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Toxicity test profile for deep eutectic solvents: A detailed review and future prospects

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HIGHLIGHTS

- Cytotoxicity, Phytotoxicity, anti-microbial activity of deep eutectic solvents.
- Computational studies for toxicity of deep eutectic solvents.
- Strategy to increase the greenness of deep eutectic solvents.
- Future aspects.

GRAPHICAL ABSTRACT

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ABSTRACT

Deep eutectic solvents (DESs) are preferable in terms of starting materials, storage and synthesis, simplicity, and component material affordability. In several industries ranging from chemical, electrochemical, biological, biotechnology, material science, etc., DES has demonstrated remarkable potential. Despite all these accomplishments, the safety issue with DES must be adequately addressed. Different DES interacts with the cellular membranes differently. It is not possible to classify all DES as easily biodegradable. By expanding the current understanding of the toxicity and biodegradation of DES, interactions between organisms and cellular membranes can be linked. The DES toxicity profile varies according to their concentration, the nature of the individual components, and how they interact with living things. Therefore, the results of this review can serve as a baseline for DES development in the future.

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1. Introduction

Organic solvents in conventional methods (Lee, 2022) were formerly utilized as entrainers but have been curtailed because of their poisonous, flammable, volatile, and non-environment-friendly characteristics (Serhan et al., 2019). In addition, ordinary salt or organic entrainers used in the conventional method may precipitate out of the solution and injure process equipment (Mahdi et al., 2015). To replace organic solvents with some new entrainer which can prove better, ionic liquids (ILs) were used to break azeotropes. But due to their toxicity and high economical value in production, the need for exploring more adaptable, reusable, eco-friendly solvents remains open (Kudłak et al., 2015; Weaver et al., 2010). When it comes to a range of real-world applications that call for challenging separation procedures, deep eutectic solvents (DESs) provide a highly promising alternative since they don't need the use of any additional organic solvent during their synthesis (Cunha and Fernandes, 2018; Maugeri and Domínguez de María, 2012). From an environmental and technological perspective, DES outperform conventional IL, providing the following advantages: 1) lower cost; 2) simple preparation with higher purity; 3) greater designability with a wide range of cations, anions, a hydrogen bond donor (HBD), and salt/HBD molar ratios (Wen et al., 2015a). The pairing of hydrogen bond acceptors (HBAs) (Craveiro et al., 2016; Smith et al., 2014), such as metal salts or substantial organic molecules with HBDs, typically a quaternary ammonium salt, synergistically generates DES (Craveiro et al., 2016; Smith et al., 2014; Tiecco et al., 2019). As a result, this combination exhibits distinct solvent characteristics and displays a lower melting point than any of its individual constituents (Ijardar et al., 2022). The term "eutectic" describes a combination whose chemical composition has the lowest melting point possible. The HBD and HBA have intense intermolecular interactions, especially hydrogen bonding, which leads to a low melting point (Oliveira et al., 2013).

DES has a vast variety of potential combinations, which is one of their critical benefits (Tomé et al., 2018). Its constituents may be chosen from many substances, enabling the DES characteristics to be adjusted to suit certain applications. A mini review on the synthesis and properties of DES was presented by Singh et al. (2021). Because of its adaptability, DES is appealing in a variety of industries (Jiang et al., 2019; Li et al., 2018; Warrag et al., 2017), including chemistry (Espino et al., 2016), materials research (Ijardar et al., 2022), and biology. Xu et al. (2017a) conducted a comprehensive evaluation of the liter ature on the possible applications of Deep Eutectic Solvents (DES) in biocatalysis (Xu et al., 2017a). Mbous et al. (2017) investigated the use of DES in biomedical and biotech engineering (Mbous et al., 2017). Zdanowicz et al. (2018) investigated the use of DES in polysaccharide processing (Zdanowicz et al., 2018), whereas Skarpalezos and Detsi (2019) conducted research on the extraction of flavonoids using DES (Skarpalezos and Detsi, 2019). Chandran et al. (2019) concentrated on the DES extraction-desulphurization process (Chandran et al., 2019). Emami and Shayanfar (2020) wrote a thorough review paper on the promising possibilities of DES in efficient drug delivery. Krishnan et al. (2020) published research on carbon capture systems involving DES, ionic liquids (IL), and polymers. Nguyen et al. (2021) compiled a comprehensive literature study on the use of DES in the pharmaceutical business (Nguyen et al., 2021), whilst Atilhan and Aparicio (2021) provided a thorough examination of DES uses in the fuel and energy industries (Atilhan and Aparicio, 2021). Ali Redha (2021) described the use of DES for phenolic compound extraction (Ali Redha, 2021). Summaries of DES uses in isolating harmful components from gases were published by Wazeer et al. (2020) (Wazeer et al., 2020) and Janjhi et al. (2023) (Janjhi et al., 2023). On the other side, Słupek et al. (2020), Ma et al. (2018b), Scholz et al. (2013) provide the insight that DES hold great potential as physical absorbents for biogas upgradation. The use of hydrophobic DES in feedstock detoxification for biofuel generation was examined by Makoś et al. (2020).

A review of the transformation of biomass into biofuels was presented by Amesho et al. (2023). Additionally, DES has exceptional solvation capabilities that enable it to dissolve a variety of organic and inorganic substances. They may be used as electrolytes in electrochemical systems, solvents for chemical reactions (Pollet et al., 2014), and extraction procedures (Cunha and Fernandes, 2018). They have also shown the capacity to stabilize and improve the reactivity of certain chemicals, allowing reactions that are difficult or impossible in conventional solvents. They may also be used to synthesize materials, such as polymers and nanoparticles.

Despite its various advantages, DES is still a very new field of study, with ongoing efforts to investigate their properties, comprehend their behavior, and improve the way they are designed for use. Due to their low volatility and low toxicity, they are often seen as being more ecologically benign and sustainable (Malolan et al., 2021; Othman et al., 2015). But a thorough analysis is necessary before we can determine if DES are indeed "green" (Wen et al., 2015a). The scientific community is actively researching and concerned about the toxicity of DES. The effects of temperature, pressure, and other variables on the characteristics of DES are all being studied by researchers, as well as various combinations of HBD and HBA (Hayyan et al., 2012). Although many conventional organic solvents are harmful and DES are usually thought to be less toxic, it is important to weigh their possible dangers and guarantee their safe usage (Azizi et al., 2012). It is also important to develop safer alternatives and help guide regulatory decision-making to maintain the health of our ecosystem. Depending on the particular pairing of HBA and HBD utilized in its formulation, the toxicity of DES may change (Mao et al., 2018a). Certain quaternary ammonium compounds employed as HBD, in particular, may have intrinsic toxicity (Hayyan et al., 2015a).

Numerous studies have been conducted to establish the toxicity of DES (Ge et al., 2017; Lapeña et al., 2021a; Torregrosa-Crespo et al., 2020a) to determine how they affect the environment and living things (Wazeer et al., 2021). These studies focus on genotoxicity, ecotoxicity, acute and chronic toxicity, and biodegradability. Those findings suggest that specific DES formulations may be more biodegradable and less harmful than typical organic solvents.

