



## Correlating the structure and composition of ionic liquids with their toxicity on *Vibrio fischeri*: A systematic study

Sylvie Viboud<sup>a</sup>, Nicolas Papaiconomou<sup>b</sup>, Aurélien Cortesi<sup>a</sup>, Grégory Chatel<sup>b</sup>,  
Micheline Draye<sup>b</sup>, Dominique Fontvieille<sup>a,\*</sup>

<sup>a</sup> Laboratoire CARTEL, UMR INRA 42, CISM, Université de Savoie, 73376 Le Bourget-du-Lac Cedex, France

<sup>b</sup> Laboratoire de Chimie Moléculaire et Environnement, CISM, Université de Savoie, 73376 Le Bourget-du-Lac Cedex, France

### ARTICLE INFO

#### Article history:

Received 17 October 2011

Received in revised form 12 January 2012

Accepted 8 February 2012

Available online 24 February 2012

#### Keywords:

Ionic liquids

Toxicity

*Vibrio fischeri*

EC<sub>50</sub>

### ABSTRACT

A systematic screening of the toxicity of ionic liquids (IL) towards *Vibrio fischeri*, a bioluminescent marine bacteria generally used in ecotoxicological bioassays, was carried out. The objectives of this work were to find hydrophilic or hydrophobic low toxicity IL and to investigate structure–toxicity relationship of IL. Toxicity of 54 IL to *V. fischeri* have been measured, some referring to new IL based on quinuclidinol or tropinol and some to generic IL (i.e., imidazolium, pyridinium, pyrrolidinium or piperidinium). For 47 of them, toxicity values have not been reported elsewhere. Water-soluble IL containing hydrophilic anions halide, thiocyanate, dicyanamide, trifluoromethanesulfonate were studied. Some IL were found to exhibit very low toxicity towards *V. fischeri*. Hydrophobic IL based on bis(trifluoromethanesulfonyl)imide, tetrafluoroborate tetraphenylborate and tetracyanoborate were also studied. Toxicity was measured in a consistent way starting from aqueous solutions saturated with IL. The least toxic hydrophobic IL found in this study was [EMIM][B(CN)<sub>4</sub>]. A multifactorial analysis was found to be convenient for finding relevant structure parameters influencing the toxicity of IL. From this analysis, the planarity of the cation ring appeared to be a relevant parameter. Finally, good linear correlations were found when toxicity of IL was plotted either against the number of aliphatic carbons surrounding a pyridinium cation or the total number of carbons of a cation.

© 2012 Elsevier B.V. All rights reserved.

### 1. Introduction

Ionic liquids (IL) are molten salts exhibiting melting points below 100 °C and composed with large organic cations and organic or inorganic anions. Because they exhibit very low vapour pressure, high thermal stability, high liquid range and relatively low viscosity, IL are currently seen as promising candidates to replace classical volatile organic compounds (VOC). Numerous studies report synthesis [1,2], properties characterization [3–5] and applications of IL to various fields of chemistry [6–11]. Considering their physico-chemical properties, they have often been referred to as “green” solvents. On the other hand, recent publications highlight their potential toxicities to the environment [12–15]. A very recent critical review has underlined the necessity of extensive and systematic studies, in order to better understand the toxicity of IL [16].

Until now, several studies have shown that even at low concentrations (10<sup>−5</sup> M) most common IL (i.e., IL containing imidazolium or pyridinium cations) exhibit a clear toxic effect to many living organisms such as bacteria [17], human cells [18], algae [19] or

freshwater snails [20]. Only few papers report toxicity of other IL families such as those containing pyrrolidinium, piperidinium, morpholinium or phosphonium cations [21]. The way the composition of an IL (i.e. cation, anion, and alkyl chain length) influences toxicity is not yet established in a definitive way.

Some applications of IL deal with the extraction of pollutants from wastewater [22]. Such processes require IL that are sufficiently hydrophobic to form a biphasic system with water. Since the solubility in water for such IL ranges between 100 and 10,000 mg L<sup>−1</sup> [23–25], this involves a potential dissemination of IL in aqueous effluents [26–29]. Besides, they can also contaminate soils through sediment deposition or bio-accumulate in living organisms. In such cases, the balance between biodegradability and toxicity can become a key factor to determine whether an IL will be harmful to the environment.

The main objective of this work was to gain further insight into the structure–toxicity relationship of IL dissolved in an aqueous phase. To that end, a systematic study on the toxicity of IL towards *Vibrio fischeri*, a well-known bioluminescent marine bacteria widely used in ecotoxicological bioassays, was carried out. The influence of three main components, namely the cation ring, the alkyl chain and the anion on the toxicity of IL was studied. The influence of cations on toxicity was examined for IL containing a

\* Corresponding author. Tel.: +33 0 479758863; fax: +33 0 479758777.  
E-mail address: [dfont@univ-savoie.fr](mailto:dfont@univ-savoie.fr) (D. Fontvieille).

bromide anion and cations imidazolium, pyridinium, pyrrolidinium, piperidinium, morpholinium, tropinium and quinuclidinium with alkyl chains varying from butyl to octyl, or a functionalised side chain. In order to investigate the influence of anions on toxicity, anions dicyanamide  $[N(CN)_2]$ , trifluoromethanesulfonate (triflate)  $[CF_3SO_3]$ , thiocyanate  $[SCN]$ , bis(trifluoromethanesulfonyl)imide  $[NTf_2]$ , tris(perfluoroethyl)trisfluorophosphate  $[FAP]$ , tetracyanoborate  $[B(CN)_4]$ , tetrafluoroborate  $[BF_4]$  and tetraphenylborate  $[BPh_4]$  were also considered.

## 2. Experimental

### 2.1. Synthesis

54 IL were used in this study. Except for  $[EMIM][FAP]$  and  $[EMIM][B(CN)_4]$  which were provided by Merck and used without further purification, all IL were synthesized according to previously reported procedures [25,30,31]. Their structure and abbreviations are given in Fig. 1. Briefly, all IL containing halide anion were synthesized by mixing 1-halogenoalkane with the corresponding amine in ethyl acetate under reflux for 72 h. Hydrophilic IL containing other anions were obtained by mixing bromide-containing IL in acetonitrile with a potassium or sodium salt containing the desired anion, or in water with a lithium salt, at room temperature for 12 h. After filtering and several washing steps, desired IL were obtained. Solubilities in water of IL containing unsaturated cations were measured by UV–Vis spectroscopy, as reported elsewhere [25]. For those containing saturated cations; solubilities in water were measured by conductivity. A calibration solution containing a known small amount of an IL was first prepared and its conductivity measured. Then, an aqueous solution saturated with the same IL was prepared. The latter solution was then diluted several times until its conductivity was found equal to that of the calibration solution. The solubility in water for the specified IL was obtained since conductivity is a linear function of ion concentration in water.

### 2.2. Toxicity bioassays

Toxicity of IL was assessed using a bioluminescent marine bacteria *V. fischeri*, which light emission is inhibited when the bacteria is grown under toxic conditions. This technique is standardized internationally [32,33]. Tests were performed according to a previously reported procedure [31]. In this work toxicity values reported in the text and in tables are expressed as  $\log_{10}$  ( $EC_{50-15min}$   $\mu M$ ) units. The  $EC_{50-15min}$  refers to the toxicity values measured 15 min after *V. fischeri* was in contact with an IL.

### 2.3. Statistics

A multiple correspondence analysis (MCA) [34,35] was performed to investigate on the underlying structure of the dataset. Multivariate analysis is a way to find relationships between variables, namely the toxicity and the structure of IL. To that end, cations were described with a set of three parameters: the number of carbons on the alkyl chain (Chain), the number of atoms in the ring (C.Cy) and the ring planarity (Plan). The type of anion (An) and the  $EC_{50}$  values (EC) were also included in the analysis. Three groups were defined for the type of anion: hydrophilic (Phil), hydrophobic (Phob) and halogenated ones (Halo). Values for the  $EC_{50}$  variable were clustered into three equal class intervals from the less  $EC:1 = [5.45, 8.25]$  to the highest toxicity  $EC:3 = [0.36, 2.05]$ . Results were interpreted on the basis of projection maps and axis contributions of the variables (squared cosine values).

