Eduardo Perales, Laura Lomba, María García-Escudero, Esther Sarasa, Carlos E. Lafuente and Beatriz Giner*

Toxicological study of some ionic liquids

https://doi.org/10.1515/gps-2017-0031
Received February 23, 2017; accepted June 6, 2017; previously published online September 5, 2017

Abstract: The increasing interest in the development of new environment-friendly solvents has led to the synthesis of new materials that minimize the impact of solvents on the environment. However, most of the published studies on green solvents focus primarily on their physicochemical properties, with limited emphasis on their toxicological risk in the environment. In this study, the acute toxicities of five ionic liquids, 1-propylpyridinium tetrafluoroborate, 1-butylpyridinium tetrafluoroborate, 1-butyl-2-methylpyridinium tetrafluoroborate, 1-butyl-3-methylpyridinium tetrafluoroborate and 1-butyl-4-methylpyridinium tetrafluoroborate, on *Vibrio fischeri* and *Daphnia magna* are evaluated. In the latter bioassay, the presence and position of a methyl group on the pyridinium ring or the length of the chain attached to the nitrogen atom seem to be the key factors for toxicity. In the *Vibrio fischeri* study, the alkyl chain attached to the nitrogen atom has a considerable influence on EC_{50} values. Moreover, quantitative structure activity relationship studies are performed to relate their physicochemical properties with their acute toxicity.

Keywords: acute toxicity; *Daphnia magna*; green chemistry; ionic liquids; *Vibrio fischeri*.

1 Introduction

Due to their unique properties, such as negligible vapor, pressure, high thermal stability, ability to solvate compounds of widely varying polarities, and the possibility of tailoring their properties, ionic liquids (ILs) have attracted growing interest [1] because they are considered "green solvents" and ideal substitutes for volatile organic compounds (VOCs) [2–6].

However, recent studies have shown that ILs may not keep all the characteristics required for a "perfect" green solvent, i.e. to be biodegradable, recyclable, cheap, readily available and especially nontoxic. The first warning signs came when the toxicities of some ILs were carefully studied and compared to those of the solvents they were supposed to replace [7]. In some cases, the toxicities of the ILs were much higher than expected [8]. Although a huge amount of environmental toxicological information on ILs exists [8–14], data on the toxicology of most of these compounds have not yet been fully evaluated in standardized bioassays.

Thus, a toxicological study in the aquatic media of five members of the N-alkylpyridinium tetrafluoroborate family has been performed. The selected ILs were the following: 1-propylpyridinium tetrafluoroborate (P), 1-butylpyridinium tetrafluoroborate (B), 1-butyl-2-methylpyridinium tetrafluoroborate (BM_2), 1-butyl-3-methylpyridinium tetrafluoroborate (BM_3) and 1-butyl-4-methylpyridinium tetrafluoroborate (BM_4). This selection allows for systematically evaluating the effect of the lengths and positions of the substituents on the ring. To the best of our knowledge, no previous studies on the toxicity of these ILs in *Daphnia magna* (*D. magna*) and *Vibrio fischeri* (*V. fischeri*) have been reported. Additionally, a quantitative structure activity relationship (QSAR) study was performed using published physicochemical properties of the studied ILs [15–19] and the toxicological and physicochemical data of previous ILs [20–32].

2 Materials and methods

2.1 Chemicals

B, BM_2, BM_3 and BM_4 with purities of 99% and P with a purity of 98% were provided by IoliTec (Heilbronn, Germany), dried for 24 h under vacuum (ca. 0.05 kPa), and stored in a desiccator.

2.2 Inhibition of bioluminescence in *V. fischeri*

Lyophilized *V. fischeri* (strain NRRL-B-11177) were purchased from Macherey-Nagel (Düren, Germany). The experimental

***Corresponding author: Beatriz Giner,** Grupo de investigación GreenLife, Facultad de Ciencias de la Salud, Universidad San Jorge, Campus Universitario, Autovía A23 km 299, 50830 Villanueva de Gállego, Zaragoza, Spain, e-mail: bginer@usj.es
Eduardo Perales, Laura Lomba, María García-Escudero and Esther Sarasa: Grupo de investigación GreenLife, Facultad de Ciencias de la Salud, Universidad San Jorge, Campus Universitario, Autovía A23 km 299, 50830 Villanueva de Gállego, Zaragoza, Spain
Carlos E. Lafuente: Departamento de Química Física, Facultad de Ciencias, Universidad de Zaragoza, 50009 Zaragoza, Spain

bioluminescence inhibition tests were performed in triplicate with re-activated bacteria according to UNE-EN ISO 2009 [33] norm, with positive (phenol, 40 mg l^{-1}) and negative controls [34]. A stock solution of each of the five ILs at concentrations of 2% NaCl were prepared and the pH adjusted to 7–7.5 with 0.1 mol l^{-1} HCl or 0.1 mol l^{-1} NaOH.

Aliquots of 0.5 ml of the reactivated bacterial suspension was transferred to each cuvette and kept for 10 min at 15°C. The first measurements were then taken to obtain the initial luminescence with a luminometer (Biofix® Lumi-10, Macherey-Nagel, Düren, Germany), equipped with an ultra-fast single photon counter detector covering the 380–660 nm spectral range, using the acute mode (Biotox B). Subsequently, 0.5 ml of each of the tested dilutions was added to the cuvette. After 30 min, the inhibition of luminescence was measured again and the percentages of inhibition were calculated.