However, it is important to keep in mind that DES toxicity is a complicated subject, and more study is still needed to fully comprehend any possible dangers (Juneidi et al., 2015a; Wen et al., 2015a). When evaluating toxicity, it is crucial to account for potential hazards linked to each constituent component as well as the entire DES structure. These findings are expected to add to our relatively limited understanding of the ecotoxicological profile of DES and provide helpful information for the development of DES that are more biocompatible, less toxic, and easily biodegradable. Furthermore, the toxicity may differ according to the mode of exposure (such as ingestion, cutaneous contact, or inhalation) and the precise application of the DES. This article provides a detailed review of the available literature on the study of DES with their impact on different type of cells and micro-organisms in terms of toxicity. Additionally, this study has identified the gaps which hold implications for both theoretical frameworks and practical applications within the field, which will provide a roadmap for advancing the knowledge base and refining methodologies for future inquiries.

2. Toxicity in consideration with different exposures

Studying multiple species enables the identification of similarities and differences in toxicological responses, which contributes to a deeper comprehension of potential hazards from DES. If researchers investigate toxicity in numerous species, they may attempt to generalize results from the study of toxicity in multiple species to humans (Fig. 1). Nonetheless, it is essential to recognize that not all animal responses correspond directly to human responses (Ferreira et al., 2022b). A detailed description of the used DES with different exposures is discussed in detail below.

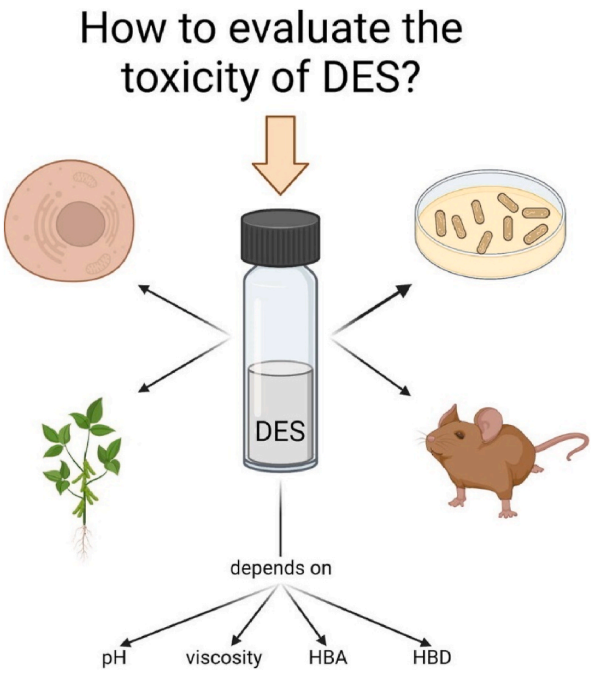

Fig. 1. Different exposures for testing toxicity of DES. (reproduced with permission for Elsevier from the work of Ferreira et al. (Ferreira et al., 2022b)).

2.1. Toxicity tests with animal/human cells

The toxicity of a DES is affected by its composition, viscosity, and concentration. In a study, where phosphonium-based aqueous DES was tested to brine shrimp (*Artemia salina*), it was found that concentrated DES are more toxic than aqueous DES. The reason was, delocalized charge compounds are more hazardous than localized charge chemicals. Research has shown the impact of HBDs on the viscosity of DES in which DES containing oxalic acid as HBD's are relatively more viscous than citric acid (Jangir et al., 2020). The cytotoxic profiles of several ChCl-based DESDs have been reported on fibroblast cells, and their toxic effect has been correlated and expressed in terms of their viscosity (Kareem et al., 2010). Although, limiting value of viscosity is not mentioned which can be used universally to identify the toxicity of any DES. But some study reveals that, high viscosity of DES was also impeding the shrimp movement, while oxygen scarcity was another possible factor, potentially leading to adverse effects on it (Hayyan et al., 2013a). Another report reveals that DES can exhibit noticeable synergistic effects, and their cytotoxicity was higher compared to their components for choline chloride (ChCl)-based DES. However, it was found that the degree of toxicity also depends on the organism such as fish cell line - channel catfish ovary (CCO) which was found to be more sensitive towards ChCl: oxalic acid (OA) DES compared to the human tumor cell line (MCF-7), though the value for the EC_{50} which lies in the same limit band (Radošević et al., 2015). The ChCl-based DES are also found to be less toxic than imidazolium- and pyridinium-based IL on fish cell CCO lines based on the decrease in cell viability (Radošević et al., 2016). They were also found to be less toxic than N, N-diethyl ammonium chloride (DAC)-based DES when tested against human cancer cells (HelaS3, AGS, MCF-7, WRL-68). The longer length of alkyl chain was found to be responsible for higher value of cytotoxicity which results in swelling of lipid bilayer (Mbous et al., 2020).

The concentration of any DES has a significant impact of its toxicity, which is indicated by LC50 (lethal concentration at 50%) values. Also, aqueous DES were clearly less harmful to *Cyprinus carpio* than their individual constituents. The type of component and their interaction with the type of cells of the organism is also discussed where it was seen that the Type I (containing organic salt and metal salt) and Type II DES (containing organic salt and hydrate metal salt) are more toxic than the Type III DES which is made up of organic salts due to the present of metal salts. In contrast to IL, the toxicity level of DES is dependent on the individual salt and its HBD, with a stronger hydrogen bond which contributes to lower toxicity (Juneidi et al., 2015b), which is again confirmed by Inayat et al. that hydrogen bonding is responsible for the toxicity level towards different organisms and cells. They evaluated that tetrabutylammonium bromide (TBAB): glycerol was shown to be less cytotoxic and have the highest antioxidant capacity than TBAB: ethylene glycol and TBAB: diethylene glycol. In the same study TBAB: diethylene glycol exhibited a higher LC50 value which is considered to be most suitable for increased cell viability percentage (Inayat et al., 2023). In literature, relative studies have been carried out by the researchers in which the comparison of the toxicity is carried out for different water content in the DES. However, the exact values are difficult to determine where the toxicity of a DES gets effected (Cao et al., 2023a; Hayyan et al., 2013b; Jangir et al., 2020; Radošević et al., 2018a).

Any toxic chemical may cause a variety of physiological and cellular reactions in animals. These pathways result in the activation of antioxidant defences, which protect the cells of the body from excessive generation of reactive oxygen species (ROS). Thus, oxidative stress is an important parameter to evaluate the toxicity. DES (citric acid:trehalose: water) containing citric acid as an HBA was tested on zebrafish and administered that concentration up to 3000 μm, is potentially safe when given by intraperitoneal injection (Ferreira et al., 2022a). While, adding organic acids to DES as HBD makes it more harmful when tested with human cell lines (HelaS3, CaOV3, B16F10, MCF-7). In this case, usage of biomaterials appears to be a valuable asset in reducing their cytotoxicity (Hayyan et al., 2016). Moreover, it is reported that ammonium-based DES are more harmful to human cells (MCF-7, A375, HT29, HepG2, PC3, OKF6 and H413), including cancer cells, than their individual components, but do not cause DNA toxicity. These results were also strengthened by carrying out the studies on ICR mice (Hayyan et al., 2015b, c) and are consistent with another research, in which the authors determined that amino-based IL are 105 times more toxic than ChCl based DES when tested on human lung tumor cell (A549) (Li et al., 2023). Whereas the toxicity of polyethylene glycol (PEG)-based DES on same lung cancer cells (A549) by considering HBD, component quantities, molar ratios, and concentrations were reported with IC50 values by CCK-8 essay method. Notably, a drop in PEG concentration was associated with a reduction in DES toxicity (Cao et al., 2023b).

