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Ecotoxicity of Cholinium-Based Deep Eutectic Solvents

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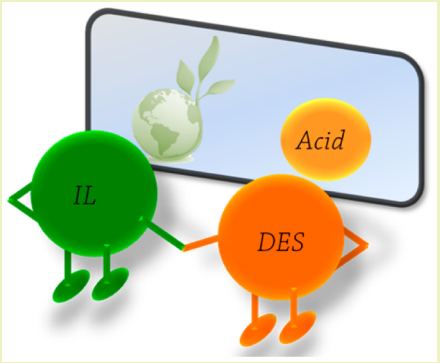
ABSTRACT: Deep eutectic solvents (DES) are mixtures of hydrogen bond acceptors (HBA) and hydrogen bond donors (HBD) with a melting point much lower than that of either of its components. They have been the focus of interest in past years due to their unique properties, low cost, and wide range of applications. Despite the attention given to DES and their claimed benignity when compared with ionic liquids, their environmental impact is poorly characterized and understood. In this work, the toxicity of some DES prepared with cholinium chloride ([Chol]Cl) as HBA and organic acids, namely acetic, citric, lactic, and glycolic acids, as HBD was measured with the Microtox toxicity test. These DES showed an intermediate toxicity when compared with the respective starting materials and can be considered as "moderately toxic," their toxicity being clearly dominated by the concentration of the acid. They are also shown to be more toxic than their corresponding ionic liquids, namely cholinium glycolate, cholinium acetate, cholinium dihydrogencitrate, and cholinium lactate. Further studies are necessary on the ecotoxicology and biodegradability of DES, namely to evaluate their "green" character and their alternative capability to both classic solvents and ionic liquids.



KEYWORDS: *Deep eutectic solvents, Microtox, Ecotoxicity, EC_{50}, Ionic liquids*

INTRODUCTION

Deep eutectic solvents (DES) are mixtures of hydrogen bond acceptors (HBA) and hydrogen bond donors (HBD) with a melting point much lower than either of their components.[1,2] The term "deep eutectic solvent" was coined by Abbott et al. in 2004,[3] while reporting a large depression of the melting temperature of a [Chol]Cl and urea mixture,[2,3] and suggested that the hydrogen-bond interactions between urea and the chloride were the main cause of the freezing-point depression of the mixture.[4] DES, however, were known for a longer time: in soldering (where eutectic mixtures of tin and lead solders have been widely used for electrical joints due to their low melting points, good wettability, good plasticity, and reasonable electrical conductivity), ice removal (the vulgar sodium chloride and water eutectic mixture), and pharmaceuticals (with the lidocaine/prilocaine eutectic mixture being the most well-known example).[2] Nowadays, the interest in DES is growing as an alternative to organic solvents, both the traditional ones and their putative green substitutes, for example the ionic liquids (ILs).[2,4] Many studies show that ILs are not intrinsically green, as often assumed;[2,5] actually, many of them are toxic[6] and poorly biodegradable,[7−9] besides costly.[6] Therefore, the search for biodegradable and low toxicity solvents continues. In Europe, REACH legislation increases the pressure to invest more in this field, expecting that "green" compounds can be economically competitive with the petrol-based ones.[8] However, when composed of unmodified, nontoxic, and non-persistent natural products, compounds are exempted from registration according to the REACH regulations; this is the case for DES.[10] The natural occurrence, renewability, low toxicity, and biodegradability of the DES starting materials suggest that these mixtures can be much "greener" and easier to register than ILs.[3,10,11] Moreover, DES are cheaper and easier to synthesize.[2] Nevertheless, concerning the seemingly low toxicity of DES, the possible synergetic effect is often neglected: the mixture toxicity could be higher than the sum of their low toxicity individual components. Therefore, the DES toxicity must be tested before any assumption since the DES toxicity and biodegradability are poorly studied.[3,11] These are fundamental aspects that must be addressed before their application on an industrial scale.[1]

Among the variety of available DES, the most popular are still those based in [Chol]Cl (used as HBA), as in the original formulation, because of their low cost, lower toxicity, and higher biodegradability and biocompatibility when compared with the most common ILs. The [Chol]Cl has been mixed with different HBDs such as polyols, carbohydrates, amides, amines, alcohols, and carboxylic acids.[2] Other combinations have been explored, in particular other salts, such as those based on the imidazolium, ammonium, and phosphonium cations.[2] When the DES starting materials are primary metabolites, namely amino acids, organic acids, sugars, or cholinium derivatives, they are called natural DES (NADES)[12] and are claimed to comply with many of the Green Chemistry Principles.[13]

