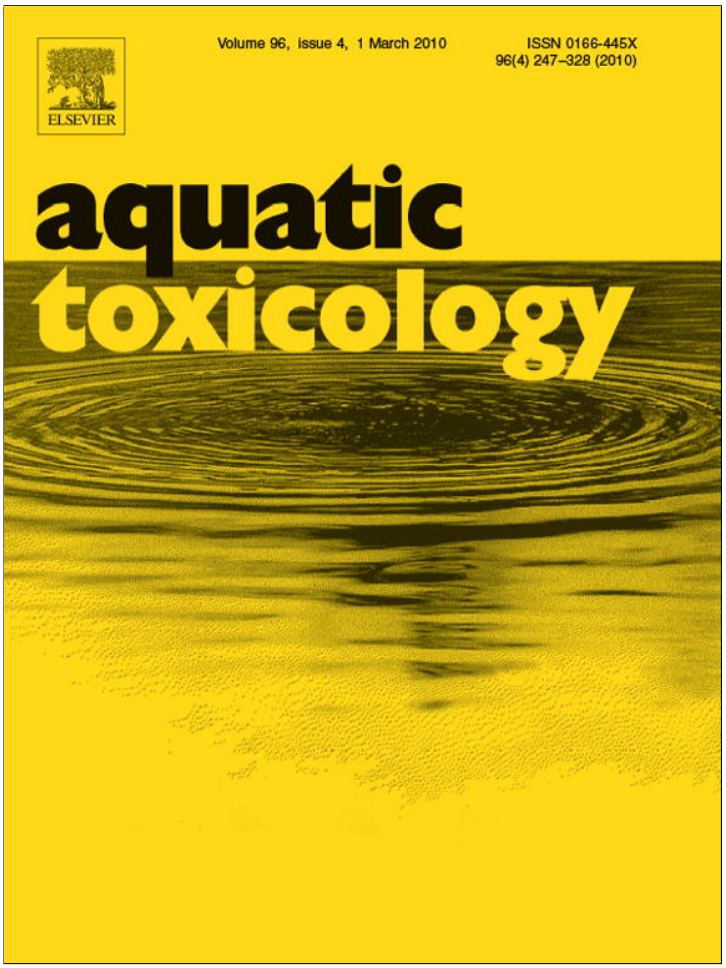

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Assessing the toxicity on $[C_3mim][Tf_2N]$ to aquatic organisms of different trophic levels

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ABSTRACT

Ionic liquids (ILs) are an exciting class of neoteric solvents that are being object of great attention as a potential replacement to conventional environmental damaging solvents in industrial applications. Despite some progress concerning ILs' toxicity and their environmental impact, the information about these compounds is still scarce.

In this work, biological tests were performed to establish the toxicity of 1-methyl-3-propylimidazolium bis(trifluoromethylsulfonyl)imide, $[C_3mim][Tf_2N]$, in five aquatic species at different trophic levels. Freshwater algal growth inhibition (*Pseudokirchneriella subcapitata* and *Chlorella vulgaris*), freshwater cladocerans' immobilization and chronic traits (*Daphnia magna* and *Daphnia longispina*) and viability of luminescent marine bacteria (*Vibrio fischeri*) were investigated. The sensitivity of the different species to the IL was compared in order to determine further repercussions in trophic food web.

It is shown that the studied IL is moderately toxic to the studied organisms. *P. subcapitata* and *D. magna* are the most tolerant species and *C. vulgaris* and *D. longispina* the most sensitive to its presence.

1. Introduction

Ionic liquids (ILs) are a novel class of technologically advanced solvents, composed by large organic cations, such as ammonium, imidazolium, pyridinium, piperidinium or pyrrolidinium with alkyl side chains that can vary in length, number and position, and organic or inorganic anions of variable nature. The huge number of different cation/anion combinations and, consequently, the potential for structure adjustability, allows property control and the design of the molecule for task-specific applications (Earl and Seddon, 2000). The most relevant and unique physicochemical properties of ILs include their negligible volatility, non-flammability, high thermal, chemical and electrochemical stabilities and the ability to be easily recycled, with favourable behaviour as a solvent, because many organic, organometallic and inorganic compounds can be dissolved in ILs (Holbrey and Seddon, 1999). Nowadays, ILs are being studied for multiple technological applications. They are seen as potential "green" substitutes of conventional organic solvents (Brennecke and Maginn, 2001). These "green" credentials are mainly derived from their negligible vapour pressures that reduce the risk of air pollution.

The information about the (eco)toxicological risk profile of ILs is still scarce and, in some cases, even nonexistent, as Ranke et al. (2007) have recently pointed out. The main conclusion was that the experimental data in several contexts, such as bioaccumulation, biodegradation of ILs in the environment, photodegradation, release to soil and water and aquatic toxicity are extremely incomplete. Thus, the main objective of this study was to generate new information about the toxicity of ILs to aquatic organisms.