An article also found that Arthrobacter simplex (TCCC 11037) was more toxic to ChCl:urea DES, which hindered bacterial growth (Mao et al., 2018b). All over, HBA in DES is the main driver of toxicity in human cells (*Keratinocytes*-HaCaT, tumor *melanocytes*- MNT-1) when evaluated through MTT assay (Macário et al., 2019). In the case of crustaceous organisms (*D. magna*), the addition of water to ChCl based DES leads to a reduction in toxicity (Lapeña et al., 2021a). Rodríguez-Juan et al. showed the selectivity of the DES efficiency in drug solvation to human cancer cells (Caco-2, HepG2, HeLa) by flow cytometry. This study reveals that DES made up of three compounds was found to be more effective in drug delivery to human cells compared to the DES made using two compounds due to their high solvation capacity and low toxicity (Rodríguez-Juan et al., 2021). Bryant et al. synthesize DES from proline and glycerol and found it to be less toxic than dimethylsulfoxide and glycerol which are very commonly used as cryoprotective agents for human cells (THP-1, HaCat, PC3, UG87-MG) because it can be cultured with the cells for almost an hour without appreciably decreasing their viability after freezing (Bryant et al., 2022). Induction of mortality, diet restriction, adipose tissue loss, excessive water consumption, plasma oxidative stress, weight loss, increase in blood lipid levels, and hepatomegaly were observed in rats when they were fed with betaine: glycerol (1:2 mol ratio) of water natural DES. However, no oxidative stress was observed in the liver. An increase in lipid peroxidation products, protein oxidation products, and glucose oxidation products were reported which are helpful against oxidative stresses but

had adverse effects on kidney weights and abnormal renal function (Benlebna et al., 2018). A summary of all human/animal cells used for testing the toxicity of DES is also given in Table 1.

2.2. Toxicity tests with plant cells

The mechanism of action of DES and their components on plants may be linked to their interactions with cellular membranes. The dependency of wheat (*T. aestivum*) growth inhibition, on the pH of ChCl-based DES was studied and seen that the ChCl:OA DES exhibited the highest growth inhibition of the wheat seedlings followed by glycerol and glucose as HBDs, because oxalic acid-based DES was prepared at 2.08 pH while the remaining two at 6 pH. Different antioxidant enzyme activities were also seen with different concentrations of ChCl: OA and observed that higher concentrations of ChCl:OA produced oxidative stress by disrupting antioxidative enzyme activities, inhibiting superoxide dismutase (SOD), guaiacol peroxidase (GPX), and catalase (CAT) activity. (Radošević et al., 2015). Compared to the HBD (urea, acetamide, glycerol, ethylene glycol) used alone, their DES with ChCl and choline acetate (ChAc) were found to be less toxic than their individual components. It is proved by shorter *hydra sinensis* lifespans and shorter roots emerging from the garlic (*Akkium sativum*) cloves, as well as the distortion and dissolution of cells at the root tip cells (Wen et al., 2015b). Lapeña et al. reported toxicity by measuring chlorophyll content in algae (*R. subcapitata*). A decrease in chlorophyll content was reported when the algae were exposed to different ChCl-based DES where ChCl:glyerol was found to be most toxic substance to algae (*S. capricornatum*) and its toxicity get increased with the addition of water to the pure DES. (Lapeña et al., 2021a). Among other variables, polarizability and hydrophobicity, have an impact on the DES toxicity. A test on algae (*Raphidocelis subcapitata)* was conducted to evaluate the toxicity of ChCl-based DES and N, N, N-triethyl-N-(2,3-dihydroxypropyl) ammonium chloride (N00Cl)-based DES. The chlorophyll content was measured in both cases and found that (N00Cl)-based DES with even number of carbons exhibited lower toxicity than the odd ones while in the case of ChCl based DES toxicity is related to properties of its constituents where fluorine atoms and ramifications both reduce toxicity (Garralaga et al., 2022). Different algae/hydra/plants used for testing the toxicity of DES are also tabulated in Table 2. Using various growth indices and stress indicators, the phytotoxicity of choline chloride (ChCl)/organic acid-based DES toward wheat (*Triticum aestivum*) seedlings was assessed. They deduced that the toxicities of DES were primarily influenced by their unique properties, which may have been eventually triggered by their HBD (Rodrigues et al., 2021).

2.3. Toxicity tests with micro-organisms

Increasing the concentration of ChCl or ChAc based DES, a more

Table 1
Toxicity test of DES with Animals/Human cells.

No.	DES	Type of cells/species	DES showing		Reference
			Minimum toxicity	Maximum toxicity	
1	ChCl:glucose (2:1), ChCl:oxalic acid (1:1), ChCl:glycerol (1:2)	Channel Catfish Ovary (CCO) cell line and Human (MCF-7) cell line	ChCl:glucose, ChCl:glycerol EC_{50} >10 mM	ChCl:oxalic acid EC_{50} = 1.64 mM	Radošević et al. (2015)
2	ChCl:glycerine (1:3), ChCl:ethylene glycol (1:3), ChCl: triethylene glycol (1:3), and ChCl:urea (1:3)	Human cells (OKF6, MCF-7, A375, HT29, PC3, HepG2, H413)	ChCl:triethylene glycol	ChCl:urea	Hayyan et al. (2015b)
3	ChCl:acetic acid (1:2, 2:1,1:1), ChCl:lactic acid (1:2, 2:1,1:1), ChCl:glycolic acid (1:2, 2:1,1:1), ChCl:citric acid (1:2, 2:1,1:1)	Marine Bacteria V. fischeri	ChCl:acetic acid	ChCl:glycolic acid	de Morais et al. (2015)
4	ChCl:Malic acid (1:1,1.5:1), ChCl:Citric acid (2:1), ChCl: lactic acid (1:1), ChCl: fructose (3:2), ChCl:xylose (2:1), ChCl:mannose (5:2)	Channel Catfish Ovary (CCO) cell line	ChCl:lactic acid	ChCl:Malic acid	Radošević et al. (2016)
5	Citric acid monohydrate:trehalose anhydrous:water (2:1:3)	Zebrafish (*Danio rerio*)	Potentially safe		Ferreira et al. (2022a)
6	Polyethylene glycol (PEG):lactic acid (4:1), PEG:p-Toluenesulfonic acid monohydrate (4:1), PEG:N-methylacetamide (4:1), PEG:acetamide (4:1), PEG: choline chloride (4:1), PEG:thiourea (4:1), PEG:urea (4:1), PEG:propanoic acid (4:1),	Lung cancer cell (A549)	PEG:N-methylacetamide	PEG:thiourea	Cao et al. (2023c)
7	ChCl:oxalic acid (1:1), ChCl:urea (1:2), ChCl:xylitol (5:2), ChCl:sorbitol (2:3), Betaine:glucose (5:2), Betaine: malic acid:proline (1:1:1), Betaine:malic acid: glucose (1:1:1), Citric acid:proline (1:1), Citric acid:glucose: glycerol (1:1:1), Citric acid:fructose: glycerol (1:1:1)	Human cell lines (i.e., HeLa, MCF-7 and HEK293T).	ChCl:xylitol, ChCl: sorbitol	ChCl:oxalic acid, ChCl: urea	Radošević et al. (2018b)
8	ChCl:butanoic acid (1:1), ChCl:hexanoic acid (1:1), ChCl:ethylene glycol (1:1), ChCl:1-propanol (1:1), and ChCl:urea (1:1), Tetramethylammonium chloride (TAC): butanoic acid (1:1), TAC:hexanoic acid (1:1), TAC: ethylene glycol (1:1), TAC:1-propanol (1:1), and TAC: urea (1:1), TAC:butanoic acid (1:1), TAC:hexanoic acid (1:1), TAC:ethylene glycol (1:1), TAC:1-propanol (1:1), and TAC:urea (1:1)	Human Keratinocytes HaCaT and Human melanoma MNT-1 cells	ChCl:butanoic acid	TAC:ethylene glycol	Macário et al. (2019)
9	N, N-diethylammonium chloride (DAC): ethylene glycol (1:2), DAC:glycerol (1:2), DAC:urea (1:2), DAC:Zinc chloride (1:2), DAC:malonic acid (1:1), ChCl:ethylene glycol (1:2), ChCl:glycerol (1:2), ChCl:urea (1:2)	Human HelaS3, A375, AGS, WRL-68, MCF-7, and PC3 cancer cell lines	ChCl:glycerol	DAC:malonic	Mbous et al. (2020)
10	ChCl:Sucrose (1:2), ChCl:1,4-butanediol (1:5), ChCl: xylitol (2:1), ChCl:1,2-propanediol (1:1), Fructose: Glucose:Sucrose (1:1:1), Betaine:Sucrose (2:1), Betaine: Sucrose (4:1), Fructose-Glucose-Sucrose (2:3.6:1), Betaine:Levunilic acid (1:2), Betaine:glycolic acid:oxalic acid (1:1.6:0.4),	Human colorectal adenocarcinoma (Caco-2), Human epithelial adecarcinoma (HeLa), Human hepatocarcinoma (HepG2) cell line, peripheral blood mononuclear cells (PBMCs)	Fructose:Glucose: Sucrose	ChCl:xylitol	Rodríguez-Juan et al. (2021)