2.3 *Daphnia magna* acute immobilization test

Daphnia magna vials (ref. DM090812, Vidrafoc, Barcelona, Spain) were stored at 4°C. OCDE 202 protocols were employed [35, 36] according to the test conditions. First, the media for the ephippia were prepared in accordance with the supplier specifications. Next, the eggs were incubated for 72 h at 20°C–22°C with 6000 lux in an incubator (model CH-0120D-AC/DC, TOXKIT, ECOTEST, Valencia, Spain). The obtained neonates were fed 2 h prior to the bioassay by adding a *Spirulina* vial.

Solutions of the studied ILs were prepared in stock solution with inorganic salts provided in vials by ECOTEST (Valencia, Spain). Positive controls were tested with $K_2Cr_2O_7$ (EC_{50} values range between 0.6 and 2.1 mg l^{-1}). Negative controls were accepted if <10% of the individuals were immobilized after the assay. The pH levels of the solutions were adjusted to fall between 7 and 7.5 using 0.1 mol l^{-1} NaOH or 0.1 mol l^{-1} HCl solutions. A total of 20 neonate organisms (aged <24 h) divided into four groups of five organisms were exposed to each concentration after 2 h of feeding. Daphnids were incubated in complete darkness for 24 h at 20°C–22°C.

The immobilization of the daphnids was measured according to the operating protocol. The daphnids were considered immobilized when they were unable to swim for 15 s after gentle stirring.

2.4 Statistics and graphical representation

The experimental results were fitted using the least squares method to EC_{50} values and standard deviations (SD):

$$\%I = 100/(1+10^{(a-\log c)b}), \quad (1)$$

where $\%I$ denotes % bioluminescence inhibition, c is the concentration (in mg l^{-1}) and a and b are adjustable parameters.

One-way ANOVA Tukey's multiple comparison tests were performed to compare between pairs of ILs in each biondicator. As for ethical approval, the biotests were carried out according to internationally accepted guidelines.

3 Results and discussion

The EC_{50} values obtained for *V. fischeri* and *D. magna* with their respective standard deviations are shown in Table 1. These results are graphically represented in Figures 1 and 2, respectively.

3.1 Toxicity of ILs in *V. fischeri*

All of the studied ILs slightly affected the bioluminescence of the bacteria, and differences in the EC_{50} values between most of the ILs could be detected. The action mechanism of the luminescence emission is related to the modification of protein and lipid biosynthetic pathways, which are relevant in cellular respiration [37].

The most toxic chemical studied was BM_2 followed by BM_3, P, B and BM_4. All the EC_{50} values were significantly different except BM_2 and BM_3, BM_2 and P, BM_3 and P, BM_4, and B (ANOVA, Tukey's multiple comparison test). The toxicity increased as the length of the substituent at nitrogen decreased. The presence of an extra methyl substituent in position 4 did not seem to substantially affect the toxicity; however, an extra methyl substituent in positions 2 or 3 increased the toxicity. Clearly, the closer the methyl substituent is to the nitrogen atom, the more toxic the IL.

According the Passino and Smith classification [38], which evaluates the acute toxicity of chemical compounds in the aquatic environment, BM_2, BM_3 and P could be considered as practically harmless for *V. fischeri* as their EC_{50} values with this biondicator are in the range of 100–1000 mg l^{-1}. Furthermore, B and BM_4 are clearly harmless because their EC_{50} values are higher than 1000 mg l^{-1}.

Table 1: Physicochemical data for ILs and their corresponding EC_{50} values on luminescence inhibition of *Vibrio fischeri* and immobilization of *Daphnia magna*.

Chemical	σ (mN · m^{-1})	C_p (J · K^{-1} mol^{-1})	μ (mPa · s)	*V. fischeri*		*D. magna*	
				EC_{50} (mg l^{-1})	n	EC_{50} (mg l^{-1})	n
P	50.97 [19]	363 [19]	119.5 [19]	790 ± 4.6	20	55 ± 4.3	21
B	46.58 [17]	395 [17]	160.3 [17, 18]	1300 ± 5.4	39	43 ± 5.6	27
BM_2	45.66 [16]	380 [16]	389.4 [16, 18]	540 ± 4.7	20	27 ± 3.9	24
BM_3	44.86 [15]	412 [15]	176.9 [15]	760 ± 4.7	27	25 ± 3.5	21
BM_4	45.45 [15]	414 [15]	199.9 [15, 18]	1400 ± 5.1	16	15 ± 3.4	18

