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The aquatic impact of ionic liquids on freshwater organisms

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ABSTRACT

Ionic liquids (ILs), also known as liquid electrolytes, are powerful solvents with a wide variety of academic and industrial applications. Bioassays with aquatic organisms constitute an effective tool for the evaluation of ILs' toxicity, as well as for the prediction and identification of possible moieties that act as toxicophores.

In this work, the acute toxicity of six ILs and two commonly used organic solvents was evaluated using freshwater organisms: *Daphnia magna*, *Raphidocelis subcapitata* and *Hydra attenuata*. The bioassays were performed by exposing the organisms to increasing concentrations of the ILs and observing *D. magna* immobilization, *R. subcapitata* growth inhibition, and the morphological or mortality effects in *H. attenuata*. The results demonstrate that the tested organisms are not equally susceptible to the ILs, e.g., bmpyr [BF_4] was the least toxic compound for *R. subcapitata*, $N_{1,1}$ [$N_{1,1,1OOH}$] for *D. magna* and emim [Tf_2N] for *H. attenuata*. This highlights the importance of applying a battery of assays in toxicological analysis. Additionally, *Hydra* proved to be the most tolerant species to the tested ILs. According to their hazard rankings, the tested ILs are considered practically harmless or moderately toxic, except $(Hex)_3(TDec)P$ [Cl], which was classified as highly toxic. The ILs were revealed to be more harmful to aquatic systems than the tested organic solvents, reaffirming the need to analyze carefully the (eco)toxicological impact of these compounds. The present study provides additional data in the evaluation of the potential hazard and the impact of ILs in the environment.

1. Introduction

Ionic liquids (ILs) are salts composed of organic cations and organic or inorganic anions with melting points below 100 °C. A large number of combinations between cations and anions are possible whereby it is possible to synthesize ILs with diverse characteristics for different applications (Plechkova and Seddon, 2008). ILs present properties such as negligible vapor pressure, chemical and thermal stability, non-flammability, high liquid range and solvation ability (Wasserscheid and Welton, 2008). The numerous possibilities of synthesis and acquisition of chemical substances with unique properties have aroused interest in the potential industrial applications of this heterogeneous group of compounds, particularly as alternatives to traditional volatile organic solvents (VOCs).

The commercialization and increasing use of ILs raises a new challenge for the sustainable development of these compounds, mainly in terms of security; hence, a detailed analysis of their toxicological impact before their release into the environment is required (Pham et al., 2010). The risk of atmospheric pollution is almost nil because ILs exhibit low vapor pressure. With regards to aquatic systems, the impact of ILs should be considered because they present some solubility in water (Freire et al., 2008). Some authors already demonstrated that the labeling of ILs as "green solvents" might not always be correct. Studies with different biological systems, such as bacteria (Romero et al., 2008), human cells (Kumar et al., 2009) and plants (Jastorff et al., 2005) revealed negative effects of ILs in those systems. As mentioned above, the eventual environmental release of ILs into the aquatic environment has concerned the scientific community. In this context, various experimental studies for the evaluation of the effect of ILs on aquatic organisms, such as fish (e.g., *Danio rerio*) (Pretti et al., 2006,

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2009), bioluminescent bacteria (e.g., *Vibrio fischeri*) (Costa et al., 2015; Pinto et al., 2012), cladocerans (e.g., *Daphnia magna*) (Bernot et al., 2005a; Wells and Coombe, 2006; Pretti et al., 2009), green algae or plants (e.g., *Raphidocelis subcapitata*) (Cho et al., 2008a), snails (e.g., *Physa acuta*) (Bernot et al., 2005b), frogs (e.g., *Rana nigromaculata*) (Li et al., 2009) and other freshwater organisms (Pretti et al., 2009) have already been published. Some of these studies discussed the toxicological effect of different cation cores, alkyl chain lengths and anions. Usually, the increase in the alkyl chain length leads to a greater negative impact (Garcia et al., 2005; Latala et al., 2009a). Regarding the cation core, some studies report that aromatic cations (imidazolium and pyridinium) normally are more toxic than non-aromatic ILs, such as pyrrolidinium, piperidinium (Ventura et al., 2013), phosphonium and ammonium (Carvalho et al., 2014). Although sometimes neglected, the toxicity of anions such as trifluoromethanesulfonate, dicyanamide (Steudte et al., 2012), tetrafluoroborate (Pinto et al., 2012) and bis(trifluoromethanesulfonyl)imide (Costa et al., 2015) has been demonstrated. In addition to the studies of the influence of the structural elements in ILs' toxicity, other parameters have been equally evaluated, namely physical and chemical properties, such as aqueous solubility, the octanol–water partition coefficient, lipophilicity and aqueous diffusivity (Deng et al., 2012). Several ILs showed poor biodegradability and consequently potential bioaccumulation in the environment (Steudt et al., 2014). Recently, various researchers designed ILs with structural elements that can potentially foment their degradation. Gathergood, for example, introduced functionalized groups in the alkyl side chain and long alkyl side chain, demonstrating that longer chains present higher degradability (Garcia et al., 2005; Gathergood et al., 2004). Hou opted for the synthesis of cholinium amino acid ILs, which were classified as "readily biodegradable" (Hou et al., 2013). From these studies, it is possible to infer that organisms from the same or different trophic levels respond differently to the same compound; thus, it becomes important to apply a battery of bioassays in the evaluation of the potential hazard of new compounds, ensuring the preference for safer compounds with lower environmental impact (Dalzell et al., 2002; Pretti et al., 2009). One of the most common aquatic invertebrates used in toxicity studies is *D. magna*, a freshwater species belonging to the *Cladocera* order. *D. magna* is used because of its high sensitivity, easy maintenance in the laboratory and short reproductive cycle. Furthermore, the evaluation of both acute and chronic bioassays can be easily performed (Bernot et al., 2005a). This species has already been applied to the evaluation of the toxicity of several ILs (Bernot et al., 2005a; Wells and Coombe, 2006). The freshwater green alga *R. subcapitata* (formerly known as *Selenastrum capricornutum* and *Pseudokirchneriella subcapitata*) is also widely used in ecotoxicological assays (Cho et al., 2008a,b). The simplicity and low cost of the assay, along with the crucial function of *Raphidocelis* as a primary producer, transferring energy to higher trophic levels make it a recurring choice in the assessment of aquatic toxicity of several xenobiotics, including ILs (Latala et al., 2005; Garcia et al., 2005; Wells and Coombe, 2006; Cho et al., 2008b). Recently, the cnidarian, *H. attenuata*, was exploited in the scope of toxicity evaluation, providing data that can complement that obtained with other organisms, leading to a more accurate analysis of the real impact of pollutants in aquatic systems. *H. attenuata* is robust, easy to manipulate and to maintain under laboratory conditions and exhibits a high reproduction rate (Blaise and Kusui, 1997). Additionally, it is widely distributed in freshwater environments and has an important role in the food chain as a secondary consumer (Demetrio et al., 2012). It has been applied in the evaluation of the toxicity of different chemical compounds, such as agrochemicals (Demetrio et al., 2012), pharmaceuticals (Quinn et al., 2009), nano-materials (Blaise et al., 2008), phenols and metabolites (Pachura-Bouchet et al., 2006), and also chlorinated water (Monteiro et al., 2014). However, there is still no information about its use in the study of ILs' toxicity.