Despite their low vapour pressure, as we previously mentioned, even the water immiscible ILs shown some solubility in water (Freire et al., 2007; Ranke et al., 2009) and some even a limited stability in presence of water (Freire et al., in press) that allows its dispersion into the aquatic systems resulting in water pollution. Furthermore, most ILs seem to be also poorly decomposed by microorganisms. The adsorption of these solvents onto a range of bacterial surfaces has also been found to be minimal, also implying that their transport through subsurface groundwater would be unimpeded (Ranke et al., 2007). Based on this information it can be hypothesized that ILs may pose environmental risks to aquatic ecosystems and accurate data on their toxicities are likely to be of foremost importance but are still scarce, especially for hydrophobic ILs. Microtox® Acute Toxicity Test is quick, simple, cost-effective and sensitive being a widely accepted method (Johnson, 2005) for toxicity determination, providing a fast yet accurate estimate of the toxicity of a compound. This test is a prokaryotic microscale toxicity bioassay using luminescent and gram negative marine bacteria.

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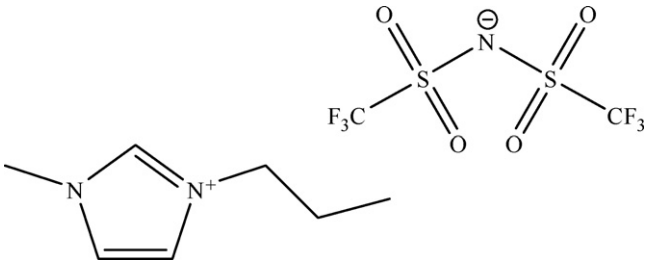

Fig. 1. Chemical structure of the IL studied: [C_3mim][Tf_2N].

The endpoint of this methodology, EC_{50}, is the effective concentration value that corresponds to the concentration of toxicant that produces 50% inhibition of light emission from a specific strain of bioluminescent bacteria. Recent studies (Couling et al., 2006; Garcia et al., 2005; Stepnowski et al., 2004) illustrate the effects of ILs in *Vibrio fischeri* (formerly *Photobacterium phosphoreum*), when the bioluminescence inhibition is studied. Some authors (Matzke et al., 2007) have studied the toxicity of imidazolium-based ILs with [Cl^-], [BF_4^-], [$(CF_3SO_2)_2N^-$], [$(CF_3)_2N^-$], octylsulfate and bis(1,2-benzenediolato)borate anions. Ranke et al. (2004) fitted the EC_{50} values of different imidazolium-based ILs with the [BF_4^-], [PF_6^-], [Br^-] and [Cl^-] anions.

This work is part of a project aiming at using water immiscible ionic liquids in biotechnological applications. Here the toxicity of a water stable and immiscible ionic liquid from the most used cation family, the imidazolium, viz. the 1-methyl-3-propylimidazolium bis(trifluoromethylsulfonyl)imide, [C_3mim][Tf_2N], shown in Fig. 1 was evaluated, thinking of the possible impact of the presence of these compounds in aquatic environments. Thus, the studied organisms were chosen to cover various trophic levels and some autochthonous species (*Chlorella vulgaris* and *Daphnia longispina*), to perform a comparative study between species, considering the same trophic level. Furthermore, these autochthonous species belongs to the Portuguese freshwater ecosystems.

There are reports about the toxicity of ILs to aquatic organisms of some trophic levels, such as to freshwater green algae – primary producers – (Latala et al., 2009a,b,c) – and to cladocerans – primary consumers (Bernot et al., 2005; Kulacki et al., 2008; Pretti et al., 2009; Wells and Coombe, 2006). The widespread distribution of algae makes these organisms ideal for toxicological studies, because they have a short life cycle and quickly respond to environmental changes (Blaise, 1993; Lewis, 1995). The cladoceran studies report the toxicity of various IL families with different anions on *Daphnia magna*, since this daphnid is a widely accepted model organism for toxicity testing because of its rapid rate of reproduction and sensitivity to different conditions. However, there is limited or no information about the effects of the ILs tested on other species of algae and cladocerans, e.g. those studied in this work—*C. vulgaris* and *D. longispina*. Moreover, dose–response bioassays on test aquatic organisms such as freshwater algae and crustaceans, are always required for defining toxicological endpoints of chemicals. The main goal of the present study is to provide further information about the toxicological impacts of an immiscible imidazolium-based IL in different species and trophic levels. We also assessed the chronic effects of [C_3mim][Tf_2N] on some population parameters of *D. magna* (clone A; Baird et al., 1989a) and *D. longispina* (clone EM7; Antunes et al., 2003).

2. Materials and methods

2.1. Test chemicals

The molecular structure of 1-methyl-3-propylimidazolium bis(trifluoromethylsulfonyl)imide, [C_3mim][Tf_2N] is provided in Fig. 1. The IL was purchased from Iolitec (Ionic Liquid Technologies, Germany). To reduce the water and other volatile compound content to negligible values, the IL was dried under constant shaking at moderate vacuum and temperature (≈353 K) for a minimum of 48 h prior to be used. After this procedure, its purity was further checked by ^{1}H NMR and ^{13}C NMR spectra and found to be >99% (w/w). The water used was ultra-pure water, double distilled, passed by a reverse osmosis system and further treated with a Milli-Q plus 185 water purification apparatus.

