Comparative Ecotoxicology Study of Two Neoteric Solvents: Imidazolium Ionic Liquid vs. Glycerol Derivative

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Abstract:

In this study we have compared the acute ecotoxicity of two solvents, with very different structure and origin, but sharing many physical-chemical properties, so they can be used for similar purposes; a well-known ionic liquid 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF_6]) and a solvent partially derived from biomass, 3-bis(2,2,2-trifluoroethoxy)propan-2-ol (BTFIP). We have used three biomodels (*Vibrio fischeri*, *Daphnia magna* and *Danio rerio*) and performed the comparison applying the Environmental, Health and Safety (EHS) hazard assessment. According to the results, ecotoxicity of [BMIM][PF_6] and BTFIP is quite similar in the simplest model *Vibrio fischeri*, while in *Daphnia magna* [BMIM][PF_6] is clearly more toxic. However, in *Danio rerio*, toxicity of these chemicals is again quite similar and both can be classified as "nontoxic". The higher index value of [BMIM][PF_6] in water mediate effect in the EHS assessment indicates that this ionic liquid is more dangerous than BTFIP, although accumulation and degradation properties have not been taken into account. Further studies will be necessary to ascertain these conclusions.

Keywords:

Solvents, ecotoxicology, ionic liquid, glycerol-derivative.

1. Introduction

The search for new solvents, coming from new sources and/or able to provide special features (often known as neoteric solvents), is a field of growing interest, especially in connection with the possibility of using renewable raw materials to produce harmless solvents, more respectful with the environment than those derived from petroleum (the so-called green solvents). For many years, ionic liquids (IL) have been considered as the "solvents of the future" (Earle and Seddon, 2000), due to their very particular combination of physical-chemical features: high polarity, almost null volatility, immiscibility with low-polar organic solvents, and, in some cases, with water as well. As a consequence, there is a huge amount of studies describing the use of IL for numerous different applications, and many IL are nowadays available from commercial sources. 1-Butyl-3-methylimidazolium hexafluorophosphate (henceforth [BMIM][PF_6]) is one of the most prominent examples of successful IL. However, as more knowledge has being gained on the toxicological profiles of this family of compounds, it has become clearer that the label of "green solvents" is not deserved in many cases (Bubalo et al., 2014; Deetlefs and Seddon, 2010; Petkovic et al., 2011; Romero et al., 2008). For instance, in the case of [BMIM][PF_6] it has been reported that the hexafluorophosphate anion can decompose in aqueous acidic medium to lead to 1-butyl-3-methylimidazolium fluoride hydrate, and hence to the toxic product HF (Holbrey et al., 2003); Swatloski et al., 2003).

On the other hand, biomass-derived chemicals are attracting a great interest in the last years, in connection with the development of the biorefinery concept. Agricultural and some industrial activities are able to generate huge amounts of raw materials, capable of being used to produce commodity and fine chemicals. In this sense, glycerol is one of the platform molecules that has received much attention in the last years (Katryniok et al., 2011; Pagliaro et al., 2007; Zhou et al., 2008). Glycerol appears as a concomitant product

in the production of biodiesel, amounting ca. 10% weight of the total output. At present, the world production of glycerol coming from vegetable oil transformations surpasses 2 million metric tons, so it constitutes a valuable starting point to obtain bio-based chemicals, useful as, for instance, solvents (Diaz-Álvarez et al., 2011; Diaz-Álvarez and Cadierno, 2013; García et al., 2014; Gu and Jerome, 2010). In this context, our research group has described the synthesis and application as solvents of a family of glycerol ethers (García et al., 2010). Some of these glycerol derivatives, namely those bearing fluoroalkyl chains, exhibited especial physical-chemical features, in some way similar to those displayed by some IL: high polarity, low vapour pressure at room temperature, an immiscibility both with hydrocarbons and with water. The most prominent example of these compounds is 1,3-bis(2,2,2-trifluoroethoxy)propan-2-ol, henceforth BTFIP, which can be efficiently prepared from trifluoroethanol and epichlorohydrin (a commodity produced from glycerol using the Solvay procedure). Table 1 gathers the comparison of some physical-chemical properties of [BMIM][PF_6] and BTFIP.