## 3. Results and discussion

In order to obtain a consistent dataset on toxicity values for IL, and to study not previously reported compounds, a total of 54 IL containing generic cations (i.e., imidazolium, pyridinium, pyrrolidinium or piperidinium) or new cations based on quinuclidinol and tropinol were studied in this work. Among them, 47 were measured for the first time. Despite IL based on imidazolium cations have been extensively studied, a comparison with our data was only possible for  $[BMIM][Br]$ , because most toxicity values reported in literature are based on IL containing a chloride ion [21].

$EC_{50}$  values for usual organic solvents and starting reactants are collected in Table 1 and that for IL in Tables 2 and 3.  $EC_{50}$  values found in literature are also presented in these tables.

In the case of IL (Tables 2 and 3), starting reactants and most of usual organic solvents (Table 1), toxicity values measured here and those previously reported in the literature differ by less than 0.4 log unit. Our data are thus in good agreement with those reported in previous studies. Nevertheless, some discrepancies are observed. For instance, the largest deviation between our measurements and previously published values [36,37] is about phenol (1 log unit). A recent publication, however, specifically devoted to the toxicity of phenol and derivatives to *V. fischeri* [37] reported a toxicity of phenol equal to the one measured in this work. A deviation of 0.6 log unit between our work and the literature is observed for sodium chloride (Table 1). In the case of sodium dicyanamide, two values reported in the literature [38,39] exhibit a difference of 0.5 log unit. The value reported here is higher by 0.4 log unit than the highest previously reported value.

Except for choline chloride, toxicities ( $EC_{50}$  in  $\mu M$ ) of IL were higher than that of acetonitrile (Table 1). Furthermore, 39 out of 54 IL exhibited toxicities higher than that of phenol. These results are in agreement with previous reports confirming the fact that IL should be considered as toxic solvents [21,38].

Most IL containing a saturated ring or an unsaturated ring with a short alkyl chain were found to be respectively less and more toxic than their corresponding starting compounds. For instance,  $[BMMOR][Br]$  is less toxic than 1-methylmorpholine by 1.5 log unit whereas  $[BMIM][Br]$  and  $[BPYR][Br]$  exhibit higher toxicities than 1-methylimidazole and pyridine.

Furthermore, the presence of an alkyl group on a pyridine ring increases the toxicity of the resulting neutral compound or IL. Pyridine is less toxic than 2,3,5-trimethylpyridine. Similarly, all IL containing a pyridinium cation without any methyl substituent are less toxic than homologues containing methyl-substituents. Similar results were previously observed by Couling et al. [38] with  $[B3M5MPYR][Br]$ .

Nevertheless, comparing the toxicity of all IL (Tables 2 and 3) with that of the corresponding starting compounds such as 1-methylimidazole, pyridine, 1-alkylpiperidine, or 1-methylpyrrolidine (Table 1) did not give any clear structure–toxicity relationships. The presence of a positive charge on the cation is expected to significantly modify the toxicity related properties of the cations as compared to those of the neutral starting compounds.

### 3.1. Ionic liquid toxicity on *V. fischeri* – multifactorial effects

A multifactorial component analysis (MCA) was carried out to gain insights into the influence of the IL structure on toxicity. Results indicated that 56% of the data total inertia could be explained by considering the first three principal components. Projections of variables on the  $PC1 \times PC2$  and  $PC1 \times PC3$  factorial plans are shown in Fig. 2. The first principal component (PC1) explains 25% of the total inertia. It clearly opposes two classes of

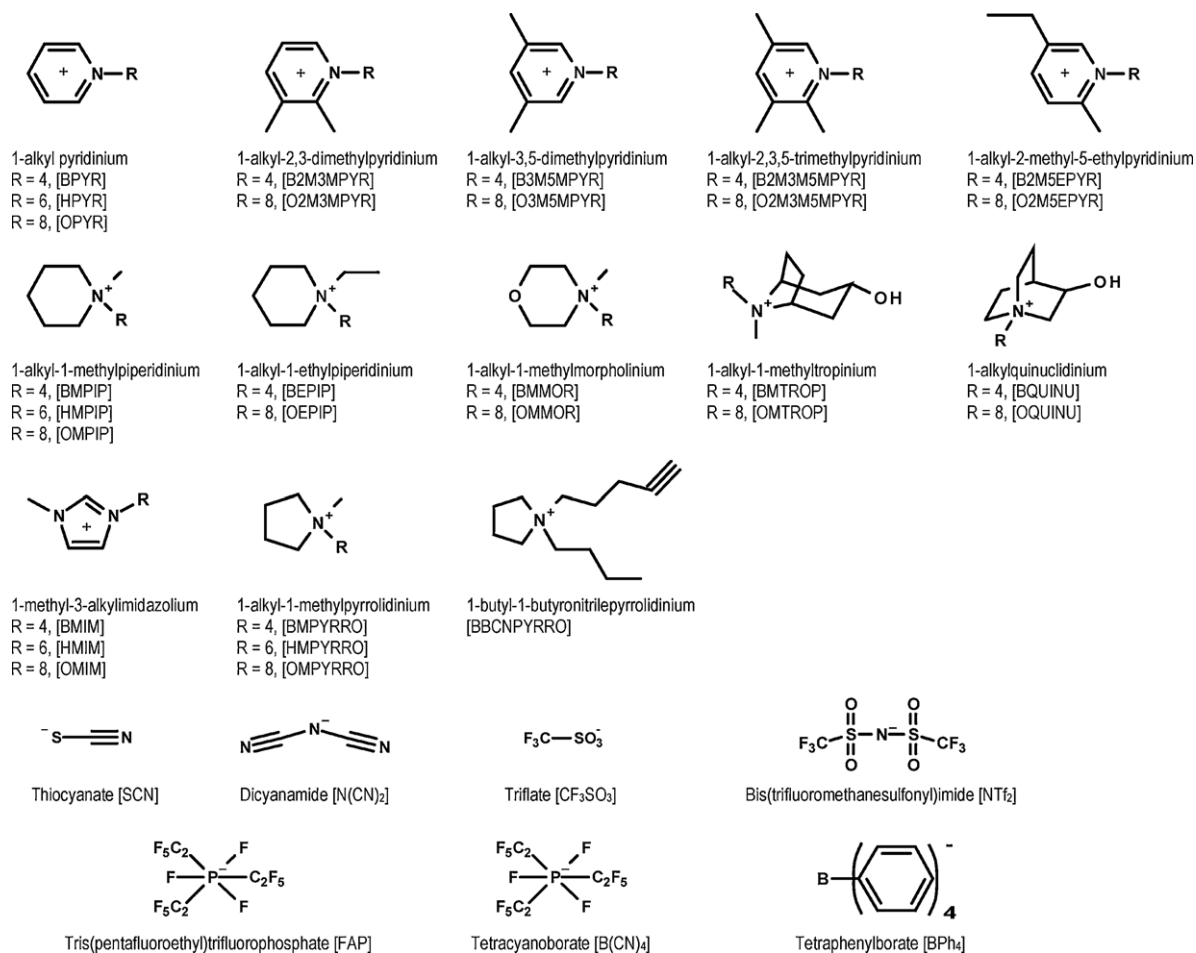


Fig. 1. Structure and abbreviations of studied ionic liquids.

**Table 1**  
*Vibrio fischeri* toxicity values for some salts, common volatile solvents and some starting compounds used for ionic liquids synthesis. "EC<sub>50,15min</sub> error" refers to standard deviation.