Table 2
Toxicity test of DES with Plants.

No.	DES	Type of cells/species	DES showing		Reference
			Minimum toxicity	Maximum toxicity	
1	ChCl:urea (1:1,1:2,2:1), ChCl:acetamide (1:1,1:2,2:1), ChCl:ethylene glycol (1:1,1:2,2:1), ChCl:glycerol (1:1,1:2,2:1), ChAc:urea (1:1,1:2,2:1), ChAc: acetamide (1:1,1:2,2:1), ChAc:ethylene glycol (1:1,1:2,2:1), ChAc:glycerol (1:1,1:2,2:1)	Garlic bulbs (*Allium sativum*)	ChCl:acetamide	ChCl:glycerol	Wen et al. (2015b)
2	ChCl:glucose (2:1), ChCl:oxalic acid (1:1), ChCl:glycerol (1:2)	Wheat seeds (*T. aestivum*)	ChCl:oxalic acid	ChCl: glucose	Radošević et al. (2015)
3	ChCl:glycerol (1:2), ChCl:urea (1:2), ChCl:ethylene glycol (1:2), ChCl:glycerol:water (1:2:1), ChCl:urea:water (1:2:1.7), ChCl:ethylene glycol:water (1:2:1)	Algae (*S. capricornatum*)	ChCl:ethylene glycol	ChCl:glycerol: water	Lapeña et al. (2021a)
4	ChCl:Sucrose (1:2), ChCl:1,4-butanediol (1:5), ChCl:xylitol (2:1), ChCl:1,2-propanediol (1:1), Fructose:Glucose:Sucrose (1:1:1), Betaine:Sucrose (2:1), Betaine:Sucrose (4:1), Fructose-Glucose-Sucrose (2:3.6:1), Betaine:Levunilic acid (1:2), Betaine:glycolic acid:oxalic acid (1:1.6:0.4)	Plant Pathogens	Fructose: Glucose:Sucrose	ChCl:xylitol	Rodríguez-Juan et al. (2021)
5	ChCl:glycerol (1:2), ChCl:glycerol ether (1:2), N, N, N-triethyl-N-(2,3-dihydroxy propyl) ammonium chloride(N00Cl):glycerol (1:2), N00Cl:glycerol ether (1:2)	Algae (*Raphidocelis subcapitata*)	ChCl:glycerol ether	N00Cl: glycerol	Garralaga et al. (2022)

deterioration effect on bacteria (*E. coli*) due to high inhibition index was reported by Wen et al. (2015b). However, ChCl-based DES was found to be more effective in the growth inhibition and killing of the bacteria (gram positive G(+), gram negative G(−)) compared to the ChAc-based DES and different techniques like disk and well diffusion method, broth dilution method, microtox assay, drop plate method and FTIR bioassay are introduced to asses DES microbial toxicity (Marchel et al., 2022a). The degree of the seriousness of the DES in killing or inhibiting the bacteria growth widely depends on the concentration of the DES used. Various cholinium-based Deep Eutectic Solvents (DES) were found to exhibit non-toxicity towards bacteria such as Bacillus subtilis, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, (Hayyan et al., 2013a; Hayyan et al., 2013a, 2013a, 2013b, 2013c, 2013c). However, contrasting findings were also reported, who identified these cholinium-based DES as having toxic effects (Wen et al., 2015c; Yang, 2018, 2019). The interaction of chloride ion (Cl^-) with the cell wall of the bacteria makes the cell wall weaker and interacts with the plasma membrane. Compared to other salts such as phosphonium (Cornmell et al., 2008) or ammonium (Petkovic et al., 2012; 2011), Cl^- ions are found to exhibit more deteriorating effects on the cell walls of the bacteria. However, the IL are not found to be more effective over the G(−) bacteria due to the presence of the extra outer lipopolysaccharide membrane on the cell wall (Samorì, 2011).

The cholinium salt and even the cholinium-based DES were found to be effective in inhibiting and successfully attacking G(−) bacteria (Russell, 2003). Charge delocalization due to the H-bonding was found to be the major contributor to arresting and killing the bacteria using ChCl-based DES (Modica-Napolitano and Aprille, 2001; Wen et al., 2015b). Citric acid as HBD (amongst acetic, lactic, and glycolic acids as HBD) in ChCl-based DES was found to be most effective in arresting the bacteria (*V. fischeri*) (de Morais et al., 2015). Increasing the acid amount in DES, effectively increases its effectiveness in arresting the bacteria, because when acidic counterpart attached in DES, reaches the cytoplasm, the delocalization of the charges happens which disturbs the functioning of the cell. However, every time it is not necessary that the delocalization effect causes damage to the cells (Macário et al., 2018b).

Using zwitterionic DES from trimethylglycine and carboxylic acids, Cardellini et al. reported the toxicity of DES due to the dehydration influence of DES, which was observed similar to the action of Calcium Chloride ($CaCl_2$) (Cardellini et al., 2014). A similar type of behaviour of twelve different DES prepared using (1S) - (+) −10-camphorsulfonic acid and aliphatic, aromatic, and amphiphilic sulfobetaine over *Saccharomyces cerevisiae* cells was also observed (Cardellini et al., 2015). The effectiveness of DES containing organic acids as HBD on the detrimental effects on bacteria, which may be partially explained by pH changes in the environment. The DES having pH < 4 has toxic effect on the metabolic and cellular proliferative activities (B.-Y. Zhao et al., 2015). The findings of the qualitative investigation on the biocompatibility of DES indicated that, DES based on amines, alcohols, and sugars were found to be non-toxic solvents. However, DES based on acids were shown to be detrimental to both G(−) bacteria (*Escherichia coli and Salmonella enteritidis*) and G(+) bacteria (*Staphylococcus aureus and Listeria monocytogenes*). Since malic acid (pH = 3.1) was noticeably observed less harmful than citric acid (pH = 2.9) successfully implies the dependency of toxicity on HBD. The biocompatibility of acid-based DES was investigated via quantitative study. The findings indicate that these DES possess antibacterial properties. However, their relatively low toxicity in comparison to conventional solvents and IL justifies their classification as “green solvents” (Wikene et al., 2017; B.-Y. Zhao et al., 2015).

