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Designing ionic liquids: the chemical structure role in the toxicity

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Abstract Ionic liquids (ILs) are a novel class of solvents with interesting physicochemical properties. Many different applications have been reported for ILs as alternatives to organic solvents in chemical and bioprocesses. Despite the argued advantage of having low vapor pressure, even the most hydrophobic ILs show some degree of solubility in water, allowing their dispersion into aquatic systems and raising concerns on its pollutant potential. Moreover, nowadays most widespread notion concerning the ILs toxicity is that there is a direct relationship with their hydrophobicity/lipophilicity. This work aims at enlarging the currently limited knowledge on ILs toxicity by addressing negative impacts in aquatic ecosystems and investigating the possibility of designing hydrophobic ILs of low ecotoxicity, by the manipulation of their chemical structures. The impact of aromaticity on the toxicity of different cations (pyridinium, piperidinium, pyrrolidinium and imidazolium) and hydrophobic anions (bis(trifluoromethylsulfonyl)imide [NTf_2] and hexafluorophosphate [PF_6]) was analysed. Concomitantly, several imidazolium-based ILs of the type [$C_nC_mC_j$im][NTf_2] were also studied to evaluate the effects of the position of the alkyl chain on the ILs' toxicity. For that purpose, standard assays were performed using organisms of different trophic levels, *Vibrio fischeri*, *Pseudokirchneriella subcapitata* and *Daphnia magna*, allowing to evaluate the consistency of the structure–activity relationships across different biological targets. The results here reported suggest the possibility of designing ILs with an enhanced hydrophobic character and lower toxicity, by elimination of their aromatic nature.

Keywords Ionic liquids · Toxicological tests · Aquatic toxicity · EC_{50} · Aromatic/aliphatic nature · Isomerism

Introduction

One of the principles of Green Chemistry aims at the reduction of the environmental toxicity of chemical compounds used in industrial processes or product formulations. Legislation concerning this subject is nowadays more stringent in Europe as REACH (EC 2006) (Registration, Evaluation, Authorization and Restriction of Chemicals) requires the registration of new commercial chemicals and holds the suppliers responsible for their products. Therefore, the optimization of technical performance must run in parallel with the minimization of hazard potentials, aiming to reduce their environmental impact and also improve their economic viability (Docherty and Kulpa 2005).

A new class of chemicals composed exclusively by ions that are liquid at room temperature, known as ionic liquids (ILs), is under development and have found favour of both academia and industry as surrogates of volatile organic compounds in many applications (Allen and Shonnard 2002;

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Earl and Seddon 2000; Wasserscheid and Welton 2007; Zhao 2006; Kragl et al. 2002; Sheldon et al. 2002; van Rantwijk et al. 2003; Freire et al. 2012). They are considered as "*designer solvents*" due to the possibility of freely manipulating their characteristics by combining these cations and anions to meet the requirements for a specific application (Plechkova and Seddon 2007, 2008). One of the main driving forces behind the interest on ILs is not only their extended performance when compared to conventional solvents, but also them being touted as "*green solvents*". The rationale for labeling them "*green*" is based on four arguments: their less hazardous synthesis (Deetlefs and Seddon 2010), their negligible vapor pressure (Deetlefs and Seddon 2006; Earle et al. 2006), their non-flammability and finally, their potentially lower (eco)toxicity (Ranke et al. 2007b; Pretti et al. 2009). While these issues are certainly important in the discussion of their industrial application, the latest point in particular, has been repeatedly challenged. Most ILs are poorly decomposed by microorganisms (Coleman and Gathergood 2010; Gathergood and Scammells 2002; Gathergood et al. 2004, 2006) and their adsorption onto a range of bacterial surfaces is also minimal (Gorman-Lewis and Fein 2004). Moreover, their properties such as resistance to photodegradation (Stepnowski and Zaleska 2005), bioaccumulation (Ventura et al. 2011), water solubilities and water stabilities (Freire et al. 2007a, b, 2008a, b, 2009, 2010), concur to strength the notion that they may pose a threat to aquatic organisms.

In what concerns their aquatic toxicity (Beadham and Gathergood 2012), there is still a great uncertainty regarding the effects and attack mechanisms of new families on different species and trophic levels. The most tested trophic levels are decomposers represented by bacteria (Ranke et al. 2004; Samorì et al. 2007; Ventura et al. 2010), producers represented by microalgae (Cho et al. 2008a, b; Kulacki and Lamberti 2008; Latala et al. 2005, 2009b; Ventura et al. 2010; Wells and Coombe 2006) and primary consumers (Pretti et al. 2009; Ventura et al. 2010; Luo et al. 2008) represented by cladocerans.

Toxicity tests with freshwater microalgae and cladocerans are highly recommended as part of the test batteries required for the ecological risk assessment of new and existing chemicals (e.g. EC 2002). Both have been for long used as model organisms in ecotoxicological studies given their generally high sensitivity to different contaminants, their key role in the energy transfer within aquatic food webs enabling protective estimation of environmentally hazardous contamination levels, and the availability and ease of use of standardized culture and test procedures. Different authors (Ventura et al. 2010; Matzke et al. 2010) tested several algae species, being concluded that in general, the cation alkyl chain length has a pronounced effect towards all these producers. Literature also suggests that the main differences in the behaviour of these algae are explained by their cell wall structure and size (Latala et al. 2009a, b, c). *Daphnia magna* is an important link between producers and higher trophic levels (Smith 2001), being of fast reproduction rate and sensitivity to environmental conditions. Literature results suggests that the alkyl chain length (Bernot et al. 2005) is the IL characteristic with higher impact on the toxicity towards cladocerans, followed by the anion moiety and the cation core (Matzke et al. 2007; Stolte et al. 2007).

The Microtox® bioassay is a bioluminescence inhibition method assessing on the bacterium *Vibrio fischeri*. It is today one of the most widespread toxicological bioassays due to its speed, simplicity and cost-effective implementation (Steinberg et al. 1995). This test was widely used in the study of the influence of the anion (Matzke et al. 2007; Ranke et al. 2004; Garcia et al. 2005), cation (Couling et al. 2006) and alkyl chain length (Garcia et al. 2005; Samorì et al. 2007; Docherty and Kulpa 2005; Matzke et al. 2007) of several ILs with results that are qualitatively in agreement with those carried for species of higher trophic levels. However, in what concerns the anion influence (Matzke et al. 2010; Ranke et al. 2007b), although it has been claimed that its modification leads to changes on their chemical and physical properties (Sheldon 2001), a less pronounced effect was observed for the toxicity (Matzke et al. 2010), being the $[NTf_2]$ anion an exception independently of the cation (Stolte et al. 2007). These results are explained by the possible intercalation of the lipophilic part of the molecules into the organism' membrane, whereas their ionic head group is, at least, partially solvated in the aqueous solution (Austin et al. 1998).

Recently, there have been attempts at understanding the relationship between the toxicity of the ILs and other properties, namely their hydrophobic nature (Salminen et al. 2007), membrane water partitioning (Stolte et al. 2007) and lipophilicity (Matzke et al. 2007; Arning et al. 2008; Ranke et al. 2007a; Stolte et al. 2007). The distinct concepts of lipophilicity and hydrophobicity are often confused. While the lipophilicity describes the ability of a compound to interact with the non-polar compounds such as lipids and cell membranes, the hydrophobicity addresses the poor affinity of the compounds towards water molecules, assessed normally by the water solubility parameter. Although often correlated, the idea that high hydrophobicity corresponds to high lipophilicity does not always holds (Matzke et al. 2007; Stolte et al. 2007; Ranke et al. 2007a).