Although their starting materials' toxicity is well-known, the toxicity of the corresponding DES was seldom studied: only

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four publications have been published to date.[1,11,13,14] In 2013, four [Chol]Cl-based DES were studied concerning their toxicity toward two Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), and to the crustacean *Artemia salina*.[11] Later that year, the toxicity of three phosphonium-based DES was studied toward the same organisms.[1] Even more recently, the effect of 11 different DES on a mouse cell line was studied and compared to two ILs.[13] In 2015, in vitro toxicity on fish and human cell lines and phytotoxicity on wheat of three [Chol]Cl-based DES was studied, as well as their biodegradability.[14] The reported results on DES toxicity were, somehow, contradictory. The [Chol]Cl-based DES were considered nontoxic toward bacteria,[11] while the phosphonium-based DES were reported to present a higher cytotoxicity than their individual components to the crustacean *Artemia salina*.[1] Other [Chol]Cl-based DES showed low to moderate cytotoxicity on fish and human cell lines and low phytotoxicity on wheat.[14] Finally, the cytotoxicity of some DES (including one of the formulations studied in this work, yet with just two molar proportions instead of the three herein studied) showed that they were less toxic than, or at worst as toxic as, the ILs,[13] as generally assumed. In terms of the comprehension behind the results, the hydrogen bonding between the mixture compounds was suspected to be the cause of the DES toxicity.[1] Nevertheless, this indicates the possible synergistic effect after DES formation.[1] Therefore, their toxicity seems to be dependent on its composition and concentration, as well on the target organisms.[1] All analysis considered, and as the cited authors suggest, further studies on this topic are necessary.[1,11,13,14]

This work represents an effort to reveal the toxicity profile of DES and aims to suppress some of the gaps on the knowledge of these compounds. For the first time, the DES ecotoxicity was assessed against the marine bacteria *Vibrio fischeri* (*V. fischeri*), using the Microtox toxicity test. The DES under study were based on the HBA [Chol]Cl and different organic acids as HBDs, therefore belonging to the so-called NADES. In this work, one of the first contributions to the barely known toxicity of these compounds, the toxicity of DES and respective mechanisms of toxicity are discussed, and a comparison between the toxic effect of DES and their ionic liquid congeners is reported, aiming at reflecting and inferring on their alleged "green" character.[15]

EXPERIMENTAL SECTION

Materials. The cholinium chloride ([Chol]Cl—purity of 98%) (Figure 1), cholinium dihydrogencitrate, and glycolic acid (99%) were purchased from Sigma-Aldrich. Lactic acid (or ethyl lactate) was purchased at SAFC (98.0%). Cholinium glycolate ([Chol][Gly]) and cholinium lactate ([Chol][Lac]) were synthesized in our laboratory, following standard procedures.[16−18] The acetic acid (AA) was purchased from JMGS (Labsolve; purity of 99.5%), citric acid (CA) from Fisher Scientific (purity of 100%), lactic acid (LA) from Riedel-de Haen (purity of 88−92%), and the glycolic acid (GA) from Sigma-Aldrich (purity of 99%; Figure 1). The DES under study were prepared in accordance with standard protocols.[19] The water content of the DES was determined by Karl Fischer titration,[19] and these values were considered in the calculation of the toxicity of the compounds (the obtained results were corrected by this percentage of water). Table 1 shows the list of DES prepared, the molar ratio, and the water content of each DES prepared.

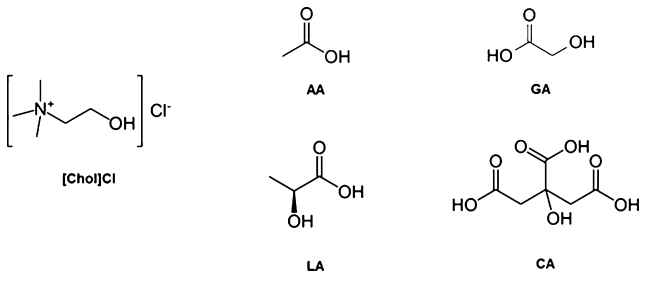

Figure 1. Chemical structure of the starting materials used in the formulation of the DES under study ([Chol]Cl, cholinium chloride; AA, acetic acid; LA, lactic acid; GA, glycolic acid; CA, citric acid).