Both [BMIM][PF_6] and BTFIP have been used as solvents for biphasic enantioselective catalysis in two comparative studies carried out by our group. In the first one, the use of BTFIP in an enantioselective conjugate reduction catalysed by chiral azabis(oxazoline)–cobalt complexes showed to superior to that of [BMIM][PF_6], allowing better recovery of the catalytic phase and better enantioselectivities (90–96% ee vs. 40–85% ee with the IL (Aldea et al., 2010). The same situation arose in the second study, where the biphasic enantioselective Kharasch–Sosnovsky allylic oxidation, based on neoteric solvents and copper complexes of ditopic ligands, was studied (Aldea et al., 2012).

The question arises as to whether BTFIP can be considered an environmentally benign solvent or not, given the total lack of experimental evidences on its toxicity and ecotoxicity. We hypothesize that the ecotoxicity of this solvent, partially originating from

biomass, is lower than the abovementioned ionic liquid. With the aim of verifying our hypothesis, the ecotoxicity of BTFIP and [BMIM][PF_6] has been obtained through the evaluation of the toxic effect in three bioindicators (bacteria, crustacean and fish) corresponding to several trophic levels.

In order to perform a comparative study, the studied solvents have been evaluated making use of Environmental, Health and Safety (EHS) hazard assessment. This method was firstly proposed by Koller et al. (2000) as an intermediate attempted to account for the problems of early design phases. Environmental, Health and Safety (EHS) aspects are assessed in several categories corresponding to environmental, health or safety related properties.

2. Materials and Methods

2.1 Materials

2.1.1 Chemicals

1-Butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF_6]) was provided by Sigma-Aldrich (purity ≥ 97 %). In order to minimizing the water content, [BMIM][PF_6] was periodically dried for 24 h under a vacuum of ca. 0.05 kPa with stirring and stored before use in a desiccator.

1,3-Bis(2,2,2-trifluoroethoxy)propan-2-ol (BTFIP) was synthesised by the following procedure:

In a round bottom flask were placed 1 mol of trifluoroethanol (100 g, aprox. 75 mL) and then 1 mol (140 g) of potassium carbonate. The flask was heated up at 70 $^{\circ}$C, and 0.5 mol of epichlorohydrin (47 g) were then dropped into the flask. After 2 hours the reaction was complete. Cooling down the flask, the mixture was filtered to remove the carbonate salt.

112 The unreacted fluorinated alcohol was removed by heating under vacuum in a rotary
113 evaporator. The remaining product was purified by vacuum distillation to yield 108 g of
114 BTFIP (84%, GC purity > 99.5%).

115 Trend analysis and quantitative structure–activity relationship (QSAR) models were
116 evaluated previously using the QSAR Toolbox 2.3 (2009) which helped to select the
117 concentrations to be tested. QSAR is based on the correlation between structural molecular
118 characteristics of series of molecules and their chemical reactivity or biological activity.
119 Additionally, a previous study was carried out to refine the range of concentrations and
120 make sure the tested concentrations within EC_{50}/LC_{50}.

121 2.2 Ecotoxicological tests

122 2.2.1 *Vibrio fischeri* (*V. fischeri*) Inhibition of Bioluminescence Test

123 The lyophilized *V. fischeri* (strain NRRL-B-11177) used for Inhibition of bioluminescence
124 test were purchased from Macherey-Nagel (ref. 945 006). This experiment was carried out
125 according with the test conditions and the operating protocol of the *V. fischeri* acute
126 toxicity test (UNE-EN-ISO 11348-3, 2007). Prior to testing, bacteria were rehydrated using
127 the corresponding reactivation solution provided by the manufacturer. Afterwards, bacteria
128 were stored at a temperature between 2–8 ºC for 5 min.

