neutralizer is a low energy electron flood source irradiating the surface of the composite with ≈ 3.6 eV electrons. The rationale for using this device is clear after observation of the shifted and distorted spectra in Fig. S1. High resolution XPS spectra were acquired at a pass energy of 40 eV using a hybrid lens and a slit aperture. O (1 s), N (1 s) and C (1 s) spectral regions were acquired at 0.1 eV/step with a dwell time of 600 ms/step.

Four nanocomposite samples were analyzed for changes in surface chemical composition using XPS analysis: plain CNT composite before and after 1380 h of exposure and amino-CNT composite before and after 1380 h of exposure. Data analysis was performed on commercially available software (Casa XPS Ltd) and elemental percentages were calculated for the charge neutralized composites using 0.78, 0.477 and 0.278 for the oxygen, nitrogen and carbon peaks, respectively (elemental RSF's provided by Kratos Analytical, Chestnut Ridge, NY). Additionally, the Si (2p) was calculated and, along with detected Co catalyst for the CNTs, was incorporated into the overall elemental percentages. All reported percent surface compositions reflect the average ± 1 standard deviation of 3 separate measurements and are from charge neutralized samples with the exception of the CNT controls.

2.5. Toxicity testing

The fly, *Drosophila melanogaster*, provides an inexpensive model for assessing the *in vivo* toxicology of ingested and inhaled CNTs at different levels of biological organization (Ahmed et al., 2010; Leeuw et al., 2007; Liu et al., 2009; Posgai et al., 2011). *D. melanogaster* is a relevant model for investigating human health, as counterparts of genes responsible for more than 700 different human genetic diseases including neurological, immunological, cardiovascular, auditory, visual, developmental and metabolic disorders are found in *Drosophila* (Reiter et al., 2001; Koh et al., 2006; Wolf et al., 2006). Its short generation time permits more sensitive toxicity assays of exposure effects on developmental rate, survivorship, and reproductive effort that are unavailable *in vitro* or impractical in mammalian models.

2.5.1. Nanocomposite preparation for toxicity testing

We performed toxicological tests on plain MWCNTs and amino-MWCNTs as free particles, and on epoxy nanocomposites containing these two nanofillers that had been exposed to UV for 1560 h. The nanocomposite was in a film form and unsuited for toxicological testing. To prepare for the ingestion study, the nanocomposites were frozen in liquid nitrogen and then ground with a mortar into micron-sized particles with a range of diameters between 5 μm and 50 μm before use (see Fig. 2). Ground nanocomposite particles were suspended in ethanol to facilitate a uniform dispersion, and added to treatment media for ingestion. An equivalent amount of ethanol was used to generate negative controls. For treatments, female flies oviposited fertilized eggs on unmodified media over a 2 h window, after which larvae were placed on treatment and negative control plates for testing (50 larvae/treatment). Time to pupation (developmental rate) and survivorship through adult eclosion at 25 °C were recorded.

2.5.2. Fly husbandry

OreRS flies were obtained from the Bloomington Stock Center, Bloomington, IN and reared on standard cornmeal–malt–yeast medium (Bloomington Stock Center recipe) at 25 °C under a 12 h day/night cycle. MWCNTs and ground composite were added to the media while cooling and decanted into 60 mm \times 15 mm Petri plates for larval feeding experiments. Control plates consisted of 20 mL standard *Drosophila* media. In treatment lines, standard media were supplemented with MWCNTs resulting in final suspensions of 10, 50, and 100 $\mu\text{g}/\text{mL}$.

2.5.3. Survivorship and developmental rate assays

Drosophila embryos were laid over a 2 h time period on control medium. Fifty embryos were collected from each plate and moved to control or treatment plates for the assay. Percent survivorship was calculated as the number of embryos that pupated divided by the total number of embryos. Developmental rate was assessed as the time from first larval instar hatch to pupation. The mean and standard deviation of percent survivorship and time to pupation were calculated and statistically analyzed using a two-tailed *t*-test with a cutoff for statistical significance of $p < 0.05$.

