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Aquatic Toxicology of Ionic Liquids (ILs)

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Introduction

The broad application of ionic liquids (ILs) as process chemicals, solvents, heat transfer and storage fluids, electrolytes, and additives is encouraging significant progress in the design of novel chemical and biotechnological processes and products [1]. Both academia and industry have been using ILs to boost established processes including laborious routes, replace nefarious chemicals, or minimize waste generation, as well as create innovative technologies and products [1–3]. What made ILs appealing was, in the first place, their recognized unique physical and chemical properties (e.g., non-flammability, non-volatility, high thermal stability, solvation ability, and structural versatility) [4]. Together with their "designer solvent" status, ILs started to be defined as "green" and, more recently, "high performance" chemicals [5, 6]. Often, however, such headlines represent nothing but overgeneralizations that lead to critical misconceptions within IL field. Likely, the most controversial IL classification is that of "green solvent". Even though ILs' nonvolatility prevents the risk of atmospheric pollution – a plus over common volatile organic solvents – their water solubility makes them prone to enter and impact on aquatic ecosystems. ILs are not inherently green, since most of them possess equivalent or even higher toxicity than traditional organic solvents [7]. At the current stage, where ILs play an expanding role in industry [1, 2], they must be regarded as emerging contaminants [8], and the full disclosure of their aquatic toxicology is imperative.

Efforts have been made along the years to clarify the toxic action of ILs over aquatic organisms. As reviewed [7, 9, 10], the set of published works follow at least one of three core strategies: (i) assessing structure-ecotoxicity relationships by changing the cation, anion, and respective alkyl chain lengths and functionalized groups; (ii) covering multitrophic bioassays, through distinct types of toxicity tests (e.g., acute toxicity and Microtox – undoubtedly the most studied – but also chronic, reproductive, and embryo toxicity); and (iii) attesting the environmental benignity of ILs designed for a specific application.

In general, the results collected so far reveal that the ILs' hazard potential can range from low to high, being mainly dependent on the cation and anion chemical structures and the aquatic organism considered. Table 1 provides a description of the battery of tests commonly used to assess the aquatic toxicity of ILs. Moreover, the European categories for hazardous substances over the aquatic organisms (i.e., fish, Crustacea, and

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algae/other aquatic plants) considered for IL toxicology studies (acute, short-term) are compiled to help characterize the ecotoxic effects reported on this entry [11].

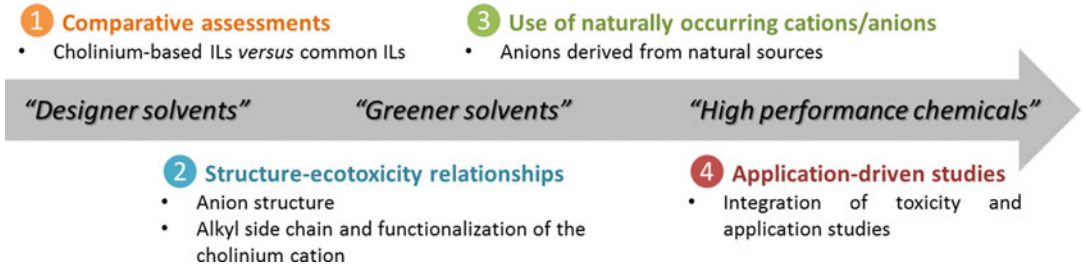
General guidelines to help defining the sustainable design of ILs are currently available [7, 9, 10]. The (eco)toxicity profiles instigated by the cation core nature, the anion moiety structure, and the introduction of aliphatic chains or functionalized groups are well-documented, although outliers may occur due to synergistic effects of the IL components and the aquatic organism tested. In earlier studies, major attention was given to nitrogen-based cyclic structures at the cation (e.g., 1-alkyl-3-butylimidazolium, $[C_nC_1im]^+$; 1-alkyl-3-methylpyridinium, $[C_nC_1pyr]^+$; 1-alkyl-1-methylpyrrolidinium, $[C_nC_1pyrr]^+$; 1-alkyl-1-methylpiperidinium, $[C_nC_1pip]^+$; and 4-alkyl-4-methylmorpholinium, $[C_nC_1mor]^+$) and halogenated anions (e.g., bis(trifluoromethylsulfonyl) imide, $[NTf_2]^-$; hexafluorophosphate, $[PF_6]^-$; tetrafluoroborate, $[BF_4]^-$; chloride, Cl^-; and bromide, Br^-). Currently, the database of ILs' aquatic toxicity is much broader and includes quaternary ammonium (e.g., tetraalkylammonium, $[N_{wxyz}]^+$; and cholinium, $[N_{111(2OH)}]^+$]), (e.g., di-alkyl-tetramethyl-guanidinium, $[C_nC_n(C_1C_1C_1C_1gua)]^+$) and phosphonium (e.g., tetraalkylphosphonium, $[P_{wxyz}]^+$) cations as well as organic anions (e.g., alkanoates, $[C_nCO_2]^-$).

Within this body of data [7, 9, 10], it is now possible to define some heuristic rules considering the cation head group, anion moiety, alkyl side-chain elongation, and functionalization to anticipate the toxicity of an IL. The impact of the cation head group is driven by the water solubility and aromaticity of the IL. Phosphonium-based and nitrogen-based aromatic cations (e.g., $[C_nC_1im]^+$ and $[C_nC_1pyr]^+$) are more toxic than nitrogen-based cyclic (e.g., $[C_nC_1pyrr]^+$, $[C_nC_1pip]^+$, and $[C_nC_1mor]^+$) and acyclic (e.g., $[C_ngua]^+$, $[N_{n444}]^+$, and $[N_{111(2OH)}]^+$) ones. The patterns were consistent along distinct microorganisms/trophic levels, e.g., *Vibrio fischeri* now *Aliivibrio fischeri (A. fischeri)*, *Daphnia magna (D. magna)*, *Raphidocelis subcapitata* (*R. subcapitata)*, and *Physa acuta (P. acuta)* [7, 9, 10]. The effect of the anion is less systematized. In general, fluorinated anions (e.g., $[NTf_2]^-$, $[PF_6]^-$, and $[BF_4]^-$) are more toxic than halides (e.g., Cl^- and Br^-) due to their hydrophobicity what facilitates their interaction with cell membranes. The patterns were consistent along distinct trophic levels, e.g., *D. magna*, *R. subcapitata*, and *Lemna minor* (*L. minor*) [7, 9, 10]. The impact of the alkyl side-chain size is ruled by the hydrophobicity. The longer the alkyl side chains of either cation or anion, the higher the toxicity, i.e., "side-chain effect" (e.g., $[C_nC_1im]^+$ or $[C_nCO_2]^-$). After a certain chain length, no further increment in toxicity seems to occur; this is known as the "cutoff effect". These patterns were consistently observed along several cation/anion pairs and distinct trophic levels, e.g., *D. magna, R. subcapitata*, and *A. fischeri* [7, 9, 10]. Again, the hydrophobicity seems to rule the impact that the insertion of hydrophilic groups has on ILs' ecotoxicity. The replacement of carbon by oxygen, chlorine, hydrogen, nitrile, and ether groups decreases the toxicity (e.g., $[C_nC_1im]^+$) [7, 9, 10].

Taking into account the literature, this entry intends to summarize the progress made within the design of more sustainable ILs and overviews the importance of aquatic toxicology to guide such a task. In this context, cholinium-based ILs are being extensively studied in a large range of applications due to their claimed "green," "biocompatible," "benign," or "nontoxic" nature, for which guidelines regarding structure-ecotoxicity relationships should be evaluated and future perspectives defined. A layout of the entry is provided in Fig. 1.