Table 1. List of DES Prepared and Analyzed in This Work

DES	mole ratio	water content (wt %)
[Chol]Cl:AA	2:1	1.08
[Chol]Cl:LA	2:1	1.13
[Chol]Cl:GA	2:1	1.04
[Chol]Cl:CA	2:1	1.01
[Chol]Cl:AA	1:1	0.92
[Chol]Cl:LA	1:1	1.00
[Chol]Cl:GA	1:1	0.86
[Chol]Cl:CA	1:1	0.88
[Chol]Cl:AA	1:2	0.71
[Chol]Cl:LA	1:2	0.87
[Chol]Cl:GA	1:2	0.63
[Chol]Cl:CA	1:2	0.77

Microtox Assay. The Microtox toxicity test (Microbics Corporation, 1992) was used to evaluate the inhibition of the luminescence in the marine bacteria *V. fischeri*. This test was performed using a range of diluted aqueous solutions (from 0 to 81.9%) of each tested compound, where 100% of the compound corresponds to a known concentration of a stock solution (generally about 1 g L^{-1}). After 5, 15, and 30 min of exposure to the compound solution, the light output of the luminescent bacteria was measured and compared with the light output of a blank control sample. The toxicity was evaluated on the basis of the percent reduction in the bacteria luminescence relative to this blank control. These records were used to estimate concentrations promoting 50% luminescence inhibition (corresponding to the median effective concentration, EC_{50}) and corresponding 95% confidence intervals for each tested IL by nonlinear regression, using the least-sqaures method to fit the data to the logistic equation. These analyses were performed with the software STATISTICA, version 8.0 (StatSoft, 2007).

RESULTS AND DISCUSSION

It is important to take into consideration the first assumption discussed in the literature regarding the toxicity profile of DES, indicating that despite being mixtures, given their nature, they cannot be studied as such (in terms of toxicity), since the authors considered that this is a paradigmatic case of the gestaltism theory: the whole is other than the sum of the parts.[20] The authors claim that the physicochemical properties of the HBD:HBA complex are different from those of the starting materials due to the hydrogen bonds created between both the HBD and HBA.[11] Taking into account the brief state-of-the-art regarding the toxicity of DES, it is considered that the DES toxicity should not be studied as the toxicity of a mixture (using, for instance, the common concentration addition and independent action models[20]) but rather as the study of the toxicity of a simple compound. Actually, despite the absence of ionic or covalent bonds, like in ILs or in hydrogen chloride (for example), respectively, the behavior of the DES seems to be

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Table 2. Values of Median Effective Concentration (EC_{50}), in mg_{DES} L^{-1}, and Respective 95% Confidence Intervals (c. i.), Obtained after 5, 15, and 30 min of Exposure of the Marine Bacteria *V. fischeri* (Microtox Toxicity Test) to Different Chemical Compounds