3. Experimental results

3.1. Morphological changes

An examination of SEM cross-sectional images of the nanocomposites (Fig. 3) suggested that amino-CNTs had a better dispersion in the epoxy matrix as compared to the plain CNTs. Fig. 4 displays SEM images of the amino-CNTs nanocomposite surfaces before and after UV exposure. Before exposure, the surface was smooth with little evidence of CNTs' presence on it (Fig. 3A). After UV exposure, a significant amount of CNTs had accumulated on the surface (Fig. 3B).

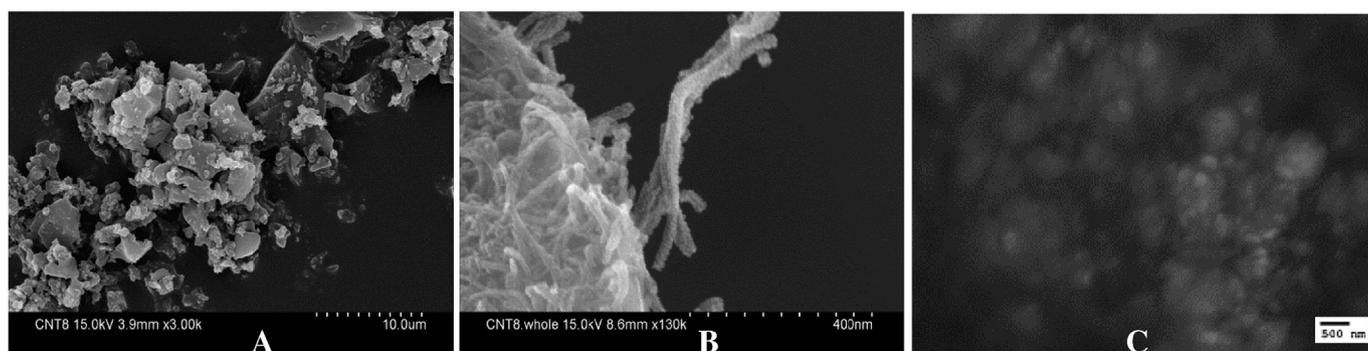


Fig. 2. A,B: SEM of mortar-ground CNT-nanocomposite used in toxicity tests. A) Individual particles are ~ 10 – 100 μm in diameter. B) Note exposed bare ends at higher magnification. C) TEM of CNT-NH₂-nanocomposite, note embedded CNTs within epoxy matrix.

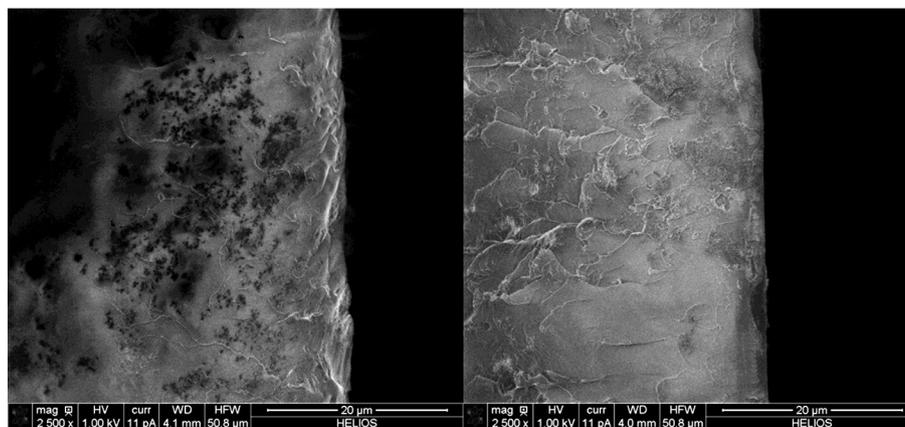


Fig. 3. SEM image of nanocomposite cross section showing CNT dispersion throughout the depth. (Left) Nanocomposite containing bare CNTs and (Right) nanocomposite containing NH₂ functionalized CNTs.