Compound	MW	Log EC <sub>50,15min</sub> (μM)	Log EC <sub>50,15min</sub> (μM) from Literature	EC <sub>50,15min</sub> (mg L <sup>-1</sup> )	EC <sub>50,15min</sub> error (mg L <sup>-1</sup> )
Acetonitrile	41	5.60	5.77 [37]	16,279	238
Ethanol	46	5.54	5.70 [37]	16,071	240
Ethyl acetate	88	5.28	4.82 [37]	16,805	650
Acetone	58	5.14	5.34 [37]	7973	457
Phenol	94	3.24	2.35 [37]; 3.24 [36]	163	4.75
Sodium bromide	103	5.89	5.29 [38]	80,090	2909
Potassium bromide	119	5.49		36,891	1548
Sodium dicyanamide	89	5.61	4.72 [38]; 5.2 [39]	36,040	601
Potassium triflate	126	5.44		35,012	913
Potassium thiocyanate	97	5.25		17,397	1087
Lithium Bis(trifluoromethylsulfonyl)imide	287	4.43	3.88 [39]	7729	541
1-Methylimidazole	82	4.54	4.17 [38]	2864	43.4
1-Methylmorpholine	101	4.36		2328	78.1
Pyridine	79	4.04	3.87 [38]; 4.43 [37]	867	14.1
1-Methylpiperidine	99	3.85		700	13.4
1-Methylpyrrolidine	85	3.76		493	21.5
2,3-Dimethylpyridine	107	3.35		238	4.17
3,5-Dimethylpyridine	107	2.79	2.64 [38]	65.9	1.92
2,3,5-Trimethylpyridine	121	2.55		43.0	2.02

toxicities (EC:1 and EC:3) appearing to be the axis that discriminates IL toxicity. Squared cosines on PC1 for EC:1 and EC:3 reached 0.63 and 0.58, respectively (Table 4). Two sets of variables are observed on the two projection maps. Group I associated to the less toxic IL includes those containing a butyl chain (Chain:4), a non-planar cation ring (Plan:1) and an hydrophilic anion (An:Phil, Fig. 2).

It is opposed to group II which includes most toxic IL containing an octyl chain (Chain:8), a planar ring (Plan:0) and a hydrophobic anion (An:Phob). The PC1 axis opposed the group formed by quinuclidinium-tropolium with a butyl chain (group I, less toxic) to dimethylpyridinium-based IL containing an octyl chain (group II, more toxic).

**Table 2***Vibrio fischeri* toxicity values for halogenated ionic liquids containing bromide, iodide or chloride anions. "EC<sub>50,15min</sub> error" refers to standard deviation.

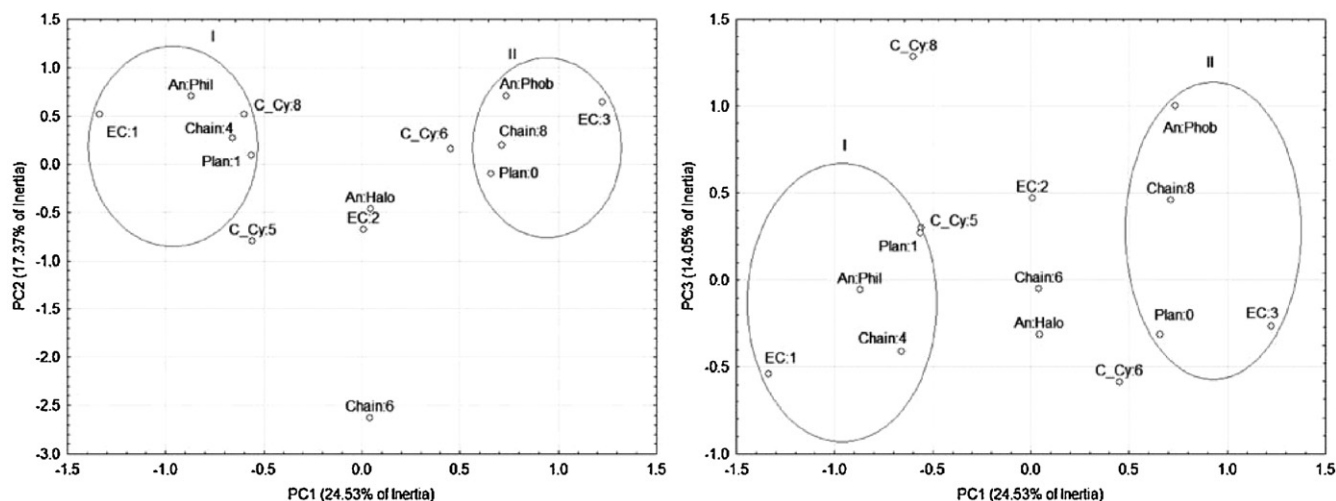
Compound	Acronym	MW	Log EC <sub>50,15min</sub> (μM)	Log EC <sub>50,15min</sub> (μM) from Literature	EC <sub>50,15min</sub> (mg L <sup>-1</sup> )	EC <sub>50,15min</sub> error (mg L <sup>-1</sup> )
Choline chloride	[Ch][Cl]	140	6.17	>5[38]	205,732	8270
1-Butyl-1-methylmorpholinium bromide	[BMMOR][Br]	238	5.45	>4.30 [21]	66,729	1777
1-Octylmorpholinium bromide	[OMMOR][Br]	294	2.56		107	5.44
1-Butyltropinium bromide	[BTROP][Br]	278	5.36		64,110	1795
1-Butyltropinium iodide	[BTROP][I]	325	4.74		17,679	765
1-Butylquinuclidinium bromide	[BQUINU][Br]	264	4.45		7400	225
1-Octylquinuclidinium bromide	[OQUINU][Br]	320	2.32		67.3	2.29
1-Butyl-1-methylpyrrolidinium bromide	[BMPYRRO][Br]	222	4.40		5525	303
1-Hexyl-1-methylpyrrolidinium bromide	[HMPYRRO][Br]	250	3.19		387	18.7
1-Octyl-1-methylpyrrolidinium bromide	[OMPYRRO][Br]	278	2.26		50.8	1.94
1-Octyl-1-methylpyrrolidinium chloride	[OMPYRRO][Cl]	234	2.17		34.7	1.62
1-Butyl-1-methylpiperidinium bromide	[BMPIP][Br]	236	4.22	4.27 [21]	3958	97.2
1-Hexyl-1-methylpiperidinium bromide	[HMPIP][Br]	264	2.94		230	6.71
1-Octyl-1-methylpiperidinium bromide	[OMPIP][Br]	292	2.00		29.3	1.54
1-Butyl-1-ethylpiperidinium bromide	[BEPIP][Br]	250	3.96		2258	60.8
1-Octyl-1-ethylpiperidinium bromide	[OEPIP][Br]	353	1.80		22.1	0.88
1-Octyl-1-ethylpiperidinium iodide	[OEPIP][I]	290	2.20		46.3	2.90
1-Butyl-1-butyronitrilepyrrolidinium bromide	[BB(CN)PYRRO][Br]	275	5.33		58,536	1404
1-Butyl-1-methylimidazolium bromide	[BMIM][Br]	219	3.66	3.43 [39]	1002	34.6
1-Hexyl-1-methylimidazolium bromide	[HMIM][Br]	247	3.13		334	9.14
1-Octyl-1-methylimidazolium bromide	[OMIM][Br]	275	2.27		50.9	1.98
1-Butylpyridinium bromide	[BPYR][Br]	216	3.72	3.39 [12]	1124	51.0
1-Hexylpyridinium bromide	[HPYR][Br]	244	2.85		175	10.3
1-Octylpyridinium bromide	[OPYR][Br]	272	1.89		21.3	1.23
1-Butyl-2,3-dimethylpyridinium bromide	[B2M3MPYR][Br]	244	2.93		208	6.30
1-Octyl-2,3-dimethylpyridinium bromide	[O2M3MPYR][Br]	300	1.08 <sup>a</sup>		3.58	0.12
1-Butyl-3,5-dimethylpyridinium bromide	[B3M5MPYR][Br]	244	2.49 <sup>a</sup>	2.69 [12]	75.8	3.25
1-Octyl-3,5-dimethylpyridinium bromide	[O3M5MPYR][Br]	300	1.12 <sup>a</sup>		4.00	0.15
1-Butyl-2,3,5-trimethylpyridinium bromide	[B2M3M5MPYR][Br]	258	2.48 <sup>a</sup>		77.8	4.33
1-Octyl-2,3,5-trimethylpyridinium bromide	[O2M3M5MPYR][Br]	300	0.64 <sup>a</sup>		1.32	0.02
1-Octyl-2-methyl-5-ethylpyridinium bromide	[O2M5EPYR][Br]	314	0.59		1.23	0.07

<sup>a</sup> Data previously published by our group [31].