chemical compound	EC_{50} (mg_{DES} L^{-1}) 5 min (95% c. i.)	EC_{50} (mg_{DES} L^{-1}) 15 min (95% c. i.)	EC_{50} (mg_{DES} L^{-1}) 30 min (95% c. i.)
[Chol]Cl[29]	**648** (525−772)	**560** (411−708)	**469** (384−555)
acetic acid (AA)	**71.8** (64.0−79.5)	**72.9** (63.8−82.0)	**73.3** (67.0−79.7)
lactic acid (LA)	**18.7** (17.9−19.5)	**17.6** (17.1−18.1)	**17.4** (17.0−17.9)
citric acid (CA)	**13.5** (12.2−14.7)	**13.1** (11.4−14.9)	**13.2** (12.2−14.3)
glycolic acid (GA)	**12.8** (11.9−13.7)	**12.0** (11.1−13.0)	**12.0** (11.3−12.6)
[Chol]Cl:AA (1:2)	**129** (117−141)	**125** (110−141)	**130** (121−138)
[Chol]Cl:LA (1:2)	**36.3** (34.0−38.6)	**34.2** (31.6−36.8)	**33.6** (32.9−34.4)
[Chol]Cl:GA (1:2)	**30.9** (29.0−32.8)	**30.4** (28.8−32.0)	**30.2** (29.4−31.0)
[Chol]Cl:CA (1:2)	**16.8** (15.5−18.1)	**15.6** (14.7−16.5)	**15.6** (14.9−16.2)
[Chol]Cl:AA	**214** (192−236)	**200** (186−214)	**197** (180−214)
[Chol]Cl:LA	**62.8** (57.7−67.9)	**63.0** (57.0−69.0)	**61.8** (50.3−73.4)
[Chol]Cl:GA	**33.9** (26.3−41.4)	**33.1** (26.9−39.2)	**32.9** (27.2−38.5)
[Chol]Cl:CA	**22.2** (20.9−23.5)	**22.5** (19.8−25.1)	**22.4** (20.0−24.8)
[Chol]Cl:AA (2:1)	**354** (308−399)	**343** (311−375)	**337** (303−371)
[Chol]Cl:LA (2:1)	**70.1** (63.9−76.2)	**68.6** (56.3−80.9)	**67.0** (40.9−93.0)
[Chol]Cl:GA (2:1)	**62.3** (50.9−73.7)	**63.0** (50.8−75.3)	**62.2** (49.9−74.6)
[Chol]Cl:CA (2:1)	**32.4** (30.5−34.2)	**31.1** (28.5−33.7)	**31.9** (27.1−36.6)