2.2. Microtox tests

Microtox® test (Microbics Corporation, 1992) was used to evaluate the inhibition of the luminescence in the marine bacteria *V. fischeri* (Beijerinck) Lehmann and Neumann. This test was performed using a range of diluted aqueous solutions (from 0 to 100%) of IL, where 100% of IL corresponds to a saturated solution with a concentration of 12.0 g L^{-1} (Freire et al., 2008). After 5 and 15 min of exposure to the IL, the light output of the luminescent bacteria was measured and compared with the light output of a blank control sample. The toxicity was evaluated and a 50% reduction in luminescence was computed using Microtox® Omni™ Software version 4.3.0.1 (Azur Environmental, 1998).

2.3. Freshwater green algae

2.3.1. Algal cultures

Cultures of *Pseudokirchneriella subcapitata* and *C. vulgaris* may be initially obtained from commercial sources and subsequently cultured using a sterile technique. Upon receipt of an algal culture not previously maintained in a facility, a period of six weeks culturing is recommended to establish the ability to successfully maintain a healthy, reproducing culture. Information on culturing algae can be found in the references listed in Gonçalves et al. (2005). Aseptic stock transfer should be maintained in or near the logarithmic growth phase and standard procedures followed (ASTM, 2002; OECD, 2002). The algal inoculum used to initiate toxicity testing was from a liquid culture shown to be actively growing in at least two subcultures lasting 7 days each prior to the start of the definitive test.

Algal cultures were reared under a 16 h^L:8 h^D photoperiod (provided by cool fluorescent white lights) and a temperature of 20 ± 2 °C.

2.3.2. Algal tests

The green algae *P. subcapitata* (Korshikov) Hindak and *C. vulgaris* Beijerinck were used to test the effects of the imidazolium on algal growth.

These two species of microalgae were obtained by Bacteria—Free protease Agar, Carolina Biological Supply Company—Burlington, North Carolina 27215 and Algal—Gro® Freshwater and Carolina Biological Supply Company—Burlington, North Carolina 27215, respectively.

Marine Biological Laboratory medium (MBL—Sterilized Hoods Hole Culture (Stein, 1973)) was used as nutritive culture medium, which was prepared in accordance with Gonçalves et al. (2005). The medium was sterilized by autoclaving. The vitamins (sterilized by filtration) were only added after medium sterilization under cold conditions. The freshwater green alga most often recommended for algal growth inhibition testing procedures is *P. subcapitata* (ASTM, 2002; OECD, 2002; USEPA, 2002). Nevertheless, the performance of *C. vulgaris* as test organism was assessed by comparison with *P. subcapitata* growth behaviour under the same conditions. For each alga, the samples were placed in 100 mL borosilicate Erlenmeyer flasks with 40 mL of final test volume (Gonçalves et al., 2005). Three days before starting the experiments, an inoculum was incubated

under the same conditions as the test cultures to adapt each alga to the test conditions and achieve exponential growth as usually recommended (OECD, 2002).

The initial cell density used for all green algae corresponded to 10^4 cells mL^{-1}. The preparation of MBL medium and flasks handling was carried out aseptically. The IL concentrations were obtained by successive dilutions of a saturated solution of $[C_3mim][Tf_2N]$ with MBL. Clean MBL medium was used as the negative control. To each treatment corresponded to a set of three replicates.

The tests were performed in an incubation chamber with continuous agitation at 100 rpm, under the same photoperiod and temperature conditions as described for algal cultures. Standard growth inhibition procedures recommend that algal growth tests should be terminated after 72 h of incubation under continuous light. In this experiment, the test was finished after 4 days of incubation (after 96 h) due to the photoperiod and the determined biomass parameter was cell counting (Gonçalves et al., 2005). Algal cell density was directly counted using a Neubauer chamber for both species of algae (APHA, 1995).

2.4. Cladocerans

2.4.1. Daphnid cultures

Monoclonal cultures of *D. magna* Straus (clone A; Baird et al., 1989a) and *D. longispina* O.F. Müller (clone EM7; Antunes et al., 2003) have been reared in our laboratory for several generations using common procedures for the standard organism (ASTM, 1997; Baird et al., 1989b; Soares et al., 1992). Daphnids were cultured in American Society for Testing and Materials hard water medium (ASTM, 1980), to which an organic additive, *Ascophyllum nodosum* extract (Baird et al., 1989b), was added. ASTM medium is prepared using sterile distilled water, adding 2.40 g of $CaCO_3$ previously dissolved in 2 L of distilled water, vitamins and 200 mL of $NaHCO_3$ (19.2 g L^{-1}), $MgSO_4{\cdot}7H_2O$ (24.57 g L^{-1}) and KCl (0.8 g L^{-1}). Cultures were reared under a $16\,h^L{:}8\,h^D$ photoperiod (provided by cool fluorescent white lights) and a temperature of $20 \pm 2\,^{\circ}C$. The medium was renewed every other day, to a total of three times per week. Cladocerans were fed with *P. subcapitata* (Korshikov) Hindak every other day, at a concentration of 3.0×10^5 cells and 1.5×10^5 cells mL^{-1} for *D. magna* and *D. longispina*, respectively. Algal ration was determined spectrophotometrically at 440 nm, as described for *C. vulgaris* Beijerinck by (Carvalho et al., 1995).