Squared cosine values on PC1 indicate that toxicity is mainly influenced by the alkyl chain length and the ring planarity, while the contribution of the anion is quite low (Table 4). This is demonstrated by values around 0.4 for Chain:4 and Chain:8, while they reach a mere 0.13 or 0.19, respectively for the anions (Phob and Phil). However it must be kept in mind that no anion containing aromatic rings or an alkyl chain was considered. Previous reports revealed that alkyl chain appended on the anion (such as in cholinium alkanooates) resulted in significant toxicity increases [40]. Besides the alkyl chain length parameter, the cation ring planarity is of a very close effect on toxicity (0.37). The number of atoms in the ring appears to have almost no effect on the IL toxicities.

Indeed squared cosine values for this parameter (C.Cy) was very low ( $\leq 0.1$ ), except for C.Cy:6. These observations were in good agreement with some of the predictions reported by Couling et al. [38] on quantitative structure–property relationship modelling.

In this study, MCA appears to be a valuable tool in understanding which parameters influence IL toxicity and in seeking for non-toxic IL. This is a simple tool compared to the complex models which are generally used, such as QSPR [38], Spectral-SAR model [41], T-SAR [42] or PLS-DA [43]. From the set of IL studied here, MCA revealed that low toxicity was related to three structure parameters: a cation containing short alkyl chain with non-planar ring combined to a hydrophilic anion.



**Fig. 2.** Projection maps (PC1 × PC2, PC1 × PC3) issued from the MCA. EC: EC<sub>50</sub> class, Chain: alkyl chain length, An: anion, C.Cy: number of carbons of the cation ring, Plan: Planarity, Phob: hydrophobic, Phil: hydrophilic.

**Table 3**  
*Vibrio fischeri* toxicity values for hydrophilic and hydrophobic ionic liquids. “EC<sub>50,15min</sub> error” refers to standard deviation.

Compound	Acronym	MW	Log EC <sub>50,15min</sub> ( $\mu$ M)	Log EC <sub>50,15min</sub> ( $\mu$ M) from Literature	EC <sub>50,15min</sub> (mg L <sup>-1</sup> )	EC <sub>50,15min</sub> error (mg L <sup>-1</sup> )	Solubility (mg L <sup>-1</sup> )
<b>Hydrophilic ionic liquids</b>							
1-Butyl-1-methylmorpholinium dicyanamide	[BMMOR][N(CN) <sub>2</sub> ]	224	4.89		17,526	595	–
1-Butyl-1-methylpyrrolidinium dicyanamide	[BMPYRRO][N(CN) <sub>2</sub> ]	208	4.26		3764	111	–
1-Octylquinuclidinium dicyanamide	[OQUINU][N(CN) <sub>2</sub> ]	306	2.26		55.3	1.65	–
1-Octyltropinium dicyanamide	[OTROP][N(CN) <sub>2</sub> ]	320	2.52		106	3.14	–
1-Butyl-1-methylmorpholinium triflate	[BMMOR][CF <sub>3</sub> SO <sub>3</sub> ]	307	4.40		7684	198	–
1-Butyl-1-methylpyrrolidinium triflate	[BMPYRRO][CF <sub>3</sub> SO <sub>3</sub> ]	291	3.98		2787	104	–
1-Butyl-2,3-dimethylpyridinium triflate	[B2M3MPYR][CF <sub>3</sub> SO <sub>3</sub> ]	313	2.84		215	7.89	–
1-Butyltropinium thiocyanate	[BTROP][SCN]	256	4.07		2982	62.0	–
1-Butyl-2,3-dimethylpyridinium thiocyanate	[B3M5MPYR][SCN]	222	2.25		39.4	1.14	–
1-Octyl-1-ethylpiperidinium thiocyanate	[OEPIP][SCN]	284	1.50		8.90	0.46	–
<b>Hydrophobic ionic liquids</b>							
1-Butyl-1-methyltropinium Bis(trifluoromethylsulfonyl)imide	[BTROP][NTf <sub>2</sub> ]	478	2.61		195	5.83	4270
1-Octyl-1-methyltropinium Bis(trifluoromethylsulfonyl)imide	[OTROP][NTf <sub>2</sub> ]	534	2.17		78.3	2.96	1630
1-Butyl-1-methylpyrrolidinium Bis(trifluoromethylsulfonyl)imide	[BMPYRRO][NTf <sub>2</sub> ]	422	2.72		219	11.52	6750
1-Octyl-1-methylpyrrolidinium Bis(trifluoromethylsulfonyl)imide	[OMPYRRO][NTf <sub>2</sub> ]	478	1.58		18.2	0.73	1030
1-Butyl-3,5-dimethylpyridinium Bis(trifluoromethylsulfonyl)imide	[B3M5MPYR][NTf <sub>2</sub> ]	444	1.86 <sup>b</sup>		31.9	1.93	3390
1-Octyl-3,5-dimethylpyridinium Bis(trifluoromethylsulfonyl)imide	[O3M5MPYR][NTf <sub>2</sub> ]	501	0.36 <sup>b</sup>		1.14	0.13	310
1-Butyl-2,3,5-trimethylpyridinium Bis(trifluoromethylsulfonyl)imide	[B2M3M5MPYR][NTf <sub>2</sub> ]	458	1.56 <sup>b</sup>		16.7	1.91	1660
1-Octyl-2,3,5-trimethylpyridinium Bis(trifluoromethylsulfonyl)imide	[O2M3M5MPYR][NTf <sub>2</sub> ]	515	0.62 <sup>b</sup>		2.16	0.16	200
1-Ethyl-1-methylimidazolium tetracyanoborate	[EMIM][B(CN) <sub>4</sub> ]	226	3.62		934	34.58	47,000
1-Octyltropinium tetrafluoroborate	[OTROP][BF <sub>4</sub> ]	341	2.77		201	11.59	nd
1-Octyl-3,5-dimethylpyridinium tetrafluoroborate	[O3M5MPYR][BF <sub>4</sub> ]	307	0.97		2.89	0.07	500
1-Octyl-1-ethylpiperidinium tetraphenylborate	[OEPIP][BPh <sub>4</sub> ]	592	– <sup>a</sup>		– <sup>a</sup>	– <sup>a</sup>	<5
1-Ethyl-1-methylimidazolium tris(pentafluoroethyl)trifluorophosphate	[EMIM][FAP]	556	1.36	< 2 [39]	12.7	1.45	320

<sup>a</sup> At half saturation concentration of ionic liquid in water, only a 15% decrease in *Vibrio fischeri* bioluminescence was observed. No EC<sub>50,15min</sub> could therefore be measured. nd: not determined.

<sup>b</sup> Data previously published by our group [31].

**Table 4**

Squared cosine values of each variable along the PC1 axis: EC, EC<sub>50</sub> class (1: less toxic, 3: more toxic); An, anion; Chain, alkyl chain length; C.Cy, number of carbons of the cation ring; Phob, hydrophobic; Phil, hydrophilic; Plan; planarity (0: planar, 1: non-planar).

	EC			An			Chain			C.Cy			Plan	
	1	2	3	Phob	Phil	Halo	4	6	8	5	6	8	0	1
cosine <sup>2</sup> value	0.63	<0.01	0.58	0.13	0.19	<0.01	0.40	<0.01	0.40	0.1	0.26	0.09	0.37	0.37

### 3.2. Cations yielding low toxicity ionic liquids

The influence of the cation on the toxicity of IL was studied with halide-based IL. In order to obtain IL exhibiting low toxicity, we used cations based on cyclic ammoniums with various alkyl chain lengths, alkyl substituents appended to the ammonium cycle, bridged structures and cycles containing heteroatoms. Structure and abbreviations used in the text are presented in Fig. 1.