3.2. Chemical degradation

3.2.1. ATR-FTIR analyses

FTIR spectra taken at different exposure times (Supplemental Fig. 1) show a decrease of peak intensity in the 1110–1120 cm^{-1} region and an increase in the peak intensity in the 1720–1730 cm^{-1} region, representing a decrease of the ether groups and an increase in the carboxyl groups, respectively, for both composites. The intensity changes of these bands as a function of exposure time for the two CNT composites and neat epoxy are displayed in Fig. 5. The intensity of the bands at 1113 cm^{-1} (due to C–O) and at 1508 cm^{-1} (due to benzene ring) of both nanocomposites and neat epoxy decreases substantially with exposure time. On the other hand, the intensity of the band at 1725 cm^{-1} of all three materials increases with exposure. These results indicate that the epoxy matrix has undergone degradation due to exposure to the UV environment. This conclusion is consistent with XPS data as will be discussed below.

A more detailed analysis of Fig. 5 might give an impression that CNT-containing composites degrade more rapidly than neat epoxy, particularly at exposure times less than 250 h. However, that is not necessarily the case. Decrease in the signal related to ether and aromatic groups might be related not only to chemical degradation of epoxy but also to the screening effect of CNTs resulting in blocking the IR-generated signals from the polymer matrix beneath. In our case, it appears the screening effect is more substantial as opposed to matrix degradation effect; some authors attribute up to 25% difference in signal strength

due to this effect (Bernard et al., 2011). As described earlier, this screening effect could also be related to a substantial absorbance of UV radiation by CNTs, thus preventing further degradation of the underlying matrix (Najafi and Shin, 2005).

3.2.2. XPS analyses

Changes in surface elemental and surface chemical composition as a result of UV exposure can be observed in the stackplot of Fig. 6 as well as Table 2 for the elemental percentages from the different CNT composites (plain and amino-CNTs). In Fig. 6, the charge neutralized C (1 s) spectra were dominated by 4 spectral features prior to UV exposure (See right panel row 1 and 3), the CC/CH and CO/CN peaks at ≈ 284.5 eV and ≈ 286.0 eV, respectively, which dominated the spectra along with smaller contributions from higher oxidation functionalities and indications of aromaticity (CON/COO and the $\pi - \pi^*$ transfer), all of which are comparable with previous reports regarding comparable epoxy used in nanocomposites (Gorham et al., 2012) and are nearly identical to the epoxy control (See Fig. 7, bottom). A spectral feature reflective of CNTs could not be identified in the unexposed nanocomposites, presumably due to one of two reasons: (1) the low surface concentration of CNTs had a spectral signal which overlapped with the CC/CH spectra (see CNT controls in Fig. 7), effectively masking it, or (2) there was a CNT-free layer of epoxy at the surface of the composite.

After UV exposure, the elemental distribution of the MWCNT composites decreased in carbon concentration and increased in surface oxygen and nitrogen concentration, as indicated in Table 2. This is consistent

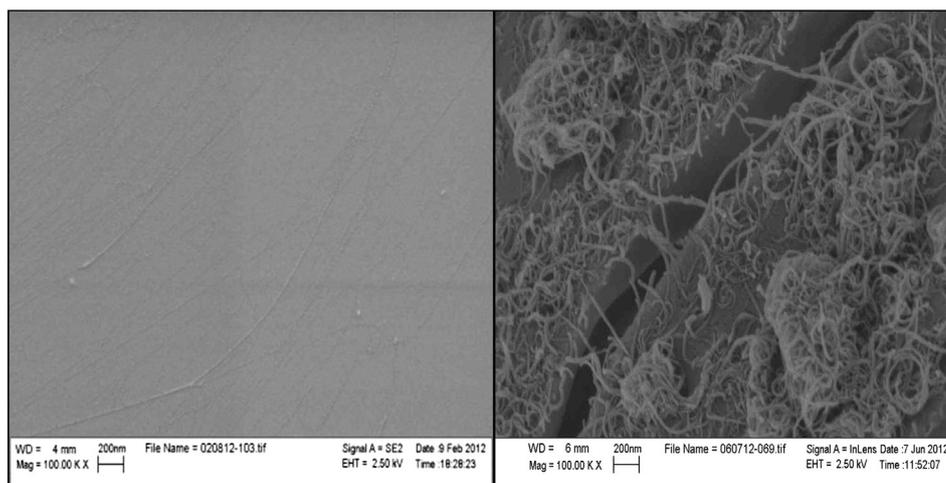


Fig. 4. Degradation of CNT-containing composites. (Left) surface of CNT-Epoxy surface before exposure to UV; (Right) magnified agglomerate of nanotubes on the surface of sample exposed to UV for 1308 h, showing cracking of the matrix and partially unprotected nanomaterials.