Boosting the Sustainable Design of ILs

In the search for "greener" and more sustainable ILs, researchers have been implementing structures arising from natural and renewable feedstocks in the preparation of ILs [12]. Cholinium chloride, formally known as (2-hydroxyethyl) trimethylammonium chloride (further abbreviated as $[N_{111(2OH)}]Cl$), is a naturally occurring essential nutrient relevant for the synthesis of vitamins and enzymes [13]. The incorporation of the cholinium



Aquatic Toxicology of Ionic Liquids (ILs), Fig. 1 Overview of the entry

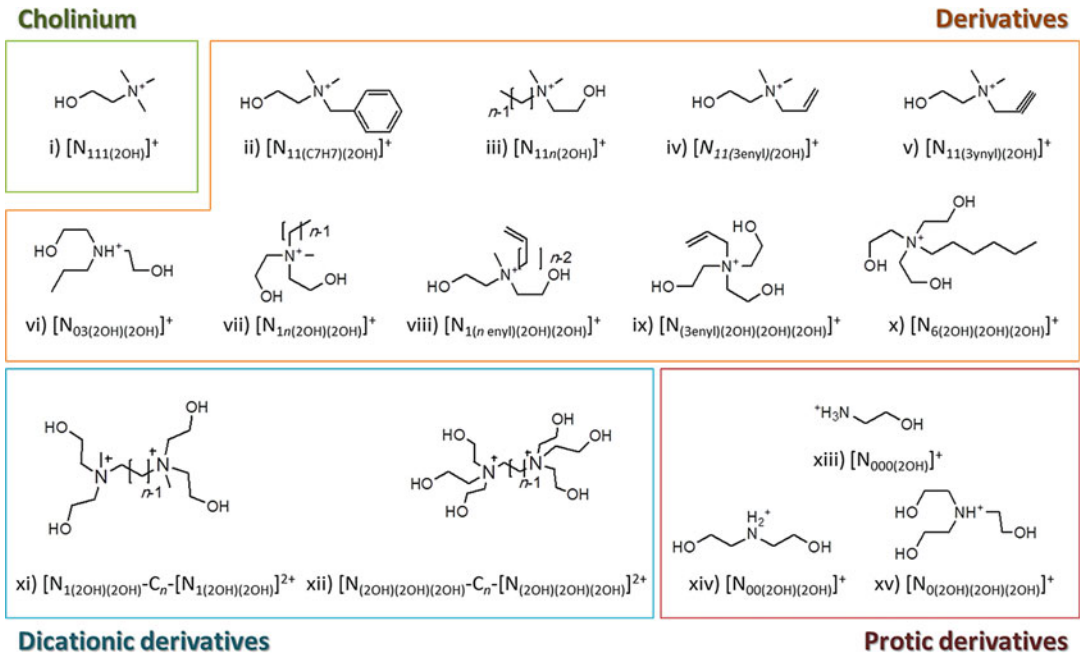
core structure as an IL cation – $[N_{111(2OH)}]^+$ – has been envisaged as a "green"/more benign/biocompatible structure, a premise followed in the most recent years. Indeed, new literature is showing cholinium-based ILs as greener alternatives for the common nitrogen-based cyclic ILs (e.g., imidazolium-based). Initially, cholinium-based ILs were only approached as a common IL family in systematic studies of several fields (aquatic toxicology included) [14–17]. Then, studies focusing the structure-ecotoxicity relationships appeared to elucidate the sustainable design of cholinium-based ILs, e.g., [18–21] and those ionic structures started to be synthesized from other natural sources (e.g., amino acids) aiming to prepare more sustainable cholinium-based ILs [22, 23]. Taking into consideration the increasing focus given to these ionic compounds, cholinium-based ILs started to be evaluated in a large range of applications [24–27], not just because they are allegedly "green" but as "high performance chemicals". Figures 2 and 3 and Table 2 provide the chemical structures, name, and abbreviations of the cations and anions considered along aquatic toxicology studies of cholinium-based ILs reviewed in this work.

Cholinium-Based ILs Versus Nitrogen-Based Cyclic ILs

The first reports on the field of IL aquatic toxicology including cholinium-based ILs date back to the first decade of the 2000s. These works have provided systematic insights on the factors responsible for ILs' toxicity on aquatic compartments along the trophic web, e.g., *A. fischeri*, *R. subcapitata, D. magna,* and *D. rerio* [14, 15].

Large batteries of structurally different ILs were investigated: (i) $[C_nC_1im]^+$, $[C_nC_1pyr]^+$, 1-alkylpyridinium $[C_npyr]^+$, $[C_nC_1pyrr]^+$, $[C_nC_1mor]^+$, trialkylsulfonium $[C_nC_nC_nS]^+$, $[N_{wxyz}]^+$, $[P_{wxyz}]^+$, and $[N_{111(2OH)}]^+$, cations, bearing distinct alkyl side-chain lengths and functionalization groups; (ii) Cl^-, Br^-, $[NTf_2]^-$, $[PF_6]^-$, dicyanamide $[N(CN)_2]^-$, diethylphosphate $[C_2C_2PO_4]^-$, and methylsulphate $[C_1SO_4]^-$ anions. In these two studies [14, 15], halogenated compounds incorporating the cholinium cation, namely, $[N_{111(2OH)}]Cl$, $[N_{111(2OH)}][NTf_2]$, and $[N_{111(2OH)}][PF_6]$, were studied. Cholinium-based ILs were generally less toxic than the aromatic-based and the surfactant-like ammonium-based ILs [14, 15], matching the heuristic rules compiled above for the role of the cation head group and alkyl side chain upon aquatic toxicity. Organisms of different trophic levels displayed distinct sensitivity upon exposure to $[N_{111(2OH)}][PF_6]$, i.e., the lower the position at the trophic hierarchy, the higher the toxic action (*R. subcapitata* > *D. magna* ≈ *D. rerio*) [15].

In the set of studies published on the use of cholinium-based ILs are two works on the embryonic development of aquatic species [16, 17]. The main results suggested the negligible impact of $[N_{111(2OH)}]Cl$ on the embryogenic development of *D. rerio* in opposition to $[C_3C_1pyrr][CF_3SO_3]$ (a fluorinated IL containing the trifluoromethanesulfonate anion, $[CF_3SO_3]^-$) and $[N_{1111}][C_1CO_2]$ [16]. Moreover, and along with $[N_{111(2OH)}][C_1CO_2]$, $[N_{111(2OH)}]Cl$ was the least hazardous IL to *Artemia salina* (*A. salina)* cysts, with $[C_4C_1im][C_1CO_2]$, $[C_4C_1im][NO_3]$, and $[N_{0001}][NO_3]$ reported as having an intermediate



Aquatic Toxicology of Ionic Liquids (ILs), Fig. 2 Chemical structure of the cation in cholinium-based ILs

