



Cite this: *Green Chem.*, 2020, **22**, 6176

Applicability of heuristic rules defining structure–ecotoxicity relationships of ionic liquids: an integrative assessment using species sensitivity distributions (SSD)[†]

Beatriz Mano,^a Fátima Jesus,^{*a,b} Fernando J. M. Gonçalves,^{id a,b}
Sónia P. M. Ventura^{id *c} and Joana Luísa Pereira^{id a,b}

The toxicity of Ionic Liquids (ILs) to aquatic organisms has been a matter of substantial interest, involving the toxicity assessment for a small number of species, which is limitative given the variation in species sensitivity to different classes of ILs. Therefore, the main objective of this study was to validate, using an integrative approach (*i.e.*, integrating the responses of several species), the heuristic rules that have been assumed for the ecotoxicity of ILs, namely the effects of the cation, elongation of the cation alkyl chain and anion moiety. For this purpose, four ILs were selected as models and their toxicity was determined for a wide variety of species, which allowed the development of species sensitivity distribution curves. The analysis of the distribution curves enables the determination of hazard concentrations affecting 5% of the represented communities (HC₅) and also the establishment of structure–ecotoxicity relationships for ILs. The median effect concentration (EC₅₀) values varied widely and the species most sensitive to each IL was variable. The integrative HC₅ values varied by five orders of magnitude, between 3.020 × 10⁻³ mg L⁻¹ representing the 1-dodecyl-3-methylimidazolium chloride, which raises concerns on the environmental hazardous potential of this IL, and 106.9 mg L⁻¹ for cholinium chloride, which confirms its low environmental toxicity. The SSD approach showed cholinium chloride as the least toxic IL, followed by cholinium dihydrogenocitrate, 1-ethyl-3-methylimidazolium and 1-dodecyl-3-methylimidazolium chloride, as the most toxic. This illustrates the minor effect of the anion on toxicity for this set of ILs, whereas the cation and the cation alkyl chain length had pronounced effects, validating the heuristic rules defining structure–ecotoxicity relationships of ILs. A very strong linear correlation between hydrophobicity and HC₅ was found ($\rho = -0.9991$). This approach allows a more efficient prediction of the potential environmental effects of ILs, thus preventing the need to comprehensively assess to the ecotoxicity of all ILs, which can be many within each family and each cation/anion possible combination. Ultimately, this will sustain the development of ILs posing less environmental hazards nonetheless retaining the desired performance.

Received 20th July 2020,
Accepted 28th August 2020
DOI: 10.1039/d0gc02486d

rsc.li/greenchem

Introduction

Ionic liquids (ILs) have been attracting much interest due to their physicochemical properties, namely their negligible vapor pressure, stability against air and moisture and non-flammability.¹ These properties granted their classification as

more biocompatible solvents and boosted their application in many chemical processes. Moreover, ILs have the advantage of being “designer solvents” as their properties can be designed to meet a specific application. Such design is achieved through modification of the cation and/or the anion by structural rearrangements: adding or replacing functional groups (*e.g.* ether, hydroxyl, among others), and/or aromatic rings; and/or by modifying the alkyl chains length.^{2,3} Hence, the number of ILs that can be synthesized is virtually countless.

Despite ILs being initially considered as green solvents, studies have been claiming that some ILs can be toxic to aquatic species.^{4,5} The need to assess ILs ecotoxicity to comply with regulatory demands (*e.g.* the REACH framework⁶ – requires read-across approaches (*e.g.* ref. 7) based on defined structure–ecotoxicity relationships. Trends ruling ILs ecotoxi-

^aDepartment of Biology, University of Aveiro, Portugal

^bCESAM – Centre for Environmental and Marine Studies, University of Aveiro, Portugal. E-mail: fatima.jesus@ua.pt

^cCICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal. E-mail: spventura@ua.pt

[†]Electronic supplementary information (ESI) available: EC₂₀ and EC₁₀ values, with the respective 95% confidence intervals (CI), obtained in the ecotoxicological tests performed in the present study. See DOI: 10.1039/d0gc02486d

city have been established as a function of the cation, cation alkyl chain length and anion. These heuristic rules rely on the effect that structural changes have on several parameters, namely on ILs lipophilicity – alterations leading to decreased lipophilicity will likely result in decreased ecotoxicity.^{8–10} For instance, the functionalization of the cation commonly leads to decreased ecotoxicity due to decreased lipophilicity.^{8,9,11} The same applies to alterations in the anion.^{9,12} On the other hand, the increase of the length of the cation alkyl side chains tends to increase ILs ecotoxicity (“side-chain effect”) until a certain threshold,^{8,9} which is also valid, although less prominently, for the anion moiety.⁴ Despite these rules apply to both the cation and the anion, alterations in the cation usually have more pronounced effects, underlying the assumption of the cation as the main driver of toxicity.^{8,13}

However, exceptions to these rules were observed depending on the biological system. For instance, the elongation of the alkyl chains of the anion moiety led to increased toxicity for *Daphnia magna*, following the heuristic rule, but not for the microalgae *Raphidocelis subcapitata* or the macrophyte *Lemna minor*.⁴ Exceptions to this heuristic rule were also found considering the cation (e.g. ref. 9) and the link between lipophilicity and toxicity of ILs has also been questioned.¹¹ Thus, it is pertinent to assess the consistency of the established heuristic rules among the responses of different organisms before assuming the feasibility of relating molecular structure to ecotoxicity in general.

In fact, the complex species-specific nature of ILs ecotoxicity hampers the use of mathematical models relating structural properties of ILs to their ecotoxicity, such as structure–activity relationships (SAR).¹⁴ An alternative to these approaches is to base the ecotoxicity variables feeding SARs in species sensitivity distributions (SSDs), which allow the determination of ecological hazard effects of chemicals in probabilistic risk assessment procedures. This method has been widely used to derive environmental quality standards by estimating the chemical concentrations which are protective of a fraction of species in the environment. Commonly, the hazardous concentration for 5% of species (HC₅) is calculated, which represents the concentration threshold under which 95% of the species are protected from toxic effects due to a chemical.

As an extension of a previous study,⁴ herein we aim to assess the consistency of the heuristic rules for the relationship between molecular structure and ILs ecotoxicity using SSDs as an integrative approach covering for the sensitivity of a wide array of ecotoxicological model species. Following a conservative approach, SSDs were built based on sublethal endpoints, since these are assumed as more protective of the aquatic biota, retrieved from testing with nine species. These species represent different functional levels of the aquatic food web, which allows appraising potential imbalances in the mass and energy transfers, thus extending individual responses to the functional regulation of the aquatic systems; also, this set of species embraces diverse uptake mechanisms, which can influence the magnitude of the toxicity.⁴ Four ILs were selected in this study as models: two based on the cholinium cation (cholinium chloride – [Chol]Cl and cholinium dihydrogenocitrate – [Chol][DHCit]) and two based on the imidazolium cation (1-ethyl-3-methylimidazolium chloride – [C₂mim]Cl and 1-dodecyl-3-methylimidazolium chloride – [C₁₂mim]Cl). Imidazolium- and cholinium-based ILs are two of the most important families of ILs, with widespread applications in many industries. The selected ILs reflect extreme positions both concerning ecotoxicity and molecular structure. Indeed, cholinium-based ILs usually exhibit lower toxicity than imidazolium-based ones [e.g. ref. 15]. Among cholinium-based ILs, [Chol]Cl is among the least toxic [e.g. ref. 15 and 16] and [DHCit][−] one of the most toxic ILs [e.g. ref. 4 and 17]. Within the imidazolium-based ILs, and following the “side-chain effect”, [C₂mim]Cl is less toxic than [C₁₂mim]Cl [e.g. ref. 18]. Concerning molecular structure, we selected a very small and a large anion, Cl[−] and [DHCit][−], respectively, in order to assess the effect of the anion moiety in the ecotoxicity of the corresponding ILs; also, we selected an aromatic and a non-aromatic cation, [C₂mim] and [Chol], respectively, to assess the effect of such cations on the ecotoxicity. This selection allows to examine the effect of the cation ([Chol]Cl vs. [C₂mim]Cl), the effect of the elongation of the cation alkyl chain ([C₂mim]Cl vs. [C₁₂mim]Cl) and the effect of the anion moiety ([Chol]Cl vs. [Chol][DHCit]). In the end, we hope to reinforce the need of developing “integrative, comparative and predictive toxicology efforts”, which “if advanced across species and scales of biological organization, are positioned to catalyze the work of chemists and engineers engaging sustainable molecular design studies”.¹⁹