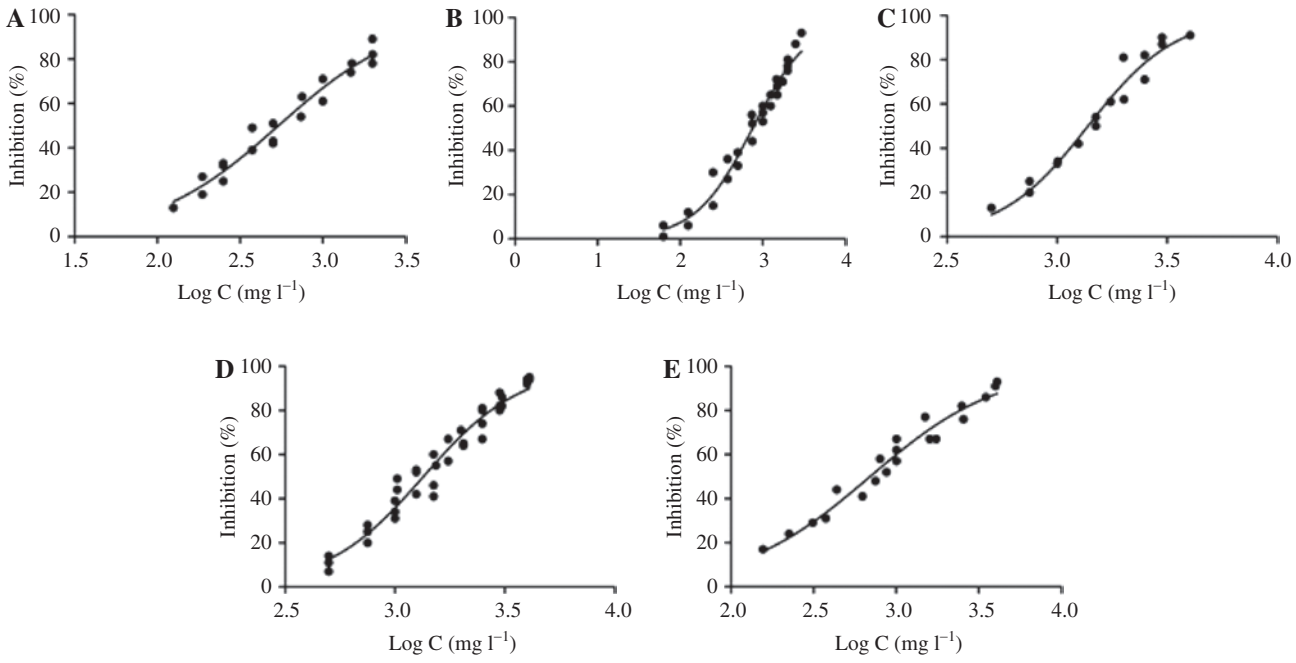

Figure 1: Dose-response in *Vibrio fischeri* for the studied ionic liquids (A) BM_2, (B) BM_3, (C) BM_4, (D) B, (E) P.

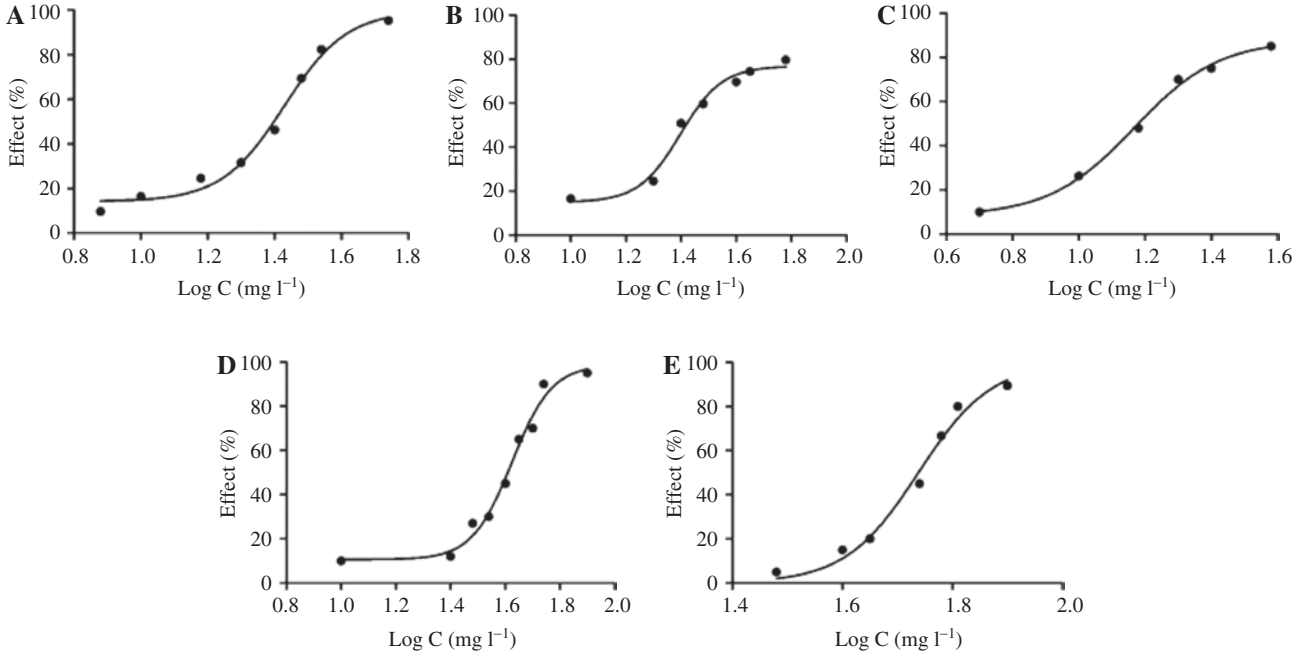

Figure 2: Dose-response in *Daphnia magna* for studied ionic liquids (A) BM_2, (B) BM_3, (C) BM_4, (D) B, (E) P.

Table 2 provides *V. fischeri* toxicity data of some conventional solvents for comparison [39] and Figure 3 shows this information in *V. fischeri*. There are solvents that are more harmful for this biondicator than the studied ILs, i.e. o-xylene, toluene or phenol. These chemicals can be classified as slightly or moderately toxic, according the Passino and Smith classification [38]. However, there are also VOCs that are much less harmful, such as methanol, acetone or dichloromethane, which are harmless for *V. fischeri*, such as BM_4 or B. Regarding other ILs [21, 22] against the studied ones, just 1-propyl-3-methylimidazolium tetrafluoroborate is less toxic for *V. fischeri* than the selected ILs. The rest of the exposed ILs are more harmful for this biondicator. According to the Passino and

Table 2: EC_{50} values and their codification (Figures 3 and 4) for different traditional solvents and imidazolium based ILs in *Vibrio fischeri* and *Daphnia magna* bioassays during 30 min and 24 h exposure, respectively.