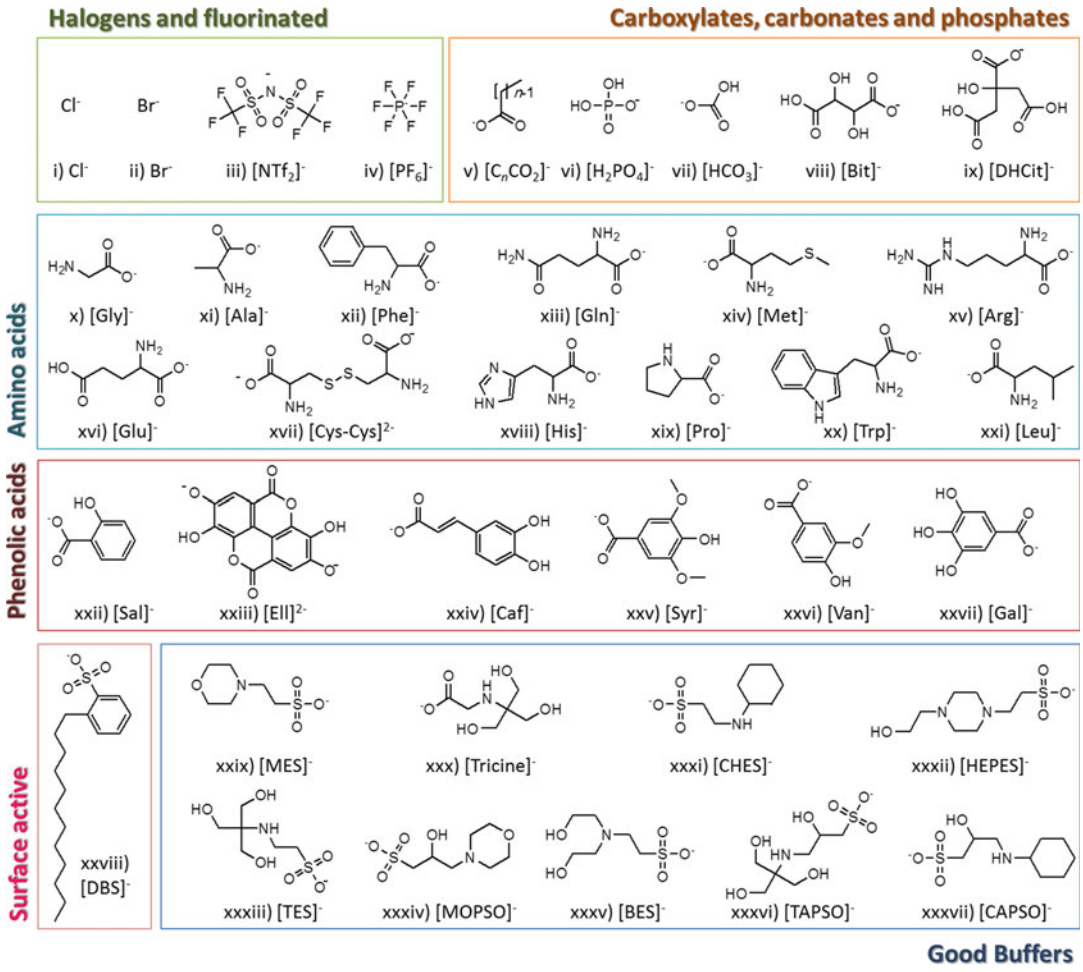
toxicity and $[N_{111(2OH)}][H_2PO_4]$ – a cholinium-based IL – consisting on the most toxic [17].

Summing up, the cholinium cation seems to represent a less toxic option for the synthesis of ILs than the widely used aromatic-based ones. Nevertheless, the scenario previously overviewed highlights the importance of avoiding overgeneralizations regarding the "nontoxic" nature of cholinium-based ILs, since it is highly contingent on the organism and anion structure.

Structure-Ecotoxicity Relationships Within Cholinium-Based ILs

Several efforts have been performed to disclose the structure-ecotoxicity relationships within cholinium-based ILs. Impacts imposed by the anion moiety [18–20] and structural modification in the cholinium cation [18–21] over aquatic compartments were assessed.

Ten distinct anions were addressed concerning their toxicity over a complete battery of organisms along the aquatic trophic web (viz., *A. fischeri, R. subcapitata*, *L. minor*, and *D. magna*) [18, 19]. Table 3 overviews the anion role on the toxicity of cholinium-based ILs, indicating a great impact upon toxicity and a lack of toxicity output patterns among distinct biological models. The toxic action mechanisms of cholinium-based ILs are somehow intricate and deviate from the patterns widely accepted for other ILs by the aquatic toxicology community. The "side-chain effect" was observed with $[C_nCO_2]^-$ anions only for *D. magna* (Table 3, entries **viii**, **xii**, and **xx**). The oxygenation role via the addition of one hydroxyl in organic acid-derived anions ($[C_1CO_2]^-$ versus $[HCO_3]^-$) has either null (*L. minor*) or positive (remaining test battery) effect on toxicity (Table 3, entries **i–iv** versus **ix–xii**), whereas further hydroxylation ($[C_1CO_2]^-$ versus $[Bit]^-$ and $[DHCit]^-$) increases the toxic action (Table 3, entries **ix–xii** versus **xxix–xxxii** and **xxxiii–xxxvi**). The anions bearing aromatic rings ($[C_1CO_2]^-$ versus $[Sal]^-$) have no constant impacts over distinct organisms (Table 3, entries **ix–xii** versus **xxv–xxviii**) [18, 19]. Additionally, branching of alkanoate anions was shown to be beneficial on the toxicity toward *A. fischeri* [straight, $[C_9CO_2]^-$, versus branched, $[C_{neo9}CO_2]^-$, anions, Table 3 entries **xxxvii** versus **xxxviii**] [28].



Aquatic Toxicology of Ionic Liquids (ILs), Fig. 3 Chemical structure of the anions in cholinium-based ILs

Table 4 provides representative toxicological data for cholinium-based ILs designed with structural modifications at the cation. Around twenty five structures were screened over *A. fischeri* in the works by Ventura and collaborators [18, 21]. The data suggested that the increase in the alkyl side or linkage chains induced toxicity, a result in good agreement with the well-documented "side-chain effect" (Table 4, entries **ix–xv** and **xvi–xix**). Moreover, by incorporating hydroxyethyl groups in the cholinium derivative structures, the authors observed an increased toxic action (Table 4, entries **xx–xxii**), on a clear opposition to the oxygenation role in common ILs. The insertion of carbon-carbon double bonds was also studied and proved to be able to decrease or maintain the toxicity, while triple bonds imposed higher toxicity levels (Table 4 entries **xxiii–xv** and **xvi–xvii**). The incorporation of a benzyl group in the cholinium cation rendered no constant impacts over distinct organisms, viz. *A. vibrio, L. minor, R. subcapitata*, and *D. magna* [18, 19] (Table 4, entries **i–iv** versus **v–viii**), being the same impact found when disclosing the role of aromatic anions (Table 3, entries **ix–xii** versus **xxv–xxviii**). Protic cations, where the methyl group linked to the central nitrogen was substituted by a hydrogen atom, were found to be more toxic than its aprotic counterparts (Table 4, entries **xxviii–xxix**). Finally, dicationic cholinium-based ILs were also investigated, these structures being defined by the presence of two cations connected by an

Aquatic Toxicology of Ionic Liquids (ILs), Table 1 Battery of tests commonly used to assess the aquatic toxicity of ILs