Experimental

Experimental

Collection of literature data

Data were collected from the literature and completed with data yield from ecotoxicity testing (section Ecotoxicological tests) to build feasible SSD models (section Data analysis) for each IL comprised in the study. Sublethal median effect concentrations (EC₅₀) corresponding to short-term exposures to each IL were the targeted yield of both the literature search and experiments. The rationale for this framing of the data input in SSD models considers that ILs, if discharged or accidentally drained into waterways, should be found there at low levels hardly promoting lethal effects; on the other hand, the industrial use of ILs is still limited, which renders continuous or even intermittent exposure in the long-term unlikely to occur.

Given the relative scarcity of sublethal ecotoxicological data published in the literature for ILs in general, the literature search followed a straightforward approach. Basic Web of Science™ (Clarivate Analytics) search was carried out through strings combining the name or synonyms of each IL and ‘ecotoxicity’, ‘aquatic toxicity’ or specifically the names of common model species used in ecotoxicological assessment focusing the aquatic compartment. All hits retrieved with these term combinations were roughly analysed through the abstract for a

primary selection accurately meeting the defined rationale above, and the final selection was fully analysed for the collection of EC_{50} and confidence intervals feeding the SSD models. Quality criteria for this selection were as follows: sufficient information on the tested solutions, allowing the unambiguous identification of the tested IL and its concentrations; sublethal endpoints read following short-term exposures; estimation of EC_{50} values by fitting appropriate predictive models (namely, non-linear regression functions, given the continuous nature of the variables), resulting in significant parameter estimation and narrow confidence intervals. Based on this search, the tests required to complete the ecotoxicological database were identified so that feasible SSD models could be built, considering that 8 datapoints are normally understood as a minimum set to feed an SSD,²⁰ and run as described below.

Chemicals

The ILs cholinium chloride (98 wt%, Sigma-Aldrich), cholinium dihydrogenocitrate (98 wt%, Sigma-Aldrich), 1-ethyl-3-methylimidazolium chloride (99 wt%, Iolitec) and 1-dodecyl-3-methylimidazolium (98 wt%, Iolitec) were tested in the present study (Table 1). For ecotoxicological tests, stock solutions of each IL were prepared in the appropriate culture media for each species and test solutions were prepared by direct dilution. Chemicals used to prepare the species culture media were analytical grade.

Bioluminescence inhibition test (Microtox)

To assess the bioluminescence inhibition promoted by the ILs in the marine bacterium *Aliivibrio fischeri*, the Microtox® Toxicity Test was used, following the basic test protocol of 81.9%.²¹ Dilutions were done following the manufacturer's protocol with the diluent supplied (2% NaCl solution) after a first step of adjusting the osmotic pressure of the samples using the supplied osmotic adjustment solution. Measurements of the luminescence emission of the bacteria were recorded and compared to the light emission of a control sample after 30 min of exposure.

Growth inhibition of microalgae

The growth inhibition of the microalgae *Raphidocelis subcapitata* and *Chlorella vulgaris* was assessed following the OECD guideline 201,²² adapted to the use of 24-well microplates.⁴ Microalgae were maintained in the laboratory as non-axenic semi-static cultures in Woods Hole MBL medium²³ and tests were performed at 23 °C, under continuous light. All treatments included an MBL blank control, an algae control and three replicates of each tested IL concentration. After 96 h of exposure, the absorbance at 440 nm was recorded (UV-1800 spectrophotometer, Shimadzu Corporation, Japan) and converted to cell density using a previously established calibration curve, except for tests with [Chol][DHCit], where the microalgae densities were determined by counting under a microscope in a Neubauer haemocytometer as this IL interferes with absorbance measurements at 440 nm. Cell densities were used for yield calculations.

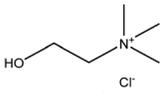
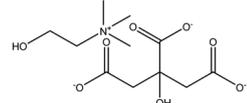
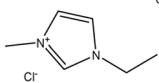
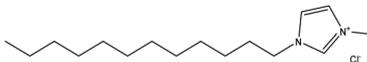
Growth inhibition of aquatic macrophytes

The growth inhibition of the macrophytes *Lemna minor* and *Lemna gibba* was assessed following the OECD guideline 221²⁴ adapted to 6-well microplates 4. Macrophytes were maintained and tested in Steinberg medium at 20 ± 2 °C under a 16 h^L:8 h^D photoperiod. In each test, individual wells were initiated with 10 mL of test solution plus three macrophyte colonies of three fronds each. Three replicated wells were established per concentration and each test included six plain-Steinberg control wells. After 7 days of exposure, the fronds in each well were counted and oven-dried (50 °C) for dry weight determination. The initial dry weight was estimated using the same procedure, by determining the dry weight in six replicates with three macrophytes colonies of three fronds each. Yield was determined for both frond number and dry weight endpoints.

Post-exposure feeding inhibition of *Daphnia magna*

Post-exposure feeding inhibition of *D. magna* was assessed following the procedure described by McWilliam and Baird.^{25,26}

Table 1 Nomenclature, CAS number, logarithm of the octanol–water partition coefficient ($\log K_{ow}$) and chemical structure of the studied ILs

Ionic liquid	Abbreviation	CAS number	$\log K_{ow}$	Chemical structure
Cholinium chloride	[Chol]Cl	67-48-1	-3.70 ⁴⁸	
Cholinium dihydrogenocitrate	[Chol][DHCit]	77-91-8	-1.32 ⁴⁸	
1-Ethyl-3-methylimidazolium chloride	[C ₂ mim]Cl	65039-09-0	-1.0177 ⁴⁹	
1-Dodecyl-3-methylimidazolium chloride	[C ₁₂ mim]Cl	81995-09-7	-0.27 ⁵⁰	

