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Toxicity assessment of various ionic liquid families towards *Vibrio fischeri* marine bacteria

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ABSTRACT

The increasing interest on the application of ionic liquids (ILs) to a wide range of processes and products has been hampered by a lack of toxicological data, mainly in what concerns novel cations, such as guanidinium, phosphonium, and functionalized and non-functionalized imidazolium-based ILs. The present study reports the toxicity of five guanidinium-, six phosphonium, and six imidazolium-based ILs, towards the luminescent marine bacteria *Vibrio fischeri*. These new results clearly show that guanidinium-, unlike the imidazolium- and phosphonium-based ILs, do not follow the trend of increasing toxicity with the increase in the alkyl chain length. Moreover, the introduction of oxygenated groups on the alkyl chains, such as ether and ester, leads to a decrease of the toxicity of guanidinium and also imidazolium compounds. In what respects the effect of the different cations, it is possible to recognize that the phosphonium-based ILs seem to be more toxic when compared to the analog imidazolium-based ILs (with the same anion and alkyl chains).

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1. Introduction

Ionic liquids (ILs) are attracting increasing attention due to their unique properties, such as negligible vapor pressure, chemical and thermal stability, non-flammability, high ionic conductivity, wide electrochemical potential window and solvation ability. A huge number of different ILs can be synthesized by the combination of different anion moieties and cation cores, or by the manipulation of their characteristics such as varying their alkyl chain length or by the introduction of oxygenated groups. This makes possible to control physical properties such as, hydrophobicity, viscosity, density, their solubility behavior, and also, to influence their biodegradation ability or toxicological features

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(Earl and Seddon, 2000; Ranke et al., 2007b; Wasserscheid and Welton, 2007; Welton, 1999).

In the light of their recent widespread commercial availability, the synthesis of ILs has been object of a huge number of developments. If in the past, the synthesis of ILs was focused on obtaining unique physico-chemical properties (1st IL generation); to achieve a specific behavior considering the potential final industrial application (2nd ILs generation); nowadays the main goal is to produce ILs with the desired biological features (3rd ILs generation) (Hough et al., 2007) for the final application and also to facilitate the REACH registration processes. Although ILs can lessen the risk of air pollution due to their virtually zero vapor pressure, they do present some water solubility (Anthony et al., 2001; Freire et al., 2007, 2008, 2009, 2010), which was already correlated with the different ILs' parameters, such as the anion (Ranke et al., 2007a) and cation hydrophobicity (Ranke et al., 2009). This is, consequently, the most likely medium for ILs to be released into the environment. In view of the crescent number of industrial applications, their accidental discharge and environmental contamination are realities to take into consideration

(Frade and Afonso, 2010; Pham et al., 2009; Ranke et al., 2007b). The properties that make them of industrial interest (high chemical and thermal stabilities and non-volatility) suggest potential problems with degradation or persistence in the environment. Moreover, due to the use of non-renewable resources as starting materials, the economic/environmental impact and the cumulative energy demand, the environmental fate and the (eco)toxicological behavior are extremely important parameters to take into account for a complete analysis of ILs sustainability, above all in order to improve the chances of large-scale applications (Kralisch et al., 2005; Zhang et al., 2008). As a consequence, the number of studies involving the environmental fate analysis (chemical degradation, bioaccumulation, biodegradability and the distribution of ILs in different environmental compartments, such as soil, water and sediments) and the toxicological effects (cytotoxicity, (eco)toxicity, phytotoxicity, and antimicrobial and antibacterial activities) of ILs are increasing (Frade and Afonso, 2010; Pham et al., 2009; Ranke et al., 2007b). The toxicological impact of ILs has been analyzed in a series of interdisciplinary studies (Jastorff et al., 2003; Matzke et al., 2007; Ranke et al., 2007b; Stolte et al., 2007a), which have underlined the importance of a preventive evaluation in the ILs design, because of the influence of different structures on pertinent technological and (eco)toxicological properties. Moreover, as previously demonstrated by our group (Gonçalves et al., 2011; Ventura et al., 2010) the need of a full understanding of the biological effects at different biological levels has a fundamental importance, and such knowledge should become a key factor in the modulation and selection of ILs features.