Trophic level	Test	Endpoint	Toxicity parameter	Duration (acute toxicity)	Toxicity categories [11]
Decomposer	Marine luminescent bacteria: *Aliivibrio fischeri*	Luminescence inhibition	EC_x (IL concentration that yields *x*% loss of luminescence)	5–30 min	Bacteria are not considered by the European classification
Primary producers	Green algae: *Raphidocelis subcapitata Scenedesmus* sp.	Growth inhibition	EC_x (IL concentration that yields *x*% growth inhibition)	24–72 h	"Acute 1": 72 h $EC_{50} \leq 1$ mg L^{-1} "Acute 2": 72 h $EC_{50} > 1$ mg L^{-1} but ≤ 10 mg L^{-1} "Acute 3": 72 h $EC_{50} > 10$ mg L^{-1} but ≤ 100 mg L^{-1}
	Duckweeds: *Lemna minor*	Growth inhibition	EC_x (IL concentration that yields *x*% growth inhibition)	96 h	"Acute 1": 96 h $EC_{50} \leq 1$ mg L^{-1} "Acute 2": 96 h $EC_{50} > 1$ mg L^{-1} but ≤ 10 mg L^{-1} "Acute 3": 96 h $EC_{50} > 10$ mg L^{-1} but ≤ 100 mg L^{-1}
Primary consumers	Freshwater crustaceans: *Daphnia magna*	Immobility	EC_x (IL concentration that yields *x*% loss of mobility)	24–48 h	"Acute 1": 48 h $EC_{50} \leq 1$ mg L^{-1} "Acute 2": 48 h $EC_{50} > 1$ mg L^{-1} but ≤ 10 mg L^{-1} "Acute 3": 48 h $EC_{50} > 10$ mg L^{-1} but ≤ 100 mg L^{-1}
	Saltwater crustaceans: *Artemia salina* (brine shrimp)	Hatchability, mortality	Hatchability, % (number of larva per number of initial cysts) LC_{50} (IL concentration that yields 50% deaths)	24–72 h	"Acute 1": 48 h $LC_{50} \leq 1$ mg L^{-1} "Acute 2": 48 h $LC_{50} > 1$ mg L^{-1} but ≤ 10 mg L^{-1} "Acute 3": 48 h $LC_{50} > 10$ mg L^{-1} but ≤ 100 mg L^{-1}
	Freshwater mollusc: *Physa acuta* (freshwater snail)	Hatchability, mortality	Mortality, % (number of dead embryos per number of total embryos); hatchability, % (number of hatched larva per number of total embryos); LC_{50} (IL concentration that yields 50% deaths)	96 h	Molluscs are not considered by the European classification
Secondary consumers	Fishes: *Danio rerio* (zebra fish)	Mortality, locomotion, hatchability	LC_{50} (IL concentration that yields 50% deaths) hatchability, % (number of hatching embryos per number of remaining embryos)	24–96 h	"Acute 1": 96 h $LC_{50} \leq 1$ mg L^{-1} "Acute 2": 96 h $LC_{50} > 1$ mg L^{-1} but ≤ 10 mg L^{-1} "Acute 3": 96 h $LC_{50} > 10$ mg L^{-1} but ≤ 100 mg L^{-1}

Aquatic Toxicology of Ionic Liquids (ILs), Table 2 Name and abbreviation of the cation-anion combinations in cholinium-based ILs

Cations			Anions		
	Name	Abbreviation		Name	Abbreviation
i	(2-Hydroxyethyl) trimethylammonium (cholinium)	$[N_{111(2OH)}]^+$	**i**	Chloride	Cl^-
ii	Benzyl(2-hydroxyethyl) dimethylammonium	$[N_{11(C7H7)(2OH)}]^+$	**ii**	Bromide	Br^-
iii	Alkyl(2-Hydroxyethyl) dimethylammonium	$[N_{11n(2OH)}]^+$	**iii**	Bis(trifluoromethylsulfonyl) imide	$[NTf_2]^-$
iv	(2-Hydroxyethyl)dimethyl (2-propenyl)ammonium	$[N_{11(3enyl)(2OH)}]^+$	**iv**	Hexafluorophosphate	$[PF_6]^-$
v	(2-Hydroxyethyl)dimethyl (2-propynyl)ammonium	$[N_{11(3ynyl)(2OH)}]^+$	**v**	Alkanoate	$[C_nCO_2]^-$
vi	Di(2-hydroxyethyl) propylammonium	$[N_{03(2OH)(2OH)}]^+$	**vi**	Dihydrogenphosphate	$[H_2PO_4]^-$
vii	Alkyldi(2-hydroxyethyl) methylammonium	$[N_{1n(2OH)(2OH)}]^+$	**vii**	Bicarbonate	$[HCO_3]^-$
viii	Alkenyldi(2-hydroxyethyl) methylammonium	$[N_{1(n\ enyl)(2OH)(2OH)}]^+$	**viii**	Bitartrate	$[Bit]^-$
ix	Tri(2-hydroxyethyl) (2-propenyl)ammonium	$[N_{(3enyl)(2OH)(2OH)(2OH)}]^+$	**ix**	Dihydrogencitrate	$[DHCit]^-$
x	Hexyltri(2-hydroxyethyl) ammonium	$[N_{6(2OH)(2OH)(2OH)}]^+$	**x**	Glycinate	$[Gly]^-$
xi	*N,N,N′,N′*-Tetra (2-hydroxyethyl)-*N, N′*-dimethyl-1, *n*-alkyldiammonium	$[N_{1(2OH)(2OH)}\text{-}C_n\text{-}[N_{1(2OH)(2OH)}]^{2+}$	**xi**	Alaninate	$[Ala]^-$
xii	*N,N,N′,N′*-Hexa (2-hydroxyethyl)-1, *n*-alkyldiammonium	$[N_{(2OH)(2OH)(2OH)}\text{-}C_n\text{-}[N_{(2OH)(2OH)(2OH)}]^{2+}$	**xii**	Phenylalaninate	$[Phe]^-$
xiii	2-Hydroxyethylammonium	$[N_{000(2OH)}]^+$	**xiii**	Glutaminate	$[Gln]^-$
xiv	Di(2-hydroxyethyl) ammonium	$[N_{00(2OH)(2OH)}]^+$	**xiv**	Methionate	$[Met]^-$
xv	Tri(2-hydroxyethyl) ammonium	$[N_{0(2OH)(2OH)(2OH)}]^+$	**xv**	Argininate	$[Arg]^-$
			xvi	Glutamate	$[Glu]^-$
			xvii	Cystinate	$[Cys\text{-}Cys]^{2-}$
			xviii	Histidinate	$[His]^-$
			xix	Prolinate	$[Pro]^-$
			xx	Tryptophanate	$[Trp]^-$
			xxi	Leucinate	$[Leu]^-$
			xxii	Salicylate	$[Sal]^-$
			xxiii	Ellagate	$[Ell]^{2-}$
			xxiv	Caffeate	$[Caf]^-$
			xxv	Syringate	$[Syr]^-$
			xxvi	Vanillate	$[Van]^-$
			xxvii	Gallate	$[Gal]^-$

(*continued*)

Aquatic Toxicology of Ionic Liquids (ILs), Table 2 (continued)

Cations			Anions		
	Name	Abbreviation		Name	Abbreviation
			xxviii	Dodecylbenzenesulfonate	$[DBS]^-$
			xxix	2-(*N*-Morpholino) ethanesulfonate	$[MES]^-$
			xxx	*N*-[Tris(hydroxymethyl) methyl]glycinate	$[Tricine]^-$
			xxxi	2-(Cyclohexylamino) ethanesulfonate	$[CHES]^-$
			xxxii	2-[4-(2-Hydroxyethyl) piperazin-1-yl] ethanesulfonate	$[HEPES]^-$
			xxxiii	2-[(2-Hydroxy-1,1-[bis (hydroxy methyl)-ethyl) amino]ethanesulfonate	$[TES]^-$
			xxxiv	2-Hydroxy-3-morpholinopropanesulfonate	$[MOPSO]^-$
			xxxv	2-(Bis(2-hydroxyethyl) amino)ethanesulfonate	$[BES]^-$
			xxxvi	*N*-[Tris(hydroxymethyl) methyl]-3-amino-2-hydroxypropanesulfonate	$[TAPSO]^-$
			xxxvii	3-(Cyclohexylamino)-2-hydroxypropanesulfonate	$[CAPSO]^-$

alkyl chain and conjugated with two anions. In this work [21], the dicationic cholinium-based ILs are described as less toxic than the parent monocationic compounds (Table 4, entries **xiii–xix**).