Monoclonal bulk cultures of *D. magna* were reared in synthetic ASTM hard water medium²⁷ supplemented with a standard organic additive and vitamins²⁸ at 20 ± 2 °C and under a $16 \text{ h}^{\text{L}}:8 \text{ h}^{\text{D}}$ photoperiod. The daphnids were fed with *R. subcapitata* (3×10^5 cells per mL) three times a week, right after medium renewal of cultures. Post-exposure feeding inhibition tests were assessed with 4 day-old organisms (4th instar).^{25,26} The exposure was carried out in glass vials containing 50 mL of test solution under the same photoperiod and temperature conditions as used for cultures. A static design was employed, using five replicates per treatment, with four organisms each. After 24 h of exposure, the organisms were transferred into new 50 mL glass vials containing clean ASTM medium with food (*R. subcapitata*, at a concentration of 3×10^5 cells per mL) and held in the dark for 4 h at 20 ± 2 °C. Following this period, the organisms were removed and the absorbance at 440 nm was recorded (UV-1800 spectrophotometer, Shimadzu Corporation, Japan), and used to estimate cell density on the basis of a previously established calibration curve. The same readings were made in 8 blank replicates (ASTM medium and food, but no daphnids) that had been added to the feeding trial to provide initial cell densities for feeding rate calculations. For [Chol][DHCit], the algal density was rather determined by microscopic counting (Neubauer haemocytometer). Algal densities were used to calculate feeding rates (cells per individual per h) according to literature.²⁹

Reproductive inhibition of *Brachionus calyciflorus*

Reproductive inhibition of *B. calyciflorus* was assessed following the guideline T90-377³⁰ using the short chronic (48 h)ROTOXKIT F® (MicroBioTests Inc., Belgium). Test organisms were obtained by hatching cysts of *B. calyciflorus* after 18 h of incubation at 25 °C. Newly hatched organisms were pre-fed with the provided RotiRich food suspension during 2 h. Exposure was carried out in 48-well microplates, with 6 treatments (5 concentrations plus a control of blank standard freshwater) and eight replicates per treatment. Each replicate consisted of one rotifer per well filled with 1 mL of test solution. The microalgae *R. subcapitata* was used as food, at a final concentration of 2.1×10^7 cells per mL. Microplates were incubated at 25 °C in the dark for 48 h. After this period, the total number of individuals per well was counted and used to estimate the population growth rate.

Feeding inhibition of *Corbicula fluminea*

Asian clams (*C. fluminea*) were harvested in a shallow freshwater ditch (Mira, Portugal; N 40° 24' 55" W 8° 45' 04"). The molluscs with shell length ranging 20–24 mm were selected for testing.³¹ These were transported to the laboratory in local water and gradually acclimated to laboratorial conditions (dechlorinated tap water under permanent aeration; 20 ± 2 °C; photoperiod: $16 \text{ h}^{\text{L}}:8 \text{ h}^{\text{D}}$) for 15 days prior to testing. Twice a week, maintenance water was renewed, and the clams were fed *ad libitum* with a concentrated suspension of *R. subcapitata*.

Tests were carried out following.³¹ Briefly, clams that had been deprived of food during 24 h were individually assigned to replicate glass vessels containing 100 mL of test solution (five concentrations plus a clean control). The test design considered 20 replicates per treatment, which were individually aerated through the test period. Tests started with the addition of food (*R. subcapitata*, reaching an initial cell density of 3×10^5 cells per mL) and a sample was immediately taken from each vial for absorbance reading at 440 nm (UV-1800 spectrophotometer; Shimadzu Corporation, Japan). After 120 min of exposure, another sample was taken for absorbance reading at 440 nm. Absorbance readings were used to estimate the corresponding cell densities through a previously established calibration curve, and further used to determine algae removal rates according to literature.³¹ The test with [Chol][DHCit] was not valid since at the highest concentrations this IL interfered with the cellular division of the microalgae, preventing the accurate estimation of the number of cells and, consequently, the determination of the removal rates.

Fish embryo test (FET) with *Danio rerio*

The effect of each IL on fish development and survival was evaluated through the FET with *D. rerio*, following the OECD guideline 236³² and described in detail in ref. 33. *D. rerio* embryos were obtained from our institutional facility, where adults are kept in carbon-filtered water supplemented with sea salt (electrical conductivity of $800 \pm 50 \mu\text{S cm}^{-1}$; pH of 7.5 ± 0.5 ; dissolved oxygen above 95% saturation), at 27 ± 1 °C under a light cycle of $14\text{h}^{\text{L}}:10\text{h}^{\text{D}}$, and fed once daily with a commercially available diet. Zebrafish eggs were collected within 30 min after natural mating of adult fish and checked under a stereoscope to discard unfertilized eggs and eggs that presented cleavage irregularities, deformations or lesions. Healthy eggs, ageing less than 4 h post-fertilization, were used in the tests, which were carried out on 24-well microplates, each well holding a replicate comprising one egg in 20 mL of test solution (IL concentration or clean system water as the control). Twenty replicates were used per treatment. The microplates were incubated at 26 ± 1 °C under a photoperiod of $16 \text{ h}^{\text{L}}:8 \text{ h}^{\text{D}}$ for 4 days. Embryos were observed daily during the exposure period under a stereoscope to record heart edema, spine/tail curvature, heart rate (at 48 h post-fertilisation), balance/equilibrium, hatching success and mortality. Only the endpoints allowing feasible EC_{50} estimation (e.g. covering concentration–response curves through at least 50% effect) were considered for further data analysis.

Data analysis

EC_x values ($x = 10, 20, 50$) and corresponding 95% confidence intervals for different test endpoints were estimated as standard ecotoxicological references, following nonlinear regression using the least-squares method to adjust the data to the logistic equation (STATISTICA, StatSoft). Using the SSD generator from the United States Environmental Protection Agency,³⁴ an SSD was built for each IL based on the EC_{50}

Table 2 Species and endpoints feeding SSDs built for each IL. EC₅₀ values and corresponding 95% confidence intervals (CI) are given as estimated in the present study, unless footnoted otherwise as indicated by the last column