The main objective of this work is to investigate sources of toxicity on the ILs structure other than those previously reported. The final goal is then to draw directions for designing ILs that combine lower toxicity with higher hydrophobicity. For that purpose, standard toxicity tests were conducted with the luminescent bacteria *V. fischeri*

(Microtox®; Azur Environmental 1998), the green microalgae *P. subcapitata* (OECD 2011), and the cladoceran zooplankter *D. magna* (OECD 2004, 2008), and corresponding EC_{50} values were estimated.

The EC_{50} parameters were compared aiming at answering three specific research questions.

First, whether the cation aromatic nature promotes the IL toxicity; this will be done by comparing the toxicity of ILs with aromatic and non-aromatic cations.

Second, if it is possible to design IL cations simultaneously more hydrophobic and less toxic; this will be addressed by comparing Microtox® toxicity records among a group of imidazolium ILs with the common $[NTf_2]$ anion but with changes in the cation alkyl chain position.

Finally, whether the Microtox® assay could be used as a general test platform within a designer solvent context i.e. if the patterns found in the relationships between chemical structure and toxicity would be unchanged as the complexity of the biological target and the endpoints focused changes. For that purpose, the responses of *P. subcapitata* (growth inhibition) and *D. magna* (immobilisation following acute exposure and reproduction following chronic exposure) will be compared to that of *V. fischeri*, using a group of ILs representative of the four different cation families holding the alkyl chain in the same position and sharing the same $[NTf_2]$ anion.

Materials and methods

Test chemicals

Figure 1 shows the molecular structures with corresponding chemical names of all ILs studied. The ILs used in this work were purchased from Iolitec (Ionic Liquid Technologies, Germany) with purities of 99 %. Before use in the toxicity tests the ILs were washed with ultra-pure water, and then dried under constant steering at high vacuum and moderate temperature ($\approx$353 K) for a minimum of 48 h. This would allow the removal of water and other volatile compounds. After this procedure, their purity was further checked by ^{1}H, ^{13}C and ^{19}F NMR spectra.

Toxicity tests

The present work reports a range of toxicological tests using the marine bacteria *V. fischeri* (Microtox® tests), the alga *P. subcapitata* and the cladoceran *D. magna*.

Microtox® tests

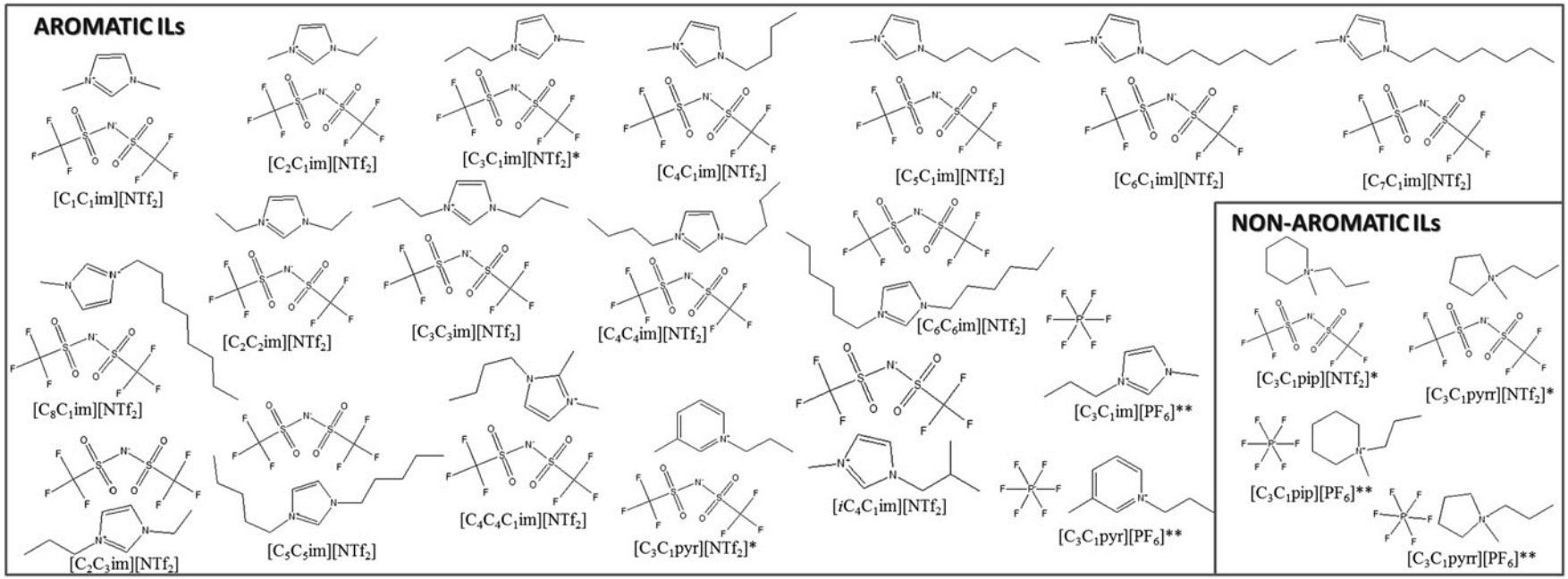
The Microtox® test (Microbics Corporation 1992) was used to evaluate the inhibition of the luminescence in the bacteria *V. fischeri*. The bacteria was exposed to a range of diluted aqueous solutions (typically from 0 to 81.9 %) of each IL, where 100 % of IL corresponds to the known concentration of a stock solution previously prepared. 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 allowing the estimation of the corresponding 5 min- and 15 min-EC_{50} values through Microtox® Omni™ Software version 4.3.0.1. (Azur Environmental 1998).

Freshwater green algae tests

Growth inhibition of the green microalgae *P. subcapitata* was assessed following exposure to four $[NTf_2]$-based ILs, each representative of a different cation family but sharing the same alkyl chain. Algae culture conditions and details on the preparation of these tests can be found in our previous work (Ventura et al. 2010) with procedures generally following guidelines by OECD (2011). 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. The inoculum concentration was adjusted immediately before setting the test, so that a fixed volume of algae suspension could be added to each treatment replicate to provide the required initial cell density of 10^4 cells mL^{-1}. The IL concentrations were obtained by successive dilutions of a stock solution with a well-known concentration previously prepared of each IL with Marine Biological Laboratory medium (MBL), and all treatments (including a MBL blank control) were run in triplicate. The tests were performed in an incubation chamber with continuous agitation at 100 rpm, under a $16h^{L}$:$8h^{D}$ photoperiod (provided by cool fluorescent white lights) and at controlled temperature of 20 $\pm$ 2 °C. Standard growth inhibition measurements were carried out after a 96 h exposure-period following Gonçalves et al. work (2005) using microscopic cell counting with a Neubauer haemocytometer as biomass surrogate.