Stolte and collaborators [20] found no well-defined trends for cholinium protic derivatives (e.g., $[N_{000(2OH)}][C_nCO_2]$, $[N_{00(2OH)(2OH)}][C_nCO_2]$, and $[N_{0(2OH)(2OH)(2OH)}][C_nCO_2]$, with $0 \leq n \leq 4$) regarding the number of hydroxyethyl groups of the cation (Table 4, entries **xxx–xxxviii**) and the alkyl side chain of the $[C_nCO_2]^-$ anion (Table 3, entries **xl–lvii**) among distinct aquatic compartments. For instance, the higher the number of hydroxyethyl groups, the higher the toxicity for *A. fischeri* (Table 4, entries **xxx** versus **xxxiii** versus **xxxvi**), what agrees with the findings by e Silva et al. using the same microorganism [21]. Still, a reverse trend is observed over *R. subcapitata*, likely due to differences at the level of the cell wall, as assumed in other studies [19]. *L. minor* was the most sensitive aquatic organism found [20], in opposition to the observations by Santos et al. [19] for aprotic, more common cholinium-based compounds where algae displayed the highest sensitivity (Table 4).

From the results found so far, the data suggested that common aprotic ILs (e.g., $[C_nC_1im]Cl$ and $[C_4pyr]Cl$) are more toxic to *A. fischeri*, *L. minor*, and *R. subcapitata* than these protic cholinium derivatives [20]. The presence of both shorter alkyl side chains and hydrophilic functional groups (e.g., hydroxyethyl and hydrogen atoms) contributes for such pattern. With regard to aprotic cholinium-based ILs, Ventura et al. [18] have recognized, however, that some of the general patterns considered in literature are distinct. The higher toxicity of the cholinium structure when compared with other IL cations (e.g., $[N_{1124}]^+$, $[C_2C_1im]^+$, all sharing the Cl^- anion) implies that for some cholinium ILs, the mechanism of toxic action may be different than that accepted for other ILs. Moreover, as revealed by Rantamäki et al.,

Aquatic Toxicology of Ionic Liquids (ILs), Table 3 Representative toxicity data for the anion role in cholinium-based ILs over different aquatic organisms

Entry	IL anion	Test organism	EC_{50} ($mg.L^{-1}$)	Categorization[a]	References
Cation: $[N_{111(2OH)}]^+$					
i	$[HCO_3]^-$	*A. fischeri*	>20,000	*Practically harmless*	[18]
ii		*R. subcapitata*	232.4	*Practically harmless*	[19]
iii		*L. minor*	483.6	*Practically harmless*	[19]
iv		*D. magna*	840.3	*Practically harmless*	[19]
v	$[C_3CO_2]^-$	*A. fischeri*	884.1	*Practically harmless*	[18]
vi		*R. subcapitata*	87.6	*Acute 3*	[19]
vii		*L. minor*	150.1	*Practically harmless*	[19]
viii		*D. magna*	637.3	*Practically harmless*	[19]
ix	$[C_1CO_2]^-$	*A. fischeri*	673.2	*Practically harmless*	[18]
x		*R. subcapitata*	124.1	*Practically harmless*	[19]
xi		*L. minor*	680.9	*Practically harmless*	[19]
xii		*D. magna*	694.6	*Practically harmless*	[19]
xiii	$[H_2PO_4]^-$	*A. fischeri*	572.7	*Practically harmless*	[18]
xiv		*R. subcapitata*	131.0	*Practically harmless*	[19]
xv		*L. minor*	1097	*Practically harmless*	[19]
xvi		*D. magna*	675.7	*Practically harmless*	[19]
xvii	$[C_2CO_2]^-$	*A. fischeri*	487.9	*Practically harmless*	[18]
xviii		*R. subcapitata*	50.17	*Acute 3*	[19]
xix		*L. minor*	149.1	*Practically harmless*	[19]
xx		*D. magna*	673.2	*Practically harmless*	[19]
xxi	Cl^-	*A. fischeri*	469.3	*Practically harmless*	[18]
xxii		*R. subcapitata*	72.51	*Acute 3*	[19]
xxiii		*L. minor*	234.2	*Practically harmless*	[19]
xxiv		*D. magna*	695.4	*Practically harmless*	[19]
xxv	$[Sal]^-$	*A. fischeri*	236.1	*Practically harmless*	[18]
xxvi		*R. subcapitata*	302.0	*Practically harmless*	[19]
xxvii		*L. minor*	110.1	*Practically harmless*	[19]
xxviii		*D. magna*	1086	*Practically harmless*	[19]
xxix	$[Bit]^-$	*A. fischeri*	37.90	*Acute 3*	[18]
xxx		*R. subcapitata*	27.26	*Acute 3*	[19]
xxxi		*L. minor*	1063	*Practically harmless*	[19]
xxxii		*D. magna*	410.5	*Practically harmless*	[19]
xxxiii	$[DHCit]^-$	*A. fischeri*	37.23	*Acute 3*	[18]
xxxiv		*R. subcapitata*	87.16	*Acute 3*	[19]
xxxv		*L. minor*	1863	*Practically harmless*	[19]
xxxvi		*D. magna*	445.0	*Practically harmless*	[19]
xxxvii	$[C_9CO_2]^-$	*A. fischeri*	28	*Acute 3*	[28]
xxxviii	$[C_{neo9}CO_2]^-$	*A. fischeri*	145	*Practically harmless*	[28]
xxxix	$[C_{i17}CO_2]^-$	*A. fischeri*	29	*Acute 3*	[28]
Cation: $[N_{00(2OH)(2OH)}]^+$					
xl	$[C_0CO_2]^-$	*A. fischeri*	800	*Practically harmless*	[20]
xli		*R. subcapitata*	976	*Practically harmless*	[20]
xlii		*L. minor*	525	*Practically harmless*	[20]
xliii	$[C_1CO_2]^-$	*A. fischeri*	1750	*Practically harmless*	[20]
xliv		*R. subcapitata*	870	*Practically harmless*	[20]
xlv		*L. minor*	631	*Practically harmless*	[20]

(*continued*)

Aquatic Toxicology of Ionic Liquids (ILs), Table 3 (continued)

Entry	IL anion	Test organism	EC_{50} ($mg.L^{-1}$)	Categorization[a]	References
xlvi	$[C_2CO_2]^-$	*A. fischeri*	650	*Practically harmless*	[20]
xlvii		*R. subcapitata*	2569	*Practically harmless*	[20]
xlviii		*L. minor*	209	*Practically harmless*	[20]
xlix	$[C_3CO_2]^-$	*A. fischeri*	800	*Practically harmless*	[20]
l		*R. subcapitata*	294	*Practically harmless*	[20]
li		*L. minor*	33	*Acute 3*	[20]
lii	$[C_{i3}CO_2]^-$	*A. fischeri*	850	*Practically harmless*	[20]
liii		*R. subcapitata*	1275	*Practically harmless*	[20]
liv		*L. minor*	79	*Acute 3*	[20]
lv	$[C_4CO_2]^-$	*A. fischeri*	350	*Practically harmless*	[20]
lvi		*R. subcapitata*	574	*Practically harmless*	[20]
lvii		*L. minor*	155	*Practically harmless*	[20]