129 Several dilutions for each of the studied solvents were prepared using a 2% NaCl stock
130 solution. The different concentrations range for these compounds have been between 500
131 and 5000 $mg \cdot L^{-1}$ (500, 1000, 1250, 2250, 2500, 3000, 3750, 4000, 4500, 5000) for
132 BMIM][PF_6] and 300-2500 $mg \cdot L^{-1}$ (300, 475, 625, 950, 1250, 1900, 2500, 5000) for
133 BTFIP. Additionally, negative and positive controls with zinc sulfate (2.2 mg/L) and
134 phenol (42.5 mg/L) were tested (Jennings et al., 2001). The pH of the solutions was
135 adjusted to 7–7.5 using either 0.1 M HCl or 0.1 M NaOH solutions.

Next, the initial luminescence of the bacteria was measured after transferring 0.5 mL of the reactivated bacterial suspension at 15 ºC to cuvettes; then 0.5 mL of each dilution to be tested was added to the cuvette. The toxicity is reflected in the ratio of the decrease in bacterial light production to the remaining light. The luminescence was measured again after 30 min. The test was repeated twice.

Luminescence was measured with a Biofix® Lumi-10 luminometer (Macherey-Nagel) using the acute mode (Biotox B) with an ultra-fast single-photon counter detector covering the 380–660 nm spectral range. The sensitivity is 10 fmol ATP when using ATP bioluminescence assays CLS II (Roche Diagnostics GmbH, Mannheim Germany).

The percentage of bioluminescence inhibition (%I) is calculated from the initial and final bacterial light intensity. Details of the specific Biofix® method used can be found elsewhere (Lomba et al., 2014).

2.2.2 *Daphnia magna* (*D. magna)* Acute Immobilization Test

The *D. magna* used in the acute immobilization test were purchased from Vidrafoc (ref. DM090812) and were stored at 4 ºC. This experiment was carried out following the guidelines of the OECD 202 test conditions and operating protocol (OECD 202, 1984 ; OC SE TG 202, 2004).

Firstly, the medium for the eggs was prepared according to the specifications of the supplier. Then, the eggs were incubated for 72 hours at 20–22 °C with 6000 lux in a TOXKIT model CH-0120D-AC/DC incubator (supplied by ECOTEST) and fed with Spirulina 2 hours prior to starting the bioassay.

Several dilutions for the studied chemicals were prepared in aqueous medium solution. The different concentrations range for these compounds have been between 3 and 100 mg/L (3,

6, 10, 20, 25, 42.5, 75, 100) for [BMIM][PF_6] and between 30 and 1500 mg/L (30, 90, 250, 500, 750, 1000, 1250, 1500) for BTFIP. Furthermore, negative and positive controls with $K_2Cr_2O_7$ (0.6–2.1 mg/L) were also tested (OECD 202, 1984 ; OC SE TG 202, 2004). The pH of the solutions was adjusted to be between 7–7.5 using 0.1 M NaOH or 0.1 M HCl solutions.

A total of 20 daphnids aged < 24 h, were exposed to the studied chemicals in complete darkness for 24 hours at 20–22°C for each concentration tested. The organisms were divided into four groups of five organisms per group. Once again, the test was repeated twice.

The immobilization of the daphnids was measured taking into account that the organisms that were unable to swim for 15 seconds after gentle stirring were considered immobile.

2.2.3 *Danio rerio* (*D. rerio*) acute toxicity test

Fish acute toxicity experiments were performed in a laboratory (The Centre de Recerca i Innovació en Toxicología from the Universitat Politècnica de Catalunya in Spain) fulfilling the criteria of Good Laboratory Practice. They were conducted in accordance with specifications of OECD 203(1992).

All of the toxicity tests were carried out at a temperature of 22±2 °C in 1.5-L aquaria with dechlorinated drinking water. The number of fishes in each experimental and control group was 7. The light regimen was 16 h light/8 h diffuse light, oxygen concentration >60% and pH = 8.3–8.5.

The acute toxicity tests of 96 h duration were run in a static exposure system (without renewing the test solution). Fish were exposed to eight and seven different concentrations

of BTFIP and [BMIM][PF_6], respectively, according to OECD indications. They were not fed during the test period and their mortality and behavioral changes were recorded at 3, 24, 48, 72 and 96 hours.