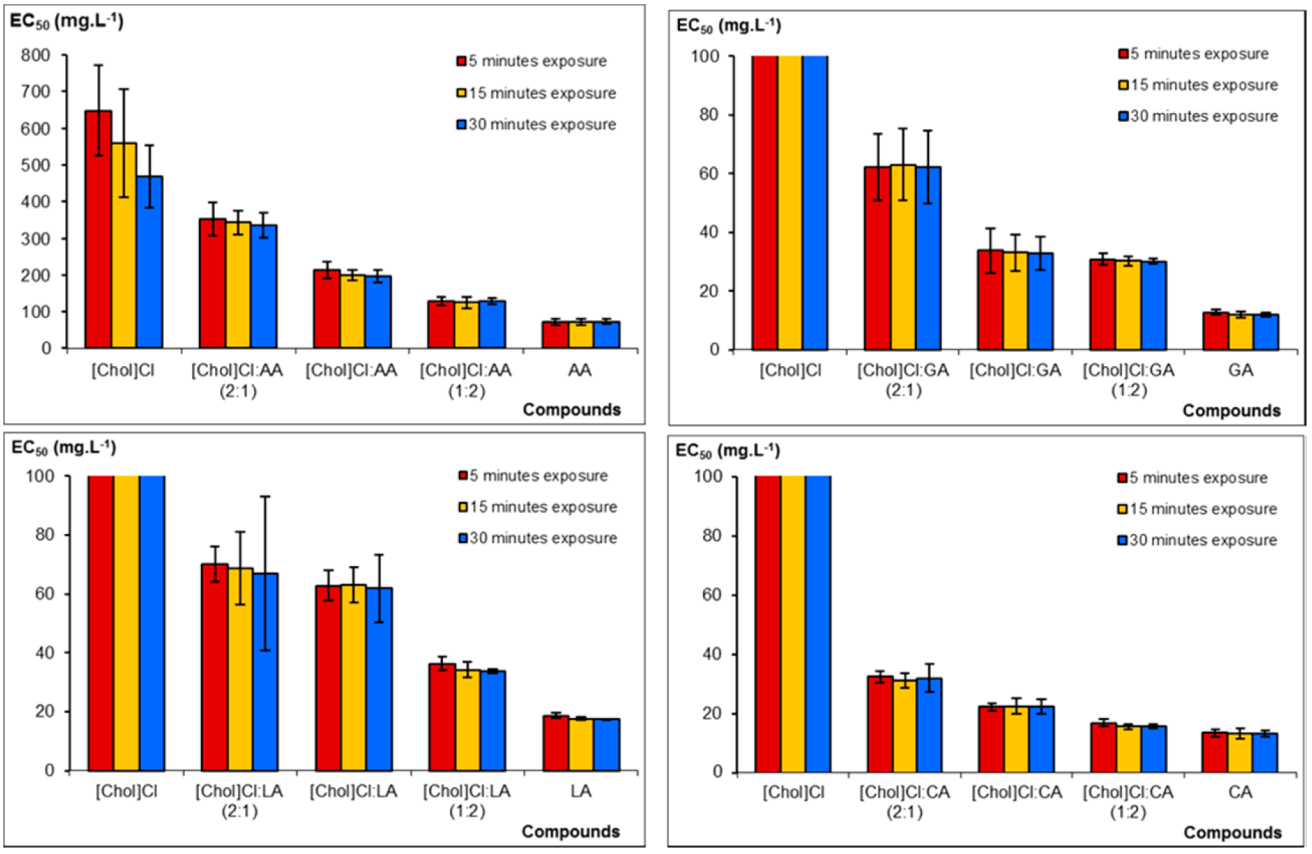

Figure 2. Values of median effective concentration (EC_{50}), in mg L^{-1}, obtained after 5, 15, and 30 min of exposure of the marine bacteria *Vibrio fischeri* (Microtox toxicity test) to different chemical compounds. Emphasis is given to the different molar proportions of each DES with the same organic acid.

closer to that of a single compound, as confirmed by Hayyan et al.[1] It must also be kept in mind that any different molar proportion may give different properties to the DES composed of the same starting materials; consequently, the number of possible DES formulations tends to be infinite. The toxicity of the DES under study, reported as EC_{50} (mg L^{-1}), and respective 95% confidence intervals (c. i.), obtained after 5, 15, and 30 min of exposure are reported in Table 2. In all cases, EC_{50} values were estimated considering significant fitting of the experimental data to the assumed distribution (chi-square testing; $p > 0.05$), and ensuring significant regression coefficients ($p < 0.05$). The consistency of the toxicity results must be stressed since all results, without exception, follow the same ascending pattern of toxicity: [Chol]Cl ≪ [Chol]Cl:acid

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Table 3. Values of Median Effective Concentration (EC_{50}), in mg_{acid} L^{-1}, and Respective 95% Confidence Intervals (c. i.), Obtained after 5, 15, and 30 min of Exposure of the Marine Bacteria *V. fischeri* (Microtox Toxicity Test) to Different Chemical Compounds

chemical compound	EC_{50} (mg_{acid} L^{-1}) 5 min (95% c. i.)	EC_{50} (mg_{acid} L^{-1}) 15 min (95% c. i.)	EC_{50} (mg_{acid} L^{-1}) 30 min (95% c. i.)
[Chol]Cl:AA (1:2)	**59.7** (54.1−65.3)	**57.8** (50.9−65.3)	**60.2** (56.0−63.9)
[Chol]Cl:LA (1:2)	**20.5** (19.2−21.8)	**19.3** (17.8−20.7)	**18.9** (18.5−19.4)
[Chol]Cl:GA (1:2)	**16.1** (15.1−17.1)	**15.9** (15.0−16.7)	**15.8** (15.3−16.2)
[Chol]Cl:CA (1:2)	**12.3** (11.4−13.3)	**11.4** (10.8−12.1)	**11.4** (10.9−11.9)
[Chol]Cl:AA	**64.4** (57.7−71.0)	**60.1** (55.9−64.4)	**59.2** (54.1−64.4)
[Chol]Cl:LA	**24.6** (22.6−26.6)	**24.7** (22.4−27.1)	**24.2** (19.7−28.8)
[Chol]Cl:GA	**12.0** (9.3−14.6)	**11.7** (9.5−13.8)	**11.6** (9.6−13.6)
[Chol]Cl:CA	**12.9** (12.1−13.6)	**13.0** (11.5−14.5)	**13.0** (11.6−14.4)
[Chol]Cl:AA (2:1)	**62.6** (54.4−70.5)	**60.6** (55.0−63.8)	**59.6** (53.6−65.6)
[Chol]Cl:LA (2:1)	**17.1** (15.6−18.6)	**16.7** (13.7−19.7)	**16.3** (10.0−22.7)
[Chol]Cl:GA (2:1)	**13.3** (10.9−15.8)	**13.5** (10.9−16.1)	**13.3** (10.7−15.9)
[Chol]Cl:CA (2:1)	**13.2** (12.4−13.9)	**12.2** (11.6−13.7)	**13.0** (11.0−14.9)
acetic acid (AA)	**71.8** (64.0−79.5)	**72.9** (63.8−82.0)	**73.3** (67.0−79.7)
lactic acid (LA)	**18.7** (17.9−19.5)	**17.6** (17.1−18.1)	**17.4** (17.0−17.9)
citric acid (CA)	**13.5** (12.2−14.7)	**13.1** (11.4−14.9)	**13.2** (12.2−14.3)
glycolic acid (GA)	**12.8** (11.9−13.7)	**12.0** (11.1−13.0)	**12.0** (11.3−12.6)