The most used methods to evaluate the toxicological risk of a substance in an aqueous media are those measuring their toxicity by an inhibition assay. Different aquatic species have been used in these inhibition measurements (daphnids, algae and fish) and also the test, which uses the *Vibrio fischeri* (formerly known as *Photobacterium phosphoreum*) bioluminescence inhibition assay. This is a rapid, cost-effective, and well-established method for toxicity determination (Steinberg et al., 1995) focusing on environmental issues, and also a standard (eco)toxicological bioassay in Europe (DIN EN ISO 11348). Several different luminescence inhibition tests of *Vibrio fischeri* have been developed so far, most being designed for analysis of aqueous samples, such as Microtox[®] Toxicity Test. This test can be used into a wide range of applications, such as the analysis of industrial effluents and discharges, waters, soils and sediments, and new products. This test is normally used as a possible approach for determination of the toxicity for both organic solvents (Lapertot and Pulgarin, 2006; Nirmalakhandan et al., 1994; Ruiz et al., 1997) and ILs (Couling et al., 2006; Docherty and Kulpa, 2005; Frade and Afonso, 2010; Garcia et al., 2005; Gonçalves et al., 2011; Luis et al., 2007; Matzke et al., 2007; Papaiconomou et al., 2010; Pham et al., 2009; Ranke et al., 2004, 2007b; Romero et al., 2008; Stolte et al., 2007a, 2007b; Ventura et al., 2010). There are different approaches in what concerns the use of Microtox[®] and ILs. The published data on ILs toxicity towards *Vibrio fischeri* were comprehensively interpreted in literature (Peraccini et al., 2007). Several authors have discussed the effect of different alkyl chain lengths, anions and different cations. Usually, the increase in the alkyl chain length leads to a pronounced augment in the toxicity (Couling et al., 2006; Docherty and Kulpa, 2005; Garcia et al., 2005; Luis et al., 2010; Ranke et al., 2004; Romero et al., 2008; Stolte et al., 2007a). This was explained by the increase in the lipophilicity of the cation that, according to the baseline toxicity, is responsible for a non-specific disturbance of the structure functioning of biological membranes (Ranke et al., 2007a). Thus, the long alkyl chains are able to be incorporated into the phospholipidic bilayer of the membranes, the same toxicity mechanism described in

literature for some surfactants (Roberts and Costello, 2003). Considering the cation core, some reports show that the aromatic cations (imidazolium and pyridinium) are, in general, more toxic than the non-aromatic ILs, such as pyrrolidinium, piperidinium, phosphonium and ammonium (Couling et al., 2006; Gonçalves et al., 2011; Luis et al., 2007, 2010; Papaiconomou et al., 2010; Pretti et al., 2009; Stolte et al., 2007a, 2007b).

The anion seems to contribute to the toxicity in spite of the large disagreement among the literature data (Bernot et al., 2005; Garcia et al., 2005; Gonçalves et al., 2011; Stolte et al., 2006); tetrafluoroborate and hexafluorophosphate were considered less toxic and bis(trifluoromethylsulfonyl)imide and octylsulfate the most toxic anions (Azimova et al., 2009; Couling et al., 2006; Docherty and Kulpa, 2005; Garcia et al., 2005; Gonçalves et al., 2011; Matzke et al., 2007; Ranke et al., 2004; Romero et al., 2008; Stolte et al., 2007a). Various authors have attempted to find a correlation between EC₅₀ and different ILs' properties, such as lipophilicity (Ranke et al., 2007a; Stolte et al., 2006; Stolte et al., 2007a, 2007b), or hydrophobicity (Ranke et al., 2009), and recently, solubility of ILs in water (Gonçalves et al., 2011).

More recently, some authors have applied Quantitative Structure–Activity Relationships (QSAR) models to data sets of ionic liquids to detect general assumptions on the toxicity and behavior of these compounds (Alvarez-Guerra and Irabien, 2011; Couling et al., 2006; Lacrămă et al., 2007; Luis et al., 2007, 2010; Putz et al., 2007). The aim is the development of predictive tools to help in the sustainable design of ILs safer for humans and for the environment (Matzke et al., 2010). The picture that emerges is that a number of characteristics play an active role on toxicity, most of them being still poorly understood (Gonçalves et al., 2011).