Choline chloride is known to yield low-toxic IL [40]. The EC<sub>50</sub> value reported here (6.17) is in agreement with these observations. It is the least toxic IL observed in our study. The EC<sub>50</sub> is in agreement with a previously reported data, which indicated a lower concentration threshold above which the EC<sub>50</sub> should stand [38]. The presence of a heteroatom on the cation yields significant toxicity decrease as compared to generic IL. [BMMOR] contains an oxygen in the saturated ring and is far less toxic than its homologue ([BMPIP][Br]). Furthermore, [BMMOR][Br] is the least toxic IL (5.45) reported so far, with a toxicity similar that of potassium bromide. A lower concentration threshold above which the EC<sub>50</sub> was expected to stand was previously reported (>4.3) [21]. Once again, our value is in agreement with this threshold.

Similarly, when a nitrile functional group is appended on the alkyl chain, such as in [BB(CN)PYRRO][Br], a significant toxicity decrease is observed. Compared to [BMPYRRO][Br] which exhibits a toxicity of 4.40, [BB(CN)PYRRO][Br] is less toxic from approximately one order of magnitude (5.33) and reaches the toxicity of potassium bromide. Such a decrease in toxicity due to nitrile group in IL had already been reported for 1-butyronitrile-1-methylpyrrolidinium bis(trifluoromethylsulfonyl)imide [18].

Toxicity measurements also reveal that more complex structures, such as those based on quinuclidinol or tropinol compounds, may yield less toxic IL than their pyrrolidinium and piperidinium homologues. It has to be pointed out though, that [BTROP][Br] and [BQUINU][Br] exhibit an –OH functional group appended to the edge of the cation ring, a design known to be associated to a lower toxicity [21,38].

Therefore it appears that adding bridged structures on a cation, and adding functional groups or heteroatom anywhere on the cation significantly decreases the toxicity of IL, down to values close to that of potassium bromide. Three IL, [BMMOR][Br], [BTROP][Br] and [BB(CN)PYRRO][Br] exhibit very low toxicity values, not far from that of choline chloride. These results expand the number of potential cations yielding low toxicity IL. In addition, since these three IL have very different structures, they are expected to exhibit very different physico-chemical properties that might be useful for specific applications.

### 3.3. Influence of the number of carbons on toxicity

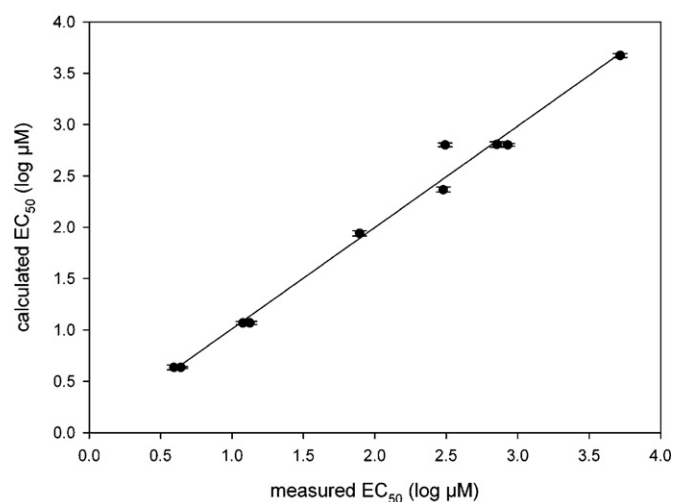
For IL containing a halide anion and a butyl chain, EC<sub>50</sub> values vary from 2.48 for [B2M3M5MPYR][Br] to 5.45 for [BMMOR][Br] (Table 2). For IL containing octyl chains, toxicities vary between 0.59 for [O2M5EPYR][Br] and 2.56 for [OMMOR][Br]. Therefore the influence of the cation on toxicity seems higher when short alkyl chains are present. Likewise, our results indicate that all IL containing octyl chains exhibit toxicities equal to or higher than any IL containing a butyl chain (Table 2). This is reasserting the significant influence of alkyl chain length on toxicity as previously

suggested by the MCA analysis and in agreement with previously reported observations [21]. For the same cation, the highest and lowest variations in toxicity between butyl and octyl homologues are respectively obtained for morpholinium (2.99 log units), and 3,5-dimethylpyridinium cations (1.37 log unit).

The influence of alkyl substituents around a pyridinium ring on the toxicity was also studied for IL containing methyl or ethyl substituents. Alkyl substituted pyridinium cations appear to be significantly more toxic than their non-substituted homologues. For instance, [B2M3MPYR][Br] or [B2M3M5MPYR][Br] are significantly more toxic than [BPYR][Br]. Similar trends are obtained for IL containing pyridinium cations and octyl chains. A careful study of the data reveals that toxicity of [HPYR][Br], which contains six carbons on the alkyl chain length, exhibits a toxicity similar to that of [B2M3MPYR][Br], which contains four carbons on the alkyl chain and two carbons on the pyridinium ring. Similarly, [B2M3M5MPYR][Br] and [B2M5EPYR][Br] exhibit similar toxicities.

Starting from these observations, EC<sub>50</sub> values of IL containing bromide and pyridinium were fitted to the number of aliphatic carbons using a linear relationship ( $EC_{50} = a + b n_C$ , where  $n_C$  is the number of aliphatic carbons and  $a$  and  $b$ , two adjustable parameters). As shown in Fig. 3, measured and calculated EC<sub>50</sub> values are in good agreement ( $R^2 = 0.934$ ). This can be interpreted in the way that methyl substituents and alkyl chain length have the same effect on toxicity of IL containing pyridinium cations. This suggests that alkyl chains, wherever around the pyridinium cation, have the same kind of interactions with some *V. fischeri* components, most probably with those of its envelopes (cell wall or cytoplasmic membrane).

Data analysis was then pushed forward plotting EC<sub>50</sub> values for IL containing a bromide anion and imidazolium, pyridinium, pyrrolidinium or piperidinium cations against the total number of carbons of the cation (Fig. 4). Here again, a good linear correlation was found ( $R^2 = 0.861$ ). A slope of  $(-0.44 \pm 0.04)$  was obtained, in agreement with previous studies on imidazolium and



**Fig. 3.** Relationship between measured and calculated values of toxicity for bromide pyridinium based ionic liquids. Predicted EC<sub>50</sub> was computed from the equation  $y = 0.434x - 0.593$  where  $x$  represents the total alkyl carbons (chain + substitutions).

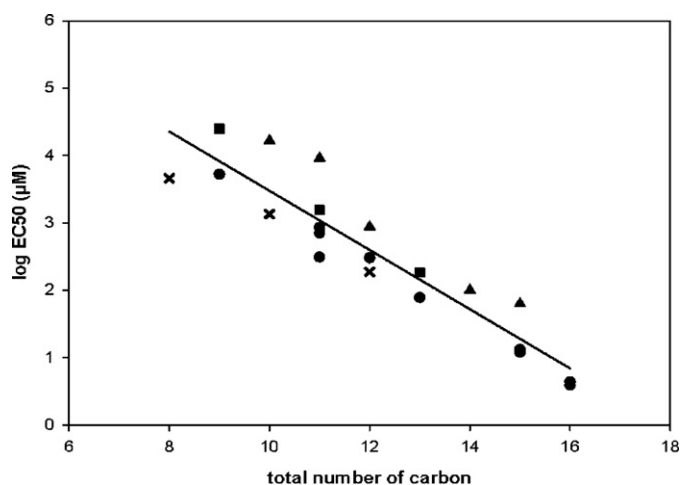


Fig. 4. Relationship between toxicity ( $EC_{50}$ ) and the total number of carbons in the cation. Regression equation:  $y = -0.4396x + 7.871$  ( $R^2 = 0.861$ ) where  $x =$  total number of carbons and  $y = \log EC_{50}$  ( $\mu\text{M}$ ). Pyridinium (●); pyrrolidinium (■), piperidinium (▲); imidazolium (✕), regression line (-).

pyridinium-based IL [17,39]. These results show that the IL toxicity can be described simply, at least as a first approximation, using the total number of carbons, whether they are aliphatic or aromatic. This means also that the saturation of a cation ring is of minor influence on IL toxicity.