2.4.2. Acute test method

Tests were performed according to standard protocols (EPA, 2002; ISO, 1996; OECD, 2000) under the same temperature and photoperiod regimes as described for rearing procedures. For both *Daphnia* species, experiments were initiated with neonates (<24-hold) obtained from the same bulk culture, born between the 3rd and 5th broods. IL concentrations were obtained by successive dilutions of the saturated solution of $[C_3mim][Tf_2N]$ in the synthetic hard water medium (ASTM). The culture medium was used as the control treatment.

Acute tests were carried out in four glass beakers per treatment containing 100 mL of test solutions. A static design was employed, using 20 neonates (randomly divided into four groups of five organisms) per control and IL concentration. *Daphnids* were exposed to different IL concentrations during 48 h without food or organic extract. Vessels were checked for immobilized individuals at 24 and 48 h for posterior determination of EC_{50} values (for immobilization).

2.4.3. Chronic test method

Chronic tests were conducted for 21 days according to standard protocols (ASTM, 1997; ISO, 2000; OECD, 1998). The body length (from the base of the spine to the top of the head) of a subsample of neonates ($n = 20$) and from the same batch was measured under stereoscope magnification in order to determine their size at the beginning of the test (l_i—initial body length). Experiments were carried out using glass beakers (10 per treatment) containing 50 mL of test solution, including organic extract, unlike in the acute tests. A semi-static design was employed, using 10 individualised organisms (specifically one per replicate) randomly assigned to the control and to each IL concentration. Daphnids were fed daily with *P. subcapitata*, and transferred to freshly prepared test solutions every other day. Cladocerans were checked every day at the same approximate hour for mortality and reproductive state (presence of eggs or offspring). When neonates were released, they were counted and discarded. A life history table was built with the mortality and fecundity data. At the end of the test, all the surviving mothers were measured (from the base of the spine to the top of the head) under stereoscope magnification (l_f—final body length). This allowed the calculation of the somatic growth rate, which was estimated from the initial (l_i, in mm) and final (l_f, in mm) body size of the daphnids, according to the following expression:

$$\text{growth rate} = \frac{\ln(l_f) - \ln(l_i)}{\Delta t} \quad (1)$$

where growth rate is expressed in day^{-1} and Δt is the time interval (21 days). Survival and fecundity estimates were also used to compute the intrinsic rate of population increase (r). This demographic parameter was iterated from the Euler–Lotka equation:

$$1 = \sum_{x=0}^{n} e^{-rx} l_x m_x \quad (2)$$

where r is expressed in day^{-1}, x is the age class ($1 \ldots n$ days), l_x is the probability of surviving to age x, and m_x is the fecundity at age x. Pseudovalues and standard errors for r were estimated using the jack-knifing technique described by the literature work (Meyer et al., 1986).

2.5. Statistical analysis

The results of the different algal treatments, expressed as optical density for both algal species, were compared using analysis of variance (ANOVA). If applicable, a Tukey multiple comparison test was applied with statistically significant differences in growth reported for $p < 0.05$ (Zar, 1996). The EC_{50} values based on inhibition concentrations were also determined by Probit analysis for each species.

The EC_{50} values for immobilization (acute tests) and fecundity (chronic tests) were calculated by Probit analysis (Finney, 1971), for both daphnids. To test the significance of the IL effect on the life history parameters, a one-way ANOVA was employed on the

Table 1
Microtox® EC_{50} values (mg L^{-1}) of $[C_3mim][Tf_2N]$ after 5, 15 and 30 min of exposure to the luminescent marine bacteria *V. fischeri*, with respective 95% confidence limits (in brackets).

Ionic liquid	EC_{50} (mg L^{-1}) (lower limit, upper limit)	Reference (time of exposure)
$[C_3mim][Tf_2N]$	480.00 (240.00; 840.00)	This study (5 min)
	240.00 (120.00; 840.00)	This study (15 min)
$[C_4mim][Tf_2N]$	125.81	Matzke et al. (2007) (30 min)