In this context, it is easy to understand the necessity of contributing to the enlargement of the toxicological databases considering the different families and structural features of ILs. Imidazolium based ILs appear as the family most studied in the toxicological field and phosphonium-based ILs being one of the less studied, despite their high industrial interest (for example in biotransformation processes such as xenobiotics-degradation (Cieniecka-Roslonkiewicz et al., 2005). Moreover, this family shows some interesting properties such as the possibility to decrease its antimicrobial activity for the longest alkyl chains (phosphonium based in alkyl chains of 8 and 14 carbons and conjugated with the chloride anion) (Cieniecka-Roslonkiewicz et al., 2005). Moreover, it was also focused that the exchange of the halide by other anions has resulted in a loss of antimicrobial activity (Cieniecka-Roslonkiewicz et al., 2005) thus rendering higher interest in this family. The apparently high toxicity of other phosphonium halides against *Vibrio fischeri* (Couling et al., 2006), *Daphnia magna* (Wells and Coombe, 2006), and *Pseudokirchneriella subcapitata* (Cho et al., 2008; Wells and Coombe, 2006) was also demonstrated. Still, the information for this family about its hazards to the environment is still scarce and not conclusive.

Recent toxicological studies have been focused on a new class of ILs with increased biodegradability through the incorporation of oxygenated alkyl chains (Coleman and Gathergood, 2010; Gathergood and Scammells, 2002; Neumann et al., 2010). The oxygenation is usually carried at two different parts of the IL structure, the cation core, represented for example by the morpholinium family, (Frade and Afonso, 2010) or at the cation alkyl chains, which can be achieved by the introduction of hydroxyl (–OH) (Docherty and Kulpa, 2005; Garcia et al., 2005; Ranke et al., 2004), ester (O–C=O) (Garcia et al., 2005) or ether (–O–) (Frade et al., 2007, 2009; Modelli et al., 2008; Ranke et al., 2007b; Samori et al., 2007, 2010; Samori et al., 2011; Stolte et al., 2007a) groups. The oxygenated imidazolium-based ILs are a promising class of

alternative solvents with some interesting properties, such as high solubility for polar substrates (Liu et al., 2005; Pinkert et al., 2009), suitable features as reaction media for some biocatalytic processes (Galletti et al., 2007) and for catalytic asymmetric reaction (Branco and Afonso, 2004), high carbon dioxide selectivity useful for gas separation processes (Bara et al., 2007) and nanoparticles stabilizing properties (Schrekker et al., 2007), which justifies the increased interest in the toxicity study of those structures. The reports previously published about oxygenated imidazolium-based ILs were focused on their biodegradability (Garcia et al., 2005), antimicrobial activity (Pernak et al., 2003) and toxicity towards *promyelocytic leukemia* cells (IPC-81) (Stolte et al., 2007a), human colon carcinoma cells (*CaCo-2* and *HT-29*) (Frade et al., 2007, 2009), *Daphnia magna* (Samori et al., 2007, 2010), marine diatoms algae (Samori et al., 2011) and the luminescent bacteria *Vibrio fischeri* (Samori et al., 2007, 2010). More recently, a review on the behavior of oxygenated imidazolium-based ILs was reported (Frade and Afonso, 2010). The results published show that the introduction of one oxygen atom into the lateral chain of imidazolium-based ILs seems to decrease the toxicity of the IL with respect to alkyl counter parts towards the crustacean *Daphnia magna* and the bacterium *Vibrio fischeri* (Samori et al., 2007). Moreover, it also increases their biodegradability using organisms of the soils, as described in literature (Garcia et al., 2005; Modelli et al., 2008). Samori et al. (2007) have described a comparison between the response of *Vibrio fischeri* and *Daphnia magna* to them. Apparently, the main contribution for the reduction on the toxicity is achieved by the introduction of a single oxygen atom in the lateral chain (Samori et al., 2010). In this work, the authors have described that the addition of more than one ethoxy moiety in the alkyl chain as a matter of fact, increases their toxicity towards the bacterium *Vibrio fischeri*, in opposition to *Daphnia magna*. They justify that the bacterium was more sensitive to an elongation of the chain than the crustacean, in spite of the increasing number of oxygen units, which should increase the overall polarity of the cation (Samori et al., 2010).