(2:1) < [Chol]Cl:acid (1:1) < [Chol]Cl:acid (1:2) < acid. In other words, any studied DES had an intermediate value of toxicity when compared to the starting materials (acids and [Chol]Cl), and the toxicity increased with the acid content (Figure 2). In what concerns the starting materials, the [Chol]Cl can be considered as practically harmless to *V. fischeri*, given the high EC_{50} values previously reported.[21,22] The four carboxylic acids (HBDs) presented a moderate toxicity, being considered slightly toxic,[21,22] in the following ascending order: AA ≪ LA < CA < GA. The level of toxicity of these different acids toward *V. fischeri* was similar, except for AA, the toxicity of this compound being 4 to 5 times lower than that of the other acids. The time of exposure had little or no impact on the toxicity of the studied compounds, despite the general tendency to a higher toxicity with a higher exposure time. The overall consistency of the results suggests a similar mechanism of toxicity for the various chemicals under study. Within the DES, the ascending toxicity pattern is related with the structure of the acid: AA < LA < GA < CA. This is in close agreement with the order found for the decreased hydrophilic tendency described by the octanol−water partition coefficients, log K_{ow} = −0.22, −0.44, −1.04, and −1.32 (Chemspider, accessed May 27, 2015), respectively. The toxicity of the acids, when isolated, followed the same tendency except for GA and CA; however, their EC_{50} values were not statistically different, and therefore, their toxicity must be considered identical. These results showed a moderate toxicity[21−23] of these DES, which is contrary to the generalized idea that DES are of low toxicity.[3,24] The EC_{50} results, reported in Table 3 as a function of the acid concentration, showed that the effect of the acid is preponderant in the toxicity, with the results for the various DES studied being essentially identical to those of the corresponding acid. The hydrogen bonding between the mixture compounds was suspected to increase the toxicity through the respective charge delocalization, since chemicals having delocalized charges are more toxic than chemicals with localized charges.[1] The HBD is expected to denature proteins, with consequences in enzymatic reactions.[25] As a result, cell activity may be affected to the point of cell death.[1] This is consistent with the known importance of the HBD on the acidity of the DES.[1] Our results are in harmony with these characteristics attributed to the HBD. Since organic acids were used as HBDs, pH values of the corresponding DES were expected to be particularly low, which was observed (pH values lower than 3).[19] As a result, a negative effect on the cell activity, through denaturation of proteins, was expected, and this may explain the moderate levels of toxicity of the studied DES (compared to the poorly toxic HBA) and the dominant role of the acid on the observed DES toxicities.

This is the fifth study on the toxicity of DES. Its results expose some contradictions to past reports. Previously, it was found that different DES ([Chol]Cl-based like those used in the present study but with three different HBDs, from the moderately toxic triethylene glycol to the less toxic ethylene glycol and to the practically harmless glycerine and urea) were of low toxicity to bacteria.[11] However, the same authors later found that phosphonium-based DES (with the same HBDs, except urea) were toxic to bacteria and even suggested their use as antibacterial agents.[1] The same DES had higher cytotoxicity than their individual components to the crustacean *Artemia salina*.[1] Three other [Chol]Cl-based DES (with the nontoxic glucose, glycerol, and the toxic oxalic acid as HBDs) showed low phytotoxicity to wheat and low to moderate cytotoxicity on fish and human cell lines,[14] with similar or higher toxicity (in the DES with oxalic acid as HBD) than the parent compounds.