Code	Chemical compound	EC_{50} (mg l^{-1})	
		V. fischeri	*D. magna*
1	O-xylene	9.2 [39]	
2	Phenol	30.8 [39]	
3	Toluene	31.7 [39]	7 [40]
4	Benzene	108 [39]	
5	Chloroform	1199 [39]	573 [41]
6	Dichloromethane	2532 [39]	223 [21]
7	Acetone	19,311 [39]	13,615 [21]
8	Methanol	101,068 [39]	22,682 [21]
9	1,4-Dioxane		8450 [42]
10	Ethanol		9847 [21]
11	Isopropanol		9959 [21]
12	Acetonitrile		10,076 [21]
13	1-Decyl-3-methylimidazolium tetrafluoroborate	0.204 [22]	
14	1-Nonyl-3-methylimidazolium tetrafluoroborate	1.55 [22]	
15	1-Octyl-3-methylimidazolium tetrafluoroborate	7.25 [22]	
16	1-Hexyl-3-ethylimidazolium tetrafluoroborate	37.8 [22]	
17	1-Heptyl-3-methylimidazolium tetrafluoroborate	73.8 [22]	
18	1-Butyl-3-ethylimidazolium tetrafluoroborate	151 [22]	
19	1-Butyl-3-methylimidazolium tetrafluoroborate	284 [21]	13.9 [21]
20	1-Pentyl-3-methylimidazolium tetrafluoroborate	331 [22]	
21	1-Pentyl-3-ethylimidazolium tetrafluoroborate	350 [22]	
22	1-Hexyl-3-methylimidazolium tetrafluoroborate	385 [22]	3.4 [21]
23	1-Propyl-3-methylimidazolium tetrafluoroborate	1850 [22]	
24	1-Octyl-3-methylimidazolium chloride		0.8 [21]
25	1-Octyl-3-methylimidazolium tetrafluoroborate		1.3 [21]
26	1-Hexyl-3-methylimidazolium chloride		2.5 [21]
27	1-Butyl-3-methylimidazolium chloride		12.4 [21]
28	1-Butyl-3-methylimidazolium bromide		13.2 [21]
29	1-Butyl-3-methylimidazolium hexafluorophosphate		31 [29]

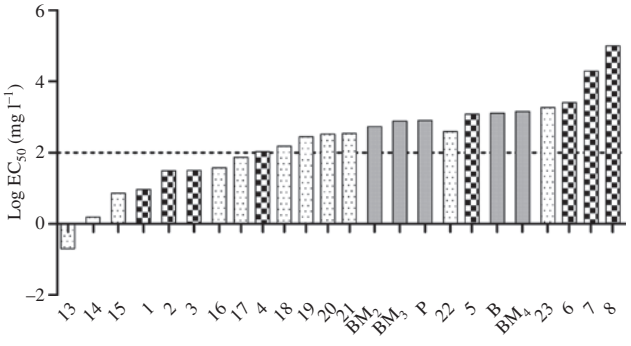

Figure 3: Log EC_{50} graphical comparison of toxicity in *Vibrio fischeri*. The codification is shown in Table 2. Pointed bars correspond with imidazolium ILs, squared bars correspond with traditional solvents and grey bars correspond with the studied ILs. The dashed line shows the limit between slightly toxic and practically harmless compounds according to Passino and Smith classification.

Smith classification [38], their aquatic toxicity includes several ranges of toxicity, i.e. 1-decyl-3-methylimidazolium tetrafluoroborate and 1-octyl-3-methylimidazolium chloride could be considered as highly toxic for *D. magna*. However, 1-hexyl-3-methylimidazolium tetrafluoroborate or 1-pentyl-3-ethylimidazolium tetrafluoroborate can be considered practically harmless.

Furthermore, according to the literature, pyridinium ILs in bioluminescence assay with *V. fischeri* are less toxic than the corresponding imidazolium, with the same anion, in QSPR studies [43]. This statement can be confirmed with the EC_{50} values of the Table 2 [21, 22], where almost all of the imidazolium ILs have lower EC_{50} values than the studied pyridinium compounds. For example, comparing the toxicity of the cations 1-butyl-3-methylimidazolium and 1-butyl-3-methylpyridinium with the same anion tetrafluoroborate, the imidazolium is more toxic than the pyridinium IL. It is also interesting to note that, although a direct correlation between the length of the alkyl chain of methylimidazolium-based ILs and toxicity has been previously shown [44], our results do not follow this trend. Thus, extrapolations of behavior in this sense are a matter of concern.

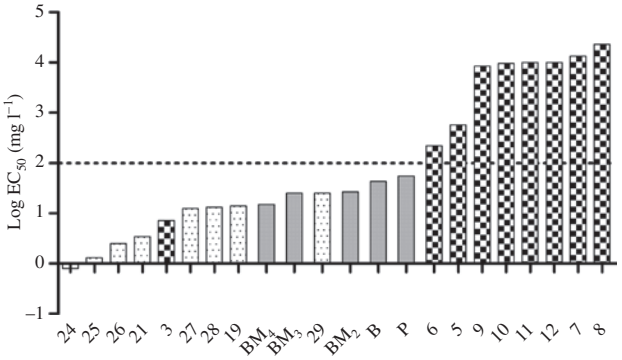

Figure 4: Log EC_{50} graphical comparison of toxicity in *Daphnia magna*.
The codification is shown in Table 2. Pointed bars correspond with imidazolium ILs, squared bars correspond with traditional solvents and grey bars correspond with the studied ILs. The dashed line shows the limit between slightly toxic and practically harmless compounds according to Passino and Smith classification.