Cladoceran tests

The cladoceran *D. magna* was acutely exposed to four $[NTf_2]$-based ILs, each representative of a different cation family but sharing the same alkyl chain. The tests were initiated with neonates (<24-h-old), born between the third and fifth broods in bulk cultures. These were monoclonal cultures of *D. magna* (clone A, sensu Baird et al. 1989) continuously reared in the laboratory in synthetic ASTM hard water medium (1980). Culture conditions as well as details on the test procedures were described previously (Ventura et al. 2010) and followed the corresponding OECD guidelines (2004, 2008). IL concentrations were obtained by successive



AROMATIC ILs: $[C_1C_1im][NTf_2]$, 1,3-dimethylimidazolium bis(trifluoromethylsulfonyl)imide; $[C_2C_1im][NTf_2]$, 1-ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide; $[C_3C_1im][NTf_2]$, 1-methyl-3-propylimidazolium bis(trifluoromethylsulfonyl)imide; $[C_4C_1im][NTf_2]$, 1-butyl-3-methylimidazolium bis(trifluoromethansulfonyl)imide; $[C_5C_1im][NTf_2]$, 1-pentyl-3-methylimidazolium bis(trifluoromethansulfonyl)imide; $[C_6C_1im][NTf_2]$, 1-hexyl-3-methylimidazolium bis(trifluoromethansulfonyl)imide; $[C_7C_1im][NTf_2]$, 1-heptyl-3-methylimidazolium bis(trifluoromethansulfonyl)imide; $[C_8C_1im][NTf_2]$, 1-methyl-3-octylimidazolium bis(trifluoromethansulfonyl)imide; $[C_2C_2im][NTf_2]$, 1,3-diethylimidazolium bis(trifluoromethylsulfonyl)imide; $[C_3C_3im][NTf_2]$, 1,3-dipropylimidazolium bis(trifluoromethylsulfonyl)imide; $[C_4C_4im][NTf_2]$, 1,3-dibutylimidazolium bis(trifluoromethylsulfonyl)imide; $[C_5C_5im][NTf_2]$, 1,3-dipentylimidazolium bis(trifluoromethylsulfonyl)imide;$[C_6C_6im][NTf_2]$, 1,3-dihexylimidazolium bis(trifluoromethylsulfonyl)imide; $C_2C_3im][NTf_2]$, 1-ethyl-3-propylimidazolium bis(trifluoromethylsulfonyl)imide; $[C_4C_4C_1im][NTf_2]$, 1-butyl-2,3-dimethylimidazolium bis(trifluoromethansulfonyl)imide; $[iC_4C_1im][NTf_2]$, 1-isobutyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide; $[C_3C_1pyr][NTf_2]$; 3-methyl-1-propylpyridinium bis(trifluoromethylsulfonyl)imide; $[C_3C_1im][PF_6]$, 1-methyl-3-propylimidazolium hexafluorophosphate; $[C_3C_1pyr][PF_6]$, 3-methyl-1-propylpyridinium hexafluorophosphate.

NON-AROMATIC ILs: $[C_3C_1pip][NTf_2]$, 1-methyl-1-propylpiperidinium bis(trifluoromethylsulfonyl)imide; $[C_3C_1pyrr][NTf_2]$, 1-methyl-1-propylpyrrolidinium bis(trifluoromethylsulfonyl)imide; $[C_3C_1pip][PF_6]$, 1-methyl-1-propylpiperidinium hexafluorophosphate; $[C_3C_1pyrr][PF_6]$, 1-methyl-1-propylpyrrolidinium hexafluorophosphate.

Fig. 1 Identification and chemical structure of all ILs studied. The *asterisks* identify the groups of ILs belonging to different families (imidazolium, pyrrolidinium, pyridinium and piperidinium) but sharing the same cation alkyl chain and the same hydrophobic anion $[NTf_2]$ (*) or $[PF_6]$ (**). *Non-marked structures* constitute the comprehensive group of imidazolium ILs with introduced variation in the cation alkyl chain position

Table 1 Solubility in water (mg L^{-1}) and EC_{50} values (mg L^{-1}) estimated for different [NTf_2] and [PF_6]-based ILs, after 5 and 15 min of exposure to the luminescent marine bacteria *V. fischeri*, with respective 95 % confidence limits (in brackets)

ILs	Solubility in water (mg L^{-1}) at 298.15 K	EC_{50} (mg L^{-1}) 5 min (lower limit; upper limit)	EC_{50} (mg L^{-1}) 15 min (lower limit; upper limit)
[C_3C_1im][NTf_2]	1.22×10^{4}[a]	480.00[b]	240.00[b]
		(240.00; 840.00)	(120.00; 840.00)
[C_3C_1pyrr][NTf_2]	9.79×10^{3}[c]	1100.00	800.00
		(600.00; 2100.00)	(200.00; 3300.00)
[C_3C_1pyr][NTf_2]	8.67×10^{3}[d]	55.00	33.00
		(38.50; 71.50)	(22.00; 49.50)
[C_3C_1pip][NTf_2]	8.64×10^{3}[c]	609.56	435.40
		(348.32; 1132.00)	(87.08; 1654.52)
[C_3C_1im][PF_6]	2.94×10^{4}	1703.00	969.40
		(1310.00; 2200.80)	(602.60; 1545.80)
[C_3C_1pyrr][PF_6]	1.01×10^{4}	3900.00	2800.00
		(3600.00; 4200.00)	(2400.00; 3300.00)
[C_3C_1pyr][PF_6]	2.40×10^{4}	(171.92; 343.84)	171.92
			(85.96; 171.92)
[C_3C_1pip][PF_6]	1.01×10^{4}	1436.40	1117.20
		(665.00; 3138.80)	(425.60; 2793.00)

[a] Solubility in water data from Freire et al. (2008a)

[b] Data from Ventura et al. (2010)

[c] Data from Freire et al. (2010)

[d] Data from Freire et al. (2007a)

Table 2 EC_{50} values and corresponding 95 % confidence limits (within brackets) estimated following exposure of the freshwater green algae *P. subcapitata* to [NTf_2]-based ILs from the four studied cation families

ILs	EC_{50} (mg L^{-1}) (lower limit; upper limit)
[C_3C_1im][NTf_2]	14.40[a]
	(6.00; 25.00)
[C_3C_1pyrr][NTf_2]	18.24
	(13.91; 26.27)
[C_3C_1pyr][NTf_2]	3.0
	(2.90; 3.20)
[C_3C_1pip][NTf_2]	14.10
	(12.48; 15.55)

[a] Data from Ventura et al. (2010)

Table 3 EC_{50} values (mg L^{-1}) of [NTf_2]-based ILs from the four studied cation families, estimated following acute and chronic exposure of *D. magna*, with the respective 95 % confidence intervals (in brackets)

ILs	Acute EC_{50} (mg L^{-1}) (lower limit; upper limit)	Chronic EC_{50} (mg L^{-1}) (lower limit; upper limit)
[C_3C_1im][NTf_2]	146.80[a]	111.56[a]
	(141.20; 153.20)	(106.40; 115.85)
[C_3C_1pyrr][NTf_2]	159.00	123.21
	(148.00; 170.00)	(118.79; 127.87)
[C_3C_1pyr][NTf_2]	98.81	–
	(96.66; 100.46)	
[C_3C_1pip][NTf_2]	117.20	–
	(89.37; 136.45)	

[a] Data from Ventura et al. (2010)

dilutions of a stock solution with a well-known IL concentration, which was previously prepared in the ASTM. Tests were performed under the same temperature and photoperiod regimes as set for cultures (Ventura et al. 2010). Acute tests were carried out in four replicated glass beakers per treatment containing 100 mL of test solutions (IL geometric dilution series plus a blank ASTM control) and five organisms each. Daphnids were exposed to different IL concentrations during 48 h and no food or organic extract was provided. Vessels were checked for immobilized individuals at 24 and 48 h, for posterior determination of corresponding EC_{50} values. The reproductive responses of *D. magna* following chronic exposure to [C_3C_1pyrr][NTf_2] were additionally assessed here. The test was run during 21 days and the appropriate OECD guidelines (2008) were followed. Briefly, ten individual replicates of newborn daphnids (<24 h; from the third to fifth brood of bulk cultures) were assigned to each treatment and each replicate was carried out in glass vessels filled with 50 mL test solution. A geometric concentration range of IL was established being the test organisms transferred to new test solutions every other day. Daphnids were fed daily with *P. subcapitata* and this and any other rearing condition was kept as described for cultures. The organisms were checked daily for mortality and reproductive output.