The mechanism of action of DES is most likely connected with its interactions with cell membranes and exhibited test system used. In a work, where antimicrobial activity is evaluated for ten DES (including ChCl, betaine, and citric acid as HBAs, as well as organic acid, sugar, sugar alcohol, amino acid, and amide as HBDs) on bacteria (*Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus*) and yeast (*Candida albicans*) reveals that lower pH acid-based DES is responsible in arresting bacteria growth. However, increasing the water content in DES decreases its toxic nature towards the bacteria due to dilution (Lapeña et al., 2021b; Radošević et al., 2018b). In a concentration-based study, acetylcholine chloride: acetamide was analysed on *E. Coli*, revealed that the lower concentration of DES has no detrimental or noticeable effects on the bacteria. Moreover, the toxicity is produced by the DES chemical makeup as well as the excessive acidity of the medium generated by DES hydrolysis during cellular development (Torregrosa-Crespo et al., 2020b). When TBAB based DES are analysed on G(+) and G(−) bacteria TBAB: glycerol was found most suitable in terms of cell viability percentage and scavenging activity (Inayat et al., 2023). Marchel et al. proposed the toxicity of the ChCl-based DES at elevated temperatures on three different strains of bacteria (*E. coli, Pseudomonas aeruginosa, Staphylococcus aureus*). The nature of the HBD adopted (considering the HBA same) determines the existence and functionality of the DES at elevated temperatures. Urea is more stable compared to glucose at higher temperatures making DES also stable at higher temperatures. Urea being acidic in nature is not necessarily that it possesses toxicity towards bacteria. The dosage along with the temperature also influences its level of toxicity. The low antibacterial property of DES was reported (Marchel et al., 2022b). Since molecules with delocalized charges are more poisonous than those with localized charges, it was hypothesized that the hydrogen bonding between the mixture compounds would enhance the toxicity via the corresponding charge delocalization. The DES’s chemical structure and physical

characteristics are mainly influenced by the hydrogen bonding between the HBD and the salt anion. But, the kind of HBD significantly influences the associated DES's toxicity (Cao et al., 2023a; Hayyan et al., 2013b, 2015b; Paiva et al., 2014). As maximum tests are carried out on microorganisms for toxicity tests of DES, a list of different used microorganisms for the same is summarized in Table 3.

The selection of toxicity testing techniques is influenced by several variables, such as the kind of material being evaluated, its intended use, moral concerns, and other requirements. To properly assess the toxicity of a chemical, tests must be carefully planned, and their findings must be critically assessed. To ensure the safety of chemicals, pharmaceuticals, and other products used in a variety of industries, toxicity testing is essential. Listed below are some of the most common techniques for testing toxicity.

3. General methods to determine toxicity

3.1. Disk test

The majority of studies employed a solid culture medium on plates with implanted DES (an antibiogram-like test)-containing disks to measure toxicity (B. Y. Zhao et al., 2015). The high density and viscosity that characterize the majority of the DES studied so far (Xu et al., 2017b) provide a significant constraint to this method. These two characteristics limit DES's diffusion from the disk to its surroundings, producing findings that could not accurately represent DES and cell interactions. This detrimental effect is reduced by analysis of toxicity in liquid media, particularly when DES are highly thick or exhibit high viscosity.

Table 3
Toxicity test of DES with microorganisms.

No.	DES	Type of cells/species	DES showing		Reference
			Minimum toxicity	Maximum toxicity	
1	ChCl:glycerol (1:2), ChCl:urea (1:2), ChCl:ethylene glycol (1:2), ChCl:glycerol:water (1:2:1), ChCl:urea:water (1:2:1.7), ChCl:ethylene glycol:water (1:2:1)	Bacteria (*A. fisheri, R. subcapitata*), crustaceans (*D. magna*)	ChCl:ethylene glycol	ChCl:urea: water	Lapeña et al. (2021a)
2	ChCl:urea (1:2), ChCl:glucose (1:1), ChCl:malonic acid (1:1)	Bacterial strains (*Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus*)	ChCl:urea	ChCl:malonic acid	Marchel et al. (2022b)
3	ChCl:urea (1:1), ChCl:acetamide (1:1), ChCl:ethylene glycol (1:1), ChCl:glycerol (1:1), ChAc:urea (1:1), ChAc:acetamide (1:1), ChAc:ethylene glycol (1:1), ChAc:glycerol (1:1)	Bacteria *(Escherichia coli)*	ChCl: acetamide	ChCl:glycerol	Wen et al. (2015b)
4	ChCl:Sucrose (1:2), ChCl:1,4-butanediol (1:5), ChCl: xylitol (2:1), ChCl:1,2-propanediol (1:1), Fructose: Glucose:Sucrose (1:1:1), Betaine:Sucrose (2:1), Betaine:Sucrose (4:1), Fructose-Glucose-Sucrose (2:3.6:1), Betaine:Levunilic acid (1:2), Betaine: glycolic acid:oxalic acid (1:1.6:0.4)	Oenological yeasts (*S.cerevisiae, S. paradoxus, H. guillermondi, H. uvarum, M. pulcherrima, S. bombicola, T. delbrueckii*), phytopathogenic microorganisms (*X. campestris, E. amylovora, E. toletana, C. michiganensis* subsp *michiganensis, C. michiganensis* subsp *insidiosus, R. radibacter, Ps. Syringae, Ps. Savastanoi*)	Fructose: Glucose: Sucrose	ChCl:xylitol	Rodríguez-Juan et al. (2021)
5	ChCl:lactic acid (1:1), ChCl:malonic acid (1:1), ChCl: maleic acid (1:1), ChCl:malic acid (1:1), ChCl:citric acid (1:1), ChCl:oxalic acid (1:1), ChCl: tartaric acid (1:1), ChCl:ethylene glycol (1:1), ChCl:propylene glycol (1:1), ChCl:glycerol (1:1), ChCl:xylitol (5:2), ChCl:sorbitol (5:2), ChCl:xylose (5:2), ChCl:glucose (5:2), ChCl:fructose (5:2), ChCl:mannose (5:2), ChCl: galactose (5:2), ChCl:sucrose (4:1), ChCl:maltose (4:1), ChCl:lactose (4:1), ChCl:raffinose (11:2)	*Lysinibacillus fusiformis* (CGMCC1347) cells	ChCl:lactose	ChCl:citric acid	Yang et al. (2017)
6	ChCl:urea (1:2), ChCl:acetamide (1:2), ChCl:ethylene glycol (1:2), ChCl:glycerol (1:2), ChCl:1,4-butanediol (1:4), ChCl:triethylene glycol (1:4), ChCl:xylitol (1:1), ChCl:D-sorbitol (1:1), ChCl:p-toluenesulfonic acid (1:1), ChCl:oxalic acid (1:1), ChCl:levulinic acid (1:2), ChCl:malonic acid (1:1), ChCl:citric acid (1:1), ChCl:tartaric acid (2:1), ChCl:xylose:water (1:1:1), ChCl:sucrose:water (5:2:5), ChCl:fructose:water (5:2:5), ChCl:glucose:water (5:2:5), ChCl:maltose: water (5:2:5)	*Staphylococcus aureus, Listeria monocytogenes, Escherichia coli* and *Salmonella enteritidis*	ChCl: triethylene glycol	ChCl:oxalic	(B.-Y. Zhao et al., 2015)
7	Acetylcholine chloride:acetamide (1:2)	*Escherichia coli*	Potentially safe at low concentrations		Torregrosa-Crespo et al. (2020b)
8	ChCl:ethylene glycol (1:2,1:1,2:1,4:1), ChCl: propionic acid (1:2,1:1,2:1,4:1),ChCl: glycerol (1:2,1:1,2:1,4:1), ChCl:urea (1:2,1:1,2:1,4:1), ChCl:1,2-propanol (1:2,1:1,2:1,4:1),	Bacteria (*Allivibrio fischeri)*	ChCl: propionic acid	ChCl:urea	Macário et al. (2018b)
9	ChCl:oxalic acid (1:1), ChCl:urea (1:2), ChCl:xylitol (5:2), ChCl:sorbitol (2:3), Betaine: glucose (5:2), Betaine:malic acid: proline (1:1:1), Betaine:malic acid: glucose (1:1:1), Citric acid:proline (1:1), Citric acid:glucose: glycerol (1:1:1), Citric acid:fructose: glycerol (1:1:1)	Bacteria (*Escherichia coli, Proteus mirabilis, Salmonella typhimurium, Pseudomonas aeruginosa, Staphylococcus aureus*), yeast (*Candida albicans*)	ChCl:xylitol, ChCl:sorbitol, Betaine: glucose	Citric acid: fructose: glycerol	Radošević et al. (2018b)
10	ChCl:glycerol (1:2), ChCl:glycerol ether (1:2), N, N, N-triethyl-N-(2,3-dihydroxy propyl) ammonium chloride(N00Cl):glycerol (1:2), N00Cl:glycerol ether (1:2)	Bacteria (*Aliivibrio Fisheri*)	ChCl:glycerol ether	N00Cl: glycerol	Garralaga et al. (2022)