IL	Species	Exposure	Endpoint	EC ₅₀ (mg L ⁻¹)	95% CI		
[Chol]Cl	<i>Aliivibrio fischeri</i>	30 min	Luminescence	469.3	383.8–554.9	^c	
	<i>Raphidocelis subcapitata</i>	96 h	Yield	72.51	52.14–92.88	^a	
	<i>Chlorella vulgaris</i>	96 h	Yield	6054	5332–6775		
	<i>Lemna minor</i>	7 d	Fronds yield	234.2	154.0–314.5	^a	
	<i>Lemna minor</i>	7 d	Dry-weight yield	1185	415.5–1955	^d	
	<i>Lemna gibba</i>	7 d	Fronds yield	486.9	440.7–533.0		
	<i>Lemna gibba</i>	7 d	Dry-weight yield	1599	829.5–2369		
	<i>Daphnia magna</i>	24 h + 4 h	Post-exposure feeding inhibition	590.2	488.1–692.3		
	<i>Corbicula fluminea</i>	2 h	Feeding inhibition	5181	3889–6473		
	<i>Danio rerio</i>	5 d	Hatching	1169	669.1–1668		
	<i>Danio rerio</i>	5 d	Yolk sac absorption	1.784 × 10 ⁴	16 302–19 368		
	<i>Danio rerio</i>	5 d	Heart edema	2759	1173–4346		
	<i>Brachionus calyciflorus</i>	48 h	Population growth	2087	1197–2976		
	[Chol][DHCit]	<i>Aliivibrio fischeri</i>	30 min	Luminescence	37.23	28.60–45.85	^c
<i>Raphidocelis subcapitata</i>		96 h	Yield	87.16	80.62–93.70	^a	
<i>Chlorella vulgaris</i>		96 h	Yield	524.0	400.3–647.8		
<i>Lemna minor</i>		7 d	Fronds yield	1863	1007–2720	^a	
<i>Lemna gibba</i>		7 d	Fronds yield	880.9	666.8–1095		
<i>Lemna gibba</i>		7 d	Dry-weight yield	1631	755.6–2506		
<i>Daphnia magna</i>		24 h + 4 h	Post-exposure feeding inhibition	570.0	494.2–645.9		
<i>Danio rerio</i>		5 d	Hatching	1411	1366–1456		
<i>Danio rerio</i>		5 d	Balance (unbalance ^a)	326.4	82.96–569.9		
<i>Danio rerio</i>		5 d	Heart edema	1411	1366–1456		
<i>Brachionus calyciflorus</i>		48 h	Population growth	253.6	215.6–291.6		
[C ₂ mim]Cl		<i>Aliivibrio fischeri</i>	30 min	Luminescence	6270	5286–7254	
		<i>Raphidocelis subcapitata</i>	96 h	Yield	265.2	161.7–368.6	
		<i>Chlorella vulgaris</i>	96 h	Yield	79.72	65.69–93.75	
	<i>Lemna minor</i>	7 d	Fronds yield	63.34	57.39–69.29		
	<i>Lemna minor</i>	7 d	Dry-weight yield	127.7	75.66–179.8		
	<i>Lemna gibba</i>	7 d	Fronds yield	51.26	44.09–58.43		
	<i>Lemna gibba</i>	7 d	Dry-weight yield	232.5	83.18–381.8		
	<i>Daphnia magna</i>	24 h + 4 h	Post-exposure feeding inhibition	310.9	279.5–342.3		
	<i>Corbicula fluminea</i>	2 h	Feeding inhibition	6576	3954–9198		
	<i>Danio rerio</i>	5 d	Yolk sac absorption	1009	9567–1.045 × 10 ⁴		
	<i>Danio rerio</i>	5 d	Tail deformities (spinal curvature)	1.476 × 10 ⁴	1.462 × 10 ⁴ –1.490 × 10 ⁴		
	<i>Danio rerio</i>	5 d	Balance (sideways ^b)	1.090 × 10 ⁴	9207–1.259 × 10 ⁴		
	<i>Brachionus calyciflorus</i>	48 h	Population growth	73.60	57.54–89.66		
	[C ₁₂ mim]Cl	<i>Aliivibrio fischeri</i>	30 min	Luminescence	0.3736	0.3504–0.3968	
<i>Raphidocelis subcapitata</i>		96 h	Yield	0.02021	0.01548–0.02494		
<i>Chlorella vulgaris</i>		96 h	Yield	0.2251	0.1900–0.2603		
<i>Lemna minor</i>		7 d	Fronds yield	3.202	2.743–3.659		
<i>Lemna minor</i>		7 d	Dry-weight yield	7.456	5.922–8.991		
<i>Lemna gibba</i>		7 d	Fronds yield	3.606	2.924–4.287		
<i>Lemna gibba</i>		7 d	Dry-weight yield	5.229	1.142–9.317		
<i>Daphnia magna</i>		24 h + 4 h	Post-exposure feeding inhibition	8.941	4.263–13.62		
<i>Corbicula fluminea</i>		2 h	Feeding inhibition	1.716	0.3302–3.102		
<i>Danio rerio</i>		5 d	Yolk sac absorption	1.633 × 10 ⁴	7017–2.564 × 10 ⁴		
<i>Danio rerio</i>		5 d	Tail deformities (spinal curvature)	1.811 × 10 ⁴	1.625 × 10 ⁴ –1.997 × 10 ⁴		
<i>Danio rerio</i>		5 d	Balance (sideways ^b)	1.134 × 10 ⁴	1.043 × 10 ⁴ –1.224 × 10 ⁴		
<i>Brachionus calyciflorus</i>		48 h	Population growth	0.2151	0.1939–0.2363		

^a Embryo showing signs of unbalance but dorsally positioned. ^b Embryo positioned sideways. ^c Ref. 17. ^d Ref. 4.

values estimated in the present study and those collected from the bibliographic search (refer to Table 2 for the list of the EC₅₀ values used to build each SSD), allowing the estimation of the HC₅ value (Hazard Concentration affecting 5% of the species). The EC₅₀ estimates were selected over other EC_x to feed the model originating each SSD given its lower relative susceptibility to differences in the formulation of the adjusted model and considering that its position on concentration–

response curves is more likely to be covered by experimental data.³⁵ Indeed, as the primary interest of this work was to validate the assumed heuristic rules for the relationship between the structure and ecotoxicity of ILs, the robustness of the input data over its protective nature as a reference for environmental toxicity was privileged. The relationship between *K*_{ow} and log HC₅ was assessed by Pearson's correlation (Statistica, StatSoft). An alpha level of 0.05 was used.

Results and discussion

The environmental toxicity of four ILs, two based on the cholinium cation and two based on the imidazolium cation, was assessed using standard organisms representing the freshwater compartment, susceptible to potential contamination by these compounds. The EC_{10} and EC_{20} values retrieved for the tested ILs are presented in Table S1 (ESI[†]), whereas the EC_{50} values are presented in Table 2. A global appraisal of the EC_{50} values suggests that these solvents may not be as “green” as widely argued. Although a strict adherence to the Global Harmonised System (GHS) of classification and labelling of chemicals by the United Nations UN³⁶ cannot be assumed due to the nature of the tests conducted herein (sublethal but comprising short-term exposures), some endpoints revealed a concerning hazardous potential of all tested ILs. For example, tests meeting the concept of both acute and chronic toxicity as defined by UN³⁶ are those regarding microalgae growth, and eventually those assessing macrophytes growth. Both cholinium-based ILs and [C₂mim]Cl can be interpreted as harmful to aquatic life (GHS classification as Acute 3) as per their EC_{50} values between 10 and 100 mg L⁻¹ for at least one microalgae species and also for *Lemna* sp. considering frond counting (Table 2). The picture on the hazardous potential of ILs is the most concerning for [C₁₂mim]Cl, with microalgae EC_{50} values below 1 mg L⁻¹ by one to two orders of magnitude, suggesting a GHS classification as Acute 1, *i.e.* very toxic to aquatic life, and macrophyte EC_{50} values between 1 and 10 mg L⁻¹, which would indicate toxicity to aquatic life (GHS classification as Acute 2).

It is noteworthy that green microalgae species can have a remarkable differential sensitivity to the ILs, as expressed by the variation in EC_{50} values by at least one order of magnitude between *R. subcapitata* and *C. vulgaris* (Table 2). Given the physiological similarity between both species, as well as considering that both have been maintained healthy for many years in our laboratory under the same incubation conditions as used in the toxicity tests (performed in the present study and in that by ref. 4), this level of sensitivity variation is difficult to ascertain, although differential sensitivity between green microalgae species to *e.g.* metals and pesticides has been observed^{37,38} and in some cases at similar magnitudes (*e.g.* ref. 39 and 40). Specific features of the IL's mechanism of toxic action and specific cell characteristics may contribute to the differential sensitivity. For example, [Chol][DHCit] caused pronounced effects in *C. vulgaris*, promoting discoloration and division of algae cells but keeping them aggregated. However, in *R. subcapitata* such effects were not observed for any of the tested ILs. Also, the two microalgae species may differ in their ability to uptake contaminants due to cell-specific morpho-physiological characteristics, such as lipid content or cell wall structure and composition, and overall size, implying differences in surface-volume ratio that constrain the internalization of chemical compounds depending on the lipophilicity of the compounds (*e.g.* ref. 39 and 41). The differential sensitivity of microalgae to the tested ILs is paramount to demonstrate that the selection of the test species within a given group of indi-

cator organisms can bias the understanding of the environmental hazardous potential of chemicals, ultimately influencing their risk assessment reports and constraining marketing authorisations.