[a]"Acute 1" $EC_{50} \leq 1$ $mg.L^{-1}$ | "Acute 2" $EC_{50} > 1$ $mg.L^{-1}$ but ≤ 10 $mg.L^{-1}$ | "Acute 3" $EC_{50} > 10$ $mg.L^{-1}$ but ≤ 100 $mg.L^{-1}$ | [11] "Practically harmless" $EC_{50} > 100$ $mg.L^{-1}$

the higher EC_{50} values of guanidinium over cholinium-based cations may lead to a stepping stone in the development of less toxic ILs [28]. Additionally, cholinium-based ILs may present a higher toxicity than conventional organic solvents (e.g., ethyl acetate and dichloromethane), often influenced by the anion structure [18], what challenges the inherently safer nature of this class of compounds.

Finally, differential scanning calorimetry (DSC) studies to correlate the EC_{50} values of cholinium-based ILs with the rupture point of biomimetic lipid bilayers were done [28]. With ILs bearing long-chain alkanoate anions, the liposomes were affected by the ILs presence correlating the EC_{50} values and the IL critical micelle concentration [28]. Works of such kind bring major progresses on understanding IL-biological membrane interactions.

Strategies to Develop Less Toxic Cholinium-Based ILs

Taking into account the heuristic rules defined in the last years for different organisms and trophic levels, the search for less toxic cholinium-based ILs continued. Cholinium structures incorporating biocompatible anions, such as amino acids (abbreviated as $[N_{111(2OH)}][AA]$), have evolved aiming at the ILs' synthesis under sustainable and environmentally friendly principles for ILs' design [12].

The aquatic toxicity of $[N_{111(2OH)}][AA]$ was addressed by covering three aquatic organisms (*R. subcapitata*, *A. salina*, and *D. rerio*) of different trophic levels (viz., green algae, brine shrimp, and zebra fish) and a plethora of amino acids, as represented in Table 5 [22, 23]. Consistency with the literature previously discussed was found related with the nontoxic nature of $[N_{111(2OH)}][AA]$ compared to their imidazolium congeners [22] and the "side-chain effect" of the anion (e.g., $[Gly]^-$ versus $[Ala]^-$ versus $[Leu]^-$ – Table 5, entries **i–iii** versus **iv–vi** versus **vii–ix**) [22, 23]. Nevertheless, the introduction of nitrogen heterocycles and benzyl groups at the amino acid anion allowed decreasing the toxicity toward brine shrimp, zebra fish, and green algae (e.g., $[Ala]^-$ versus $[His]^-$, $[Pro]^-$, $[Phe]^-$, and $[Trp]^-$ in Table 5) – a result of the lower IL lipophilicity when compared to the aliphatic correspondent [23]. Such a phenomenon is opposite to what has been observed for the toxicity of common ILs, but in agreement with some of the patterns disclosed for cholinium-based ILs [18, 19].

Balancing the "Innocuous Nature" and "High Performance" with Cholinium ILs

Above, insights on the ecotoxicity of cholinium-based ILs were collected and discussed. Although

Aquatic Toxicology of Ionic Liquids (ILs), Table 4 Representative toxicity data for the cation role in cholinium-based ILs and over different aquatic organisms

Entry	IL cation	Test organism	EC_{50} (mg. L^{-1})	Categorization[a]	References
Anion: Cl^-					
i	$[N_{111(2OH)}]^+$	*A. fischeri*	469.3	*Practically harmless*	[18]
ii		*R. subcapitata*	72.51	*Acute 3*	[19]
iii		*L. minor*	234.2	*Practically harmless*	[19]
iv		*D. magna*	695.4	*Practically harmless*	[19]
v	$[N_{11(C7H7)(2OH)}]^+$	*A. fischeri*	1498	*Practically harmless*	[18]
vi		*R. subcapitata*	196.2	*Practically harmless*	[19]
vii		*L. minor*	11.86	*Acute 3*	[19]
viii		*D. magna*	217.5	*Practically harmless*	[19]
Anion: Br^-					
ix	$[N_{112(2OH)}]^+$	*A. fischeri*	25619.07	*Practically harmless*	[21]
x	$[N_{113(2OH)}]^+$		33972.39	*Practically harmless*	[21]
xi	$[N_{114(2OH)}]^+$		13442.88	*Practically harmless*	[21]
xii	$[N_{115(2OH)}]^+$		3016.96	*Practically harmless*	[21]
xiii	$[N_{116(2OH)}]^+$		746.30	*Practically harmless*	[21]
xiv	$[N_{118(2OH)}]^+$		162.96	*Practically harmless*	[21]
xv	$[N_{1112(2OH)}]^+$		0.81	*Acute 1*	[21]
Anion: $2(Br^-)$					
xvi	$[N_{1(2OH)(2OH)}\text{-}C_6\text{-}[N_{1(2OH)(2OH)}]^{2+}$	*A. fischeri*	6117.15	*Practically harmless*	[21]
xvii	$[N_{1(2OH)(2OH)}\text{-}C_8\text{-}[N_{1(2OH)(2OH)}]^{2+}$		5579.82	*Practically harmless*	[21]
xviii	$[N_{1(2OH)(2OH)}\text{-}C_{10}\text{-}[N_{1(2OH)(2OH)}]^{2+}$		388.95	*Practically harmless*	[21]
xix	$[N_{1(2OH)(2OH)}\text{-}C_{12}\text{-}[N_{1(2OH)(2OH)}]^{2+}$		97.89	*Acute 3*	[21]
Anion: Br^-					
xx	$[N_{116(2OH)}]^+$	*A. fischeri*	746.30	*Practically harmless*	[21]
xxi	$[N_{16(2OH)(2OH)}]^+$		276.96	*Practically harmless*	[21]
xxii	$[N_{6(2OH)(2OH)(2OH)}]^+$		19.74	*Acute 3*	[21]
xxiii	$[N_{113(2OH)}]^+$		33972.39	*Practically harmless*	[21]

(*continued*)

Aquatic Toxicology of Ionic Liquids (ILs), Table 4 (continued)

Entry	IL cation	Test organism	EC_{50} (mg. L^{-1})	Categorization[a]	References
xxiv	$[N_{11(3enyl)(2OH)}]^+$		20798.81	*Practically harmless*	[21]
xxv	$[N_{11(3ynyl)(2OH)}]^+$		235.92	*Practically harmless*	[21]
xxvi	$[N_{13(2OH)(2OH)}]^+$		1601.29	*Practically harmless*	[21]
xxvii	$[N_{1(3enyl)(2OH)(2OH)}]^+$		2745.90	*Practically harmless*	[21]
xxviii	$[N_{13(2OH)(2OH)}]^+$		1601.29	*Practically harmless*	[21]
xxix	$[N_{03(2OH)(2OH)}]^+$		370.07	*Practically harmless*	[21]
Anion: $[C_3CO_2]^-$					
xxx	$[N_{000(2OH)}]^+$	*A. fischeri*	2239	*Practically harmless*	[20]
xxxi		*R. subcapitata*	104	*Practically harmless*	[20]
xxxii		*L. minor*	59	*Acute 3*	[20]
xxxiii	$[N_{00(2OH)(2OH)}]^+$	*A. fischeri*	800	*Practically harmless*	[20]
xxxiv		*R. subcapitata*	294	*Practically harmless*	[20]
xxxv		*L. minor*	33	*Acute 3*	[20]
xxxvi	$[N_{0(2OH)(2OH)(2OH)}]^+$	*A. fischeri*	501	*Practically harmless*	[20]
xxxvii		*R. subcapitata*	1287	*Practically harmless*	[20]
xxxviii		*L. minor*	178	*Practically harmless*	[20]