Statistical analysis

Alga treatments, expressed as the yield in cell density, were compared using analysis of variance and, if applicable, a

Table 4 Solubility in water (mg L^{-1}) and Microtox® EC_{50} values (mg L^{-1}) of $[C_nC_m\text{im}][NTf_2]$-based ILs after 5 and 15 min of exposure to the luminescent marine bacteria *V. fischeri*, with the respective 95 % confidence limits (in brackets)

ILs	Solubility in water (mg L^{-1}) at 298.15 K	EC_{50} (mg L^{-1}) 5 min (lower limit; upper limit)	EC_{50} (mg L^{-1}) 15 min (lower limit; upper limit)
$[C_1C_1\text{im}][NTf_2]$	2.99×10^{4}[a]	3597.51	2362.78
		(3044.74; 4150.27)	(1989.08; 2736.48)
$[C_2C_1\text{im}][NTf_2]$	1.74×10^{3}[a]	440.27	330.23
		(313.85; 566.68)	(227.83; 432.62)
$[C_3C_1\text{im}][NTf_2]$	1.19×10^{3}[a]	480.36[b]	240.18[b]
		(240.00; 840.00)	(120.00; 840.00)
$[C_4C_1\text{im}][NTf_2]$	6.79×10^{3}[a]	141.99	141.99
		(70.99; 425.96)	(70.99; 141.99)
$[C_5C_1\text{im}][NTf_2]$	4.53×10^{3}[a]	46.87	46.87
		(46.87; 93.74)	(46.87; 46.87)
$[C_6C_1\text{im}][NTf_2]$	2.23×10^{3}[a]	23.07	22.80
		(19.55; 26.60)	(17.27; 28.33)
$[C_7C_1\text{im}][NTf_2]$	1.28×10^{3}[a]	23.76	19.25
		(20.22; 27.93)	(15.41; 24.05)
$[C_8C_1\text{im}][NTf_2]$	8.53×10^{2}[a]	7.20	6.44
		(3.13; 13.20)	(2.93; 17.70)
$[C_2C_2\text{im}][NTf_2]$	1.19×10^{4}	345.89	230.59
		(230.59; 576.48)	(0.00; 3343.58)
$[C_3C_3\text{im}][NTf_2]$	4.46×10^{4}	58.42	35.93
		(31.56; 108.18)	(15.13; 85.29)
$[C_4C_4\text{im}][NTf_2]$	$\approx 1.93 \times 10^{3}$	78.05	59.35
		(41.77; 145.90)	(51.76; 68.05)
$[C_5C_5\text{im}][NTf_2]$	–	28.19	30.21
		(21.18; 37.50)	(24.89; 36.66)
$[C_6C_6\text{im}][NTf_2]$	–	42.75	46.64
		(32.45; 56.62)	(28.85; 75.41)
$[C_2C_3\text{im}][NTf_2]$	–	120.50	87.82
		(99.15; 146.50)	(68.14; 113.20)
$[C_4C_1C_1\text{im}][NTf_2]$	4.86×10^{3}	130.85	87.23
		(87.23; 218.08)	(0.00; 610.62)
$[iC_4C_1\text{im}][NTf_2]$	8.30×10^{3}	442.93	283.81
		(223.57; 585.99)	(210.95; 381.88)

[a] Data from Freire et al. (2008a)

[b] Data from Ventura et al. (2010)

Tukey multiple comparison test was run with statistically significant differences between treatments in growth reported for $p < 0.05$. The EC_{50} values and corresponding 95 % confidence intervals for luminescence inhibition in *V. fischeri* were estimated using the Microtox® Omni™ Software version 4.3.0.1 (Azur Environmental 1998). All other EC_{50} values (algal yield, daphnid immobilization and daphnid fecundity) were estimated by Probit analysis (Finney 1971).

The significance level (α) used in all analyses was 0.05.

Results and discussion

One of the most appealing characteristics of ILs is the capacity to design them with a specific set of properties to meet the multiple requirements of a specific potential application (Plechkova and Seddon 2008, 2007; Brennecke and Maginn 2001). In previous works, the mutual solubilities of ILs with water were investigated (Freire et al. 2007a). It was found that aromatic cations, such as imidazolium and pyridinium, have a larger solubility in water than non-aromatic cations, namely pyrrolidinium and piperidinium. The water solubilities of the simpler ILs based in the anions $[PF_6]$ and $[NTf_2]$ addressed in this study are reported in Table 1. Water solubilities for $[C_3C_1\text{pyrr}][PF_6]$ and $[C_3C_1\text{pip}][PF_6]$ were measured using a methodology previously described in literature (Freire et al. 2010). The toxicological data obtained from tests with *V. fischeri*, *P. subcapitata* and *D. magna* are reported in Tables 1, 2, 3 and 4 (the molar concentrations are also reported in Supporting Information—Tables A1, A2, A3, A4).

The Microtox® bioassays reported in Table 1 were carried using imidazolium, pyrrolidinium, pyridinium and piperidinium-based ILs with the same alkyl chain length (propyl) and conjugated with the hydrophobic anions [NTf_2] and [PF_6] as presented in Fig. 1. Under the conditions used in this work, the hydrolysis of [PF_6] can be considered negligible (Freire et al. 2010). The EC_{50} values obtained show that the ILs based on the aromatic imidazolium and pyridinium cations are always more toxic than those based on non-aromatic cations such as pyrrolidinium and piperidinium. Also, cations with 6 member rings are always more toxic than 5 member ring cations, as well as [NTf_2]-based ILs are more toxic than [PF_6]-based, as already suggested by other authors (Romero et al. 2008; Garcia et al. 2005; Matzke et al. 2007; Stolte et al. 2007). For both anions [NTf_2] and [PF_6] the increase in toxicity follows the tendency:

$$[C_3C_1pyrr] < [C_3C_1pip] < [C_3C_1im] < [C_3C_1pyr]$$

The relation between the ILs toxicities measured by Microtox® and the respective water solubilities is shown in Fig. 2. The results clearly indicate that the studied ILs can be divided in two groups presenting very different dependencies of the toxicity with the water solubility. One of the groups comprises the non-aromatic ILs, based on the piperidinium and pyrrolidinium, while the other comprises the aromatic ILs based on the imidazolium and pyridinium cations. The non-aromatic ILs present a much lower water solubility and toxicity than the aromatic ILs, while both [PF_6] and [NTf_2] anions have little impact on the toxicities, having a similar toxicity dependency on the water solubility. While for both groups the toxicity does increase with the hydrophobicity (EC_{50} values decrease with the increase in water solubility), following a generally accepted trend (Matzke et al. 2010), the differences in behavior between these two groups show that it is possible to design ILs of enhanced hydrophobicity while decreasing their toxicity, in a paradigmatic example of the "*designer solvent*" character of ILs. A compilation of toxicity data from the literature reported in Tables A5 and A6 (see Supporting Information) further supports this hypothesis as all the pairs of imidazolium/pyrrolidinium (aromatic) and pyridinium/piperidinium (non-aromatic) ILs available are in good agreement with our observations.