The guanidinium cations are the basis of a bioactive family of ILs, yet poorly studied (Frade and Afonso, 2010; Frade et al., 2009; Stolte et al., 2007a). The toxicological reports for this family are scarce (Frade and Afonso, 2010) and, at the same time the knowledge of their physico-chemical properties is also very limited (Carrera et al., 2010a, 2010b; Rosatella et al., 2009). The toxicity observed for guanidinium-based ILs seems to be very anion dependent, hexafluorophosphate [PF₆]⁻ anion being responsible for the most negative effect, when compared with dicyanamide [N(CN)₂] and bis(trifluoromethylsulfonyl)imide [NTf₂] (Frade et al., 2007, 2009). Accordingly, the introduction of an ether group in the guanidinium cation core is responsible for a remarkable decrease of the effect upon *CaCo-2* cells viability (Frade et al., 2007, 2009).

This work addresses the measurement of new toxicological data for the luminescent marine bacterium *Vibrio fischeri* for three distinct IL families: guanidinium-, phosphonium- and imidazolium-based ILs, being the latest family used to determine the influence of cation functionalization on the ionic liquid toxicity.

2. Material and methods

2.1. Test chemicals

The molecular structure of the ILs used is provided in Fig. 1. The ILs used were: di-butyl-tetramethyl-guanidinium iodide [TMGC₄]I, di-heptyl-tetramethyl-guanidinium iodide [TMGC₇]I, di-dodecyl-tetramethyl-guanidinium iodide [TMGC₁₂]I, tetrahexyl-dimethyl-guanidinium chloride [(di-h)₂DMG]Cl, N,N'-dimethyl-N,N,N',N'-tetra-(2-methoxyethyl)-guanidinium chloride [(C₃O)₄DMG]Cl, 3-methyl-1-(ethoxycarbonyloctyl)imidazolium bromide [C₁₀C(O)OEtmim]Br,

1-(2-(2-methoxyethoxy)ethyl)-3-methylimidazolium chloride [C₅O₂mim]Cl, trihexyltetradecylphosphonium bromide [P_{6,6,6,14}]Br (purity > 97 percent), trihexyltetradecylphosphonium chloride [P_{6,6,6,14}]Cl (purity > 98 percent), trihexyltetradecylphosphonium methanesulfonate [P_{6,6,6,14}][CH₃SO₃] (purity > 98 percent), tetrabutylphosphonium bromide [P_{4,4,4,4}]Br (purity > 98 percent), and tributyl(methyl)phosphonium methylsulfate [P_{4,4,4,1}][CH₃SO₄] (purity > 99 percent), triisobutyl(methyl)phosphonium tosylate [P_{4,4,4,1}][TOS] (purity > 99 percent), 1-butyl-3-methylimidazolium bromide [C₄mim]Br (purity > 99 percent), 1-butyl-3-methylimidazolium methanesulfonate [C₄mim][CH₃SO₃] (purity > 99 percent), and 1-butyl-3-methylimidazolium tosylate [C₄mim][TOS] (purity > 98 percent).

The guanidinium- and oxygenated imidazolium-based ILs were synthesized according to reported procedures: [(C₃O)₄DMG]Cl (Rosatella et al., 2009), [(di-h)₂DMG]Cl (Mateus et al., 2003), [C₅O₂mim]Cl (Branco et al., 2002), [TMGC₇]I (Rosatella et al., 2009), [TMGC₁₂]I (Rosatella et al., 2009), [TMGC₄]I (Rosatella et al., 2009) and [C₁₀O₂mim]Br (Lourenço and Afonso, 2007). The phosphonium-based ILs were supplied by Cytec Industries and the hydrophilic imidazolium based ILs were purchased at IoLiTec (Ionic Liquid Technologies, Germany) and their mass fraction purities were further confirmed by ¹H NMR and ¹³C NMR.

2.2. Microtox[®] tests

The Microtox[®] Toxicity Test (Microbics Corporation, 1992) was used to evaluate the inhibition of the luminescence in the marine bacteria *Vibrio fischeri*. This test was performed using a range of diluted aqueous solutions (from 0 to 100 percent) of each IL, where 100 percent of IL corresponds to a known concentration of a stock solution. After 5, 15, and 30 min of exposure to the IL solution (depending of 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 percent reduction in luminescence was computed using Microtox[®] Omni[™] Software version 4.3.0.1. (Azur Environmental, 1998).