It must be stated that, in this study, the DES with oxalic acid (also a carboxylic acid) was clearly more toxic than the other DES to any target organism.[14] Finally, the cytotoxicity of 11 DES (including [Chol]Cl:CA but with only two of the molar proportions herein studied) was assessed toward a mouse fibroblast-like cell line in comparison to that of two ILs (1-butyl-3-methylimidazolium acetate and 1-butyl-3-methylimidazolium chloride).[13] The results showed that the DES were as toxic as, or less toxic than, the studied ILs, as generally assumed. Contrarily to our findings, the equimolar [Chol]Cl:CA was reported to be less cytotoxic, to be precise, than the 2:1 proportion. This DES, [Chol]Cl:CA (2:1), was among the four more cytotoxic DES studied, with their cytotoxicity comparable to that of the two ILs; all seven other DES were not so cytotoxic.[13]

More than comparing the DES toxicity with model or archetypical ILs, it is important to compare the toxicity of the

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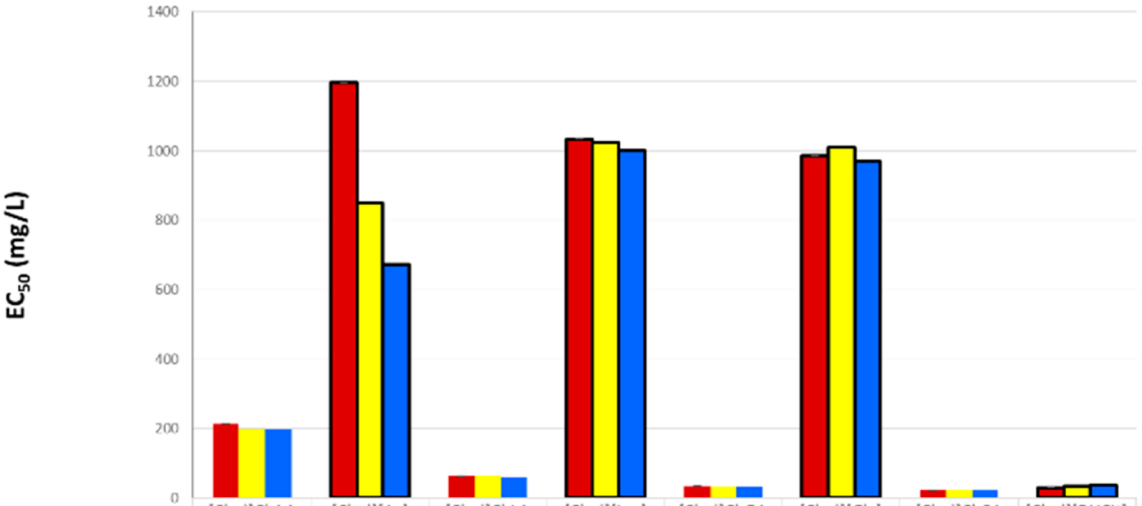

	[Chol]Cl:AA	[Chol][Ac]	[Chol]Cl:LA	[Chol][Lac]	[Chol]Cl:GA	[Chol][Gly]	[Chol]Cl:CA	[Chol][DHCit]
5 minutes exposure	214	1196.71	62.8	1034.27	33.9	986.55	22.2	28.75
15 minutes exposure	200	850.74	63.0	1025.07	33.1	1009.98	22.5	35.1
30 minutes exposure	197	673.21	61.8	1001.18	32.9	970.22	22.4	37.23

Figure 3. Values of median effective concentration (EC_{50}), in mg L^{-1}, obtained after 5, 15, and 30 min of exposure of the marine bacteria *V. fischeri* (Microtox toxicity test) to different cholinium-based DES (this work) and the correspondent ILs (dark black line): results of [Chol][DHCit] and [Chol][Ac] adapted from ref 21; data of [Chol][Lac] and [Chol][Gly] measured in this work.

DES under study with their "equivalent" ILs, namely choline acetate ([Chol][Ac]), choline dihydrogencitrate ([Chol][DHCit]), choline glycolate ([Chol][Gly]), and choline lactate ([Chol][Lac]), as shown in Figure 3. This comparison showed that the ILs are less toxic than their DES "counterparts." Only [Chol][DHCit], a vulgar dietary supplement, presents toxicity values comparable to the values obtained for the DES [Chol][CA]. This suggests that it might be necessary to reconsider the general assumption of the "green" character of DES and its alternative capability to ILs, at least in what concerns their ecotoxicity toward bacteria. Nevertheless, additional studies are required to understand the differences in toxicity behavior of these similar compounds.