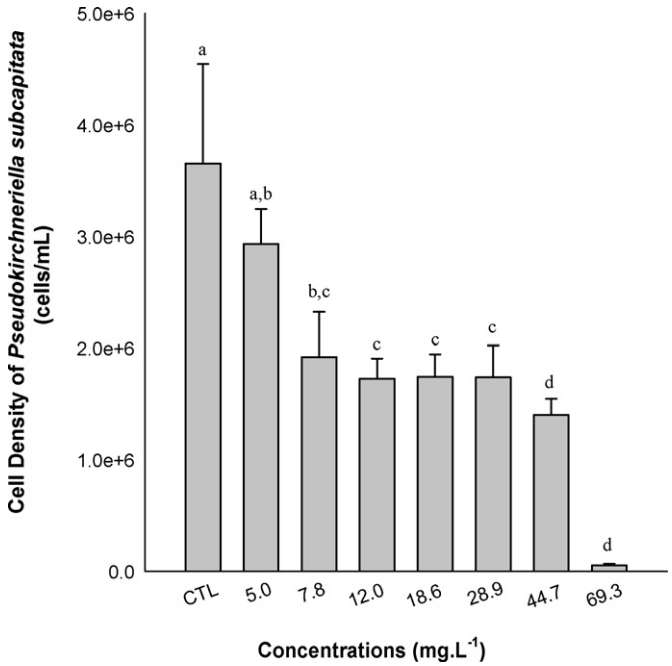

Fig. 2. Growth inhibition of *P. subcapitata* when exposed to successive dilutions of [C_3mim][Tf_2N], after 96 h of incubation. Error bars represent standard error and different letters above the bars indicate significant differences between the treatments ($p < 0.05$).

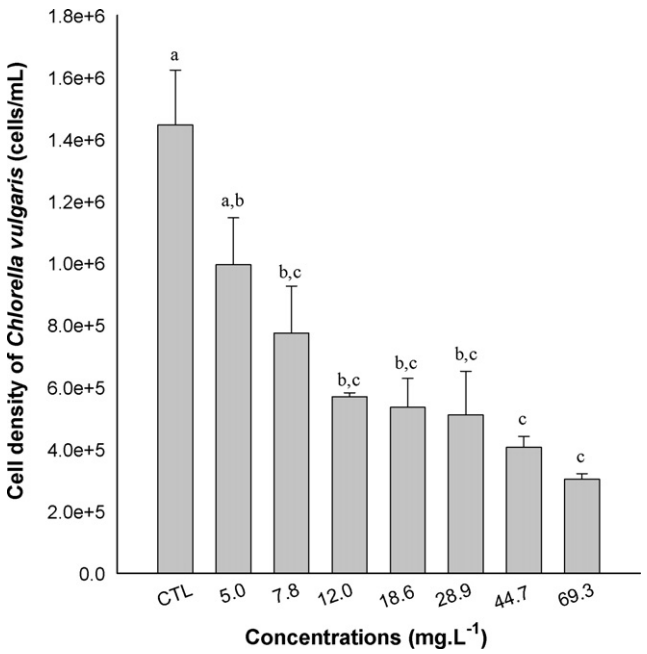

Fig. 3. Growth inhibition of *C. vulgaris* when exposed to successive dilutions of [C_3mim][Tf_2N], after 96 h of incubation. Error bars represent standard error and different letters above the bars indicate significant differences between the treatments ($p < 0.05$).

chronic test data. When the ANOVAs were statistically significant, a Dunnett test was then applied to the data in order to determine which concentrations were significantly different from the control group. A significance level (α) of 0.05 was used in the analyses.

3. Results and discussion

The experimental EC_{50} values determined by Microtox® bioassays for 5 and 15 min of IL exposure are presented in Table 1. These results indicate that [C_3mim][Tf_2N] can be considered moderately toxic. Various imidazolium-based ILs were previously tested and reported in the literature (Couling et al., 2006; Samori et al., 2007). However, only Matzke et al. (2007) have presented the EC_{50} for an imidazolium-based IL with a [Tf_2N] anion. Despite of the differences on the alkyl chain length and time of exposure between the two works the toxicity values are consistent. These results show that, as the time of exposure of *V. fischeri* increases, a reduction of EC_{50} value is observed.

Having established the toxicity of [C_3mim][Tf_2N] towards marine luminescent bacteria it is important to evaluate its relative toxicity when compared with organic solvents that the ILs are supposed to replace. The EC_{50} results relatively to some common organic solvents for 30 min of exposure are presented in Table 2. Although the exposure time is not the same and knowing that the longer the exposure time the lower the EC_{50} value, it was observed that [C_3mim][TF_2N] presents a somewhat lower toxicity than other common solvents.

Table 2
EC_{50} values (mg L^{-1}) of organic solvents after 30 min of exposure to the luminescent marine bacteria *Photobacterium phosphoreum*, by other studies.

Compound	EC_{50} (mg L^{-1})	Reference
Phenol	30.76	
Toluene	31.74	
Benzene	108.05	
Ethylene glycol	621.00	Kaiser and Palabrica (1991), Steinberg et al. (1995)
Chloroform	1199.33	
Dichloromethane	2532.33	
Ethyl acetate	5822.00	
Acetone	19, 311.14	
Methanol	101, 068.50	

Table 3
EC_{50} values (mg L^{-1}) of [C_3mim][Tf_2N] for freshwater microalgae (*P. subcapitata* and *C. vulgaris*).

Species	EC_{50} (mg L^{-1})
P. subcapitata	14.40 (6.00; 25.00)
C. vulgaris	10.29 (7.53; 13.02)

The growth of both algal species is affected by the presence of the IL as shown in Figs. 2 and 3. *P. subcapitata* was more tolerant (EC_{50} = 14.40 mg L^{-1}) than *C. vulgaris* (EC_{50} = 10.29 mg L^{-1}) to the IL (Table 3). No previous EC_{50} values are reported for

Table 4
EC_{50} values of organic solvents calculated by other studies to freshwater green algae.