[a]"Acute 1" $EC_{50} \leq 1$ mg.L^{-1} | "Acute 2" $EC_{50} > 1$ mg.L^{-1} but ≤ 10 mg.L^{-1} | "Acute 3" $EC_{50} > 10$ mg.L^{-1} but ≤ 100 mg.L^{-1} | [11] "Practically harmless" $EC_{50} > 100$ mg.L^{-1}

ILs were previously seen as "greener" substitutes for nitrogen-based cyclic ILs (e.g., [$C_nC_1$im]-based) [14–17], studies on structure-ecotoxicity relationships called the attention for the nefarious effects associated to both cation and anion structures [18–23]. Still, the studies here discussed provide useful information for the design of less toxic cholinium-based ILs but fail in integrating their ecotoxicological profile and respective applications [14–23]. Examples of cases combining an evaluation of ecotoxicity with the application in the pharmaceutical (e.g., formulation of drugs [24]), chemical (e.g., formulation of detergents [25]), and biotechnological (e.g., production and extraction of biomolecules [26, 27]) fields will be overviewed, with the data presented in Table 6.

ILs bearing active pharmaceutical ingredients (IL-APIs) represent an auspicious strategy to overcome the polymorphism and bioavailability issues of common APIs; yet, the toxicity of some ILs is still limiting their real application. Within this framework [24], Sintra et al. synthesized antioxidant IL-APIs exclusively obtained from natural sources. By pairing the cholinium cation and phenolic acids as the anion moiety, the authors provided a complete physical, chemical, and biological characterization [24]. It was possible to deliver a new set of IL-API chemicals, displaying better bioactivity (antioxidant and anti-inflammatory) and bioavailability (water solubility) than the parent acidic compounds. Such achievements assemble comparable cytotoxic

Aquatic Toxicology of Ionic Liquids (ILs), Table 5 Representative toxicity data for $[N_{111(2OH)}][AA]$ over different aquatic organisms

Entry	$[AA]^-$ anion	Test organism	LC_{50}/EC_{50} (mg.L^{-1})	Categorization[a]	References
i	$[Gly]^-$	*A. salina*	15952.10	*Practically harmless*	[23]
ii		*D. rerio*	226.33	*Practically harmless*	[23]
iii		*R. subcapitata*	5766.63	*Practically harmless*	[23]
iv	$[Ala]^-$	*A. salina*	9968.91	*Practically harmless*	[23]
v		*D. rerio*	179.57	*Practically harmless*	[23]
vi		*R. subcapitata*	2474.40	*Practically harmless*	[23]
vii	$[Leu]^-$	*A. salina*	9156.29	*Practically harmless*	[23]
viii		*D. rerio*	160.86	*Practically harmless*	[23]
ix		*R. subcapitata*	1011.56	*Practically harmless*	[23]
x	$[Met]^-$	*A. salina*	6515.87	*Practically harmless*	[23]
xi		*D. rerio*	155.16	*Practically harmless*	[23]
xii		*R. subcapitata*	1031.31	*Practically harmless*	[23]
xiii	$[His]^-$	*A. salina*	19213.91	*Practically harmless*	[23]
xiv		*D. rerio*	274.46	*Practically harmless*	[23]
xv		*R. subcapitata*	9997.21	*Practically harmless*	[23]
xvi	$[Pro]^-$	*A. salina*	11186.72	*Practically harmless*	[23]
xvii		*D. rerio*	184.76	*Practically harmless*	[23]
xviii		*R. subcapitata*	4343.72	*Practically harmless*	[23]
xix	$[Phe]^-$	*A. salina*	15720.64	*Practically harmless*	[23]
xx		*D. rerio*	203.52	*Practically harmless*	[23]
xxi		*R. subcapitata*	3952.16	*Practically harmless*	[23]
xxii	$[Trp]^-$	*A. salina*	15383.49	*Practically harmless*	[23]
xxiii		*D. rerio*	194.27	*Practically harmless*	[23]
xxiv		*R. subcapitata*	3723.89	*Practically harmless*	[23]

[a]"Acute 1" $LC_{50}/EC_{50} \leq 1$ mg.L^{-1} | "Acute 2" $LC_{50}/EC_{50} > 1$ mg.L^{-1} but ≤ 10 mg.L^{-1} | "Acute 3" $LC_{50}/EC_{50} > 10$ mg.L^{-1} but ≤ 100 mg.L^{-1} | [11] "Practically harmless" $LC_{50}/EC_{50} > 100$ mg.L^{-1}

profiles and lower ecotoxicity, showcasing the promising applicability in dermatological formulations and oral drugs [24]. The aquatic toxicity studies using *A. fischeri* revealed that all are practically harmless to the environment (Table 6, entries **i–vi**) [11]. Regarding the structure-ecotoxic relationships, the increased number of methoxy groups in the aromatic rings of the anion (i.e., $[Van]^-$ versus $[Syr]^-$, Table 6 entry **i** versus **iii**) was found to induce higher toxic effects over *A. fischeri* [24]. These findings support the idea that oxygenation may limit the benign nature of cholinium-based ILs [18–21], in opposition to what has been reported for $[C_nC_1im]$-based ILs.

Gehlot et al. [25] focused on surface-active ILs (SA-ILs) as candidates to overcome surface activity and water solubility issues of the conventional surfactants (e.g., sodium dodecylbenzenesulfonate, Na[DBS]). Envisioning their application in detergent formulations, the synthesis and full characterization of $[N_{111(2OH)}][DBS]$ was performed (e.g., surface activity, enzymatic activity, and ecotoxicity) [25]. These studies revealed enhanced surface-active properties and lower critical micelle concentration than Na[DBS]. At the same time, the SA-IL synthesized allowed the improvement of enzymatic activity while being revealed as non-toxic for the freshwater microalgae *Scenedesmus* sp. (microalgae growth was not affected as shown in Table 6, entry **vii**) [25].