Also noteworthy in this context is the difference in sensitivity between the two groups of producers tested, microalgae and macrophytes, the former being generally more sensitive than the latter to the tested ILs (Table 2). The macrophytes were almost invariably more tolerant to the ILs than the most sensitive microalgae species (except for [C₂mim]Cl) despite they sustain chemical uptake both *via* surface contact and systemically (*via* roots).

The systemic uptake is an additional route for chemical internalization, especially considering compounds with low lipophilicity, which is the case of the ILs tested herein (low to very low octanol–water partition coefficients, as represented by the log K_{ow} in Table 1). Thus, the potentiation of the effect of systemic uptake in addition to the uptake by surface contact to increase toxicity to macrophytes would expectably minor as well, and our data are consistent with this rationale: *Lemna* sp. were more tolerant (EC_{50} values higher by at least one order of magnitude; Table 2) than the most sensitive microalgae to the least lipophilic IL, [Chol]Cl, but also than both microalgae species to the most lipophilic IL, [C₁₂mim]Cl.

The built SSD curves (Fig. 1), supported by the toxicity values presented in Table 2, reveal a wide variation in the median effect concentration (EC_{50}) values for each IL, more pronounced for the imidazolium-derived ILs. The widest variation of the EC_{50} values was observed for [C₁₂mim]Cl (more than 800 000-fold), whereas the narrowest variation was observed for [Chol][DHCit] (50-fold). The SSD curves for each IL and the derived HC₅ (Fig. 1) allow a summarised view on the relative toxicity of each IL to the species considered, as well as a comprehensive validation of the assumed heuristic rules for ILs ecotoxicity.

As expected, the rank order of sensitivity of species for each IL varied accordingly to the tested IL, thus confirming that the toxicity of each IL depended on the species. For instance, the species *L. minor*, concerning the endpoint yield, was the least sensitive species for [Chol][DHCit] but the second most sensitive for [Chol]Cl. Also noteworthy is the fact that the microalgae *R. subcapitata* was amongst the most sensitive species to the tested ILs, except to [C₂mim]Cl.

On the other hand, the fish *D. rerio* was amongst the least sensitive species. It is worth noting that the most and least sensitive species belong to different trophic and functional levels. The fact that the producer *R. subcapitata* is among the most sensitive species suggests that phytoplankton, which form the basis of aquatic trophic webs, might be strongly affected if these ILs reach aquatic systems; consequently, such deleterious effects will reflect easily throughout the food webs as a result of decreased availability and/or quality of food for higher trophic levels.

Structure–ecotoxicity relationships for the tested ILs can be appraised in the present study by comparing extreme pairs tested. The effect of the cation can be observed by comparing

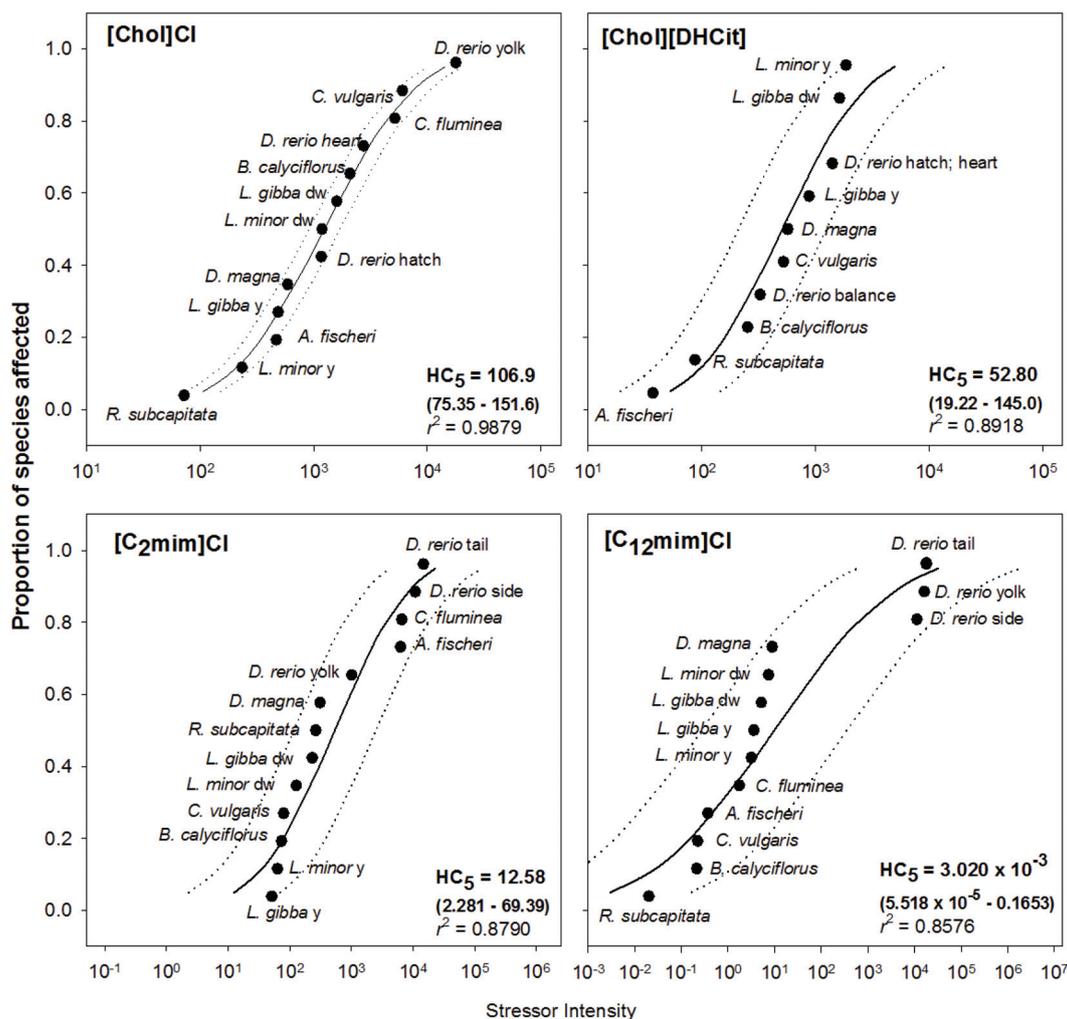


Fig. 1 Species sensitivity distribution (SSD) curves for sublethal endpoints considering exposure to [Chol]Cl, [Chol][DHCit], [C₂mim]Cl and [C₁₂mim]Cl. When appropriate, the endpoint used was coded after the name of the species: dw – dry weight; balance – embryo unbalanced position; hatch – egg hatching; heart – heart edema; side – embryo positioned sideways; tail – tail deformities; y – yield; yolk – yolk sac absorption. The estimated HC₅ benchmarks are given for each IL, with corresponding 95% confidence intervals within brackets and coefficient of determination.