Having established the toxicity of the ILs towards marine luminescent bacteria it is important to compare their toxicity with common organic solvents. The EC_{50} values for the bacteria *V. fischeri* reported in literature for some common organic solvents (Kaiser and Palabrica 1991) are within 30–100 mg L^{-1}, meaning that ILs have a much lower toxicity than these compounds. The Microtox® toxicity screening thus apparently supports their widespread classification as green solvents.

EC_{50} toxicity parameters for the standard algae *P. subcapitata* were also determined and are reported in Table 2. The EC_{50} values obtained indicate that the ILs were considered moderately toxic, except the pyridinium-based IL, which may be considered only slightly toxic according to the hazard ranking proposed by Pretti and his collaborators (2009) and adapted from earlier literature (Passino and Smith 1987).

Figure 3 describes the algae growth for increasing concentrations of each IL, and the statistics confirmed that this parameter was significantly affected by the presence of all these ionic structures. Analysing the EC_{50} results (Table 2) for *P. subcapitata*, was possible to recognize that the relation between the cation toxicity and the different cations was the same as described above for the Microtox® assays. The toxicity of the ILs here studied towards *P. subcapitata* as compared with common solvents such as dichloromethane (EPA 2002), dimethylformamide (Cho

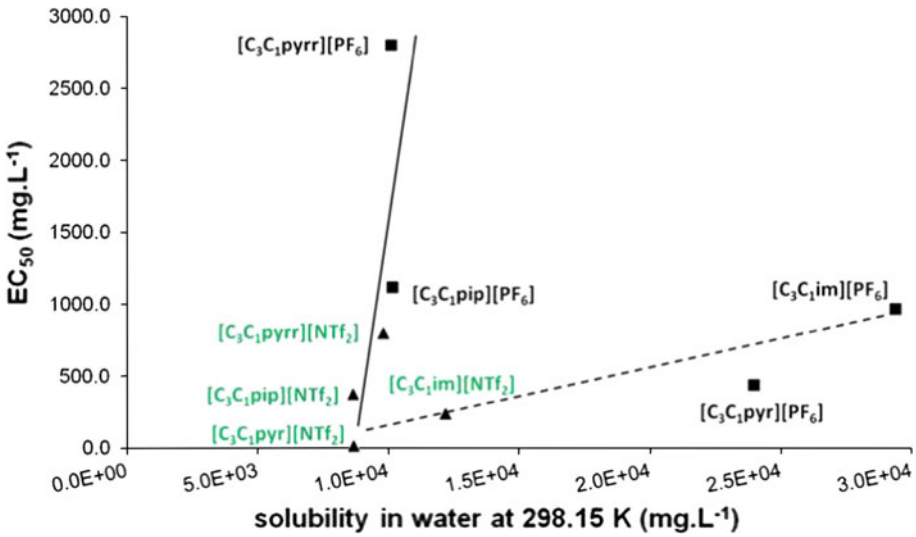


Fig. 2 Relationship between the water solubility of the different (aromatic and non-aromatic) ILs and their toxicity parameters (EC_{50} values) obtained by Microtox® assays. The lines in the figure are only for eye guide

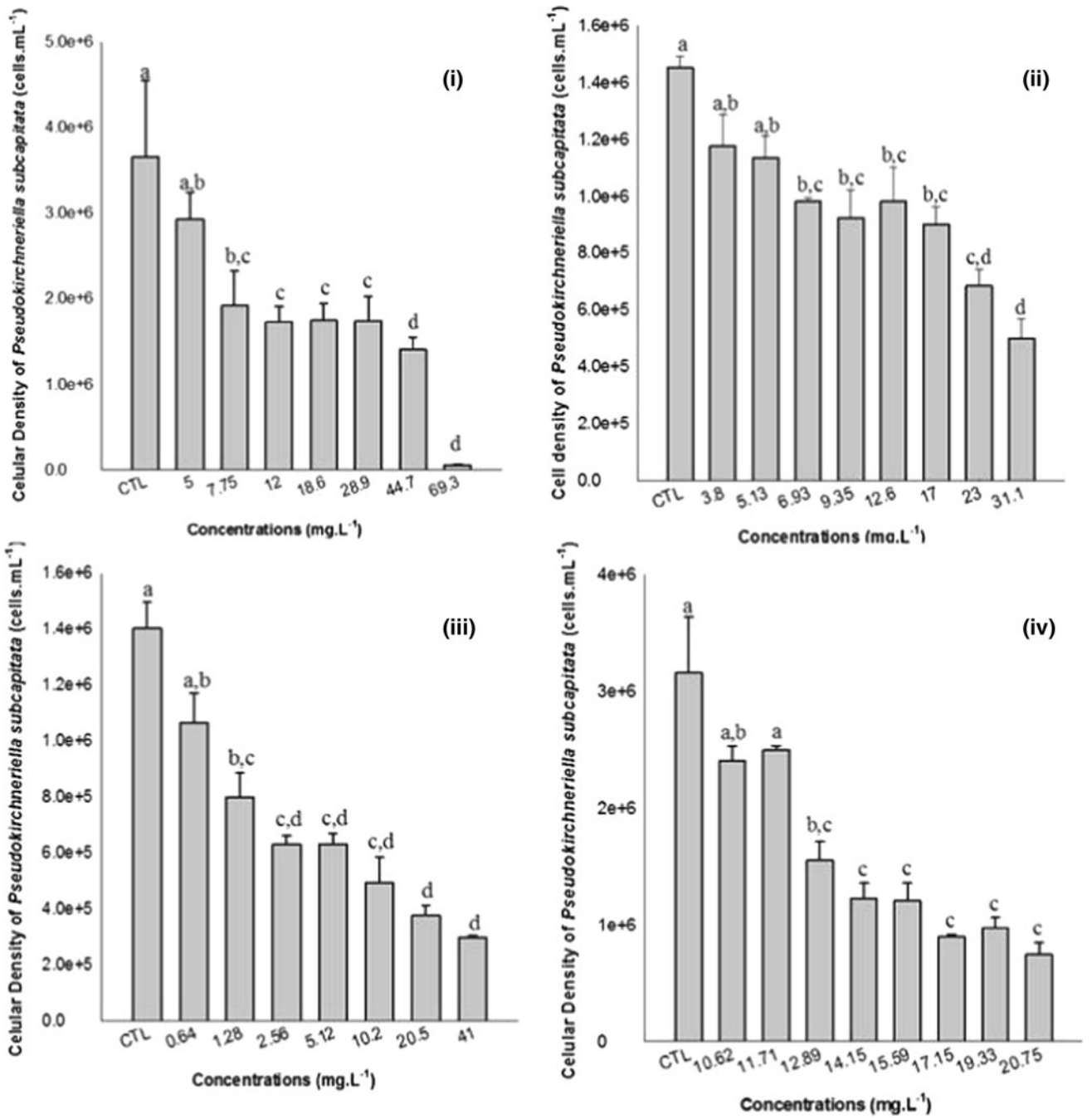


Fig. 3 Growth inhibition of *P. subcapitata* when exposed to successive dilutions of: (*i*) $[C_3C_1im][NTf_2]$, (*ii*) $[C_3C_1pyrr][NTf_2]$, (*iii*) $[C_3C_1pyr][NTf_2]$ and (*iv*) $[C_3C_1pip][NTf_2]$, after 96 h of incubation. Those values represent the mean of at least three replicates. Error bars represent standard error and the letters correspond to significant differences between the treatments ($p < 0.05$)

et al. 2008a), methanol (Cho et al. 2008a) and aniline (Ranke et al. 2009) is somewhat higher with the exception of aniline that presents a toxicity comparable to our ionic compounds. This comparative assessment raises concerns on the environmental safety of ILs and denotes the need for conspicuous risk assessment of these newly developed alternatives to common organic solvents.