3.2. Close bottle test

A water sample, such as one obtained from a wastewater treatment facility and diluted with distilled water, serves as the inoculum (i.e., a source of microorganisms) for this test (Azzouz and Hayyan, 2023a). The substance of interest is then dissolved in a saline solution and added to the container containing the inoculum. The dissolved oxygen is measured repeatedly after the bottle is closed to calculate the biochemical oxygen demand (BOD), or how much oxygen is used by organisms as they metabolize organic materials. This is used to compute the percentage of the theoretical oxygen demand (ThOD), which is then compared to the value obtained from a blank mixture (i.e., one that doesn't include the tested chemical) (Coleman and Gathergood, 2010). When a reference molecule achieves a ThOD of 60% after 14 days, the biodegradation test is often regarded as genuine. Chemicals are judged readily biodegradable when they acquire a ThOD of 60% or greater within 28 days (Biodegradability, 1992). These factors are typically not taken into account in the biodegradability tests using DES that have been published so far.

3.3. Cellular preadaptation

When determining the degree of toxicity of DES, Crespo et al. have shown that the kind of culture used to evaluate toxicity, preadaptation of the cells used as a model to examine cytotoxicity, or the procedures used to sterilize DES may all have a major impact on the outcomes (Torregrosa-Crespo et al., 2020a). When 600 mM DES is present, the cells become hazardous in single experiments. However, growth may be observed when they were already used to this dosage.

The main problem with the existing toxicity data is that different authors employed different methodologies. Therefore, it is important to create a consistent technique so that it can compare the various parameters in terms of toxicity. To determine how to assess a DES toxicity, its prospective use is crucial. Ferreira et al. provides a framework for the investigation of DES toxicity that may aid the scientific community in determining which investigations should be conducted in light of the intended use. According to them, the proposed framework requires updating when new scientific data on the toxicity of DES and the variety of potential toxicological testing is published (Ferreira et al., 2022b).

For experimental analysis, one requires pricey tools, voluminous data collecting, or lengthy investigations. Due to a lack of availability of resources and also because of technological improvements, researchers are progressively switching from experimental analysis to computational analysis. To comprehend real-world situations, validate computer models, and provide ground truth data for the accuracy and application of computational conclusions, experimental analysis is essential. But, to get a deeper knowledge of the subject, it is frequently used to combine computational and experimental results. Additionally, the computational analysis may direct the design of experiments by assisting researchers in determining the crucial parameters and situations to concentrate on while analysing toxicity.

4. Computational analysis of toxicity

With the help of different machine learning algorithms, namely support vector regression, random forest regression, Multilayer Perception- Artificial Neural Network, and Multiple Linear Regression Quantitative Structure-Activity Relationships (QSAR) can be used for studying the toxicity of 72 different types of natural DES over *Aliivibrio Fischeri* bacteria (Chirico and Gramatica, 2011). Importance of the Vanderwall surface area, dipole moment, the number of nitrogen atoms, molar refractivity, and molecular mass for shaping the toxicity of DES and their components towards *A. fis cheri* was highlighted (Halder and Cordeiro, 2019a). In the QSAR analysis of cytotoxicity for ChCl-based DES against human embryonic kidney cells (HEK-293) carried out and reported that the constitutional descriptors and the molar ratio of HBA to HBD of natural DES play important roles in determining the cytotoxicity of natural DES towards cancer cells. The count of HBD carbons and the molar ratio of HBA to HBD exhibited negative effects (Ahmadi et al., 2018). The possibility of a multitasking-Quantitative Structure Toxicity Relationships (mtk-QSTR) strategy to provide a discriminant model for testing the toxicity of DES across several biological targets was investigated (Halder and Cordeiro, 2019b). The model also emphasizes the significant contributions of topology, HBD, polarizability, and electronegativity to DES toxicity. Additionally, distinct HBD components were graded according to their hazardous contributions to DES with the use of the developed mtk-QSTR model. A Technique for Order of Preference by Similarity to the Ideal Solution (TOPSIS) was used to calculate additive effects and rank DES (Fig. 2) components according to how green they are. DES, which is created by combining straight-chain alcohol, sugars, and amides, may be a promising green solvent, as opposed to those that contain metal ions and organic acids, according to comprehensive assessments that take into account safety, biodegradability, and toxicological criteria (Bystrzanowska and Tobiszewski, 2021a). In Silico-Methods for Predicting Drug Toxicity is also introduced based on Toxicokinematic and pharmacokinetic models (Benfenati Editor, 2016).

Both the toxicity and biodegradability of DES are interconnected in their environmental impact and their effect on different species. DES which is poisonous and difficult to biodegrade can linger in the environment for a long time and have negative impacts on a variety of creatures and ecosystems. On the other hand, a DES environmental effect and possible harm can be reduced if it is biodegradable and can be spontaneously broken down by microbes. To guarantee their safe use and reduce their environmental effect, studying the biodegradability of DES is also important. Understanding the connection between these two elements makes it feasible to create and employ DES that is less hazardous and more eco-friendly, promoting sustainability and ecologically friendly chemical operations.