The development of self-buffering and biocompatible ILs has led to remarkable progresses in biotechnological and biopharmaceutical applications. The studies reported are recurrently

Aquatic Toxicology of Ionic Liquids (ILs), Table 6 Representative toxicity data for cholinium-based ILs used for distinct applications

Entry	Anion/precursor	Test organism	IL EC_{50} ($mg.L^{-1}$)	IL categorization[a]	Precursor EC_{50} ($mg.L^{-1}$)	Precursor categorization[a]	References
IL-APIs							
i	$[Van]^-$/vanillic acid	*A. fischeri*	1000.6	*Practically harmless*	27.5	*Acute 3*	[24]
ii	$[Caf]^-$/caffeic acid		856.3	*Practically harmless*	n.d.[b]	–	[24]
iii	$[Syr]^-$/syringic acid		568.5	*Practically harmless*	32.5	*Acute 3*	[24]
iv	$[Gal]^-$/gallic acid		1725.4	*Practically harmless*	32.1	*Acute 3*	[24]
v	$[Sal]^-$/salicylic acid		221.1–236.1	*Practically harmless*	15.2	*Acute 3*	[18, 24]
vi	$[Ell]^{2-}$/ellagic acid		n.d.[b]	–	n.d.[b]	–	[24]
SA-ILs							
Vii	$[DBS]^-$/n.a.[c]	*Scenedesmus* sp.	Maximum number of live cells unaffected	–	n.a.[c]	–	[25]
GB-ILs							
viii	$[TES]^-$/TES	*A. vibrio*	>60,000	*Practically harmless*	661.17	*Practically harmless*	[26, 29]
ix	$[HEPES]^-$/HEPES		19,584	*Practically harmless*	8684.08	*Practically harmless*	[26, 29]
x	$[CAPSO]^-$/CAPSO		19504.46	*Practically harmless*	3068.88	*Practically harmless*	[27]
xi	$[MES]^-$/MES		9789	*Practically harmless*	214.74	*Practically harmless*	[26, 29]
xii	$[BES]^-$/BES		7327.89	*Practically harmless*	2225.00	*Practically harmless*	[27]
xiii	$[MOPSO]^-$/MOPSO		6132.40	*Practically harmless*	1245.47	*Practically harmless*	[27]

xiv	$[Tricine]^-$/Tricine		4588	*Practically harmless*	6040.57	*Practically harmless*	[26, 29]
xv	$[TAPSO]^-$/ TAPSO		3439.25	*Practically harmless*	965.42	*Practically harmless*	[27]
xvi	$[CHES]^-$/CHES		208.65	*Practically harmless*	16497.82	*Practically harmless*	[26, 29]

[a]"Acute 1" $EC_{50} \leq 1$ mg L^{-1} | "Acute 2" $EC_{50} > 1$ mg.L^{-1} but ≤ 10 mg.L^{-1} | "Acute 3" $EC_{50} > 10$ mg.L^{-1} but ≤ 100 mg.L^{-1} | [11] "Practically harmless" $EC_{50} > 100$ mg.L^{-1}
[b]Not determined due to solubility issues
[c]Not assessed

investigating purification processes by the implementation of aqueous biphasic systems (i.e., water-rich liquid-liquid extraction systems formed by aqueous solutions of two incompatible solutes, such as IL-salt, IL-polymer, IL-carbohydrate) [26, 27]. The literature available covers nine cholinium-based ILs bearing anions derived from Good's buffers (further abbreviated as $[N_{111(2OH)}][GB]$) and their applicability in the purification of antibodies from hen egg yolk [26] and lipase from fermentation broth [27]. Regardless of the $[GB]^-$ anion structure, all $[N_{111(2OH)}][GB]$ are categorized as "practically harmless" (EC_{50} >100 $mg.L^{-1}$, *A. fischeri* – Table 6, entries **viii–xvi**) and exhibit enhanced environmental features compared to other classes of ILs (e.g., $[N_{4444}]^+$ and $[P_{4444}]^+$) and their GBs congeners (except $[CHES]^-$and $[Tricine]^-$ – cf. Table 6, entries **viii–xvi**) [26, 27, 29]. In line with the controversy with the oxygenation role in the toxicity of cholinium-based ILs [18–21, 24], the structure-ecotoxicity relationships dictate that the taurine derivative lacking oxygenated substituent groups (e.g., $[CHES]^-$ – Table 6, entry **xvi**) is by far the most toxic $[N_{111(2OH)}][GB]$ [26]. In opposition, within $[GBs]^-$ oxygenated via the addition of hydroxyl groups, a proportional relationship between the number of oxygenated elements and toxicity is observed (e.g., $[MOPSO]^-$, $[TAPSO]^-$, and $[CAPSO]^-$ – Table 6, entry **x** versus **xiii** versus **xv**) [27]. $[N_{111(2OH)}][GB]$-based structures were shown to offer relevant advantages to the proposed applications, namely, wide buffering capacity, good stabilizing power for biomolecules, and no toxicity, while yielding enhanced extraction efficiency and selectivity data [26, 27].

Remarkably, all studies discussed above were able to find a good compromise between the structure-ecotoxicity-application relationships by applying cholinium-based ILs [24–27]. The cautious design of cholinium-based ILs as APIs, additives, or solvents allowed either keeping or improving the performance while lowering the environmental impact of current applications. Still, such studies [24–27] lack comprehensive ecotoxic evaluations, revealing the need for further investigation for other organisms of the aquatic trophic chain and other types of toxicity studies.

Conclusions

Considering the toxicity displayed by some ILs, particularly the most common nitrogen-based cyclic of aromatic nature, e.g., those based on $[C_nC_1im]^+$ and $[C_nC_1pyr]^+$, cholinium-based ILs emerge as less toxic and more sustainable chemicals. Such notion is firstly supported by aquatic toxicology studies including cholinium-based ILs as part of the ILs' battery investigated, mainly using halogenated anions (e.g., $[N_{111(2OH)}]Cl$, $[N_{111(2OH)}][NTf_2]$, and $[N_{111(2OH)}][PF_6]$). Systematic studies devoted to structure-ecotoxicity relationships controlling the toxicity of cholinium-based ILs, where the role of both the anion and cation structures was evaluated, are reviewed. The well-documented "side-chain effect" was consistently observed by elongating the alkyl chains of the cation (e.g., $[N_{11n(2OH)}]^+$), while deviations were observed for the $[C_nCO_2]$-based anions. The effects noticed for both the inclusion of oxygenated and aromatic groups on the ecotoxicity were intricate and highly dependent on the cation/anion combinations and the organism under study. The introduction of naturally occurring amino acids as anions is a feasible route to create eco-friendly cholinium-based ILs, but there must be a careful choice of the amino acid used.

Based on the summarized results, it can be concluded that depending on the cationic and anionic parts combined, some cholinium-based ILs may exhibit higher toxicity than nitrogen-based cyclic of aromatic nature, namely, those based on $[C_nC_1im]^+$, and some volatile organic solvents. The data available highlights a different mode of toxic action for this class of ILs and challenges its "benign" nature often claimed a priori by many researchers. Under this scenario, the overgeneralized idea that cholinium-based ILs are safe and environmentally innocuous chemicals should definitely be avoided. At least, aquatic toxicity data covering organisms along different levels of the trophic web and different

types of toxicity tests other than acute toxicity (e.g., chronic, reproductive, developmental, and embryo toxicity) as well as cytotoxicity, terrestrial toxicity, and antimicrobial activity profiles should be investigated before considering cholinium-based ILs as nontoxic. However, given the variety and variability of ILs, this task of an empirical evaluation of the toxicity of all ILs is not feasible and thus mathematical models [e.g., quantitative structure-activity relationships (QSARs) and quantitative structure-property relationships (QSPRs)] must be developed and applied (e.g., [14, 30]).

The environmental impact of ILs, however, must not be only based on toxicity data. The whole life cycle of cholinium-based ILs should be carefully evaluated in a way that all steps, meaning, from their synthesis/production and application until their discharge, should be contemplated. It should be highlighted that simpler and cleaner synthetic routes have been reported, based on the use of naturally occurring precursors [22, 24]. As recently highlighted by Jessop [31], the lack of life cycle assessments of ILs-mediated applications is precluding the evolution of the field, and, therefore, these are urgent. The challenge now is to ally both "designer" and "green" concepts to develop "high performance" and safer processes and products.

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