Results were validated and repetitions were not necessary. Validation criteria included the maintenance of constant assay conditions, mortality of control under 10% and diluted oxygen concentration at least 60% of air saturation value.

2.3 Statistics and graphical representation

Experimental results obtained have been fitted using the least squares method to the following function to obtain the corresponding EC_{50}/LC_{50} values and standard deviations (SD):

$$\%I = 100/(1 + 10^{(logEC_{50} - logc)a})$$

where $\%I$ denotes % bioluminescence inhibition for *V. fischeri*, % immobilization for *D. magna* and % death for *D. rerio*, $logEC_{50}$ and a are the adjustable parameters.

The statistical analysis was performed using the SPSS 18.00 software (IBM® SPSS software). A threshold of p=0.05 has been set to accept or reject the null hypothesis.

2.4 EHS assessment

EHS method includes a total of 11 effects corresponding to three categories: environment, health and safety. In this case, according to the nature of the study which aims to conduct an environmental assessment of the risks associated with the use of the two solvents under study, it has been decided to assess only the Environment category.

The assessment of the EHS aspects is divided into different effects. If these effects can be analysed in a similar way, they are combined to the so called dangerous properties. There

206 are two values defined for each dangerous property: an index ($IndVal_{ij}$) and a physical
207 value ($PhysVal_{m,j}$).

208 We have compared the environmental risk taking into account the effective dangerous
209 property (EDP_{ij}) of each solvent (eq.1).

210 $$EDP_{ij} = IndVal_{ij} + F_{ij} \quad (1)$$

211 where $IndVal_{ij}$ is defined as:

212 $$IndVal_{ij} = \max(IndVal_{ij,m}) \quad (2)$$

213 being $IndVal_{ij,m}$ the index value of the substance *j* defined for each the *i* dangerous
214 properties of each of the *m* categories.

215 In this case, the selected dangerous properties for assessing the Environment category are
216 water-mediated effects (LC_{50}/EC_{50} acute), degradation (half-life in environment) and
217 accumulation (log k_{ow}).

218 F_{ij} is set to 0 for dangerous property accumulation and degradation while for water-
219 mediated effects for organic substances is defined as follows:

220 $$F_{ij} = 0.2xlog(PhysVal_{degradation,j} x PhysVal_{accumulation,j}) \quad (3)$$

221 Being $PhysVal_{m,j}$ the physical value.

222 From the original EHS approach (Koller et al., 2000), several correlations between the
223 experimental or calculated properties used for the evaluation and the index ($IndVal_{ij}$) and
224 physical value $PhysVal_{m,j}$. The information needed to carry out the method is gathered in
225 Table 2.

226

3. Results and discussion

3.1 Ecotoxicology tests

EC_{50}/LC_{50} values obtained in *V. fischeri*, *D. magna* and *D. rerio* with their respective standard deviations are gathered in Table 3. Furthermore, results are graphically represented in Fig. 1–3.