Acute EC_{50} values are reported in Table 3 for the crustacean *D. magna*. The acute EC_{50} values were determined for $[C_3C_1im][NTf_2]$, $[C_3C_1pyrr][NTf_2]$, $[C_3C_1pip][NTf_2]$ and $[C_3C_1pyr][NTf_2]$ while the chronic EC_{50} values were determined for $[C_3C_1pyrr][NTf_2]$ as a complement to earlier studies (Ventura et al. 2010). These data show that the chronic toxicity seems to be slightly lower than the acute

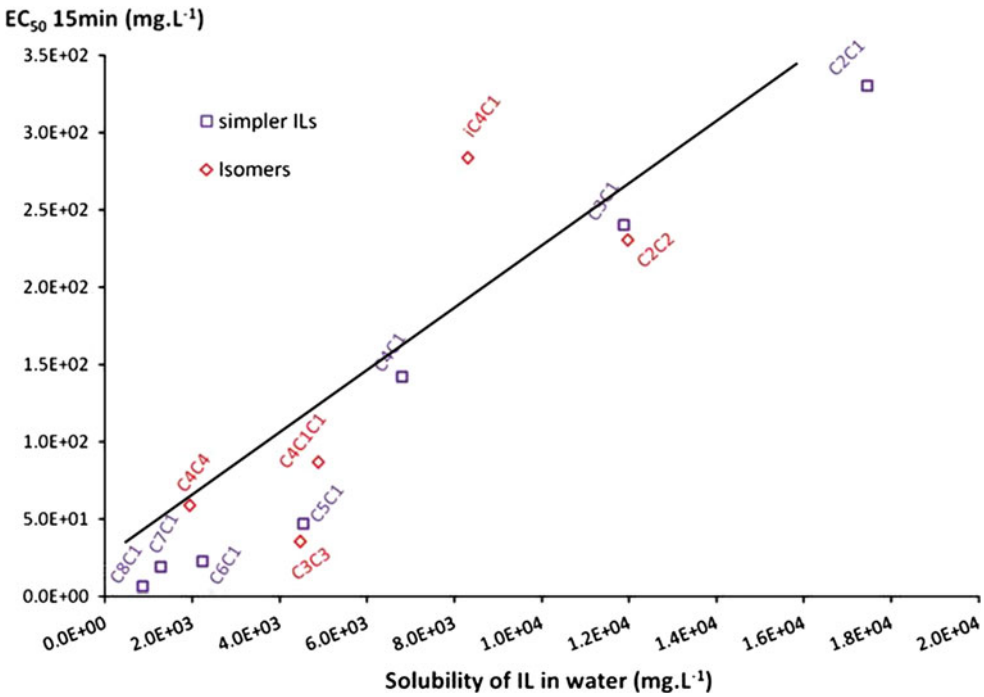


Fig. 4 Relationship between the water solubility of imidazolium (simpler and isomers)-based ILs and their EC_{50} parameters obtained by Microtox® tests

toxicity, suggesting that the latter can provide a good guide for the environmental effects of chronic exposures to ILs. Provided that a parallelism can be traced between the output of these two distinct test methodologies, the time- and cost-effective acute toxicity assays should be assumed safe tools for the environmental risk assessment of ILs, particularly at screening stages. *D. magna* was affected by the presence of all ILs (considering the acute tests) with the most remarkable effects corresponding to the exposure to $[C_3C_1pyr][NTf_2]$, which is considered, according to the aforementioned hazard ranking (Passino and Smith 1987) as only "slightly toxic" ($EC_{50} < 100$ mg L^{-1}). The acute EC_{50} results present the same tendency as the changes as shown by the algae. A similar tendency was observed on *D. magna* for the butyl analogues of the ILs here studied (Pretti et al. 2009). When compared with common solvents the toxicity of these ILs is equivalent to aromatic compounds such as aniline (EC_{50} = 80–380 mg L^{-1}) (Pretti et al. 2009) or nitrogen containing compounds, namely amines (EC_{50} = 200 mg L^{-1}) (Kaniewska-Prus 1982), and much less toxic than phenol (EC_{50} = 10–17 mg L^{-1}) (Cowgill and Milazzo 1991) or ammonia (EC_{50} = 2.90–6.93 mg L^{-1}) (Kaniewska-Prus 1982). In crustaceans, algae and bacteria, the mechanism of toxicity induced by ILs is not yet known. Nevertheless, some authors have suggested that this property could be related to enzymatic inhibition and a membrane disruption of the daphnids (Matzke et al. 2010).

Together with the aromatic versus aliphatic effect on the toxicity towards aquatic microorganisms, the effect of the isomerism was also assessed, being used the $[iC_4C_1im][NTf_2]$ and several other imidazolium-based ILs of the type $[C_nC_mC_jim][NTf_2]$, with the subscripts ranging from $1 < n < 8$, $1 < m < 6$ and $0 < j < 1$ (Fig. 1). The toxicity of these ionic compounds was also evaluated according to their solubilities in water (Table 4), aiming to evaluate the possibility of using the isomerism on the cation alkyl chains to promote the design of safer ILs. The first step was to investigate the effect of distributing the cation alkyl chains between the two nitrogen atoms in both what concerns their solubilities in water and EC_{50} values, towards the marine bacterium *V. fischeri* (Table 4; Fig. 4). According to the results here obtained the general trend, commonly known as "*side chain effect*", is observed for both the structures of ILs ($[C_nC_1im][NTf_2]$, and $[C_mC_mim][NTf_2]$). The location of the cation alkyl chain does not affects the water solubility as expected, since this is primarily related with the cation molar volume (Freire et al. 2010) but surprisingly, it also does not affect the toxicity with the $[C_nC_mim][NTf_2]$ presenting identical EC_{50} values to the $[C_nC_1im][NTf_2]$ for which $m + m = n + 1$. The same still holds for the $[C_4C_1C_1im][NTf_2]$ that falls in the same correlation as the other ILs here studied as shown in Fig. 4. The only isomer that seems to have an impact on the toxicity is $[iC_4C_1im][NTf_2]$. A branched alkyl chain seems to increase the EC_{50}. This approach seems promising to control the toxicity of the ILs but further studies are required to confirm this result for a larger range of ILs.

The toxicity data here reported show that the ILs studied present similar or even lower toxicities than commonly used organic solvents. It was also shown that their toxicity can be further lowered by an appropriate manipulation of the IL chemical structures to reduce their toxicity. Our

results suggest that while the distribution of the cation alkyl chain by the two nitrogens does not seem to have an impact on the toxicity, the use of branched chain may be relevant. However a significant capacity of the non-aromatic cations to increase the hydrophobicity while lowering the toxicity was disclosed. Finally, this work demonstrates that it is possible to design task-specific ILs, not only to achieve improved physico-chemical properties, but also to obtain inherently environmentally safer ILs.