3.2 Toxicity of ILs in *Daphnia magna*

Each of the studied ILs strongly affected the mobility of *D. magna*. Although the mechanisms of action are still unknown, several authors have noted that ILs have the potential to cause enzyme inhibition, disruption of membrane permeability, structural damage and oxidative stress [9, 45].

The most toxic IL studied was BM_4, followed by BM_2/BM_3, B and P. Only the BM_2 and BM_3 EC_{50} values are not significantly different from each other (ANOVA, Tukey's multiple comparison test). In general, the presence of an extra methyl substituent increased the observed toxicity. As it pertains to the length of the substituent of at nitrogen, B is moderately more toxic than P. All of these ILs are included in the category of "slightly toxic" for *D. magna*, according the Passino and Smith classification [38].

The *D. magna* toxicities of conventional solvents are shown in Table 2 [21, 40–42] and compared to one another in Figure 4. The EC_{50} values of the studied ILs are lower to chloroform and dichloromethane, although higher than toluene. In comparison with other ILs, as shown in Table 2 [21, 29], pyridinium compounds are, at least, as toxic for the aquatic environment as imidazolium ILs, i.e. 1-butyl-3-methylimidazolium tetrafluoroborate, 1-butyl-3-methylimidazolium chloride and 1-butyl-3-methylimidazolium bromide. These are in the same range of aquatic toxicity than the studied pyridinium chemicals according the Passino and Smith classification [38]. Others, like 1-hexyl-3-methylimidazolium chloride and 1-octyl-3-methylimidazolium chloride, are considered as moderately toxic or highly toxic, respectively. These results confirmed the Couling QSAR modeling [43] as those equations predicted that the toxicity increases with the number of aromatic atoms in the cation ring.

In general, our results agree with the reported toxicity behavior of ILs in *D. magna*. There is a well-established link between toxicity and alkyl-chain length [8]. Furthermore, our results also agree with quantitative structure–toxicity relationship (QSTR) modelling, which predicts an increase in toxicity due to the methylation of the aromatic carbons [46].

3.3 Toxicity of ILs according other biomodels

There are not much available data about these ILs with biomodels corresponding to other trophic levels. Ranke et al. [47] analyzed BM_2, BM_3, BM_4 and B in IPC-81 rat cell model during 48 h, obtaining their EC_{50} values. According to these results, the distance of the methyl group to the nitrogen in the pyridinium cation does not have a direct relationship with the toxicity, with BM_4 being the most cytotoxic IL (221 mg l^{-1}), followed by B (322 mg l^{-1}), BM_2 (421 mg l^{-1}) and BM_3 (473 mg l^{-1}). On the other side, there are also previous data for the acetylcholinesterase inhibition assay for BM_2, BM_3 and B [8]. In this case, the progression of the toxicity is in full agreement with our results in *V. fischeri*. Stock et al. [48] also performed the acetylcholinesterase inhibition assay with several ILs, including two pyridinium-based ILs, 1-butyl-3-methylpyridinium tetrafluoroborate (BM_3) and hexafluorophosphate. The EC_{50} values found for this biomarker are very similar to each other (8 mg l^{-1} and 8.3 mg l^{-1}, respectively), and are also lower than in our studied biomodels. Thus, there is no clear relationship among biomodels with this type of ILs. These observations indicate the high disparity of the results in this type of solvents and, therefore, remarking the relevance to evaluate the toxicity in several biomodels to ensure the harmlessness of future industrial solvents [49].

3.4 QSAR studies

The development of QSAR helps in understanding how the toxicities, properties, or activities of chemicals vary with structural composition. We have applied the QSAR concept to provide a mathematical model derived from the available physicochemical properties [50, 51], with respect to two specific endpoints in *V. fischeri* and *D. magna*. As a first approximation, the relationships between several physicochemical properties (viscosity, surface tension

Table 3: Physicochemical and toxicological (*Vibrio fischeri*) properties used in the QSAR for previously studied ILs.

Chemical	*V. fischeri* EC_{50} (mg l^{-1})	σ (mN · m^{-1})	C_p (J · K^{-1} mol^{-1})	η (mPa · s)
1-Ethyl-3-methylimidazolium tetrafluoroborate	6236.06 [20]	54.4 [23]	308.1 [26]	66.5 [27]
1-Butyl-3-methylimidazolium tetrafluoroborate	284.54 [21]	46.6 [24]	367.1 [26]	279.86 [28]
1-Hexyl-3-methylimidazolium hexafluorophosphate	46.18 [21]	43.4 [24]	425 [26]	585 [24]
1-Octyl-3-methylimidazolium tetrafluoroborate	7.25 [22]	32.7 [25]	497.8 [26]	325 [25]
1-Octyl-3-methylimidazolium hexafluorophosphate	3.03 [21]	36.5 [24]	851 [26]	682 [24]

Table 4: Physicochemical and toxicological (*Daphnia magna*) properties used in the QSAR for previous studied ILs.