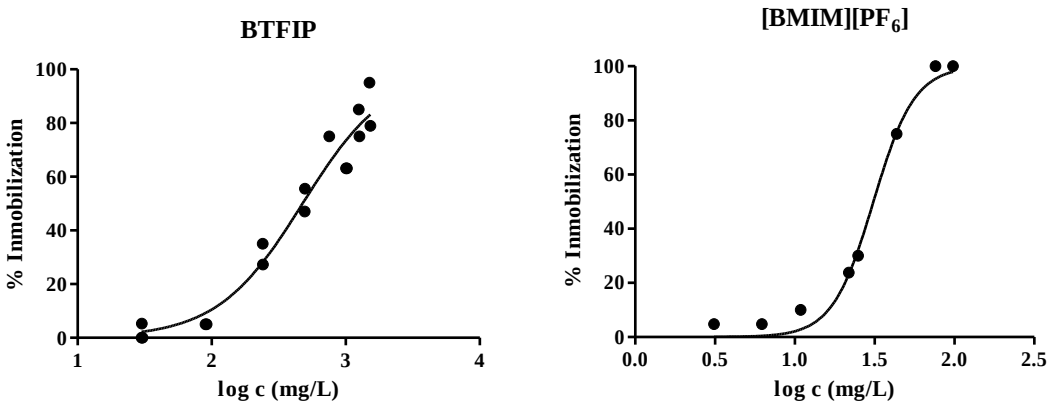

Fig. 1: Results for *D. magna*

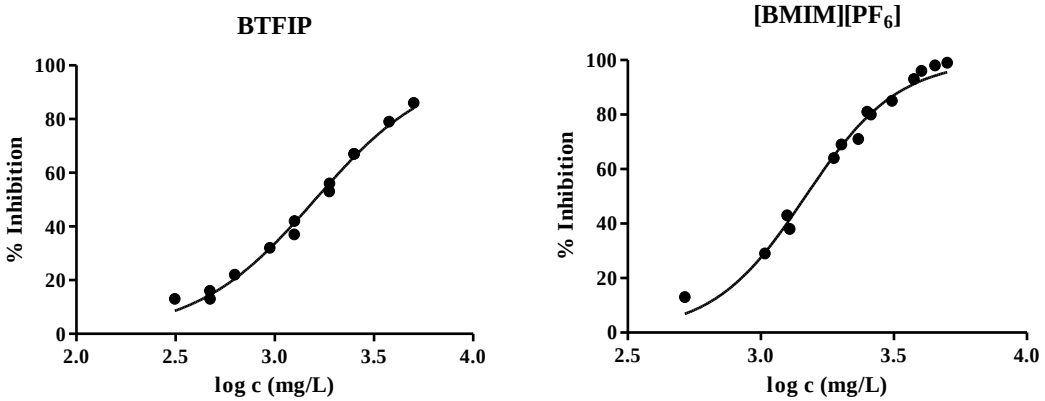

Fig. 2: Results for *V. fischeri*

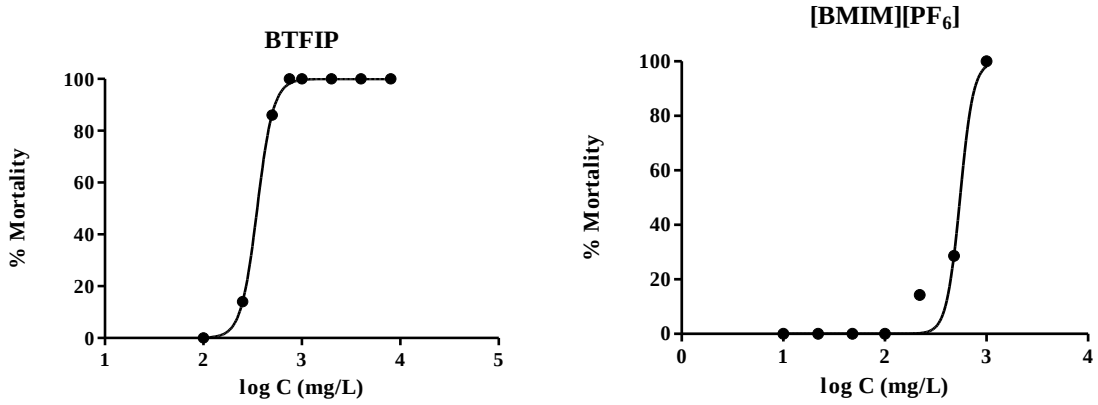

Fig. 3: Results for *D. rerio*

The proposed hypothesis has been partially verified; ecotoxicity of the [BMIM][PF_6] is higher compared to BTFIP except for *D. rerio*.

In the case of *V. fischeri*, EC_{50} obtained values are quite similar for both chemicals and, in general, none of them can be considered as toxic for the environment using this bioindicator (United Nations, 2011). However, the ionic liquid [BMIM][PF_6] is slightly more ecotoxic. Although the action mechanism is still unidentified, it is known that the bacterial bioluminescence reactions are coupled to the electron transport system in cellular respiration and are indicative of cellular metabolism (Onorati and Mecozzi, 2004). In that sense, lower bioluminescence implies decreased cellular respiration. Thus, [BMIM][PF_6] causes a slightly higher effect in the cellular respiration than BTFIP.