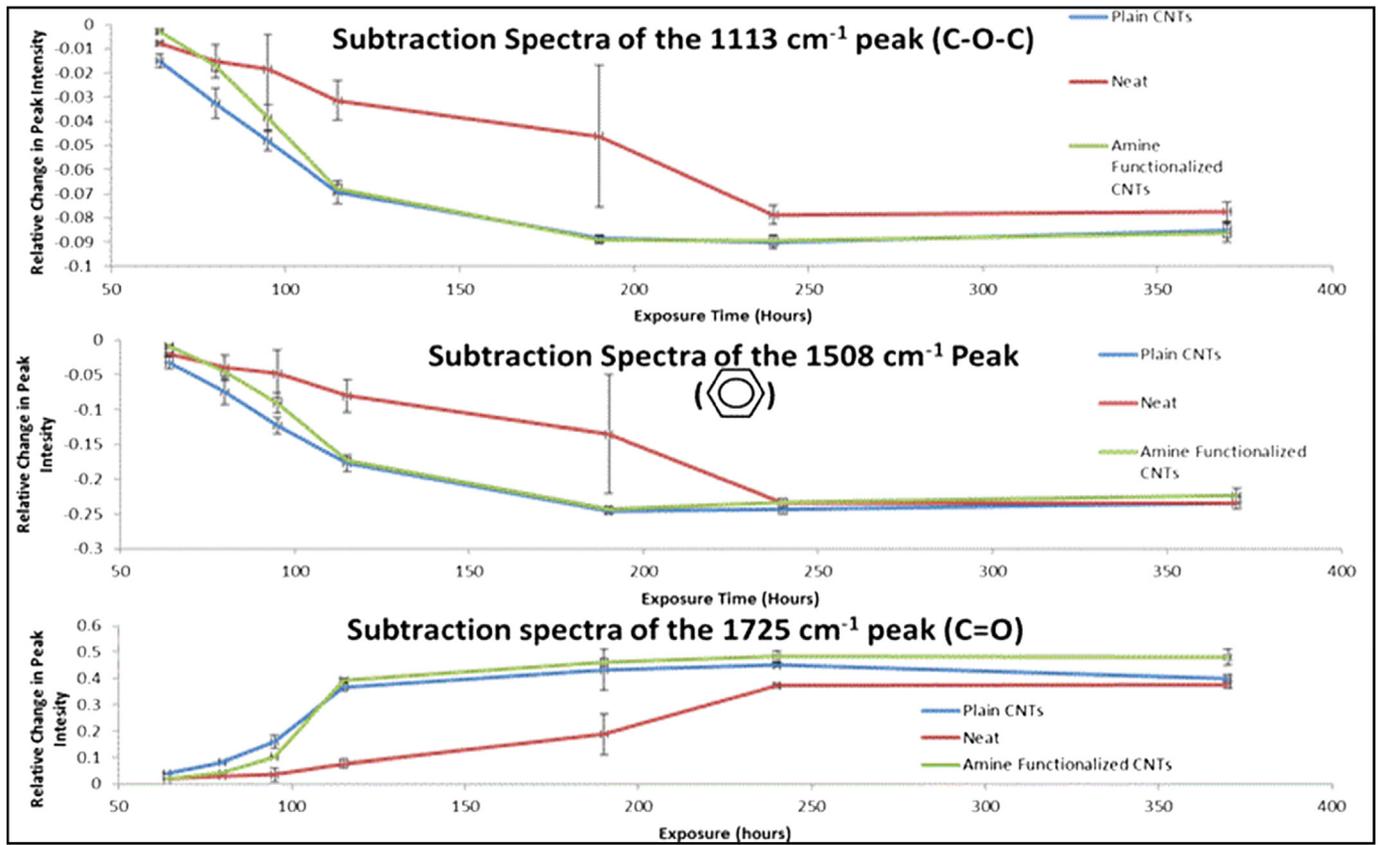


Fig. 5. FTIR analysis of the samples showing a decrease in the FTIR peak assigned to C–O bonds with a matching increase in that of C=O bonds.