5. Biodegradability of DES

In the course of biodegradation, DES or their by-products may be transformed, degraded, or accumulate inside cells. The more fundamental DES appear to be easily biodegradable, however using organic acids as HBDs and substituting choline with another HBA (although only two other HBAs have been tested so far) have a detrimental effect on biodegradability (Abranches and Coutinho, 2023). It is unknown how water affects biodegradability, although it appears to have a considerable influence. There are still many things to learn. To develop heuristics that might aid design toward biodegradability, a larger database would be necessary. For the DES predecessors, this information is accessible, but it is unclear how the biodegradability of DES compares to that of its antecedents. When a substance passes particular screening procedures for ultimate biodegradability, it is referred to as being "readily biodegradable" as per the rules set up by Organisation for Economic Co-operation and Development (OECD) (Biodegradability, 1992). Most of the researchers uses the Close bottle test but only Lapeña et al. (2021a) used the manometric respirometry test (Biodegradability, 1992). A review of the biodegradability of DES is also available (Azzouz and Hayyan, 2023b).

Chen and Mu (2021) proposed 13 points-based strategies for classifying any IL or DES to be environmentally friendly or not. Compared to IL, DES is not readily biodegradable. Many classical IL with shorter alkyl side chains is safer but can stay in the ecosystem for long, whereas some with long chains are readily biodegradable but are toxic in nature. However, for choline-based IL, the balance between toxicity and biodegradability was reported in the literature (Hou et al., 2013; Macário et al., 2018a). More than 60 % biodegradation of ChCl-based DES within 7 days (Radošević et al., 2015). Sugar and amide-based DES as HBD can be degraded easily by nature with more than 69 % degradation at 28 days. Hydroxyl, amines, and carboxyl groups provide

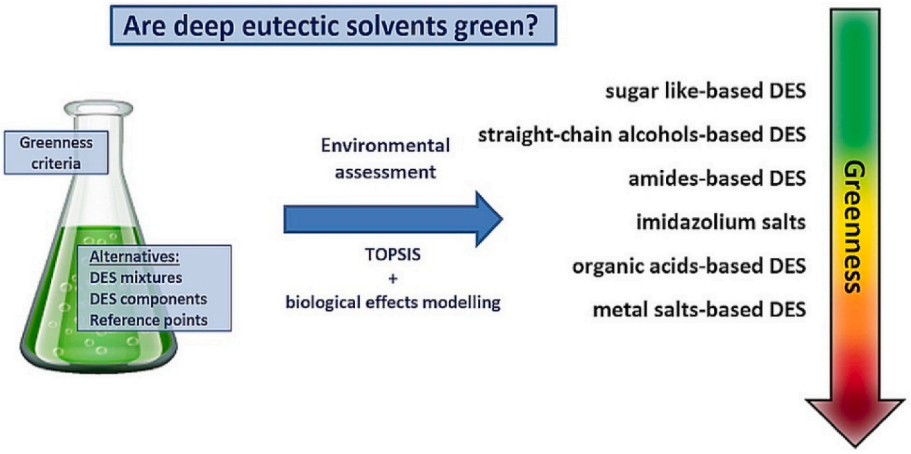

Fig. 2. Multicriteria decision analysis (technique for order of preference by similarity to the ideal solution-TOPSIS ranking) along with biological effects modelling for DES (Reproduced with permission for Elsevier from the work of Bystrzanowska et al. (Bystrzanowska and Tobiszewski, 2021c)).

excellent sites for starting the degradation reaction effectively. Compared to metallic ions and organic acid-based DES, DES formed by mixing sugars alcohols, straight-chain alcohol, sugars, and amides were found to be greener (Bystrzanowska and Tobiszewski, 2021b). Goss et al. show the difference in measurements for field and lab-based biodegradation tests in terms of dissolved oxygen concentration, conductivity, pH, and temperature (Goss et al., 2020).

To determine if a DES qualifies as a green solvent, green analytical chemistry (GAC) principles, and tools should be used for its analyzation. The twelve principles of GAC set out its own objectives, since not all of the concepts of green chemistry are entirely relevant to analytical chemistry. As time goes on, different green metric tools (Fig. 3) have been used to determine the degree of greenness and eco-friendliness of DES as well as their implications on safety, the environment, and human health. Certain ones are tailored to certain types of analytical techniques, whilst others are universal and suitable for most analytical methods. Now a days, for the critical evaluation of greenness, metric tools like life cycle assessment (LCA), analytical eco-scale (AES), green analytical procedure index (GAPI), analytical greenness (AGREE), national environmental methods index (NEMI), environment assessment tool (EAT), analytical method volume intensity (AMVI), analytical method greenness score (AMGS) etc. are being used (Alqarni et al., 2023; Kowtharapu et al., 2023; Sajid and Płotka-Wasylka, 2022; Zaib et al., 2022). A characteristic description for a green biodegradable DES is depicted in Fig. 4. It is also noteworthy that DES research is still in its infancy and that attempts are always being made to create specialized scales and procedures for more accurate evaluation of their green quality. The evaluation scales and criteria may grow to include a wider

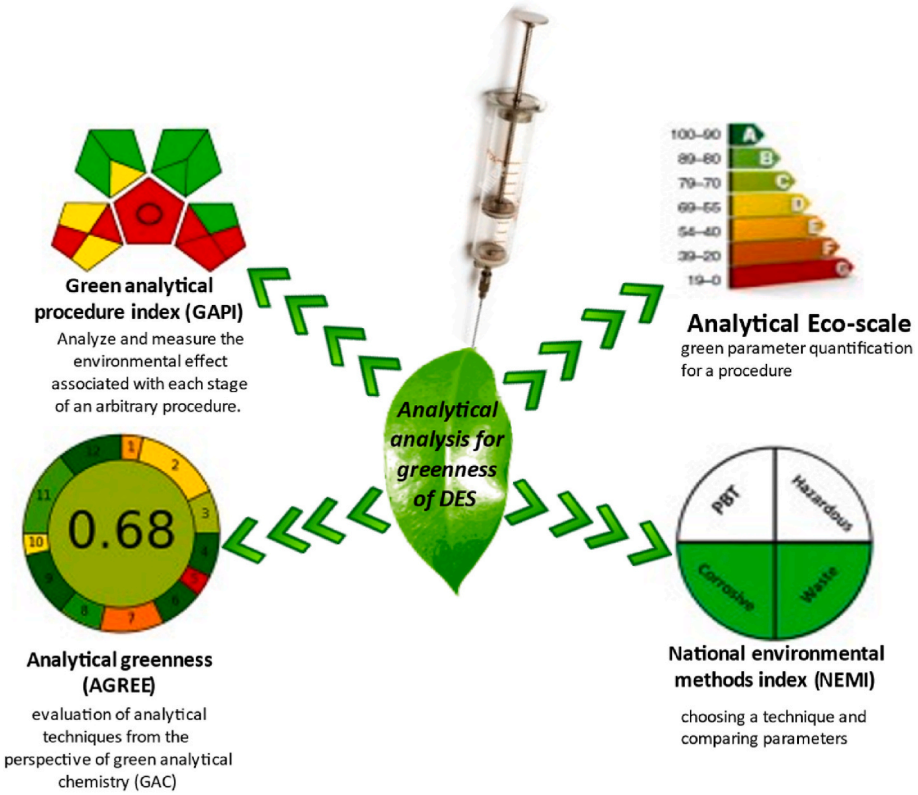

Fig. 3. Some of the most frequently used analytical metric tools to assess greenness along with their purpose to use.