Chemical	*D. magna* EC_{50} (mg l^{-1})	σ (mN · m^{-1})	C_p (J · K^{-1} mol^{-1})	η (mPa · s)
1-Butyl-3-methylimidazolium bromide	13.2 [21]	39.4 [30]	311 [26]	1486.49 [28]
1-Butyl-3-methylimidazolium tetrafluoroborate	13.9 [21]	46.6 [24]	367.1 [26]	279.86 [28]
1-Butyl-3-methylimidazolium hexafluorophosphate	31 [29]	43 [31]	408.7 [26]	273.94 [31]
1-Hexyl-3-methylimidazolium tetrafluoroborate	3.4 [21]	40.4 [32]	431 [26]	220 [32]
1-Octyl-3-methylimidazolium tetrafluoroborate	1.3 [21]	32.7 [25]	497.8 [26]	325 [25]

and heat capacity, at 298.15 K) and the acute effective concentrations for the *V. fischeri* bioindicator and *D. magna* were investigated. Then, a multiregression expression was derived. For this study, we have included both the toxicological information obtained in this work and previous bibliographic data of other ILs in order to increase the number of input data and make the model reliable. To select the properties that give higher signals in the multiregression analysis, we performed an initial screening using different combinations of the physicochemical properties. Finally, the selected properties were surface tension (mN · m^{-1}) and viscosity (mPa · s). All of the data required for the calculations are presented in Table 1 for the studied compounds [15–19], and Tables 3 (for *V. fischeri*) and 4 (for *D. magna*) for the previous referenced ILs [20–32].

The simple relationships between the selected properties and acute effective concentrations for the *V. fischeri* bioindicator and *D. magna*, as well as the R coefficient, are shown in the following individual structure-projected toxicity-parameter expressions:

$$\mathrm{Log\ EC}_{50\,V.\,fischeri}^{\mathrm{QSAR1}} = 0.15\sigma - 4.4 \quad R = 0.81 \tag{2}$$

$$\mathrm{Log\ EC}_{50\,V.\,fischeri}^{\mathrm{QSAR2}} = -0.0057c_p + 4.9 \quad R = 0.65 \tag{3}$$

$$\mathrm{Log\ EC}_{50\,V.\,fischeri}^{\mathrm{QSAR3}} = -0.0044\eta + 3.7 \quad R = 0.68 \tag{4}$$

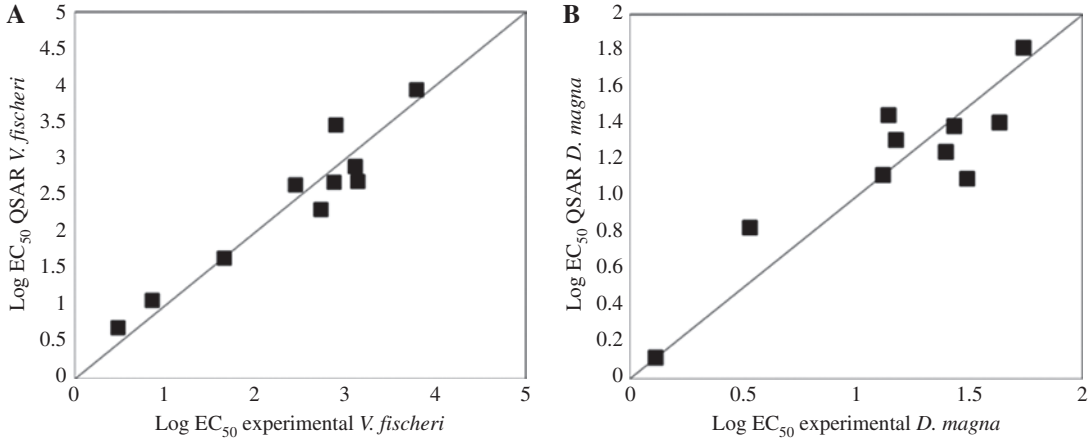

Figure 5: Plots of predicted vs. experimental values of log EC_{50} as calculated through multiregression analysis in *Vibrio fischeri* (A) and *Daphnia magna* (B).

$$\text{Log EC}_{50\ D.\ magna}^{\text{QSAR1}} = 0.087\sigma - 2.6 \quad R = 0.76 \tag{5}$$

$$\text{Log EC}_{50\ D.\ magna}^{\text{QSAR2}} = -0.0065c_p + 3.8 \quad R = 0.40 \tag{6}$$

$$\text{Log EC}_{50\ D.\ magna}^{\text{QSAR3}} = -0.00015\eta + 1.2 \quad R = 0.014 \tag{7}$$

The global multiregression expressions, along with the statistical indicator R^2 are as follows:

$$\text{Log EC}_{50\ V.\ fischeri}^{\text{QSAR}} = -1.7 + 0.10\sigma - 0.0022\eta \quad R^2 = 0.91 \tag{8}$$

$$\text{Log EC}_{50\ D.\ magna}^{\text{QSAR}} = -3.1 + 0.096\sigma + 0.00031\eta \quad R^2 = 0.81 \tag{9}$$

In Figure 5, plots of the experimental values versus those calculated with the regression models are shown. It may be noted that small correlations for direct relationships between each physicochemical property are found, except for surface tension that seems to carry the strongest signal. The property is closely related to intermolecular interactions between the ions. With regards the global multiregression analysis, despite the small sample size and the very different molecular structures tested, the overall correlation using basic QSAR displays acceptable values for the coefficients of determination, with better results found for the bioindicator *V. fisheri*. The IL showing the highest deviations in the correlation study are *P* in the case of *V. fischeri* and 1-butyl-3-methylimidazolium hexafluorophosphate in the case of *D. magna*.

4 Conclusions

Toxicity of several pyridinium-based ILs with tetrafluoroborate anions was evaluated with *D. magna* and *V. fischeri*. As it pertains to *V. fischeri* toxicity, the shorter the alkyl chain is, the more toxic the molecule is. The presence of a methyl group in the aromatic ring increases the toxicity of the ILs, with the exception of BM_4, for which no relevant changes is observed with respect to the parent compound. Specifically, the methyl group in position 2 induces higher toxicity than when it is placed in position 3.