### 3.4. Influence of the planarity of cation rings

At equal length of the alkyl chain, all IL bearing a planar unsaturated ring exhibited higher toxicities than those bearing a non-planar saturated ring. These results are in agreement with previous studies [13,21,38] referring to the saturation of the cation ring rather than its planarity. With an octyl chain, IL containing a planar ring exhibit toxicities ranging from 0.59 for [O2M5EPYR][Br] to 2.27 for [OMIM][Br]. For IL based on a non-planar ring, toxicity values range from 1.80 for [OEPIP][Br] to 2.56 [OMMOR][Br].

This observation is even clearer for butyl chain based IL, since [BPYR][Br] or [BMIM][Br] exhibit toxicities two orders of magnitude higher than those holding saturated rings, such as [BMMOR][Br] and [BMPYRRO][Br] (Table 2). Comparing the toxicities of IL containing planar pyridinium cations and those of non-planar piperidinium cations with the same number of carbons gives similar results. Toxicities of unsaturated pyridinium, such as [B2M3MPYR][Br] are found to be only 1.3 log unit higher than that of saturated piperidinium, such as [BMPIP][Br].

The planarity factor of cations also explains the significant variations in toxicity observed for [BQUINU][Br] and [BTROP][Br]. Structures of [BQUINU][Br] and [BTROP][Br] are close, as shown in Fig. 1. However, unlike [BTROP][Br], [BQUINU][Br] exhibits a stiff and planar structure and is 0.9 log more toxic than [BTROP][Br]. These results thus suggest that, besides the effect of alkyl chain length and ring substitutions, the planarity of the ring seems to play a significant part in the toxicity of IL to *V. fischeri*.

### 3.5. Hydrophilic anions yielding low toxic ionic liquids

IL containing halide anions generally exhibit high melting points or are very viscous at room-temperature. To decrease both viscosity and melting points, other anions such as dicyanamide ( $[\text{N}(\text{CN})_2]$ ), trifluoromethanesulfonate (triflate,  $[\text{CF}_3\text{SO}_3]$ ) or thiocyanate ( $[\text{SCN}]$ ) anions are used [44,45]. We therefore studied the toxicity of ten IL containing the latter anions. All IL were found to be liquid at or near room-temperature. Results are collected

in Table 3. For comparison purposes, toxicities of salt precursors, namely potassium thiocyanate, potassium triflate and sodium dicyanamide, were measured and reported in Table 1.

Surprisingly, it appears that sodium dicyanamide exhibits a very low toxicity (5.61), similar to that of potassium bromide. The following order for toxicity of salts applies here:



Toxicities of IL containing anions  $[\text{N}(\text{CN})_2]$ ,  $[\text{CF}_3\text{SO}_3]$  or  $[\text{SCN}]$  followed the trend observed for the alkaline salts. IL containing the  $[\text{CF}_3\text{SO}_3]$  anion tend to be more toxic than those featuring  $[\text{N}(\text{CN})_2]$ . For instance, [BMMOR][ $\text{CF}_3\text{SO}_3$ ] exhibits a higher toxicity than [BMMOR][ $\text{N}(\text{CN})_2$ ].

IL containing an  $[\text{SCN}]$  anion exhibit higher toxicities than their triflate or thiocyanate homologues. For instance, despite the similar toxicities of [BTROP][Br] and [BMMOR][Br], [BTROP][SCN] exhibits higher toxicity than [BMMOR][ $\text{N}(\text{CN})_2$ ] and [BMMOR][ $\text{CF}_3\text{SO}_3$ ]. IL containing dicyanamide anion exhibit the lowest toxicities. Values of 4.89 and 4.26 have been obtained for [BMMOR][ $\text{N}(\text{CN})_2$ ] and [BMPYRRO][ $\text{N}(\text{CN})_2$ ], respectively. To the best of our knowledge, this is the first example of room-temperature IL exhibiting low-toxicity to *V. fischeri* reported so far. This is also demonstrating that low toxicity IL can be designed based on proper ion combinations. It is interesting to observe that toxicity of [BMMOR][ $\text{N}(\text{CN})_2$ ] is similar to the one of ethyl acetate, which is considered as a non-harmful solvent, and is only twice as low the one of potassium bromide. It also appears that the toxicity [BMMOR][ $\text{N}(\text{CN})_2$ ] is not far from that of choline chloride.

The toxicity of the four most toxic hydrophilic IL namely [B2M3MPYR][ $\text{CF}_3\text{SO}_3$ ], [B3M5MPYR][SCN], [OEPIP][SCN] and [OQUINU][ $\text{N}(\text{CN})_2$ ] could be described with the following simple additive rule:

$$EC_{50}[M^+X^-] = EC_{50}[M^+Br^-] + EC_{50}[K^+X^-] + EC_{50}[K^+Br^-]$$

This rule did not hold for IL based on [TROP] or [MMOR] cations. In these cases, replacing bromide with  $[\text{N}(\text{CN})_2]$  or  $[\text{CF}_3\text{SO}_3]$  yielded increases in toxicity significantly higher than those observed between KBr and  $\text{KN}(\text{CN})_2$  or  $\text{KCF}_3\text{SO}_3$ , respectively.

### 3.6. Hydrophobic anions yielding low toxic ionic liquids

In this work, toxicities of hydrophobic IL were measured starting from IL-saturated aqueous solutions. The  $EC_{50}$  value was then obtained as a percentage of the IL saturation concentration in water. Measuring the solubility in water of the corresponding IL yielded an  $EC_{50}$  value in  $\mu\text{M}$  units.

All hydrophobic IL based on  $[\text{NTf}_2]$  anion exhibited toxicities significantly higher than those of their bromide homologues. This can be explained by the high toxicity of  $\text{NTf}_2^-$  anion compared to that of bromide.

Nevertheless, no systematic increase in toxicity is found when  $[\text{NTf}_2^-]$  is used instead of a bromide anion. This emphasizes on the fact that specific interactions between the ions of an IL also play a significant role in toxicity. Previous studies [46,47] suggested that due to its hydrophobicity, the  $[\text{NTf}_2^-]$  anion might interact closely with the membrane and induce a starting intrusion of the alkyl chain of the IL cation into the lipidic bilayer.

In order to find hydrophobic IL with low toxicities and low solubilities in water, IL based on hydrophobic anions [FAP],  $[\text{B}(\text{CN})_4]$ ,  $[\text{BF}_4]$  and  $[\text{BPh}_4]$  and various alkyl chain lengths were studied.

The [FAP] anion is a perfluorinated phosphate anion that is known to be very hydrophobic. Despite the presence of a short ethyl chain on the imidazolium cation, the solubility in water of [EMIM][FAP] is very low ( $320 \text{ mg L}^{-1}$ ), similar to that of [O3M5MPYR][ $\text{NTf}_2$ ]. It is thus interesting to compare toxicities of

these two IL regarding their similar solubility in water and their difference in structure. [EMIM][FAP] turns out to be as toxic as [O3M5MPYR][NTf<sub>2</sub>]. This shows that [FAP] anion yields much more toxic IL than [NTf<sub>2</sub>] does, a result that is consistent with previous work [39]. In addition, our study provides a precise EC<sub>50</sub> value for [EMIM][FAP] in agreement with the concentration threshold (<2) found in the literature [39].

With a [BF<sub>4</sub>] anion associated to pyridinium or tropinium cations, IL appear to be less toxic than their [NTf<sub>2</sub>] homologues. This is consistent with the assumption that perfluorocarbon-based cation have an important effect on toxicity. Similar trends were previously observed on bacteria [19,31] or eukaryotic cells [18].

On the other hand, [EMIM][B(CN)<sub>4</sub>] exhibits a high solubility in water (approximately 47,000 mg L<sup>-1</sup>) and a low toxicity in water (3.62). Thus, using a short alkyl chain and a hydrophobic non-fluorinated anion can yield hydrophobic IL with low toxicity.