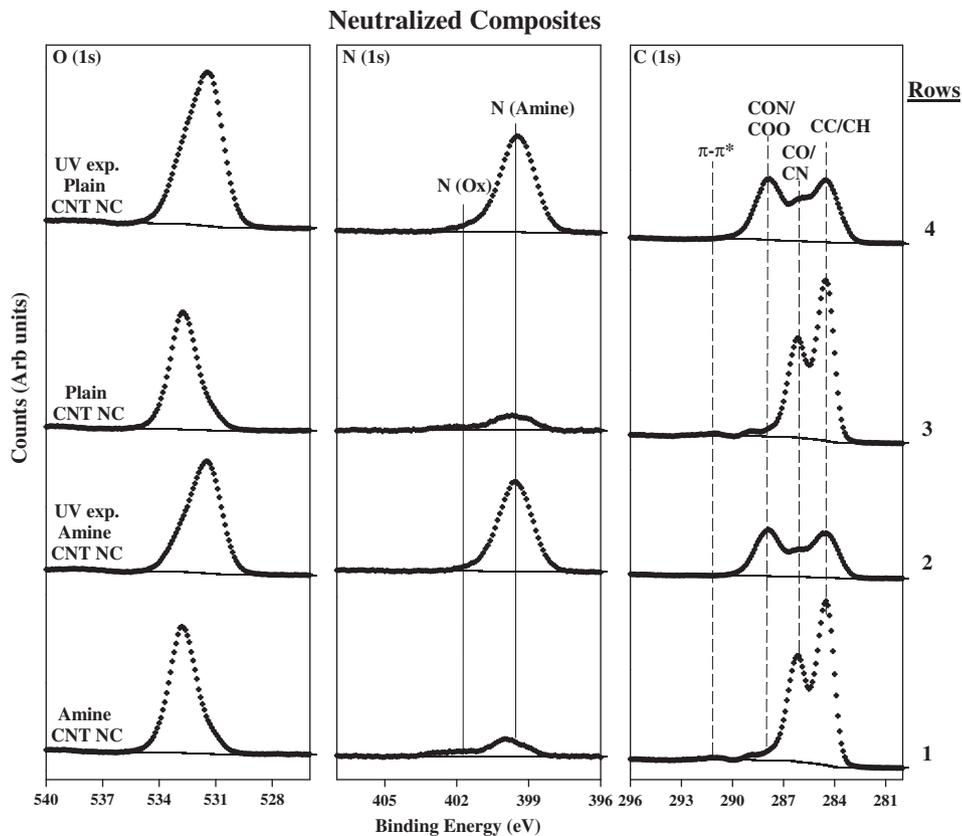


Fig. 6. Representative spectra from amine and plain CNT-Nanocomposite before and after 1380 h of UV exposure. (From left to right) The spectra presented are the O (1 s) region, N (1 s) and C (1 s) elemental regions.

Table 2
Percent surface concentrations of carbon, oxygen, and nitrogen.

	C%	St. Dev	O %	St. Dev	N %	St. Dev
Amine: 0 h exp	78.6	0.14	19.0	0.2	2.4	0.1
Amine: 1380 h exp	58.1	0.3	28.8	0.7	13.1	0.5
Plain: 0 h exp	78.9	0.2	18.8	0.1	2.3	0.1
Plain: 1380 h exp	59.1	0.6	30.3	0.3	10.6	0.9

Percent surface concentrations of the elements presented. Each set of percentages is derived from the average of 3 unique measurements ± 1 standard deviation.

with the change in elemental distribution of the plain epoxy due to UV exposure, as displayed in Table 1. Furthermore, there was a clearly visible decline in the CC/CH and CO/CN components of the C (1 s) region, as is shown in Fig. 6 (Rows 2 and 4), while the highly oxidized carbon spectral feature (COO/CON) grew substantially into a broad, dominant spectral feature at ≈ 287.8 eV. Regardless, an increase in the oxidation of the carbon within the nanocomposite is also consistent with the O (1 s) region, which increased with UV exposure and with the UV exposed epoxy control (Fig. 7). Lastly, the N (1 s) spectra did increase for both CNT composites and remained at a peak maximum roughly constant with unexposed CNT composite at ≈ 399.5 eV.