3. Results and discussion

The present work shows the impact of various ILs features, such as the elongation of the alkyl chain length (guanidinium and phosphonium families), the influence of different cation cores and anions (non-functionalized imidazolium and phosphonium families), the increase in the number of long alkyl chains (guanidinium), and finally the effect of the side chains' functionalization with oxygenated groups (ester and ether structures) in distinct cation cores (guanidinium and imidazolium) in their toxicity towards the marine luminescent bacteria *Vibrio fischeri*.

Table 1 shows the EC₅₀ values for the guanidinium- and oxygenated imidazolium-based ILs. It can be observed that the increase in the toxicity of guanidinium family follows the tendency [TMGC₄]I < [TMGC₁₂]I < [TMGC₇]I, which is not in agreement with the established idea about the influence of the alkyl chain length on the toxicity. From [TMGC₄]I to [TMGC₇]I there is an increase in toxicity as expected. This effect is currently known as the "side chain effect" (Matzke et al., 2010), and describes the increase in the toxicity derived from the elongation of the IL' alkyl side chain. There are a few works (Docherty and Kulpa, 2005; Garcia et al., 2005; Ranke et al., 2004; Romero et al., 2008) reporting this effect also for the bioluminescent bacteria *Vibrio fischeri*. The EC₅₀ values reported in those articles shows that there is a correlation between the toxicity and the number of carbons on the alkyl chains, independently of the exposure period. However, there is a change in the toxicity tendency when [TMGC₇]I and [TMGC₁₂]I were compared, since in this case the elongation of the alkyl chain leads to the decrease in the IL toxicity. In fact, it is also described in literature that the dependence between the increase in both the alkyl side chain length and toxicity no longer holds true for very long alkyl chains. At a certain chain length, the toxicity cannot be increased any further, this phenomenon being described as the "cut-off" effect (Matzke et al., 2010; Stolte et al., 2007b). As discussed in literature (Matzke et al., 2010), different explanations for this phenomenon are proposed based either on insufficient solubility (nominal concentration deviating from real test concentration) or kinetic aspects (uptake is slowed because of steric effects for compounds