All these results are in agreement with other reports showing that the higher the lipophilicity, the higher the toxicity of an IL [21]. Since lipophilicity is related to hydrophobicity, we can expect that the more hydrophobic the anion or the alkyl chain, the more toxic the IL.

The last anion tested was tetraphenylborate [BPh<sub>4</sub>], known to yield IL with very low solubility in water [25]. [EOPIP][BPh<sub>4</sub>] exhibits a solubility in water below 5 mg L<sup>-1</sup>. At 50% of its saturation concentration in water, this IL induces a 15% decrease in the bioluminescence of *V. fischeri*. No EC<sub>50</sub> measurement after 15 min time exposure could then be carried out with this IL. Such a compound can therefore be less harmful for aqueous environments, because its solubility in water is lower than the EC<sub>50</sub>. However it may reach sediments of rivers and lakes where it can accumulate [48,49]. It may even bind to suspended organic matter and bio-accumulate in plankton or other living organisms, such as fish, then representing long term hazards for biocoenosis. In fact, this problem can be generalized to all types of IL, including the hydrophilic ones that exhibit low toxicity such as [BMMOR][Br].

#### 4. Conclusions

54 IL containing various cation rings, side chains, and anions were studied in this work. Toxicities ranged from 9.4 10<sup>-6</sup> to 0.28 M. IL containing cations based on tropinium or quinuclidinium were tested for the first time. This study confirmed that IL cannot be considered as a whole new class of either green or toxic compounds. Their structure has indeed a strong effect on toxicity. Our work demonstrates that it is possible to dramatically decrease the toxicity of an IL by carefully choosing the cation and anion, thus paving a way towards the design of environmentally friendly room-temperature IL.

Except for choline chloride, all IL were found to be more toxic than acetonitrile. Three of them ([BMMOR][Br], [BTROP][Br] or [BB(CN)PYRRO][Br]) were less toxic than ethyl acetate. Moreover, IL containing a butyl chain appeared to be less toxic than phenols. The five least toxic IL containing bromide anion were based on choline, [BMMOR], [BTROP], [BB(CN)PYRRO], and [BQUINU] cations.

As expected, the length of the alkyl chain had a significant influence on the toxicity of IL. Surprisingly we also found that the methyl groups appended to a pyridinium cation had the same effect on toxicity as the alkyl chain length. A good linear correlation was obtained between EC<sub>50</sub> values and the number of aliphatic carbons surrounding pyridinium cations. Furthermore, a fairly good correlation was found between EC<sub>50</sub> values and the total number of carbons on cations imidazolium, pyridinium, pyrrolidinium or piperidinium.

Toxicity decreased significantly when a functional group, such as nitrile, hydroxyl or ether was added onto the cation, either the alkyl chain or the cation ring.

Hydrophilic IL containing low toxicity cations exhibited relatively low toxicity. Among them, [BMMOR][N(CN)<sub>2</sub>] appeared to have the lowest toxicity value, close to that of potassium chloride.

As expected, all hydrophobic IL based on [NTf<sub>2</sub>] anion were toxic. Decreasing the solubility in water by changing the nature of the cation ring or the alkyl chain length led to a systematic increase in toxicity. Using other hydrophobic anions such as [FAP] anion resulted in equally toxic IL. Only [B(CN)<sub>4</sub>] anion yielded an IL, [EMIM][B(CN)<sub>4</sub>], exhibiting a fairly low toxicity. [EOPIP][BPh<sub>4</sub>] displayed such a low solubility in water that no EC<sub>50</sub> value could be measured. It was shown finally that for a given set of IL, MCA could be quite helpful in finding relevant parameters influencing their toxicity.

#### Acknowledgement

We would like to kindly thank Dr. Cristina Silva Pereira (ITQB, Instituto de Tecnologia Química e Biológica, Portugal) for her valuable help and very useful discussions regarding this work.

#### References

- [1] J.G. Huddleston, A.E. Visser, W.M. Reichert, H.D. Willauer, G.A. Broker, R.D. Rogers, Characterization and comparison of hydrophilic and hydrophobic room temperature ionic liquids incorporating the imidazolium cation, *Green Chem.* 3 (2001) 156–164.
- [2] D. Gerhard, S.C. Alpaslan, H.J. Gores, M. Uerdingen, P. Wasserscheid, Trialkylsulfonium dicyanamides – A new family of ionic liquids with very low viscosities, *Chem. Commun.* (2005) 5080–5082.
- [3] D.R. MacFarlane, P. Meakin, J. Sun, N. Amini, M. Forsyth, Pyrrolidinium imides: a new family of molten salts and conductive plastic crystal phases, *J. Phys. Chem. B* 103 (1999) 4164–4170.
- [4] J.M. Crosthwaite, M.J. Muldoon, J.K. Dixon, J.L. Anderson, J.F. Brennecke, Phase transition and decomposition temperatures, heat capacities and viscosities of pyridinium ionic liquids, *J. Chem. Thermodyn.* 37 (2005) 559–568.
- [5] P. Kilaru, G.A. Baker, P. Scovazzo, Density and surface tension measurements of imidazolium-, quaternary phosphonium-, and ammonium-based room-temperature ionic liquids: data and correlations, *J. Chem. Eng. Data* 52 (2007) 2306–2314.
- [6] O. Hiroyuki, Y. Masahiro, M. Tomonobu, Ionic conductivity, in: O. Hiroyuki (Ed.), *Electrochemical Aspects of Ionic Liquids*, John Wiley & Sons, Inc., 2005, pp. 75–81.
- [7] V.I. Parvulescu, C. Hardacre, Catalysis in ionic liquids, *Chem. Rev.* 107 (2007) 2615–2665.
- [8] F. Van Rantwijk, R.A. Sheldon, Biocatalysis in ionic liquids, *Chem. Rev.* 107 (2007) 2757–2785.
- [9] N.V. Plechkova, K.R. Seddon, Applications of ionic liquids in the chemical industry, *Chem. Soc. Rev.* 37 (2008) 123–150.
- [10] A. Albinet, N. Papaiconomou, J. Estager, J. Suptil, J.L. Besombes, A new ozone denuder for aerosol sampling based on an ionic liquid coating, *Anal. Bioanal. Chem.* 396 (2010) 857–864.
- [11] A. Albinet, N. Papaiconomou, J. Estager, J. Suptil, M. Draye, J.L. Besombes, Erratum to: a new ozone denuder for aerosol sampling based on an ionic liquid coating, *Anal. Bioanal. Chem.* 397 (2010) 887.
- [12] K.M. Docherty, C.F. Kulpa, Toxicity and antimicrobial activity of imidazolium and pyridinium ionic liquids, *Green Chem.* 7 (2005) 185–189.
- [13] D. Zhao, Y. Liao, Z. Zhang, Toxicity of ionic liquids, *Clean – Soil, Air, Water* 35 (2007) 42–48.
- [14] M. Petkovic, J. Ferguson, A. Bohn, J. Trindade, I. Martins, M.B. Carvalho, M.C. Leitaó, C. Rodrigues, H. Garcia, R. Ferreira, K.R. Seddon, L.P.N. Rebelo, C. Silva Pereira, Exploring fungal activity in the presence of ionic liquids, *Green Chem.* 11 (2009) 889–894.
- [15] N. Wood, G. Stephens, Accelerating the discovery of biocompatible ionic liquids, *Phys. Chem. Chem. Phys.* 12 (2010) 1670–1674.
- [16] M. Petkovic, K.R. Seddon, L.P.N. Rebelo, C. Silva Pereira, Ionic liquids: a pathway to environmental acceptability, *Chem. Soc. Rev.* 40 (2011) 1383–1403.
- [17] A. Romero, A. Santos, J. Tojo, A. Rodríguez, Toxicity and biodegradability of imidazolium ionic liquids, *J. Hazard. Mater.* 151 (2008) 268–273.
- [18] R.A. Kumar, N. Papaiconomou, J.-M. Lee, J. Salminen, D.S. Clark, J.M. Prausnitz, In vitro cytotoxicities of ionic liquids: effect of cation rings, functional groups, and anions, *Environ. Toxicol.* 24 (2009) 388–395.
- [19] M. Matzke, S. Stolte, K. Thiele, T. Juffernholz, J. Arning, J. Ranke, U. Welz-Biermann, B. Jastorff, 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 (2007) 1198–1207.