[C₂mim]Cl and [Chol]Cl, as these compounds differ by the cation: 1-ethyl-3-methylimidazolium *versus* cholinium, respectively. Greater toxicity of [C₂mim]Cl than [Chol]Cl was evidenced, with HC₅ estimates of 12.58 and 106.9 mg L⁻¹, respectively, which represents a 8-fold variation. This variation illustrates increased toxicity with increased hydrophobicity of the cation, which agrees with the log *K*_{ow} variation presented in Table 1 (higher log *K*_{ow} represents increased hydrophobicity and likely consequent increased toxicity). Indeed, lower hydrophobicity of the cholinium cation was expected due to the presence of the hydroxyl group, meaning that it will have a higher affinity for interacting with the water and lower affinity for the more hydrophobic structure of organisms. This was already reported by testing the interaction of some of the ILs studied in this work towards Langmuir monolayers of two types of phospholipids and cholesterol.⁴² The trend of

increased toxicity with increased hydrophobicity of the cation is in line with previous findings (*e.g.* ref. 43 and studies cited therein).

To assess the effect of alkyl chain length on ILs toxicity, the data of [C₂mim]Cl and [C₁₂mim]Cl should be compared, as these compounds differ only in the cation alkyl chain length: ethyl radical *versus* dodecyl radical, respectively. The IL [C₁₂mim]Cl showed a much greater toxicity than [C₂mim]Cl, with HC₅ values of 3.020 × 10⁻³ and 12.58 mg L⁻¹, respectively, which represents a 4166-fold variation. The extension of the cationic alkyl chain was translated in increased toxicity, which corroborates previous results (*e.g.* ref. 4 and 43 and references cited therein⁴⁴). Such increased toxicity is likely associated with the higher hydrophobicity of [C₁₂mim]Cl as indicated by the log *K*_{ow} values (Table 1). Increased alkyl chain length has been argued to increase the interaction with biological mem-

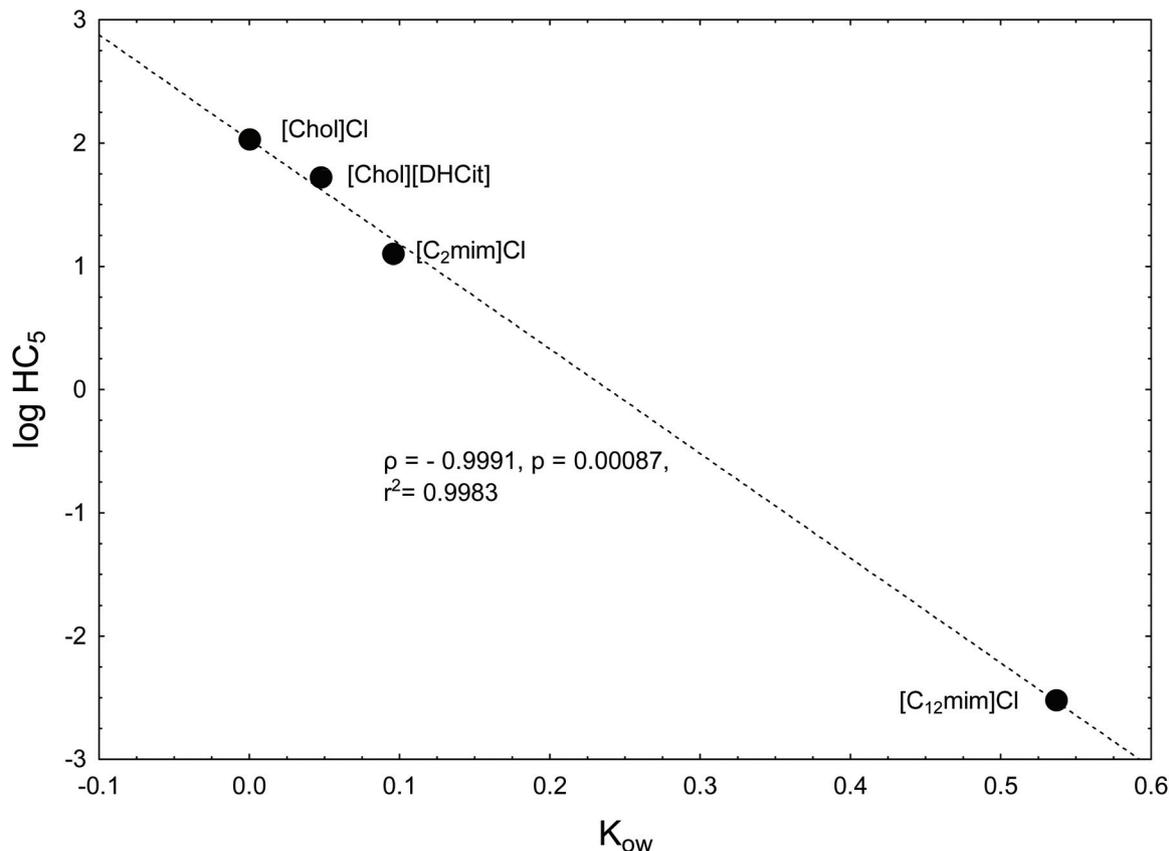


Fig. 2 Simple linear correlation between the octanol–water partition coefficient (K_{ow}) and the logarithm of HC_5 (hazard concentration for 5% of the species, derived from SSDs curves).

branes, favouring their disruption.⁸ The trend of increased toxicity with increasing cationic alkyl chain is not, however, valid for all ILs, such as guanidinium-based ILs.⁹

Concerning the effect of the anion on the ILs toxicity, it can be deduced by comparing data of [Chol]Cl and [Chol][DHCit], as these compounds differ by the anion: chloride (Cl^-) versus dihydrogenocitrate [$C_3H_5O(COO)^{3-}$], respectively. [Chol][DHCit] showed slightly higher toxicity than [Chol]Cl, with HC_5 values of 52.80 and 106.9 $mg\ L^{-1}$, respectively, which agrees with the effect expected by increasing hydrophobicity (Table 1). Despite the HC_5 values show a 2-fold variation, their confidence intervals largely overlap, reinforcing the mild effect of the anion in constraining ILs toxicity, herein at the community level. However, at the species level, the effect of the anion was significant for some species – for instance [Chol][DHCit] was 12-fold more toxic for *A. fischeri* than [Chol]Cl, whereas [Chol]Cl was 8-fold more toxic than [Chol][DHCit] for *L. minor* concerning the endpoint fronds yield. For other species, such as *R. subcapitata* and *D. magna* (post-exposure feeding inhibition), the effect of the anion was indeed not relevant (Table 1). This duality observed at the species level is consistent with what has been pictured previously, *i.e.* cases where the anion has no significant effect (*e.g.* ref. 13, 45 and 46) and

others where the anion has a significant effect (*e.g.* ref. 4, 9, 12 and 35).