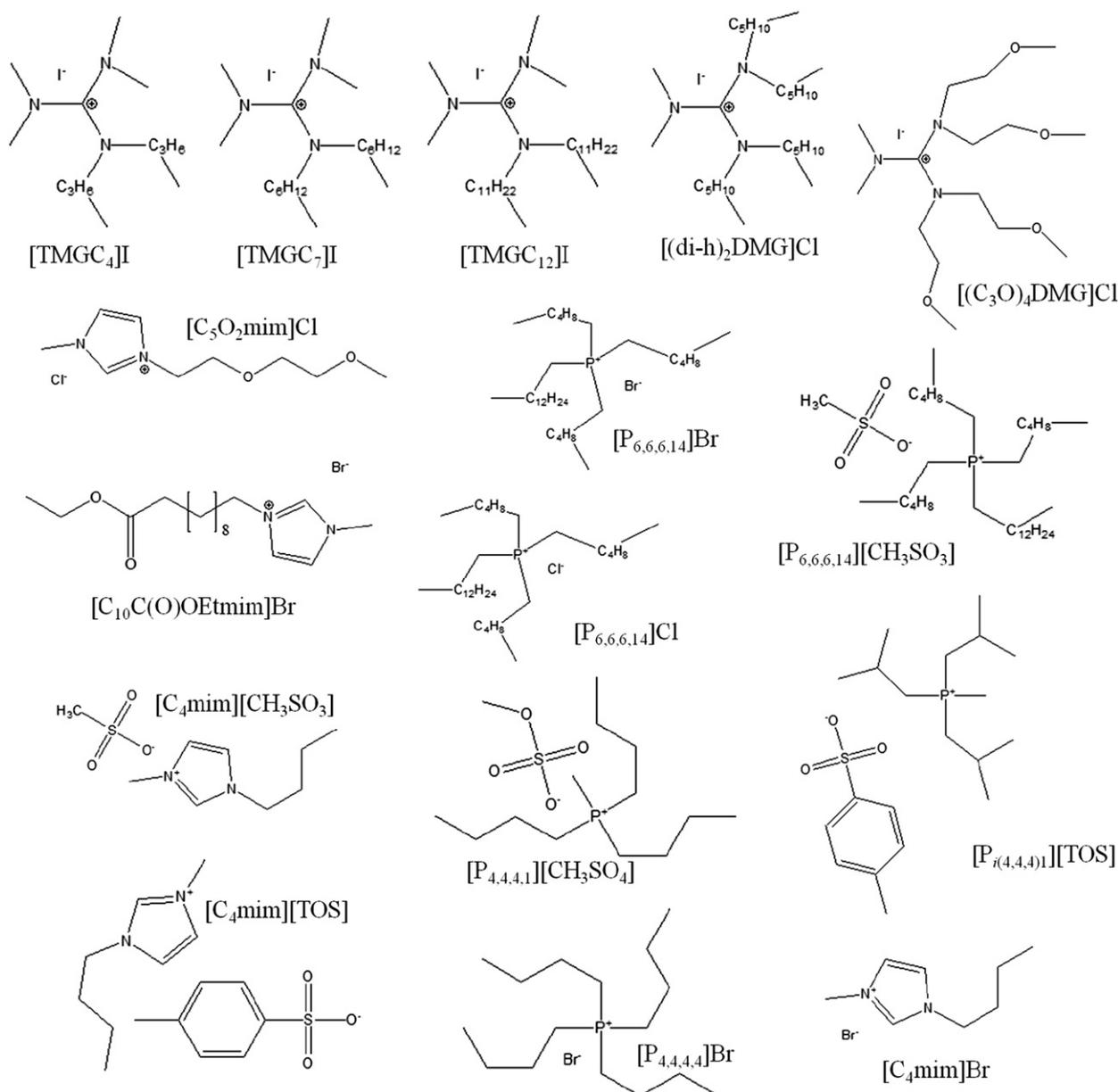


Fig. 1. Chemical structures of the ILs studied.

Table 1

Microtox[®] EC₅₀ values (mg L⁻¹) for all the ILs tested (guanidinium, imidazolium, and guanidinium) after 5, 15 and 30 min of exposure to the luminescent marine bacteria *Vibrio fischeri*, with respective 95 percent confidence limits (in brackets) obtained in the fit of the data.

Ionic liquid	EC ₅₀ (mg L ⁻¹) 5 min (lower limit; upper limit)	EC ₅₀ (mg L ⁻¹) 15 min (lower limit; upper limit)	EC ₅₀ (mg L ⁻¹) 30 min (lower limit; upper limit)
[TMGC ₄]I	61.20 (51.00; 71.40)	30.60 (20.40; 51.00)	–
[TMGC ₇]I	4.96 (1.24; 17.36)	3.72 (0.00; 24.80)	–
[TMGC ₁₂]I	26.00 (15.60; 52.00)	15.60 (5.20; 57.20)	–
[(C ₃ O) ₄ DMG]Cl	139.17 (106.43; 180.11)	98.24 (65.49; 147.36)	–
[(di-h) ₂ DMG]Cl	8.47	3.81	–
[C ₅ O ₂ mim]Cl	34.76 (11.59; 75.31)	40.55 (5.79; 144.83)	–
[C ₁₀ C(O)OEtMim]Br	5.51 (4.03; 7.54)	4.49 (1.67; 12.08)	–
[P _{6,6,6,14}]Br	i.d.	6.38	–
[P _{6,6,6,14}][CH ₃ SO ₃]	i.d.	7.43 (3.68; 15.23)	2.67 (0.87; 8.26)
[P _{6,6,6,14}]Cl	i.d.	7.10 (1.13; 44.40)	2.95 (0.92; 11.49)
[P _{4,4,4,1}][TOS]	169.60 (84.80; 275.60)	169.60 (21.20; 1462.80)	–
[P _{4,4,4,1}][CH ₃ SO ₄]	–	237.60 (99.18; 569.60)	232.20 (75.04; 720.10)
[P _{4,4,4,4}]Br	216.00 (21.60; 1382.40)	172.80 (0.00; 3218.40)	–
[C ₄ mim]Br	1651.68 (1228.03; 2221.48)	735.93 (494.69; 1094.83)	–
[C ₄ mim][CH ₃ SO ₃]	1123.54 (645.87; 1955.26)	901.99 (435.22; 1717.93)	–
[C ₄ mim][TOS]	957.60 (604.20; 1517.50)	653.22 (511.00; 835.10)	–

i.d.—impossible to determine.