- [20] A.S. Wells, V.T. Coombe, On the freshwater ecotoxicity and biodegradation properties of some common ionic liquids, *Org. Process Res. Dev.* 10 (2006) 794–798.
- [21] S. Stolte, M. Matzke, J. Arning, A. Böhen, W.R. Pitner, U. Welz-Biermann, J. Ranke, Effects of different head groups and functionalised side chains on the aquatic toxicity of ionic liquids, *Green Chem.* 9 (2007) 1170–1179.
- [22] I. Billard, A. Ouadi, C. Gaillard, Liquid–liquid extraction of actinides, lanthanides, and fission products by use of ionic liquids: from discovery to understanding, *Anal. Bioanal. Chem.* 400 (2011) 1555–1566.
- [23] A. Chapeaux, M.A. Stadherr, J.F. Brennecke, Liquid phase behavior of ionic liquids with water and 1-octanol and modeling of 1-octanol/water partition coefficients, *J. Chem. Eng. Data* 52 (2007) 2462–2467.
- [24] M.G. Freire, C.M.S.S. Neves, P.J. Carvalho, R.L. Gardas, A.M. Fernandes, I.M. Marrucho, L.M.N.B.F. Santos, J.A.P. Coutinho, Mutual solubilities of water and hydrophobic ionic liquids, *J. Phys. Chem. B* 111 (2007) 13082–13089.
- [25] N. Papaiconomou, J. Salminen, J.-M. Lee, J.M. Prausnitz, Physicochemical properties of hydrophobic ionic liquids containing 1-octylpyridinium, 1-octyl-2-methylpyridinium, or 1-octyl-4-methylpyridinium cations, *J. Chem. Eng. Data* 52 (2007) 833–840.
- [26] R. Germani, M.V. Mancini, G. Savelli, N. Spreti, Mercury extraction by ionic liquids: temperature and alkyl chain length effect, *Tetrahedron Lett.* 48 (2007) 1767–1769.
- [27] Y. Yan-Ying, Z. Wei, C. Shu-Wen, Extraction of ferulic acid and caffeic acid with ionic liquids, *Chin. J. Anal. Chem.* 35 (2007) 1726–1730.
- [28] P. Giridhar, K.A. Venkatesan, S. Subramaniam, T.G. Srinivasan, P.R. Vasudeva Rao, Extraction of uranium (VI) by 1.1 M tri-n-butylphosphate/ionic liquid and the feasibility of recovery by direct electrodeposition from organic phase, *J. Alloys Compd.* 448 (2008) 104–108.
- [29] N. Papaiconomou, J.-M. Lee, J. Salminen, M. von Stosch, J.M. Prausnitz, Selective extraction of copper, mercury, silver, and palladium ions from water using hydrophobic ionic liquids, *Ind. Eng. Chem. Res.* 47 (2008) 5080–5086.
- [30] J. Salminen, N. Papaiconomou, R.A. Kumar, J.-M. Lee, J. Kerr, J. Newman, J.M. Prausnitz, Physicochemical properties and toxicities of hydrophobic piperidinium and pyrrolidinium ionic liquids, *Fluid Phase Equilib.* 261 (2007) 421–426.
- [31] N. Papaiconomou, J. Estager, Y. Traore, P. Bauduin, C. Bas, S. Legeai, S. Viboud, M. Draye, Synthesis, physicochemical properties, and toxicity data of new hydrophobic ionic liquids containing dimethylpyridinium and trimethylpyridinium cations, *J. Chem. Eng. Data* 55 (2010) 1971–1979.
- [32] 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, ISO, Geneva, 1998.
- [33] ASTM, Test Method for Assessing the Microbial Detoxification of Chemically Contaminated Water and Soil Using a Toxicity Test with a Luminescent Marine Bacterium, ASTM international Designation: D5660-96, West Conshohocken, PA, 2009.
- [34] J.P. Benzécri, L'analyse des données: l'analyse des correspondances, Dunod, Paris, 1973.
- [35] M. Greenacre, Theory and Applications of Correspondence Analysis, Academic Press Inc., London, Orlando, FL, 1984.
- [36] V. Aruoja, M. Sihtmäe, H.C. Dubourguier, A. Kahru, Toxicity of 58 substituted anilines and phenols to algae *Pseudokirchneriella subcapitata* and bacteria *Vibrio fischeri*: Comparison with published data and QSARs, *Chemosphere* 84 (2011) 1310–1320.
- [37] K.L.E. Kaiser, V.S. Palabrica, Photobacterium phosphoreum toxicity data index, *Water Pollut. Res. J. Can.* 26 (1991) 361–431.
- [38] D.J. Couling, R.J. Bernot, K.M. Docherty, J.K. Dixon, E.J. Maginn, Assessing the factors responsible for ionic liquid toxicity to aquatic organisms via quantitative structure–property relationship modeling, *Green Chem.* 8 (2006) 82–90.
- [39] S. Stolte, J. Arning, U. Bottin-Weber, M. Matzke, F. Stock, K. Thiele, M. Uerdingen, U. Welz-Biermann, B. Jastorff, J. Ranke, Anion effects on the cytotoxicity of ionic liquids, *Green Chem.* 8 (2006) 621–629.
- [40] M. Petkovic, J.L. Ferguson, H.Q.N. Gunaratne, R. Ferreira, M.C. Leitao, K.R. Seddon, L.P. Rebelo, C.S. Pereira, Novel biocompatible cholinium-based ionic liquids–toxicity and biodegradability, *Green Chem.* 12 (2010) 643–649.
- [41] A.-M. Lacrămă, M. Putz, V. Ostafe, A spectral-SAR model for the anionic–cationic interaction in ionic liquids: application to *Vibrio fischeri* ecotoxicity, *Int. J. Mol. Sci.* 8 (2007) 842–863.
- [42] B. Jastorff, R. Störmann, J. Ranke, Thinking in structure–activity relationships – a way forward towards sustainable chemistry, *Clean – Soil, Air, Water* 35 (2007) 399–405.
- [43] M. Alvarez-Guerra, A. Irabien, Design of ionic liquids: an ecotoxicity (*Vibrio fischeri*) discrimination approach, *Green Chem.* 13 (2011) 1507–1516.
- [44] D.R. MacFarlane, J. Golding, S. Forsyth, M. Forsyth, G.B. Deacon, Low viscosity ionic liquids based on organic salts of the dicyanamide anion, *Chem. Commun.* (2001) 1430–1431.
- [45] M.J. Deng, P.Y. Chen, T.I. Leong, I.W. Sun, J.K. Chang, W.T. Tsai, Dicyanamide anion based ionic liquids for electrodeposition of metals, *Electrochem. Commun.* 10 (2008) 213–216.
- [46] K. Evans, Supported phospholipid bilayer interaction with components found in typical room-temperature ionic liquids – a QCM-D and AFM study, *Int. J. Mol. Sci.* 9 (2008) 498–511.
- [47] S.R.T. Cromie, M.G. Del Po'polo, P. Ballone, Interaction of room temperature ionic liquid solutions with a cholesterol bilayer, *J. Phys. Chem. B* 113 (2009) 11642–11648.
- [48] W. Mrozik, C. Jungnickel, T. Ciborowski, W. Pitner, J. Kumirska, Z. Kaczynski, P. Stepnowski, Predicting mobility of alkylimidazolium ionic liquids in soils, *J. Soils Sed.* 9 (2009) 237–245.
- [49] P. Stepnowski, W. Mrozik, J. Nichthausser, Adsorption of alkylimidazolium and alkylpyridinium ionic liquids onto natural soils, *Environ. Sci. Technol.* 41 (2006) 511–516.