Overall, the HC_5 values derived from the SSD curves confirmed that the heuristic rules governing ILs ecotoxicity, namely regarding the effects of the cation and of the elongation of the cation alkyl chain, are valid at the community level, at least for the tested ILs. Thus, based on the HC_5 values the toxicity of the tested ILs follows the order [Chol]Cl \leq [Chol][DHCit] $<$ [C₂mim]Cl \ll [C₁₂mim]Cl, which meets the rule of increased toxicity with increased hydrophobicity of the IL. The correlation between K_{ow} and $\log HC_5$ (Fig. 2) supports the strong association of both variables ($\rho = -0.9991$; $p = 0.00087$; $r^2 = 0.9983$). Such a strong correlation suggests that ILs ecotoxicity to aquatic communities is linearly associated to ILs hydrophobicity. However, this correlation is based on a limited number of observations and thus further studies with ILs from other families and with diverse functionalizations should be assessed before such a correlation between these two variables can be broadly assumed to feed structure–activity relationships envisaging the environmental hazard assessment of these compounds.

An important addition to the reasoning of heuristic rules for IL ecotoxicity was confirmed in the present study and

relates to the species-specificity of the ecotoxicological trends. For instance, the cation effect would not have been observed if toxicity assessment was performed only for *R. subcapitata*; the alkyl chain length effect would have been missed if toxicity was assessed only for *A. fischeri*. This highlights the importance of assessing toxicity in an integrated manner, representing potentially affected communities, instead of assessing with a single or a limited number of indicator species for a feasible establishment of structure–ecotoxicity relationships. Such an approach is also more ecologically relevant from an environmental perspective.⁴⁷ In the end, we hope to convince researchers and industrial players on the importance of using these and other integrative toxicological approaches to understand the “whole picture” regarding the environmental hazardous potential of the solvents they specifically want to develop and license.

Conclusions

Despite the touting of ILs as green solvents, their toxicity can be quite high, as herein confirmed for [C₁₂mim]Cl using a broad set of indicator species. In fact, at concentrations within the $\mu\text{g L}^{-1}$ range, this IL is likely to affect 5% of the species within an aquatic community (HC₅). Such high toxicity contradicts the proclaimed “green” character of ILs. The major outcome of the present study is however the validation of postulated heuristic rules for structure–ecotoxicity relationships within ILs when integrating the responses of (multi-species) communities (SSD approach) and the confirmation of a biasing effect in the rules driven by the use of single-species indicators of ecotoxicity. We observed the mild effect of the anion in inducing appreciable ecotoxicity variations, as [Chol] Cl is generally as toxic as [Chol][DHCit]; the moderate role of the cation, *i.e.* the family, in the relative positioning of IL ecotoxicity, by finding significantly lower HC₅ benchmarks for imidazolium cations compared to the cholinium cation; the high relevancy of the elongation of the alkyl chain (and related increase in hydrophobicity) of the cation in constraining ecotoxicity, as [C₁₂mim]Cl was markedly more toxic than [C₂mim] Cl. As per its ecological relevancy, feasibility and considering that it is widely used to support prospective risk assessment requirements before chemical licensing (*e.g.* REACH directive in the EU), the SSD approach should be extended to other ILs, confronting structural variables (number and functionalization of the alkyl chains) and/or related properties (*e.g.* *K*_{ow}), particularly when there is no consistency between the responses given by the different biological systems tested, as it has been commonly reported. This naturally facilitates a feasible appraisal of the environmental hazardous potential of these designer solvents, supporting trustful read-across methods (that decrease the need of actual experimental assessment) applying to authorisation dossiers for compliance with environmental protection regulation.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

Thanks are due to FCT/MCTES for the financial support to CESAM (UIDP/50017/2020 + UIDB/50017/2020) and CICECO (UIDB/50011/2020 & UIDP/50011/2020). The authors also acknowledge the support by FCT/MCTES through the project PTDC/ATP-EAM/5331/2014 to the work and the research grant of F Jesus (BI/UI88/3307/2017). JL Pereira is funded by National Funds (OE), through FCT, I.P., in the scope of the framework contract foreseen in the numbers 4, 5 and 6 of the article 23, of the Decree-Law 57/2016, of August 29, changed by Law 57/2017, of July 19.

Notes and references

- 1 C. Ruß and B. König, Low melting mixtures in organic synthesis—an alternative to ionic liquids?, *Green Chem.*, 2012, **14**(11), 2969–2982.
- 2 M. G. Freire, *et al.*, Mutual solubilities of water and hydrophobic ionic liquids, *J. Phys. Chem. B*, 2007, **111**(45), 13082–13089.
- 3 W. L. Hough and R. D. Rogers, Ionic liquids then and now: from solvents to materials to active pharmaceutical ingredients, *Bull. Chem. Soc. Jpn.*, 2007, **80**(12), 2262–2269.
- 4 J. Santos, *et al.*, Environmental safety of cholinium-based ionic liquids: assessing structure–ecotoxicity relationships, *Green Chem.*, 2015, **17**(9), 4657–4668.
- 5 P. J. Carvalho, *et al.*, Understanding the impact of the central atom on the ionic liquid behavior: phosphonium vs ammonium cations, *J. Chem. Phys.*, 2014, **140**(6), 064505.
- 6 European Commission, Regulation (EC) No 1907/2006 of the European parliament and of the council, European Commission, Editor, Official Journal of the European Union, 2006.
- 7 ECHA, *Read-Across Assessment Framework (RAAF)*. 2017, European Chemicals Agency, Helsinki, Finland.
- 8 S. Stolte, *et al.*, Effects of different head groups and functionalised side chains on the aquatic toxicity of ionic liquids, *Green Chem.*, 2007, **9**(11), 1170–1179.
- 9 S. P. Ventura, *et al.*, Simple screening method to identify toxic/non-toxic ionic liquids: agar diffusion test adaptation, *Ecotoxicol. Environ. Saf.*, 2012, **83**, 55–62.
- 10 S. P. M. Ventura, *et al.*, Ecotoxicological risk profile of ionic liquids: octanol-water distribution coefficients and toxicological data, *J. Chem. Technol. Biotechnol.*, 2011, **86**(7), 957–963.
- 11 J. Arning, *et al.*, Qualitative and quantitative structure activity relationships for the inhibitory effects of cationic head groups, functionalised side chains and anions of