with a large molecular size). Despite the inexistence of literature toxicity data for *Vibrio fischeri*, these guanidinium-based ILs were previously tested in the human colon cancerous cells *CaCo-2* (Frade et al., 2009) and the same trend was observed for these cells. [TMGC₄]I is considered as non toxic, but the EC₅₀ values reported for [TMGC₇]I and [TMGC₁₂]I were, respectively < 750 and 955 μM (Frade et al., 2009). Also for this biological system the same “cut-off” effect, which supports our results was observed.

The EC₅₀ values for [TMGC₇]I and [(di-h)₂DMG]Cl, presented in the same table, can be used to study the effect on the toxicity of the number of long alkyl chains in the guanidinium core. It was not possible to compare directly the influence of TMG- and DMG-based ILs because those have distinct anions, which may be responsible for significant differences in the ILs' toxicity Matzke et al., 2007; Gonçalves et al., 2011. However, and despite the difference of one carbon in the alkyl chains of [TMGC₇]I and [(di-h)₂DMG]Cl, the toxicity results described for both the ILs in Table 1, seem to indicate that the increase in the number of long alkyl chains (2 alkyl chains for [TMGC₇]I and four alkyl chains for [(di-h)₂DMG]Cl) is responsible for a slight increase in the toxicity (the EC₅₀=3.72 (0.00; 24.80) for the [TMGC₇]I and EC₅₀=3.81 at 15 min of exposure). This could be explained by the increase in the hydrophobic/lipophilic nature of the cation core, when the number of substituted alkyl chains was enhanced. According to the hazard ranking described by Passino's group (Passino and Smith, 1987) and already used (Table 2), the level of toxicity for both the ILs is not different, since both are considered as “slightly toxic”. Again, this seems to be in good agreement with the results reported by Frade et al. (2007, 2009) for the cytotoxicity data towards the human colon carcinoma cells (*CaCo-2* cells and *HT-29*).

Considering the oxygenation of the side chain of the cation, and aiming at understanding the effect of these oxygen groups in different cation cores, two different imidazolium-based ILs and one guanidinium were also studied. Table 1 shows the toxicity results for [(C₃O)₄DMG]Cl, [C₅O₂mim]Cl and [C₁₀C(O)OEt_{mim}]Br for 5 and 15 min of exposure time. The guanidinium can be classified as “practically harmless”, [C₅O₂mim]Cl can be classified as “moderately toxic” while [C₁₀C(O)OEt_{mim}]Br is “slightly toxic” to *Vibrio fischeri*, according to literature (Passino and Smith, 1987). For these compounds no statistical differences are observed between the two exposure times and no other works reporting their toxicological profiles towards different organisms or (biological) test systems were described. However, it was expected that the inclusion of those oxygenated groups in the side chains leads to the decrease in their toxicity as was explained by Frade and Afonso (2010). These authors reported that the introduction of the group (O–C=O), can be considered advantageous in the reduction of the ILs' toxicity, making them biocompatible. This behavior is not exclusive for the guanidinium- or imidazolium-based ILs, as described in different works (Jastorff et al., 2005; Matzke et al., 2010; Pernak et al., 2001; Petkovic et al., 2011; Pretti et al., 2006; Putz et al., 2007; Samori et al., 2010; Stolte et al., 2007b). In this context, a compilation of toxicity data reported in

Table A.1 (Appendix A) was carried aiming at showing that the effect of the oxygenation of the side chain is normally independent of the cation core and the biologic test system used.