Conclusions

This work suggests the possibility of to overcome the conflicting goals of the toxicity minimization and hydrophobic nature maximization of ILs as required for their use in various processes. In the present study, toxicological data for different aquatic species, namely *V. fischeri*, *P. subcapitata* and *D. magna*, were analysed. The results clearly show that the toxic character separates the ILs studied in two groups, the aromatic and the non-aromatic ILs, presenting very different dependencies of toxicity with the water solubility. While in general the toxicity increases with the hydrophobicity, the response differences between both the aromatic and non-aromatic groups show that the design of ILs by enhancing their hydrophobic character while simultaneously decreasing their toxicity could be a real approach in a near future. This work reports a paradigmatic example of the "*designer solvent*" character of the ILs. If the potential of this "*designer solvent*" character is fully understood and applied, the full potential of ILs may be released with solvents not only with enhanced performances, but also with a lower environmental impact and a "*greener*" character.

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Conflict of interest The authors declare that they have no conflict of interest.

References

Allen DT, Shonnard DR (2002) Green engineering. Prentice Hall, Upper Saddle River

Arning J, Stolte S, Boschen A, Stock F, Pitner W-R, Welz-Biermann U, Jastorff B, Ranke J (2008) Qualitative and quantitative structure activity relationships for the inhibitory effects of cationic head groups, functionalised side chains and anions of ionic liquids on acetylcholinesterase. Green Chem 10:47–58

ASTM (1980) Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates and amphibians, E 729–80. American Society for Testing and Materials, Philadelphia

Austin RP, Barton P, Davis AM, Manners CN, Stansfield MC (1998) The effect of ionic strength on liposome-buffer and 1-octanol-buffer distribution coefficients. J Pharm Sci 87:599–607

Azur Environmental (1998) Microtox manual. Carlbad CA, USA

Baird DJ, Barber I, Bradley MC, Calow P, Soares AMVM (1989) The *Daphnia* bioassay: a critique. Hydrobiologia 188/189: 403–406

Beadham MG, Gathergood N (2012) Handbook of Green chemistry. In: Boethling R, Voutchkova A (eds) Designing safer chemicals—ionic liquids, Chap. 6, vol 9, 1st edn. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, pp 137–158

Bernot RJ, Brueseke MA, Evans-White MA, Lamberti GA (2005) Acute and chronic toxicity of imidazolium-based ionic liquids on *Daphnia magna*. Environ Toxicol Chem 24:87–92

Brennecke JF, Maginn EJ (2001) Ionic liquids: innovative fluids for chemical processing. AIChE J 47:2384–2389

Cho C-W, Jeon Y-C, Pham TPT, Vijayaraghavan K, Yun Y-S (2008a) The ecotoxicity of ionic liquids and traditional organic solvents on microalga *Selenastrum capricornutum*. Ecotoxicol Environ Saf 71:166–171

Cho C-W, Pham TPT, Jeon Y-C, Yun Y-S (2008b) Influence of anions on the toxic effects of ionic liquids to a phytoplankton *Selenastrum capricornutum*. Green Chem 10:67–72

Coleman D, Gathergood N (2010) Biodegradation studies of ionic liquids. Chem Soc Rev 39:600–637

Couling DJ, Bernot RJ, Docherty KM, Dixon JK, Maginn EJ (2006) Assessing the factors responsible for ionic liquid toxicity to aquatic organisms via quantitative structure–property relationship modeling. Green Chem 8:82–90

Cowgill UM, Milazzo DP (1991) The sensitivity of *Ceriodaphnia dubia* and *Daphnia magna* to seven chemicals utilizing the three-brood test. Arch Environ Contam Toxicol 20:211–217

Deetlefs M, Seddon KR (2006) Ionic liquids: fact and fiction. Chim Oggi Chem Today 24:16–23

Deetlefs M, Seddon KR (2010) Assessing the greenness of some typical laboratory ionic liquid preparations. Green Chem 12: 17–30. doi:10.1039/b915049h

Docherty KM, Kulpa CFJ (2005) Toxicity and antimicrobial activity of imidazolium and pyridinium ionic liquids. Green Chem 7:185–189

Earle MJ, Seddon KR (2000) Ionic liquids. Green solvents for the future. Pure Appl Chem 72:1391–1398

Earle MJ, Esperanca J, Gilea MA, Lopes JNC, Rebelo LPN, Magee JW, Seddon KR, Widegren JA (2006) The distillation and volatility of ionic liquids. Nature 439:831–834. doi:10.1038/nature04451

EC (2002) European Commission Guidance document on aquatic ecotoxicology. Under Council directive 91/414/EEC. SANCO/3268/2001 Rev 4

EC (2006) http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:396:0001:0849:EN:PDF. Accessed 14 Feb 2012

EPA (2002) Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms, 4th edn. EPA-821-R-02-013

Finney DJ (1971) Probit Analysis. Cambridge University Press, Cambridge

Freire MG, Neves CMSS, Carvalho PJ, Gardas RL, Fernandes AM, Marrucho IM, Santos LMNBF, Coutinho JAP (2007a) Mutual solubilities of water and hydrophobic ionic liquids. J Phys Chem B 111:13082–13089

Freire MG, Santos LMNBF, Fernandes AM, Coutinho JAP, Marrucho IM (2007b) An overview of the mutual solubilities of water-imidazolium-based ionic liquids systems. Fluid Phase Equilib 261:449–454

Freire MG, Carvalho PJ, Gardas RL, Marrucho IM, Santos LMNBF, Coutinho JAP (2008a) Mutual solubilities of water and the [$C_n$mim][Tf_2N] hydrophobic ionic liquids. J Phys Chem B 112: 1604–1610

Freire MG, Carvalho PJ, Gardas RL, Santos LMNBF, Marrucho IM, Coutinho JAP (2008b) Solubility of water in tetradecyltrihexylphosphonium-based ionic liquids. J Chem Eng Data 53: 2378–2382. doi:10.1021/je8002805

Freire MG, Carvalho PJ, Silva AMS, Santos LMNBF, Rebelo LPN, Marrucho IM, Coutinho JAP (2009) Ion specific effects on the mutual solubilities of water and hydrophobic ionic liquids. J Phys Chem B 113:202–211

Freire MG, Neves CMSS, Marrucho IM, Coutinho JAP, Fernandes AM (2010) Hydrolysis of tetrafluoroborate and hexafluorophosphate counter ions in imidazolium-based ionic liquid. J Phys Chem A 114:3744–3749

Freire MG, Cláudio AFM, Araújo JMM, Coutinho JAP, Marrucho IM, Canongia Lopes JN, Rebelo LPN (2012) Aqueous Biphasic Systems: a boost brought about by using ionic liquids. Chem Soc Rev. 41:4966–4995

Garcia MT, Gathergood N, Scammells PJ (2005) Biodegradable ionic liquids Part II. Effect of the anion and toxicology. Green Chem 7:9–14

Gathergood N, Scammells PJ (2002) Design and preparation of room-temperature ionic liquids containing biodegradable side chains. Aust J Chem 55:557–560

Gathergood N, Scammells PJ, Garcia MT (2004) Biodegradable ionic liquids: Part I. Concept, preliminary targets and evaluation. Green Chem 6:166–175

Gathergood N, Scammells PJ, Garcia MT (2006) Biodegradable ionic liquids Part III. The first readily biodegradable ionic liquids. Green Chem 8:156–160

Gonçalves AMM, de Figueiredo DR, Pereira MJ (2005) A low-cost methodology for algal growth inhibition tests using three freshwater green algae. Fresenius Environ Bull 14:1192–1195

Gorman-Lewis DJ, Fein JB (2004) Experimental study of the adsorption of an ionic liquid onto bacterial and mineral surfaces. Environ Sci Technol 38:2491–2495

Kaiser KLE, Palabrica VS (1991) *Photobacterium phosphoreum* toxicity data index. Water Pollut Res J Can 26:361–431