In this work, it was intended to compare the sensitivity of *H. attenuata* to ILs with other freshwater species commonly used in (eco)toxicological bioassays. The aquatic impact of different families of ILs was assessed utilizing three different organisms, by means of relatively simple and inexpensive bioassays. It is expected that the results obtained in this study can provide more detailed and extensive information on ILs' aquatic toxicity and complement the data described in the literature regarding the potential hazards of ILs, particularly to aquatic systems.

2. Materials and methods

2.1. Test compounds

All solutions were prepared using chemicals of analytical reagent grade and high purity water (Milli-Q) with a specific conductance <0.1 $\mu S\ cm^{-1}$.

The tested ILs (Fig. 1) were purchased from Aldrich: bmim [PF_6] (1-butyl-3-methylimidazolium hexafluorophosphate; ⩾98.5%), bmpyr [BF_4] (1-butyl-1-methylpyrrolidinium tetrafluoroborate; ⩾97.0%), $N_{1,1}$ [$N_{1,1,1OOH}$] (dimethylammonium dimethylcarbamate; ⩾97.0%), $N_{4,4,4,4}$ [BF_4] (tetrabutylammonium tetrafluoroborate; ⩾99.0%), emim [Tf_2N] (1-ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide; ⩾97.0%) and $(Hex)_3(TDec)P$ [Cl] (trihexyltetradecylphosphonium chloride; ⩾95.0%). Methanol and acetonitrile were obtained from Merck. All of the tested ILs were stored at room temperature in a carefully controlled anhydrous environment.

2.2. Maintenance of test organisms

R. subcapitata, *D. magna* and *H. attenuata* strains were cultured and maintained according to the recommended protocols (Blaise et al., 2000; OECD/OCDE, 2012; Trottier et al., 1997) in the laboratory of Applied Ecology of CENA/USP, Piracicaba, SP, Brazil.

The growth medium AAM (algal assay medium), composed of micro and macronutrients, described by Blaise and co-authors (Blaise et al., 2000), was used to cultivate *R. subcapitata* algae. Weekly, 1–2 mL of algae culture was subcultured in a new algal culture medium under aseptic conditions, guaranteeing that the organisms used in the toxicity tests were in the logarithmic phase of growth. The culture was kept at 24 °C under continuous agitation and illumination of 4000% lux.

A *D. magna* culture of approximately 20 females, up to one month old, was cultivated in 2 L containers. The microcrustaceans grew in Elendt M-4 Medium, which contained a total hardness of 250 $mg\ mL^{-1}$ as $CaCO_3$ with a pH adjusted to 7.0. The culture was kept in an incubator at 22 °C, under a 12/12 h light/dark photoperiod. The culture medium was renewed three times a week and was fed with an algae suspension of *R. subcapitata* (1.5×10^6 cells mL^{-1}), dry yeast (5 $g\ L^{-1}$) and trout flake food (5 $g\ L^{-1}$) (EPA, 2002).

The *Hydra* culture was maintained in circular glass containers (20 cm diameter) filled with two-thirds of adequate medium. The medium was composed of 2.95 g of $CaCl_2 \cdot H_2O$ and 2.2 g of TES buffer (N-tris[hydroxymethyl]methyl-1-[2-aminoethanesulphonic acid]) dissolved in 1 L of distilled water and the pH was adjusted to 7.0. Subsequently, the mixture was topped off to 20 L with distilled water. The culture was kept in an incubator that was used to maintain the culture at 20 °C and to guarantee a photoperiod of 12 h light and dark, alternately. The *Hydra* were fed three times a week with freshly hatched iodine-disinfected *Artemisia salina*. Approximately 2 h after feeding, the medium was replaced by a