On the other hand, the same Table 1 shows the results for various phosphonium-based ILs conjugated with different anions and alkyl chains, for 5, and 15 min of exposure. The toxicity results obtained for the phosphonium family, suggest that the long alkyl chains promote higher toxic effects towards the bacterium, which is shown for example in the toxicity differences observed for [P_{6,6,6,14}]Br EC₅₀=6.38 mg L⁻¹ and [P_{4,4,4,4}]Br EC₅₀=172.80 mg L⁻¹, respectively. This behavior falls into the conventional “side chain effect” previously discussed (Matzke et al., 2010). These compounds had previously been studied by Couling et al. (2006) and although a good agreement was observed for [P_{6,6,6,14}]Br its value for [P_{4,4,4,4}]Br of 0.51 mg L⁻¹ seems to be far underestimated and does not make sense in what concerns the effect of the alkyl chain elongation.

At the same table are reported EC₅₀ values for the [P_{6,6,6,14}] cation moiety, conjugated with different anions, such as bromide, Br, chloride, Cl, and methanesulfonate, [CH₃SO₃]. In this case, the anions seem to have an insignificant influence in the EC₅₀ results, independent of the exposure time. The toxicity in this case, was also determined for 30 min of exposure time—Table 1. It is clear from the results of Table 1 that the increase in the time of exposure of the *Vibrio fischeri* to ILs is responsible for the increase on toxicity. This can be shown by the comparison between the toxicity values described for [P_{6,6,6,14}]Cl and [P_{6,6,6,14}][CH₃SO₃]. The marginal influence of the anions was also shown for other systems in literature (Ranke et al., 2004; Stolte et al., 2007b). These results can be explained on one hand, by the poor affinity of the anions to the *Vibrio fischeri* structure and, on the other hand, by the overlapping effect of the long alkyl chains substituted in the cation core.

The remaining results reported in this table show the effect of three different imidazolium-based ILs and phosphonium with short alkyl chains, composed by three different anions. In this context, and comparing the effect of the phosphonium and imidazolium families, it was demonstrated that the toxic effect of the acyclic family is significantly higher when compared to the toxic effect of the imidazolium-cation core. This is shown when comparing the results here reported for the pairs [P_{4,4,4,4}]Br/[C₄mim]Br, [P_{6,6,6,14}][CH₃SO₃]/[C₄mim][CH₃SO₃], and [P_{4,4,4,1}][TOS]/[C₄mim][TOS]. These results support those previously reported by other authors (Couling et al., 2006; Stolte et al., 2007b). [P_{4,4,4,1}][CH₃SO₄] was also studied, however, due to the differences on the alkyl chain and anion moiety, a comparison was not possible since this work is the first reference describing its toxic effect towards the luminescent bacterium *Vibrio fischeri*. However, it was possible to observe that, one more time, the exposure time is an important condition in what concerns the toxicity effects of the ILs in general (Table 1). Also for the imidazolium cation, the effect of three different anions was assessed. It was observed that the anion effect is more pronounced for this cation than for the phosphonium, in particular for the [CH₃SO₃] anion, probably due to the short alkyl chains of the cation core.

Table 2

Description of the hazard ranking described by Passino and Smith (1987).

Description	Concentration of IL (mg L ⁻¹)
Practically harmless	100–1000
Moderately toxic	10–100
Slightly toxic	1–10
Highly toxic	0.1–1

4. Conclusions

The present study was focused on the toxicity determination of various guanidinium-, phosphonium- and imidazolium-based ILs towards the bioluminescent marine bacteria *Vibrio fischeri*. The guanidinium-based ILs seem to follow the established trend that the increase in the alkyl chain length ([TMGC₄]I and [TMGC₇]I) promotes the increase in the toxicity of the IL, normally designated by “side chain effect”, but seems to suffer also the “cut-off” effect ([TMGC₁₂]I).

The introduction of ether or ester groups in the IL side chain leads to the decrease of the toxicity, independent of the cation core (phosphonium or guanidinium).

The generality of the ILs studied (imidazolium, guanidinium and phosphonium) are shown to be “moderately” or “slightly toxic” towards the marine bacteria. The toxicity for the phosphonium-based ILs was shown to increase with the alkyl chain from [P_{4,4,4,4}] to [P_{6,6,6,14}]. For the [P_{6,6,6,14}] cation the anion effect on the toxicity was observed to be residual. Finally, according to the results here reported was possible to conclude that the phosphonium-based ILs are more toxic than the analog imidazolium-based ILs (same anion and alkyl chain length).

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Appendix A. Supplementary toxicological data from literature

Supplementary materials associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2011.10.006.

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