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Assessment and prediction of joint algal toxicity of binary mixtures of graphene and ionic liquids

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HIGHLIGHTS

- Isolated ILs in graphene-IL mixtures were the main contributors to toxicity.
- GO-IL mixtures resulted in more severe joint toxicity than iG-IL mixtures.
- Mechanism may be driven by adsorption, stability, and oxidative stress.
- Joint toxicities of graphene and ILs were additive or antagonistic.
- Predictions made using CA and IA models were close to observed joint toxicities.

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ABSTRACT

Graphene and ionic liquids (ILs) released into the environment will interact with each other. So far however, the risks associated with the concurrent exposure of biota to graphene and ILs in the environment have received little attention. The research reported here focused on observing and predicting the joint toxicity effects in the green alga *Scenedesmus obliquus* exposed to binary mixtures of intrinsic graphene (iG)/graphene oxide (GO) and five ILs of varying anionic and cationic types. The isolated ILs in the binary mixtures were the main contributors to toxicity. The binary GO-IL mixtures resulted in more severe joint toxicity than the binary iG-IL mixtures, irrespective of mixture ratios. The mechanism of the joint toxicity may be associated with the adsorption capability of the graphenes for the ILs, the dispersion stability of the graphenes in aquatic media, and modulation of the binary mixtures-induced oxidative stress. A toxic unit assessment showed that the graphene and IL toxicities were additive at low concentration of the mixtures but antagonistic at high concentration of the mixtures. Predictions made using the concentration addition and independent action models were close to the observed joint toxicities regardless of mixture types and mixture ratios. These findings provide new insights that are of use in the risk assessment of mixtures of engineered nanoparticles and other environmentally relevant contaminants.

1. Introduction

Carbon nanomaterials (CNMs) are becoming popular because they have special properties that allow them to be used in various unique applications (Galloway et al., 2010; Geim and Novoselov, 2007; Nowack et al., 2013; Qu et al., 2013; Yang and Xing, 2010). Intrinsic graphene (iG) and its derivatives such as graphene oxide (GO) have the most fascinating nanostructures of all the CNMs (Geim, 2009), and they have exceptional properties (Novoselov et al., 2012; Wang et al., 2014a, 2015). The wide range of applications of iG and GO nanomaterials could lead to nanoparticulate release into the environment and result in unexpected hazards on human and environmental health (Hu and Zhou, 2013; Wu et al., 2016). Interest in the chemical behavior, environmental fate, and

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ecotoxicological effects of graphene and its derivatives has only recently started to increase.

Environmental contaminants are almost always present as mixtures (Jahnke et al., 2016; Jia et al., 2015), and concern about the risks posed by mixtures of nanomaterials and co-contaminants is increasing. The effects of exposure to mixtures of nanoparticulates and other contaminants have only recently started to be studied (Liu et al., 2016; Sanchís et al., 2016; Song et al., 2014; Tong et al., 2015; Wang et al., 2016a,b; Yang et al., 2014). It has been found that interactions between CNMs and other contaminants in the environment can influence the environmental fate (Linard et al., 2015; Wang et al., 2016a), bioavailability (Baun et al., 2008; Kim et al., 2010; Linard et al., 2015; Schwab et al., 2013), and ecotoxicity of CNMs and the other contaminants investigated (Song et al., 2014; Tong et al., 2015; Sanchís et al., 2016). For example, our previous study (Wang et al., 2016a) found that low-molecular-weight organic acids probed to disperse graphene nanoplatelets in an aqueous medium. Kim et al. (2010) concluded that the addition of nontoxic concentration of surface-modified single-walled carbon nanotubes enhanced the uptake and toxicity of copper to *Daphnia magna*. Sanchís et al. (2016) also stated that synergistic and antagonistic effects of binary mixtures composed of fullerene soot and organic co-contaminants on *Daphnia magna* and *Vibrio fischeri* were assessed.

Ionic liquids (ILs) are new, purely ionic, salt-like materials that are composed only of cations and anions (Hayes et al., 2015). The unique physicochemical properties (e.g., unusually low melting points, relatively low viscosities, and negligible vapor pressures) of ILs mean that there is enormous potential for industrial applications of ILs (Ge et al., 2011; Pham et al., 2010). The IL field is developing rapidly, so it is important to consider the potential effects of an IL on environmental, ecological, and human health at the design stage to determine how acceptable the IL is likely to be in the long term (Amde et al., 2015; Mehrkesh and Karunanithi, 2016). Recent physicochemical studies have suggested that ILs have characteristics that make them as ideal dispersing solvents for CNMs such as graphene materials (Khare et al., 2013; Zhou et al., 2010). It is therefore reasonable to assume that the continuing development and use of ILs as solvents for CNMs will lead to ILs and CNMs being accidentally discharged into the environment and that mixtures of ILs and CNMs will pose risks to biota. Furthermore, sewage systems and municipal wastewater treatment plants have become important intermediate pathways for CNMs and ILs transfer into the environment since industrial and municipal wastewater are considered to be the main source of CNMs (Brar et al., 2010; Suárez-Iglesias et al., 2017) and ILs (Markiewicz et al., 2013; Pham et al., 2010). Once released into the environment, CNMs may also interact with ILs. Theoretically, the presence of ILs will strongly affect the behavior of CNMs such as single-walled carbon nanotubes (Wang et al., 2008) and fullerene (Wang et al., 2014b) in aqueous solutions. However, little is currently known about the effects on the aquatic environment of mixtures of graphene nanomaterials and ILs, and, in particular, very little is known about the joint toxicity of graphene nanomaterials and ILs to biota.