The post-degradation amino-CNT samples showed 3% higher levels of nitrogen at their surface as compared to those with plain nanotubes, indicating the potential for CNTs exposed at the surface. For further qualitative evidence regarding surface accumulation of CNTs (both amino functionalized and plain), XPS data was acquired without the use of the charge neutralizer.

3.3. Toxicological testing

Following the protocols described in the experimental section, we found no effect of free MWCNT or MWCNT-nanocomposite ingestion on fly developmental rate (not shown). However, we did find effects on the survivorship, and the results are presented in Fig. 8. Flies ingesting free amino-CNTs had significantly reduced survivorship at each dose tested. As predicted, the amino-CNTs were not toxic when embedded in nanocomposite. The epoxy embedding nullifies the high aspect ratio of CNTs, the feature most associated with CNT toxicity (Ali-Boucetta et al., 2011). The greater toxicity of the free amino-CNTs

vs. free plain CNTs can be attributed to the amino group facilitating CNT uptake by the cells. The increase in survival rate for some CNTs exposed samples as compared to control samples has some precedence in observed beneficial effects of CNT exposure in plant models (Tripathi et al., 2011) although the mechanism of this phenomenon in published and our experimental data are unclear.

4. Discussion

4.1. Morphological changes

Several possible forms of CNTs were found on the composite surface by UV irradiation: completely unprotected and agglomerated CNTs; partially exposed CNTs fractured due to the crack formation originating from exposure; CNTs still encapsulated in the matrix; and fragments of the matrix (not shown but implied due to appearance of the cracks), which can be potentially released in the environment due to extensive combined UV and moisture attacks. The SEM micrograph of Fig. 3B suggests that CNTs could be released into the environment after the polymer matrix is degraded. It should be mentioned that this release process takes time, and the rate of release, which is a complex function of material and environmental factors, needs to be quantified.

4.2. XPS analysis

Although ATR-FTIR provides invaluable chemical information towards identifying the mechanisms of composites degradation, the XPS analysis is also instructive in confirming some of the above mentioned conclusions. Given the surface sensitivity of XPS the results below are informative in identifying chemical changes to approximately 10 nm from the top surface layer as opposed to up to 2500 nm for ATR-FTIR technique, depending on the wave length. The charged data presented in the SI Fig. S1 illustrates the insulating effect of the epoxy component, on the C (1 s) region of the CNT nanocomposite as clearly demonstrated by the shift in binding energy by >20 eV in both amine functionalized and plain CNTs prior to UV exposure. Upon UV exposure, the dominant, charged features of the C (1 s) region both shift to lower binding energies indicative of an alleviation of some of the charging phenomenon. Additionally, a new feature developed in both of the CNT composites. Indeed, upon magnifying the C (1 s) regions (SI Fig. S1, right) one can