Compound	Algal species	Chronic EC_{50} (mg L^{-1})	Reference
Dichloromethane	*P. subcapitata*	662.0	EPA (1995)
Aniline	*P. subcapitata*	19.0	Cho et al. (2008), Freire et al. (2009)
Toluene	*P. subcapitata*	>100	Wells and Coombe (2006)
Xylenes	*P. subcapitata*	~5	Wells and Coombe (2006)
Methanol	*P. subcapitata*	11.1	Cho et al. (2008)
2-propanol	*P. subcapitata*	11.92	Cho et al. (2008)
Phenol	*C. vulgaris*	370.0	Pretti et al. (2009)

Table 5
Acute and chronic EC_{50} values (mg L^{-1}) of [C_3mim][Tf_2N] for cladocerans species (*D. magna* and *D. longispina*), with respective 95% confidence intervals (in brackets).

Species	Acute EC_{50} (mg L^{-1}) (lower limit, upper limit)	Chronic EC_{50} (mg L^{-1}) (lower limit, upper limit)
D. magna	146.80 (141.20–153.20)	111.56 (106.40–115.85)
D. longispina	74.41 (69.56–80.01)	i.d.

i. d.—impossible to determine, since the mortality was inferior to 20%.

Table 6
Acute EC_{50} values of organic solvents to *D. magna*, determined by other studies.

Compound description	Acute EC_{50} (mg L^{-1})	Reference
Methanol	24.50	Pretti et al. (2009)
Dichloromethane	1.68	Pretti et al. (2009)
Acetonitrile	3.60	Pretti et al. (2009)
Aniline	80–380	Pretti et al. (2009)
Triethylamine	200	Pretti et al. (2009)
Chlorine	0.12–0.15	Kaniewska-Prus (1982)
Ammonia	2.90–6.93	Kaniewska-Prus (1982)
Phenol	10–17	Cowgill and Milazzo (1991)