- ionic liquids on acetylcholinesterase, *Green Chem.*, 2008, **10**(1), 47–58.
- 12 A. Cieniecka-Roslonkiewicz, *et al.*, Synthesis, anti-microbial activities and anti-electrostatic properties of phosphonium-based ionic liquids, *Green Chem.*, 2005, **7**(12), 855–862.
 - 13 J. Ranke, *et al.*, Biological effects of imidazolium ionic liquids with varying chain lengths in acute *Vibrio fischeri* and WST-1 cell viability assays, *Ecotoxicol. Environ. Saf.*, 2004, **58**(3), 396–404.
 - 14 N. Weyhing-Zerrer, *et al.*, Predictability of ionic liquid toxicity from a SAR study on different systematic levels of pathogenic bacteria, *Ecotoxicol. Environ. Saf.*, 2017, **139**, 394–403.
 - 15 M. Petkovic, *et al.*, Exploring fungal activity in the presence of ionic liquids, *Green Chem.*, 2009, **11**(6), 889–894.
 - 16 K. D. Weaver, *et al.*, Cyto-toxicity and biocompatibility of a family of choline phosphate ionic liquids designed for pharmaceutical applications, *Green Chem.*, 2010, **12**(3), 507–513.
 - 17 S. P. Ventura, *et al.*, Ecotoxicity analysis of cholinium-based ionic liquids to *Vibrio fischeri* marine bacteria, *Ecotoxicol. Environ. Saf.*, 2014, **102**, 48–54.
 - 18 J. Zhang, *et al.*, The time-dependent hormetic effects of 1-alkyl-3-methylimidazolium chloride and their mixtures on *Vibrio qinghaiensis* sp. -Q67, *J. Hazard. Mater.*, 2013, **258–259**, 70–76.
 - 19 B. W. Brooks, Greening chemistry and ecotoxicology towards sustainable environmental quality, *Green Chem.*, 2019, **21**(10), 2575–2582.
 - 20 P. L. TenBrook, *et al.*, *Methods for deriving pesticide aquatic life criteria*, in *Reviews of Environmental Contamination and Toxicology*, ed. D. E. Whitacre, Springer, Boston, MA, 2008, pp. 19–109.
 - 21 Azur Environmental, *The Microtox® Acute Basic, DIN, ISO and Wet Test Procedures*, Azur Environmental, Carlsbad, CA, USA, 1998.
 - 22 OECD, Test No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test, 2011.
 - 23 J. R. Stein, J. A. Hellebust and J. Craigie, *Handbook of phycollogical methods: culture methods and growth measurements*, Cambridge University Press, 1973.
 - 24 OECD, Test N221: Lemna sp. Growth Inhibition Test, in *OECD Guidelines for the Testing of Chemicals*, Organisation for Economic Co-operation and Development, Paris, 2006, p. 22.
 - 25 R. A. McWilliam and D. J. Baird, Application of postexposure feeding depression bioassays with *Daphnia magna* for assessment of toxic effluents in rivers, *Environ. Toxicol. Chem.*, 2002, **21**(7), 1462–1468.
 - 26 R. A. McWilliam and D. J. Baird, Postexposure feeding depression: A new toxicity endpoint for use in laboratory studies with *Daphnia magna*, *Environ. Toxicol. Chem.*, 2002, **21**(6), 1198–1205.
 - 27 ASTM, Standard Guide for Conducting *Daphnia magna* Life-cycle Toxicity Tests, in *ASTM E1193 - 97(2004)*, American Society for Testing and Materials, Pennsylvania, 2004.
 - 28 C. Loureiro, *et al.*, Performance of standard media in toxicological assessments with *Daphnia magna*: chelators and ionic composition versus metal toxicity, *Ecotoxicology*, 2011, **20**(1), 139–148.
 - 29 Y. Allen, P. Calow and D. J. Baird, A mechanistic model of contaminant-induced feeding inhibition in *Daphnia magna*, *Environ. Toxicol. Chem.*, 1995, **14**(9), 1625–1630.
 - 30 AFNOR, *French Standard T90-377: Water quality—determination of chronic toxicity to *Brachionus calyciflorus* in 48 hours— population growth inhibition test*, Association Française de Normalization, 2000.
 - 31 B. B. Castro, *et al.*, Feeding inhibition in *Corbicula fluminea* (OF Muller, 1774) as an effect criterion to pollutant exposure: perspectives for ecotoxicity screening and refinement of chemical control, *Aquat. Toxicol.*, 2018, **196**, 25–34.
 - 32 OECD, Test N236: Fish Embryo Acute Toxicity (FET) Test, in *OECD Guideline for testing of chemicals*, Organisation for Economic Co-operation and Development, Paris, 2013, p. 22.
 - 33 R. Oliveira, *et al.*, Effects of triclosan on zebrafish early-life stages and adults, *Environ. Sci. Pollut. Res.*, 2009, **16**(6), 679–688.
 - 34 USEPA, *Methods/indicators for determining when metals are the cause of biological impairments of rivers and streams: species sensitivity distributions and chronic exposure-response relationships from laboratory data*, Office of Research and Development, Cincinnati, OH, USA, 2005.
 - 35 C.-W. Cho, *et al.*, Influence of anions on the toxic effects of ionic liquids to a phytoplankton *Selenastrum capricornutum*, *Green Chem.*, 2008, **10**(1), 67–72.
 - 36 United Nations, *Globally Harmonized System of Classification and Labelling of Chemicals (GHS) - fifth revised edition*, United Nations, New York and Geneva, 2013, p. 536.
 - 37 J. F. Fairchild, D. S. Ruessler and A. R. Carlson, Comparative sensitivity of five species of macrophytes and six species of algae to atrazine, metribuzin, alachlor, and metolachlor, *Environ. Toxicol. Chem.*, 1998, **17**(9), 1830–1834.
 - 38 M. Pereira, *et al.*, Differences in the effects of metals on growth of two freshwater green algae (*Pseudokirchneriella subcapitata* (Korshikov) Hindak and *Gonium pectorale* Müller), *Bull. Environ. Contam. Toxicol.*, 2005, **75**(3), 515–522.
 - 39 L. Queirós, *et al.*, Ecotoxicological assessment of the herbicide Winner Top and its active substances—are the other formulants truly inert?, *Ecotoxicology*, 2018, **27**(7), 945–955.
 - 40 T. Vidal, *et al.*, Acute and chronic toxicity of Betanal® Expert and its active ingredients on nontarget aquatic organisms from different trophic levels, *Environ. Toxicol.*, 2011, **27**(9), 537–548.
 - 41 J. Tang, K. D. Hoagland and B. D. Siegfried, Uptake and bioconcentration of atrazine by selected freshwater algae, *Environ. Toxicol. Chem.*, 1998, **17**(6), 1085–1090.

- 42 C. M. Mendonça, *et al.*, Understanding the interactions of imidazolium-based ionic liquids with cell membrane models, *Phys. Chem. Chem. Phys.*, 2018, **20**(47), 29764–29777.
- 43 S. n. P. Ventura, *et al.*, Imidazolium and pyridinium ionic liquids from mandelic acid derivatives: Synthesis and bacteria and algae toxicity evaluation, *ACS Sustainable Chem. Eng.*, 2013, **1**(4), 393–402.
- 44 S. P. Ventura, *et al.*, Toxicity assessment of various ionic liquid families towards *Vibrio fischeri* marine bacteria, *Ecotoxicol. Environ. Saf.*, 2012, **76**, 162–168.
- 45 R. J. Bernot, *et al.*, Acute and chronic toxicity of imidazolium-based ionic liquids on *Daphnia magna*, *Environ. Toxicol. Chem.*, 2005, **24**(1), 87–92.
- 46 K. M. Docherty and C. F. Kulpa Jr., Toxicity and antimicrobial activity of imidazolium and pyridinium ionic liquids, *Green Chem.*, 2005, **7**(4), 185–189.
- 47 K. V. Brix, D. K. DeForest and W. J. Adams, Assessing acute and chronic copper risks to freshwater aquatic life using species sensitivity distributions for different taxonomic groups, *Environ. Toxicol. Chem.*, 2001, **20**(8), 1846–1856.
- 48 J. F. Pereira, *et al.*, Molecular interactions in aqueous biphasic systems composed of polyethylene glycol and crystalline vs. liquid cholinium-based salts, *Phys. Chem. Chem. Phys.*, 2014, **16**(12), 5723–5731.
- 49 M. Montalbán, *et al.*, Experimental measurements of octanol-water partition coefficients of ionic liquids, *J. Adv. Chem. Eng.*, 2015, **5**, 1000133.
- 50 U. Domańska, E. Bogel-Lukasik and R. Bogel-Lukasik, 1-Octanol/Water Partition Coefficients of 1Alkyl-3-methylimidazolium Chloride, *Chem. – Eur. J.*, 2003, **9**(13), 3033–3041.