The mechanism of toxicity must be similar for the various DES tested as suggested by the consistency of the results presented in Table 1, but principally by the data reported in Table 2, the similar character of the DES studied (same HBA and carboxylic acids as HBDs) and the small influence of the exposure time. An important factor in the mechanism of DES toxicity may be related with the cellular organization of the organisms. Previous results on DES toxicity include Gram-negative and Gram-positive bacteria (though the authors did not consider the differences in the cell wall in the interpretation of the results)[1,11] and eukaryotic cells.[13,14] Paiva and collaborators[13] explained the toxicity of DES toward bacteria by the disruption of the cell walls of the bacteria due to the presence of delocalized charges in these liquids,[13] referring to the work of Hayyan et al.[1,11] Apart from the interpretation of phytotoxicity results,[14] no further discussion on the toxic effect of DES on the cell wall was presented by the authors. So, at this point, the review of Warnecke and Gill, on organic acids toxicity in *Escherichia coli* (also with a Gram-negative cell wall), is probably the most relevant reference for this discussion.[26] One of the primary factors contributing to the toxicity of organic acids is their ability to diffuse across cellular membranes when nondissociated.[26] This might be the case of the DES under study given the relevant effect of the HBDs on the toxicity of DES and also their pH, which is lower than the pK_a of their respective organic acids.[19] The data here reported in Table 2 suggests that the DES have a similar toxicity and toxic effect to that of their corresponding organic acids. The cholinium species seem to have little effect besides acting as a solvent for the acid, the concentration of which seems to be the main driving force behind the toxicity of the DES.

Although it is known that lipophilic ILs can disrupt cell membranes,[27] it is unlikely that the same happens with DES and the corresponding HBDs under study in this work. Given the high hydrophilicity of DES, it seems more reasonable to assume that DES may pass the cell membrane and exert its toxic effect (mainly attributed to the acid counterparts) in the cytoplasm, where they may disrupt both the pH (which is about 7.5 in the bacteria cytoplasm) and the anion pool of the cytoplasm, like in the Gram-negative bacteria *Escherichia coli*.[26] The resulting increase in the internal acidity may result in negative effects on the integrity of purines, denaturation of enzymes, and oxidative stress, all factors negatively affecting cell viability.[26] Therefore, this possible toxicity mechanism must be kept in mind when formulating new DES, namely those with organic acids as HBDs.

The cytotoxicity of the phosphonium-based DES studied by Hayyan and collaborators[1] was much higher than that of their individual components. The authors stated that this indicates the synergistic effect after DES formation. The relevant toxic effect on bacteria led to consider the potential use of DES as antibacterial agents.[1] Our results are contrary to these findings and hypotheses. Precaution is advised in the formulation of such speculative considerations. The huge range of different possible DES formulations will, probably, prevent any generalization on DES toxicity without a higher volume of data available, considering different groups of individual components (HBDs and HBAs). This need for more data on DES toxicity (namely, the study of toxicity toward organisms at different trophic levels) is in close agreement with the conclusions of the previous works on this topic,[1,11,13,14] and it is mandatory prior

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to DES application on the industrial scale. Therefore, and before these assessments, the DES cannot be labeled "green."[14] The design of DES, which are as tunable as the ILs, may be crucial in the quest for "greener" solvents while assuring their desirable physicochemical properties.[14] Furthermore, this ecotoxicological evaluation will not be complete without biodegradation studies,[14] whether to prove their readily biodegradability assumption[13] or to assess the metabolites formed in the biodegradation process,[28] which could be more toxic than the not so toxic DES.

The results on DES toxicity obtained so far suggest some selectivity in what concerns the cell type, therefore with a potential in cancer therapy, as claimed by Hayyan and coauthors.[11] Our results confirm this assumption, but more data are also necessary in this field.

CONCLUSIONS

Toxicity testing is still mandatory before any assumption on the toxicity of DES based solely on the characteristics of their starting materials, to avoid misinterpretations and over-generalizations. Our results suggest a "moderate toxicity" of the DES, dominated by the toxicity profile of the corresponding acids (HBD). According to experimental evidence, these DES are more toxic than the congener ILs, and thus it is concluded that DES may not be as "green" as generally supposed. The ecotoxicological evaluation of DES must continue because the data available are not enough to predict their behavior in the environment. Future research must include biodegradability studies and toxicity assays to different trophic levels.

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Notes

The authors declare no competing financial interest.

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