Kaniewska- Prus M (1982) The effect of ammonia, chlorine and chloramines toxicity on the mortality of *Daphnia magna* Straus. Pol Arch Hydrobiol 29:607–624

Kragl U, Eckstein M, Kaftzik N (2002) Enzyme catalysis in ionic liquids. Curr Opin Biotechnol 13:565–571

Kulacki KJ, Lamberti GA (2008) Toxicity of imidazolium ionic liquids to freshwater algae. Green Chem 10:104–110

Latala A, Stepnowski P, Nedzi M, Mrozik W (2005) Marine toxicity assessment of imidazolium ionic liquids: acute effects on the Baltic algae *Oocystis submarina* and *Cyclotella meneghiniana*. Aquat Toxicol 73:91–98

Latala A, Nedzi M, Stepnowski P (2009a) Acute toxicity assessment of perfluorinated carboxylic acids towards the Baltic microalgae. Environ Toxicol Pharmacol 28:167–171

Latala A, Nedzi M, Stepnowski P (2009b) Toxicity of imidazolium and pyridinium based ionic liquids towards algae. *Bacillaria paxillifer* (a microphytobenthic diatom) and *Geitlerinema amphibium* (a microphytobenthic blue green alga). Green Chem 11:1371–1376

Latala A, Nedzi M, Stepnowski P (2009c) Toxicity of imidazolium and pyridinium based ionic liquids towards algae. *Chlorella vulgaris*, *Oocystis submarina* (green algae) and *Cyclotella meneghiniana*, *Skeletonema marinoi* (diatoms). Green Chem 11:580–588

Luo Y-R, Li X-Y, Chen X-X, Zhang B-J, Sun Z-J, Wang J-J (2008) The developmental toxicity of 1-methyl-3-octylimidazolium bromide on *Daphnia magna*. Environ Toxicol 23:736–744

Matzke M, Stolte S, Thiele K, Juffernholz T, Arning J, Ranke J, Welz-Biermann U, Jastorff B (2007) The influence of anion species on the toxicity of 1-alkyl-3-methylimidazolium ionic liquids observed in an (eco)toxicological test battery. Green Chem 9:1198–1207

Matzke M, Arning J, Ranke J, Jastorff B, Stolte S (2010) Design of inherently safer ionic liquids: toxicology and biodegradation. In: Handbook of green chemistry. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, pp 225–290. doi:10.1002/9783527628698.hgc069

Microbics Corporation (1992) Microtox® manual—a toxicity testing handbook. Microbics Corporation, Carlsbad, pp 1–5

OECD (2004) *Daphnia* sp., acute immobilisation test—test guideline 202. Organization for the Economic Cooperation and Development, Paris

OECD (2008) *Daphnia magna* reproduction test–test guideline 211. Organization for the Economic Cooperation and Development, Paris

OECD (2011) Freshwater alga and cyanobacteria, growth inhibition test—test guideline 201. Organization for the Economic Cooperation and Development, Paris

Passino DRM, Smith SB (1987) Acute bioassays and hazard evaluation of representative contaminants detected in Great Lakes fish. Environ Toxicol Chem 6:901–907

Plechkova NV, Seddon KR (2007) Ionic liquids: "Designer" solvents for green chemistry, in methods and reagents for green chemistry, vol 38. John Wiley & Sons, Inc, pp 103–130

Plechkova NV, Seddon KR (2008) Applications of ionic liquids in the chemical industry. Chem Soc Rev 37:123–150

Pretti C, Chiappe C, Baldetti I, Brunini S, Monni G, Intorre L (2009) Acute toxicity of ionic liquids for three freshwater organisms: *Pseudokirchneriella subcapitata*, *Daphnia magna* and *Danio rerio*. Ecotoxicol Environ Saf 72:1170–1176

Ranke J, Molter K, Stock F, Bottin-Weber U, Poczobutt J, Hoffmann J, Ondruschka B, Filser J, Jastorff B (2004) Biological effects of imidazolium ionic liquids with varying chain lengths in acute *Vibrio fischeri* and WST-1 cell viability assays. Ecotoxicol Environ Saf 58:396–404

Ranke J, Muller A, Bottin-Weber U, Stock F, Stolte S, Arning J, Stormann R, Jastorff B (2007a) Lipophilicity parameters for ionic liquid cations and their correlation to in vitro cytotoxicity. Ecotoxicol Environ Saf 67:430–438. doi:10.1016/j.ecoenv.2006.08.008

Ranke J, Stolte S, Stormann R, Arning J, Jastorff B (2007b) Design of sustainable chemical products the example of ionic liquids. Chem Rev 107:2183–2206. doi:10.1021/cr050942s

Ranke J, Othman A, Fan P, Müller A (2009) Explaining ionic liquid water solubility in terms of cation and anion hydrophobicity. Int J Mol Sci 10:1271–1289

Romero A, Santos A, Tojo J, Rodríguez A (2008) Toxicity and biodegradability of imidazolium ionic liquids. J Hazard Mater 151:268–273

Salminen J, Papaiconomou N, Kumara A, Lee J-M, Kerr J, Newmana J, Prausnitz JM (2007) Physicochemical properties and toxicities of hydrophobic piperidinium and pyrrolidinium ionic liquids. Fluid Phase Equilib 261:421–426

Samorì C, Pasteris A, Galletti P, Tagliavini E (2007) Acute toxicity of oxygenated and nonoxygenated imidazolium-based ionic liquids to *Daphnia magna* and *Vibrio fischeri*. Environ Toxicol Chem 26:2379–2382

Sheldon R (2001) Catalytic reactions in ionic liquids. Chem Commun 2399–2407

Sheldon RA, Lau RM, Sorgedrager MJ, van Rantwijk FV, Seddon KR (2002) Biocatalysis in ionic liquids. Green Chem 4:147–151

Smith DG (2001) Pennak's freshwater invertebrates of the United States: Porifera to Crustacea, 4th edn. Wiley, New York

Steinberg SM, Poziomek EJ, Engelmann WH, Rogers KR (1995) A review of environmental applications of bioluminescence measurements. Chemosphere 30:2155–2197

Stepnowski P, Zaleska A (2005) Comparison of different advanced oxidation processes for the degradation of room temperature ionic liquids. J Photochem Photobiol A 170:45–50

Stolte S, Arning J, Bottin-Weber U, Muller A, Pitner W-R, Welz-Biermann U, Jastorff B, Ranke J (2007) Effects of different head groups and functionalised side chains on the cytotoxicity of ionic liquids. Green Chem 9:760–767

van Rantwijk FV, Madeira Lau R, Sheldon RA (2003) Biocatalytic transformations in ionic liquids. Trends Biotechnol 21:131–138

Ventura SPM, Gonçalves AMM, Gonçalves F, Coutinho JAP (2010) Assessing the toxicity on [C_3mim][Tf_2N] to aquatic organisms of different trophic levels. Aquat Toxicol 96:290–297

Ventura SPM, Gardas RL, Gonçalves F, Coutinho JAP (2011) Ecotoxicological risk profile of ionic liquids: octanol–water distribution coefficients and toxicological data. J Chem Technol Biotechnol 86:957–963

Wasserscheid P, Welton T (2007) Ionic liquids in synthesis, 2nd edn. Wiley-VCH-Verlag, Weinheim

Wells AS, Coombe VT (2006) On the freshwater ecotoxicity and biodegradation properties of some common ionic liquids. Org Process Res Dev 10:794–798

Zhao H (2006) Innovative applications of ionic liquids as "green" engineering liquids. Chem Eng Commun 193:1660–1677