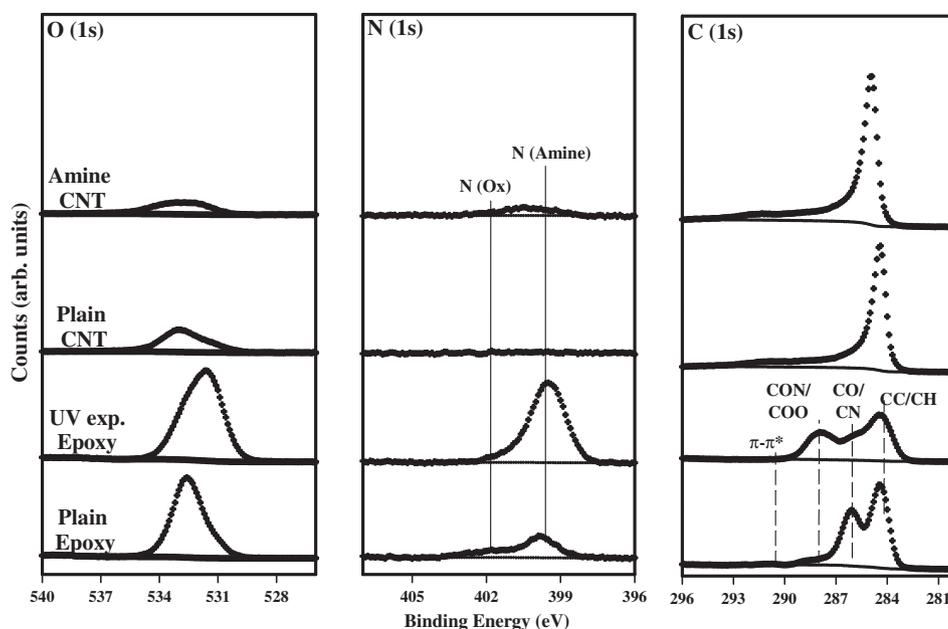


Fig. 7. Spectra of plain and amine functionalized CNT powders, as well as an epoxy control and a UV irradiated epoxy control. 2.

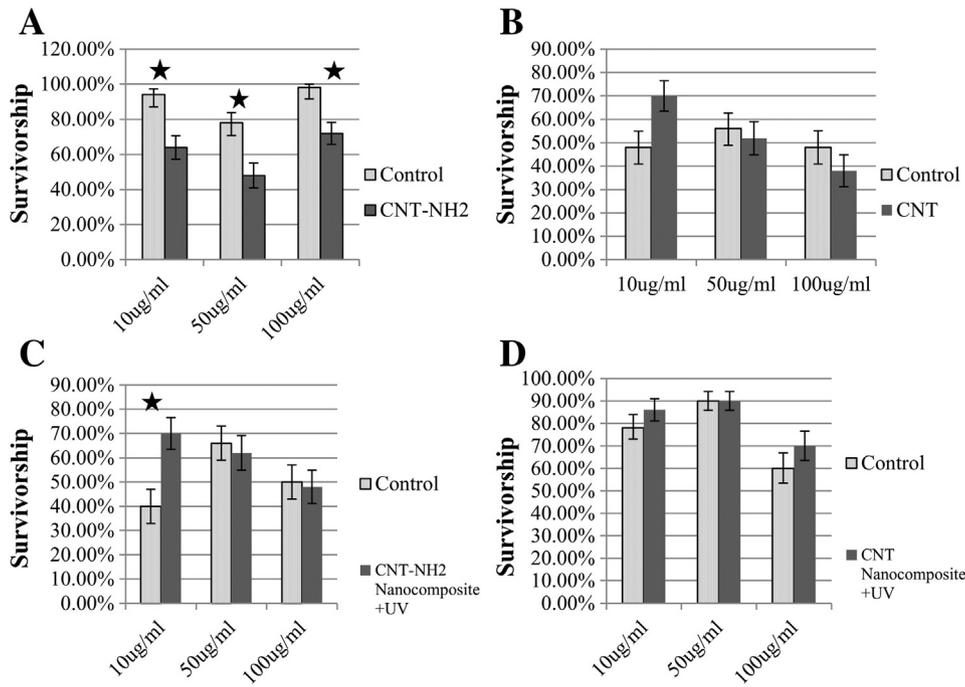


Fig. 8. Proportion of larvae surviving to pupation (mean ± SdV) is presented for control and treatment lines as described in experimental section in detail. A) Free amino-CNTs, B) Free plain CNTs, C) Ground 1380-h UV-exposed amino CNT-epoxy composite, and D) Ground 1380-h UV-exposed plain CNT-epoxy composite. N = 50 larvae/treatment. *Significant difference under 2-tailed *t*-test ($p < 0.05$).

clearly observe a feature developing at ≈ 284.5 eV which was previously assigned to the CC/CH functionality in the neutralized spectra (Fig. 6). However, due to the insulating nature of the epoxy matrix and based on our previous XPS spectral results, it is more likely that the new feature is largely due to a small surface accumulation of the conductive CNTs upon the UV-irradiated nanocomposite surface which also possess a peak maximum near 284.5 eV. Furthermore, this is consistent with the images of CNTs on the surface after UV exposure, as demonstrated in Fig. 4. Qualitatively, this suggests that one of two possible reactions has occurred to the CNT nanocomposites due to UV radiation: (1) The epoxy matrix has been selectively, photo-oxidatively removed, leaving

behind an increased surface concentration of CNTs or (2) the CNTs have migrated to the surface due to the UV radiation. However, due to the clear oxidation of the composite and loss of the initial components demonstrated by both techniques (Figs. 4 and 5), preferential photo-oxidative removal of the epoxy component of the nanocomposite is the most probable cause of enhance CNT surface concentration (Fig. 9).