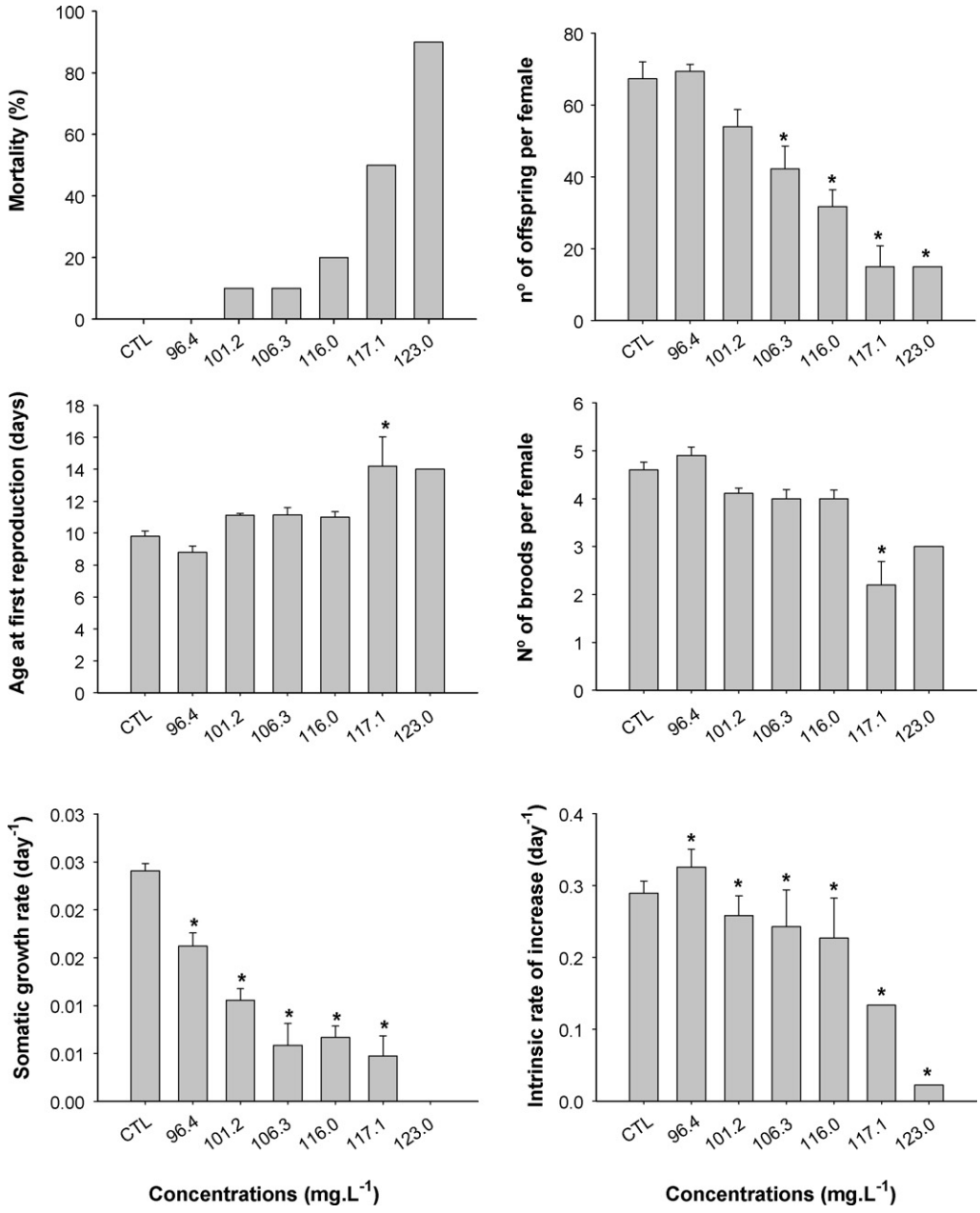

Fig. 4. Life history parameters of *D. magna* exposed to different IL concentrations for 21 days. Error bars represent standard error and * indicates statistically significant differences (Dunnett test, $p \leq 0.05$) between the test concentrations and the control (CTL).

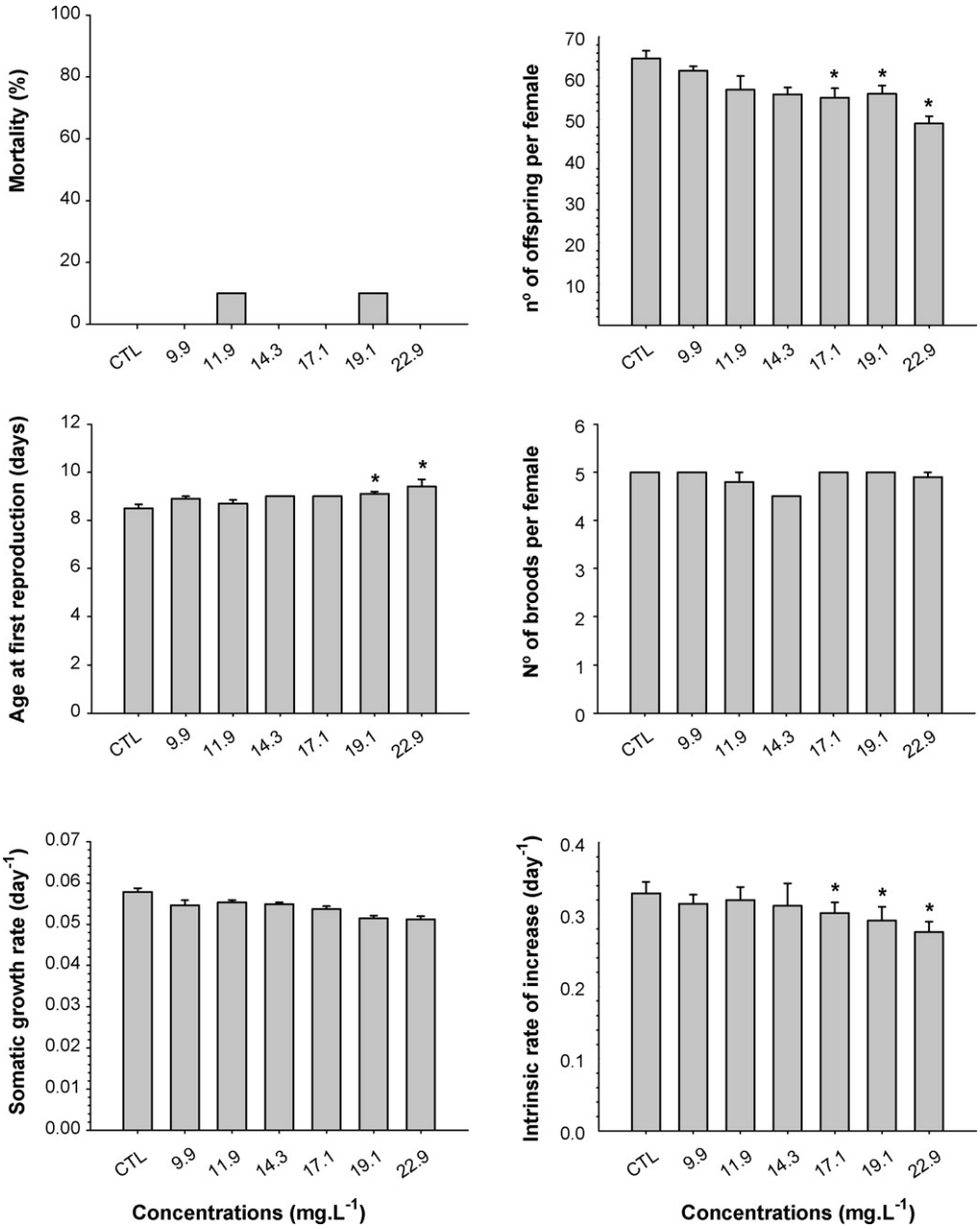

Fig. 5. Life history parameters of *D. longispina* exposed to different IL concentrations for 21 days. Error bars represent standard error and * indicates statistically significant differences (Dunnett test, $p \leq 0.05$) between the test concentrations and the control (CTL).