The aim of the study presented here was to assess and predict the joint toxicity of iG/GO and five different ILs. The green algae *Scenedesmus obliquus* was used as a model organism for evaluating the toxic effects of the individual compounds and the mixtures as green algae are a major constituent of the aquatic food chain and a primary agent for global biogeochemical cycles (Chen et al., 2012). Molecular simulations were carried out to estimate the interactions between the graphenes and the ILs at the microscopic scale. The dispersion stability of the graphenes in the presence of the ILs in an aqueous medium was evaluated at the mesoscopic scale. The joint algal toxicities of the binary mixtures at two different mixture ratios and their induced antioxidant activities in the algae were quantified using toxicity tests and biochemical assays, respectively. The joint toxicities were assessed using the toxic unit (TU) approach, and forecasted using the concentration addition (CA) and the independent action (IA) model.

2. Materials and methods

2.1. Test materials

The iG powder used in the tests was purchased from Plasma-Chem GmbH (Berlin, Germany). The iG was 1–4 nm thick, and the particles were 2 μm in diameter. GO powder was purchased from Aladdin Industrial Co. (Shanghai, China). The GO was 0.55–1.20 nm thick, and the particles were 0.5–3.0 μm in diameter. Detailed information on the iG and GO was provided by the manufacturers and is shown in Table S1. Five commonly used and representative ILs, 1-butyl-3-methyl imidazolium tetrafluoroborate ([BMIM][BF_4]), 1-butyl-3-methyl imidazolium hexafluorophosphate ([BMIM][PF_6]), 1-butyl-3-methyl imidazolium chloride ([BMIM][Cl]), 1-butyl-3-methyl pyridinium chloride [BMPy][Cl], and 1-butyl-1-methyl pyrrolidinium chloride [BMPyr][Cl], each with a purity of >97%, were purchased as powders from Aladdin Industrial Co.

2.2. Preparation of the test materials

Stock aqueous suspensions of iG and GO (both at 5 g L^{-1}) were prepared by dispersing particles of the graphene in ultrapure water. The stock aqueous suspensions were sonicated for 30 min to obtain a stable suspension with well-dispersed particles. A graphene suspension for use in a test was prepared by adding an aliquot of a stock suspension dropwise to an aliquot of an algal culture medium. An aqueous stock solution of each IL at a concentration of 5 g L^{-1} was prepared using ultrapure water. The test concentrations of the [BMIM][BF_4], [BMIM][PF_6], [BMIM][Cl], and [BMPy][Cl] were between 0.1 and 400 mg L^{-1}, and the test concentrations of the [BMPyr][Cl] were between 0.1 and 1600 mg L^{-1}. No aggregation was observed at these concentrations. A graphene–IL complex suspension was prepared by mixing an aliquot of a graphene stock suspension with an aliquot of a dilute IL solution, followed by stirring the mixture for 30 min in the dark.

2.3. Physicochemical characterization

The morphologies of the graphenes in the absence and presence of the ILs were characterized by transmission electron microscopy (TEM) using a Tecnai G2F20 instrument (FEI, Hillsboro, OR, USA). The zeta potentials (ZPs) and the hydrodynamic diameters (D_H) of the graphene particles in suspensions in the absence and presence of ILs were measured using phase analysis light scattering (PALS) (Corbett et al., 2012) and dynamic light scattering (DLS) (Nickel et al., 2014) techniques, respectively. A Nano ZS90 ZetaSizer (Malvern Instruments, Malvern, UK) was used to perform both PALS and DLS. The actual graphene and IL concentrations were determined using an ultraviolet–visible spectrophotometer (UV1102; Shanghai Tian Mei Scientific Instrument Co., Shanghai, China). The graphene concentrations in the test samples were determined using a seven-point standard calibration curve, which had a correlation coefficient $R^2 > 0.99$ (Fig. S1). The binary mixtures of the graphenes and ILs (pH = 7.8 ± 0.2) for adsorption experiments were equilibrated by stirring for 24 h at 25 °C in a dark room. Afterwards each test sample was passed through a syringe filter with 0.02 μm pore diameter (Antop 25, Whatman) and the corresponding control experiments were paired. The equilibrium IL concentrations in the samples were determined using a series of standard curves