4.3. Future work

The next logical step of this research would be to test nanocomposite following longer exposures to moisture/UV in the QUV chamber. The

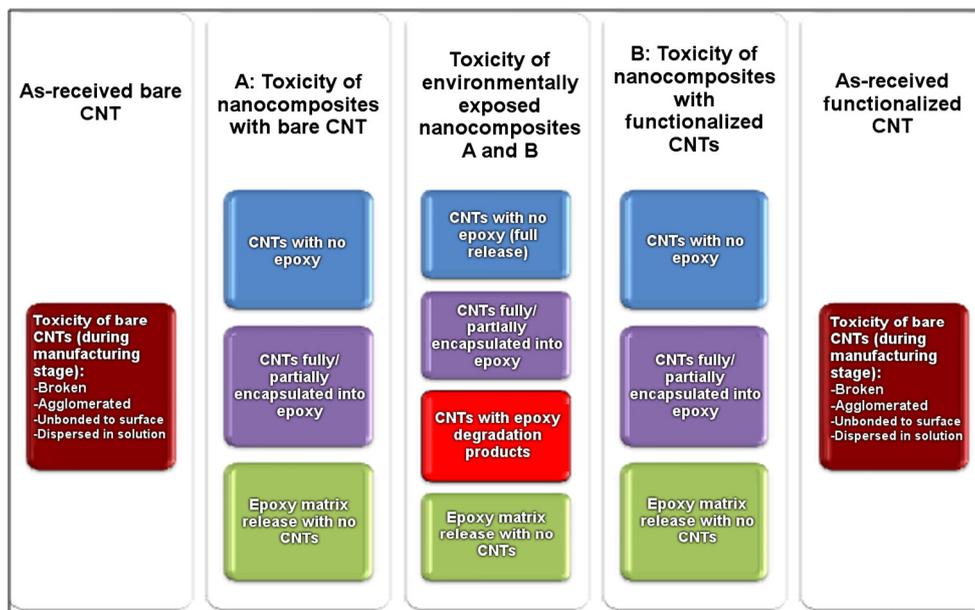


Fig. 9. A conceptual scheme of different types of toxicity associated with nanocomposites degradation.

ground particles from the UV-aged samples will be much smaller in size (high nano-range) and may show a different toxicity than the ground nanocomposite tested here, which were in micrometer range. This will simulate the worst case scenario of UV exposure, deprotection and subsequent abrasion, which will potentially result in the most significant release of the deprotected and/or partially encapsulated CNT. The key factors in this will be if the bare CNT ends remain encapsulated under this treatment, and if these smaller particles generate oxidative stress.

It is also important to highlight the issues of a reliable detection of deprotected CNTs. One strategy to reliably determine the release of CNTs is the use of ^{14}C isotopically labeled CNTs, which has been used in several environmental toxicology studies but not in nanocomposites systems (Petersen et al., 2009, 2011a,b).

5. Conclusions

In this study we have evaluated the stability of epoxy composites containing CNTs under environmentally relevant weathering conditions. We found that composites underwent a significant degradation under combined effects of moisture and UV, resulting in partial CNT deprotection from the polymer matrix, with the potential for environmental release. Based on evaluation of spectroscopic and microscopy data, we have developed a conceptual framework concerning various environmental release and toxicological scenarios. Although a preliminary study on the effects of exposure to nanomaterials imbedded in the polymer matrix based on *in vivo* Drosophila model did not detect a significant increase in toxicity, this could have been due to the fact that a majority of CNTs collected in abraded samples were still encapsulated in the matrix. This scenario is very different than described in Section 3.3, when only free nanotubes were examined. However, under certain conditions, a combined UV exposure and mechanical abrasion can potentially lead to more pronounced release. As such, further studies are needed to evaluate the potential toxicological impact CNTs released from polymer composites.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scitotenv.2013.11.135>.

Conflict of interest

We do not have any conflicts of interest.

Acknowledgments

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