$[C_3mim][Tf_2N]$. However, the toxicity for $[C_4mim][Tf_2N]$ on *P. subcapitata* (EC_{50} = 26.49 mg L^{-1}) was reported in the literature (Pretti et al., 2009). This is in good agreement with our study. Similarly, Fritz and Braun (2006) obtained comparable results using chemical compounds that alter processes universally important, such as oxidative phosphorylation. Toxicants like herbicides that are designed to inhibit the photosynthesis electron transport process have a mode of action that makes them potentially lethal to a wide variety of non-target species of primary producers in which freshwater microalgae are included (Oliveira et al., 2007). Moreover, algal size is also an important factor in the interaction between algae and toxicants (Latala et al., 2009b).

In order to compare ILs and organic solvents, the toxicity of some common solvents towards *P. subcapitata* and *C. vulgaris* is presented in Table 4. Again, the toxicity of this IL is comparable to other aromatic solvents such as aniline, methanol and 2-propanol and lower than the toxicity of xylenes.

The acute and chronic EC_{50} values reported in Table 5 for *D. magna* and *D. longispina* show that the former species is more tolerant to IL concentrations than the latter one. Indeed, *D. magna* appears to tolerate IL concentrations twice as high as those tolerated by *D. longispina*. This behaviour can be explained by the smaller size of the latter species, and the consequent greater surface-to-volume ratio (Lilius et al., 1995), which would lead to increased exposure of the organism to the chemical. This pattern has been observed by several authors when comparing acute toxicities of different chemicals to the two daphnids (Antunes et al., 2004; Marques et al., 2004a,b; Pereira et al., 2007). The outcome of acute assays, i.e. a lower sensitivity of the larger species, was confirmed in the chronic exposures.

No previous EC_{50} values (for immobilization) have been reported for $[C_3mim][Tf_2N]$ but Pretti et al. (2009) report such values for $[C_4mim][Tf_2N]$ on *D. magna* (EC_{50} = 18.91 mg L^{-1}). However, the acute EC_{50} value of *D. magna* when exposed to $[C_4mim][Tf_2N]$

demonstrates this IL is more toxic than [C_3mim][Tf_2N] for this cladoceran species. The comparison between the acute toxicity of [C_3mim][Tf_2N] and organic solvents for *D. magna* reported in Table 6 shows that this IL toxicity compares favourably with the toxicity of some common solvents.

In general, all life history parameters of both daphnids were affected by [C_3mim][Tf_2N] with the exception of the number of broods and somatic growth rate (SGR) for *D. longispina* (Figs. 4 and 5). The mortality of *D. magna* increased in the two higher concentrations of IL (50 and 90%, correspondingly). For *D. longispina*, the mortality was below 20%, which explains the absence of the corresponding LC_{50} value (EC_{50} value for immobilization approximates to LC_{50}). A concentration-dependent decrease in the number of offspring was observed for both species, but it was more notable for *D. magna*. This decrease was accompanied by a developmental delay, indicating an increase in the age at first reproduction. A decrease in the somatic growth rate of both species was also observed. Also it was more pronounced in *D. magna*. The number of broods produced per female was also significantly reduced in *D. magna*. As a consequence of the lower fecundity and of the developmental delay, the intrinsic rate of increase was significantly reduced in both species. The effect was more obvious for *D. magna*. This reduction was preceded by a stimulation of reproduction and development at low concentration of [C_3mim][Tf_2N]—96.37 mg L^{-1}. IL is a contact product and its main intake route is likely to be through body surface rather than via filtration-related mechanisms. Actually, *D. longispina* is smaller than *D. magna*, with consequent greater surface-to-volume ratio.

As mentioned in the introduction, once in industrial use, the main pathway of ILs into the environment is through aqueous effluents. Previous studies (Deng et al., 2009; Freire et al., 2009) have shown that the concentration of ILs in aqueous solutions can be reduced significantly by treating them with salts. They show that a number of salts have a strong salting-out ability allowing them to be used in water treatment. Assays carried by us show that using aluminium sulfate concentrations of 0.8 mol L^{-1}, it is possible to reduce the solubility of [C_3mim][Tf_2N] from 75,000 to 500 ppm. We are currently carrying out studies to identify the possibility of using inorganic salts in the treatment of effluents contaminated with ILs. Whether this treatment can bring the concentration of the IL below the desired value or can be used as a primary treatment that must be followed by adsorption or another option it remains to be seen. Both the moderate toxicity of the IL studied and the possibility of treating the aqueous effluents contaminated with ILs open good perspectives to their industrial application.

4. Conclusions

The toxicity of the ionic liquid [C_3mim][Tf_2N] for different species at different trophic levels was studied and it showed moderate toxicity equivalent to other aromatic industrial solvents.

This study demonstrates that the sensitivity to the [C_3mim][Tf_2N] differs among species. Within the species of the same trophic levels *P. subcapitata* is shown to be somewhat more tolerant than *C. vulgaris* when exposed to the IL. When compared with *D. longispina*, *D. magna* is shown to be far more sensitive to the presence of the IL.

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