Both solvents affected the mobility of *D. magna*. Although, once again, the action mechanisms are not known yet, several authors have suggested that solvents could cause enzyme inhibition, disruption of membrane permeability, structural damage and oxidative stress (Bernot et al., 2005). In this case, the results obtained for *D. magna* indicate that the glycerol-derived solvent BTFIP is much less toxic than the ionic liquid [BMIM][PF_6].

256 Thus, it is possible to categorize [BMIM][PF_6] as belonging to the Category: Acute III and
257 as "moderately toxic" whereas BTFIP can be classified as nontoxic or "practically
258 harmless", according to the United Nations classification (United Nations, 2011) or
259 Passino and Smith (Passino and Smith, 1987) classification respectively.

260 According to the lethal concentrations in *D. rerio*, both solvents are quite similar and
261 would be classified as Category: Acute III by the United Nations classification (United
262 Nations, 2011) or as "practically harmless" by Passino and Smith (Passino and Smith,
263 1987), although LC_{50} is higher in [BMIM][PF_6] than in BTFIP.

264 Nevertheless, fishes died during the first hour of exposition to concentrations of BTFIP
265 higher than 2000 mg/L. Additionally, behavioral alterations of fish were observed during
266 the assay, including immobility and periods of swimming on their back followed by erratic
267 movements and death, when exposed to higher concentrations of BTFIP (at 500 mg/L and
268 750 mg/L concentrations). This result suggested some kind of alteration of the central
269 nervous system. Normal behavior was only observed when fishes were exposed to
270 concentrations of BTFIP lower than 250 mg/L.

271 No alterations of behavior of zebrafish were observed in expositions to [BMIM][PF_6].

272 Based on the EC_{50} and LC_{50} values calculated, toxicity of BTFIP is higher in *D. rerio* than
273 in the other two biomodels. In contrast, the crustacean *D. magna* is the most sensitive
274 organism to [BMIM][PF_6], followed by the fish *D. rerio* and finally by the bacteria *V.*
275 *fischeri*. This clearly shows the substantial difference in the sensitivities of the different
276 organisms studied.

277 It should be emphasized that single bioassays have limitations, so transfer or prediction of
278 ecotoxicological data obtained with different biomodels is not always valid. It is hard to
279 make predictions from a lower level of organization to higher ones. (Lange et al., 1998).

For this reason, it is necessary to include in the study organisms from different levels to allow a better understanding of possible effects of the studied chemicals in ecosystems.

3.2 ESH assessment

It is important to note that ecotoxicity data are not enough to completely assess the environmental risk of the studied compounds. The evaluation of other properties, important from the environmental point of view, such as bioavailability or biodegradability, provide a more accurate view of the associated risk, when combined with ecotoxicity data. ESH assessment takes into account these parameters.

According to this approach, an index value of value of 0 represents harmless substances whereas a value of 1 indicates dangerous substances regarding the considered ESH effect. It is worth mentioning, that the calculated indexes for accumulation are negative values. This means that none of the studied compounds show a risk from the viewpoint of mobility and bioaccumulation in biological systems, since the threshold value of partition coefficient that corresponds to the minimum index value is set to 3. In the case of the dangerous property water mediate effect, the higher index value is obtained for [BMIM][PF_6], indicating a more dangerous substance than BTFIP. However, taking into account accumulation and degradation dangerous properties, the ionic liquid [BMIM][PF_6] would be less dangerous.

When the index value water mediated effect is modified with the relevant fate index to obtain the effective dangerous property, BTFIP results more dangerous than the ionic liquid. The effective dangerous property is reduced if the substance is degradable or increased if the substance has an accumulation potential. In this case, although index value for water mediated effect is higher for [BMIM][PF_6], degradation rate of BTFIP modifies the index value significantly. However, it should be pointed out that experimental

degradation data of the studied solvents are not available, so the information needed to perform the approach has been obtained from Biowin models (US EPA, 2012) .