In the case of *D. magna* toxicity, longer alkyl chains lead to more toxic ILs. The presence of a methyl group on the aromatic ring increases the toxicity, with BM_4 being the most toxic compound. The position of the methyl substituent on the aromatic ring and the length of the alkyl attached to the nitrogen slightly influences the toxicity of these compounds in *D. magna*.

The QSAR study indicates that small correlations are found between the individual physicochemical properties of the ILs and (eco)toxicity, except for surface tension. The overall correlation allows the description of (eco)toxic behavior with high coefficient of determination values.

Acknowledgments: The GREENLIFE research group acknowledges financial support from MINECO (CTQ2013-44867-P), Dirección General de Aragón (Grupo consolidado E105), and FEDER. We also gratefully acknowledge the support provide by Pinares de Venecia División Energética and Brial (ENATICA). Both business groups are committed to sustainable developments through environmental studies.

Conflict of interest statement: The authors declare no conflicts of interest.

References

[1] Zhang S, Sun N, He X, Lu X, Zhang X. *J. Phys. Chem. Ref. Data*. 2006, 35, 1475–1517.
[2] Earle MJ, Seddon KR. *Pure Appl. Chem*. 2000, 72, 1397–1398.
[3] Rakita P. In *Ionic Liquids as Green Solvents,* Rogers RD, Seddon KR, Eds., American Chemical Society: Washington, DC, 2003.
[4] Rogers RD, Holbrey JD, Spear SK, Turner MB. *Abstr. Pap. Am. Chem. S.* 2004, 227, U310.
[5] Yang Z, Pan WB. *Enzyme Microb. Technol.* 2005, 37, 19–28.
[6] Zhao H, Xia HSQ, Ma PS. *J. Chem. Technol. Biotechnol.* 2005, 80, 1089–1096.
[7] Swatloski RP, Holbrey JD, Rogers RD. *Green Chem.* 2003, 5, 361–363.
[8] Pham TPC, Cho CW, Yun YS. *Water Res*. 2010, 44, 352–372.
[9] Bernot RJ, Brueseke MA, Evans-White MA, Lamberti GA. *Environ. Toxicol. Chem*. 2005, 24, 87–92.
[10] Bernot RJ, Kennedy EE, Lamberti GA. *Environ. Toxicol. Chem*. 2005, 24, 1759–1765.
[11] Bubalo MC, Radosevic K, Redovnikovic IR, Halambek J, Sreck VG. *Ecotoxicol. Environ. Saf.* 2014, 99, 1–12.
[12] Cho CW, Pham TPT, Jeon YC, Yun YS. *Green Chem.* 2008, 10, 67–72.
[13] Deetlefs M, Seddon KR. *Green Chem.* 2010, 12, 17–30.
[14] Stolte S, Matzke M, Arning J, Böschen A, Pitner WR, Welz-Biermann U, Jastorff B, Ranke J. *Green Chem.* 2007, 9, 1170–1179.
[15] Bandrés I, Giner B, Royo FM, Lafuente C. *J. Phys. Chem. B* 2008, 112, 3077–3084.
[16] Bandrés I, Pera G, Martín S, Castro M, Lafuente C. *J. Phys. Chem. B* 2009, 113,11936–11942.
[17] Bandrés I, Royo F, Gascón I, Castro M, Lafuente C. *J. Phys. Chem. B* 2010, 114, 3601–3607.
[18] Bandrés I, Alcalde C, Lafuente C, Atilhan M, Aparicio S. *J. Phys. Chem. B* 2011, 115, 12499–12513.
[19] Bandrés I, López MC, Castro M, Barbera J, Lafuente C. *J. Chem. Thermodyn*. 2012, 44, 148–153.
[20] Yu ZY, Mo LY, Zhang J, Liu SS. *Chemosphere*. 2016, 163, 452–460.