In addition to the physicochemical and ecotoxicological properties of the solvents, there are also other important factors affecting their greenness: uses and applications of the solvents, their lifecycle, production processes or removal rate in depuration processes.

4. Conclusions

This work provides experimental data on ecotoxicity potentials of BTFIP and [BMIM][PF_6], two solvents with very different structure and origin, but sharing many physical-chemical properties, so they can be used for similar purposes. Although raw data based only in the calculations of effect and lethal concentrations suggests that BTFIP (a solvent partially derived from biomass) is less harmful than [BMIM][PF_6] (an ionic liquid), specially for *D. magna* (since for the rest of biomodels, none of studied solvents can be considered toxic). This could justify the substitution of the ionic liquid by the glycerol derivative. However, the alteration of behavior of the vertebrate biomodel and the ESH assessment points to the fact that BTFIP could be more harmful than suspected for the environment, so further studies will be necessary to ascertain this point.

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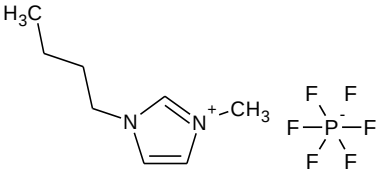
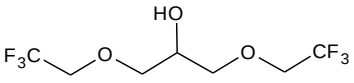
Property	Solvent	
Name	1-butyl-3-methylimidazolium hexafluorophosphate	1,3-bis(2,2,2-trifluoro-ethoxy)propan-2-ol
Code	[BMIM][PF_6]	BTFIP
Structure		
Molecular mass (g mol^{-1})	284.18	256.14
Density (g cm^{-3})	1.365[a]	1.384[b]
Refraction index	1.411[a]	1.352[b]
m.p. (ºC)	–8 ºC[a]	–6 ºC
b.p. (ºC)	>350 ºC	197 ºC[b]
Vap. P at r.t. (mm Hg)	~0	0.4[b]
Viscosity at r.t. (cP)	312[a]	8.14[b]
Water solubility (wt./wt.)	0.0230[c]	0.0284[b]
Solvatochromic polarity parameters:		

E_T^N	0.64–0.69[d]	0.70[b]
π*	0.89–1.04[d]	0.38[b]
α	0.63–0.68[d]	0.82[b]

a (Carda-Broch et al., 2003) b (García et al., 2010) c (Chapeaux et al., 2007) d (Jessop et al., 2012)

Table 1: Studied solvents and some of their relevant physical-chemical properties

	Water mediated effects			Accumulation		Degradation	
	Mean value EC_{50} (mg/L)	$IndVal_{ij}$	EDP_{ij}	Log k_{ow}	$EDP_{ij} = IndVal_{ij}$	Halflife (days)	$EDP_{ij} = IndVal_{ij}$
BTFIP	809	0.02	-0.44	1.424[a]	-0.79	21[c]	0.66
[BMIM][PF_6]	685	0.04	-1.39	-1.66[b]	-2.33	4[c]	0.30

a (Garcia et al., 2010) b (Ropel et al., 2005) c Using Ultimate and Primary Biodegradation Models (Biowin 3 and 4) from EPIWEB 4.1 (US EPA, 2012) $IndVal_{ij} = -0.109 * \ln(Mean\ value\ EC_{50}) + 0.75$ for water mediate effects. $IndVal_{ij} = 0.5 * (\text{Log kow}) - 1.5$ and $PhysVal = 0.001 * e^{(2.3026 * \text{Log kow})}$ for accumulation. $IndVal_{ij} = 0.2171 * \ln(Halflife)$ and $PhysVal = 0.01 * Halflife - 0.037$ for degradation.

Table 2: Substance data used for ESH assessment and results.

	V. fischeri		*D. magna*		*D. rerio*	
	EC_{50}	SD	EC_{50}	SD	LC_{50}	SD
BTFIP	1597	2.375	477	6.978	353	0.773
[BMIM][PF_6]	1473	3.293	31	4.538	550	6.348

Table 3: Effect concentrations and lethal concentration in mg/L and their corresponding standard deviations.