[21] García MT, Gatherwood N, Scamells PJ. *Green Chem.* 2005, 7, 9–14
[22] Ranke J, Mölter K, Stock F, Bottin-Weber U, Poczobutt J, Hoffmann J, Ondruschka B, Filser J, Jastorff B. *Ecotox. Environ. Safe.* 2004, 58, 396–404.
[23] Zhou ZB, Matsumoto H, Tatsumi K. *ChemPhysChem* 2005, 6, 1324–1332.
[24] Huddleston JG, Visser AE, Reichert WM, Willauer HD, Broker GA, Rogers RD. *Green Chem.* 2001, 3, 156–164.
[25] Restolho J, Serro AP, Mata JL, Saramago B. *J. Chem. Eng. Data* 2009, 54, 950–955.
[26] Paulechka YU. *J. Phys. Chem. Ref. Data* 2010, 39, 33108.
[27] Bogdanov MG, Kantelehner W. *Z. Naturforsch.* 2009, 64, 215–222.
[28] Kim KS, Shin BK, Lee H. *Korean J. Chem. Eng.* 2004, 21, 1010–1014.
[29] Perales E, García CB, Lomba L, Aldea L, García JI, Giner B. *Ecotox. Environ. Safe.* 2016, 132, 429–434.
[30] Lisal M. *J. Chem. Phys.* 2013, 139, 214701.
[31] AlTuwaim MS, Alkahldi KHA, Al-Jimaz AS, Mohammad AA. *J. Chem. Eng. Data* 2014, 59, 1955–1963.
[32] Beigi AAM, Abdous M, Yousefi M, Pourmortazavi SM, Vahid A. *J. Mol. Liq.* 2013, 177, 361–368.
[33] International Standard Organisation (ISO). *11348-3 Water Quality, Determination of the Inhibitory Effect of Water Samples on the Light Emission of Vibrio fischeri (Luminescent Bacteria Test), Part 3: Method Using Freeze-Dried Bacteria*. Geneva, Switzerland, 2009.
[34] Jennings VL, Rayner-Brandes MH, Bird DJ. *Water Res.* 2001, 35, 3448–3456.
[35] OC SE TG 202 2004 (European C2 method as described in the EU Regulation 440/2008).
[36] OECD 202 (1984) Guideline for Testing of Chemicals No. 202, Daphnia sp., acute immobilisation test and reproduction test. Paris, France.
[37] Onorati F, Mecozzi M. *Chemosphere* 2004, 54, 678–687.
[38] Passino DRM, Smith SB. *Environ. Toxicol. Chem.* 1987, 6, 901–907.
[39] Docherty KM, Kulpa CF. *Green Chem.* 2005, 7, 185–189.
[40] Galassi S, Mingazzini M, Vigano L, Cesareo D, Tosato ML. *Ecotoxicol. Environ. Saf.* 1988, 16, 158–169.
[41] Lilius H, Hästbacka T, Isomaa B. *Environ. Toxicol. Chem.* 1995, 14, 2085–2088.
[42] Bringmann G, Kühn R. *Z. Wasser. Abwass. For.* 1982, 15, 1–6.
[43] Couling DJ, Bernot RJ, Docherty KM, Dixon JK, Maginn EJ. *Green Chem.* 2006, 8, 82–90.
[44] Romero A, Santos A, Tojo J, Rodríguez A. *J. Hazard. Mater.* 2008, 151, 268–273.
[45] Yu M, Wang SH, Luo YR, Han YW, Li XY, Zhang BJ, Wang JJ. *Ecotoxicol. Environ. Saf.* 2009, 72, 1798–1804.
[46] Roy K, Das RN. *J. Hazard. Mater.* 2013, 254–255, 166–178.
[47] Ranke J, Müller A, Bottin-Webber U, Stock F, Stolte S, Arning J, Störmann R, Jastorff B. *Ecotoxicol. Environ. Saf.* 2007, 67, 430–438.
[48] Stock F, Hoffmann J, Ranke J, Störmann R, Ondruschka B, Jastorff B. *Green Chem.* 2004, 6, 286–290.
[49] Levet A, Bordes C, Clément Y, Mignon P, Morell C, Chermette H, Marote P, Lantéri P. *J. Mol. Model.* 2016, 22, 288.
[50] Lacrama AM, Putz MV, Ostafe V. *Int. J. Mol. Sci.* 2007, 8, 842–863.
[51] Putz MV, Lazea M, Sandjo LP. *Molecules*. 2011, 16, 6603–6620.

Bionotes

Eduardo Perales

Eduardo Perales obtained his degree in Pharmacy (2013) from the Universidad San Jorge. He also received a Master's degree in Environmental Management for Business (2014). Currently, he is a PhD student at the Universidad San Jorge and is part of the GREENLIFE research group. He is an author of three manuscripts in international journals and an author of 19 national and international congress publications. Furthermore, he has participated in one R&D&I project funded in competitive calls.

Laura Lomba

Laura Lomba holds degrees in Chemistry (2008) and Pharmacy (2013) and obtained a PhD Degree in Environmental Sciences at the Universidad San Jorge (2014). She is a lecturer at Universidad San Jorge and is part of the GREENLIFE research group. She is an author of 18 manuscripts in scientific journals and 32 publications of national and international congresses. Furthermore, she has participated in two R&D&I projects funded in competitive calls.

María García-Escudero

María García-Escudero received her degree in Pharmacy from Universidad San Jorge (Zaragoza, Spain). She holds a Master's degree in Pharmaceutical Industry and Parapharmacy from CESIF (Barcelona). She is an expert at Clinical Diagnostic Laboratory, Universidad Complutense (Madrid) and has experience with animal and human cell culture experiments. Her professional life is linked to the pharmaceutical industry sector, where she has developed her competencies in R&D, validation of manufacturing processes, and quality assurance.

Esther Sarasa

Esther Sarasa obtained her degree in Biochemistry at Universidad de Zaragoza in 2009. She is a laboratory technician at Universidad San Jorge and is part of the GREENLIFE research group. She is an author of 12 national and international congress publications and obtained two awards for the best poster. Her main research is focused on the ecotoxicological and toxicological characterization of drugs and solvents.

Carlos E. Lafuente

Carlos E. Lafuente studied Chemistry at the University of Zaragoza where he completed his PhD degree in 1995. After a post-doctoral stay at Centre de Recherche Paul Pascal (CNRS, Bordeaux), he became an Associate Professor of Physical Chemistry at the University of Zaragoza in 2000. His research is mainly focused on thermophysical properties of liquid systems (single liquids and mixtures). He has co-authored more than 170 publications in peer-reviewed journals.

Beatriz Giner

Beatriz Giner obtained her PhD Degree in Physical Chemistry at the Universidad de Zaragoza in 2007. Currently, she is a lecturer at Universidad San Jorge and is the principal investigator of the GREENLIFE research group. She has participated in seven R&D&I projects funded in competitive calls. She is an author of 64 peer-reviewed publications in scientific journals from SCR (40% in Q1) and three book chapters. Her scientific network is supported by publications with 40 distinct authors from diverse affiliations.