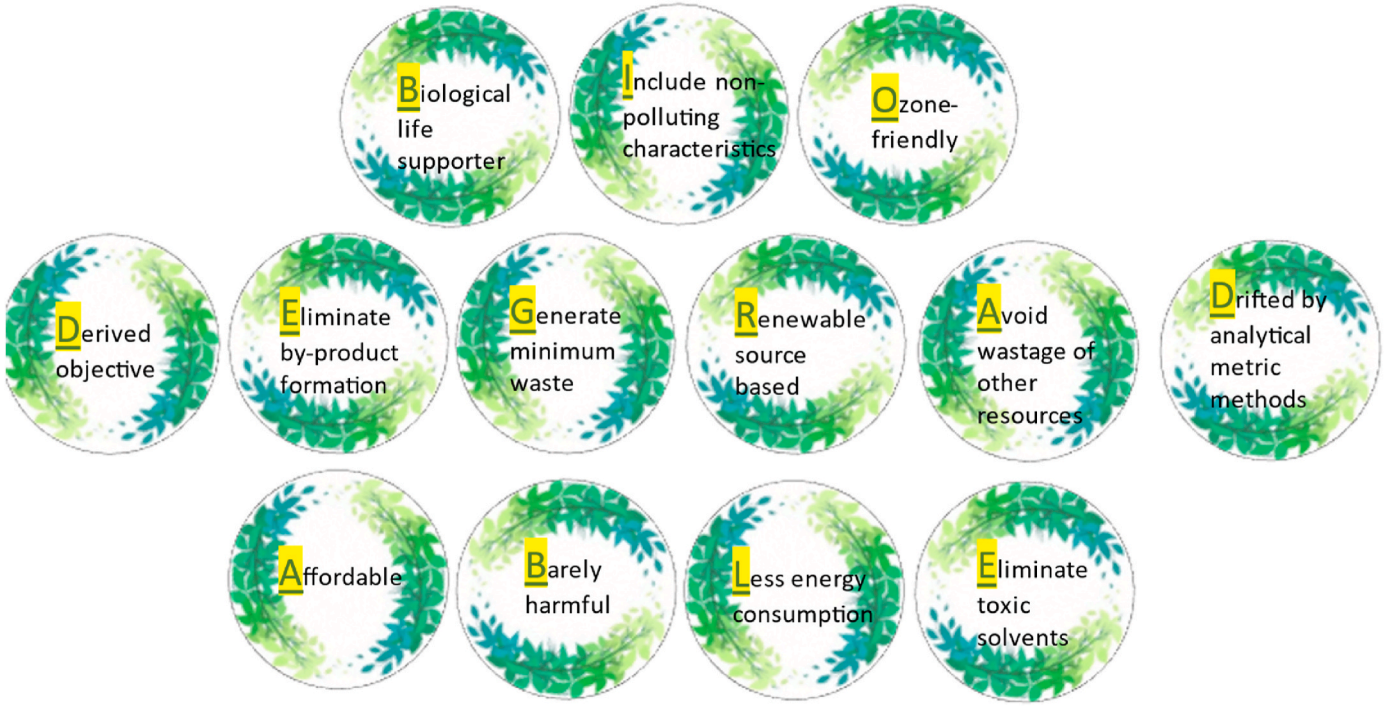

Fig. 4. Characteristic assessment for a green and biodegradable Deep Eutectic solvent. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

variety of DES-specific aspects when new breakthroughs come to light.

6. Future research

This review has highlighted the toxicity and biodegradability of DES, but there are still several areas that might use additional research to help us understand these solvents on a more in-depth level.

Toxicological Studies: The toxicity investigations have not taken into account cross-reactions between DES and the salts or nutrients of the culture medium. When DES are hydrolysed in liquid culture media, cross-reactions between the DES hydrolyzation products and the salts, amino acids, carbohydrates, etc. in the culture medium are promoted (Hammond et al., 2017; Kumari et al., 2018; Ma et al., 2018a). To obtain correct outcomes when characterizing DES toxicity, new suggestions and guidelines (Torregrosa-Crespo et al., 2020a) must be put into practice. Liquid culture media as opposed to solid media or antibiograms; preadapted cells, tissues, or seeds as opposed to non-preadapted ones; continuous monitoring of pH, temperature, radiation, etc. during cellular incubation in the presence of DES as opposed to the absence of continuous monitoring of these parameters; buffered culture media as opposed to unbuffered cultures; and sterilization of DES by UV or filtration (0.22 μm filters) as opposed to sterilization by autoclave. More investigation is required to assess a particular test we apply to hydrophilic DES and which to hydrophobic DES. Studies should specify the viscosity levels for which a particular toxicity test can be used. This will make it possible to design safer substitutes and detect any potential dangers related to their usage.

Environmental Fate and Transport: Analysing the dissemination and possible effects on ecosystems of DES in the soil, water, and air environmental compartments will provide light on their fate and transport. To evaluate the persistence and build-up of DES in the environment, long-term studies are also required. Understanding the biodegradation pathways of DES is essential for improving their biodegradability. Future studies should concentrate on finding the microorganisms and enzymes involved in the breakdown of DES and improving the circumstances that support their activity. Although a lot of studies have been done focussing on the toxicity with different HBA, further studies are required for analysing the toxicity with different HBD.

Design of Sustainable DES: A top objective should be giving new DES formulations enhanced biodegradability and decreased toxicity. The performance of greener and more sustainable DES components should be compared to that of conventional solvents as part of ongoing research. By filling up these research gaps, we can open the door for the safe and long-term use of DES across a range of sectors, helping to create a future that is greener and more ecologically friendly.

Biodegradability of DES: Application of in silico techniques to forecast DES biodegradation and associated routes also requires effort. Utilizing in silico methods will minimize the number of trials and allow beginning with more environmentally friendly DES. Another issue is the dearth of experimental data needed to develop, examine, and evaluate the machine learning and Artificial intelligence (AI) algorithms used to forecast DES deterioration.

7. Conclusion

Overall, even though DES provide several benefits over conventional solvents, such as lesser toxicity, it is crucial to take care while using them and to continue researching for any possible hazards. To guarantee the safe and responsible use of DES in diverse applications, thorough toxicity evaluations and adherence to safety guidelines are required. The requirement for toxicity assessment when bringing new compounds to the marketplace and the industry served as the primary inspiration for this review. There is little information about the toxicity of DES and how it has been assessed, and it is dispersed throughout several research reports. In summary, this review article has given a thorough understanding of the toxicity tests done so far and biodegradability of DES. We have determined via thorough examination of the current literature that although DES has several benefits as a prospective substitute for traditional solvents, it also presents certain environmental problems.

According to their chemical makeup and particular use, DES may display varied levels of toxicity. Before contemplating their widespread usage, it is essential to thoroughly assess the toxicity profiles of various

DES. It is important to note that certain DES have been demonstrated to have lower toxicity levels than conventional organic solvents, making them suitable alternatives for applications that are ecologically benign and sustainable. To reduce possible risks, it's crucial to choose DES components and concentrations carefully. It is also concluded that HBD structural characteristics are also important in determining the degree of biodegradation, with the inclusion of a hydroxyl group imparting a negligible improvement in biodegradability. It's critical to pick the components and concentrations of DES with care to minimize any potential dangers. When handling and utilizing DES, relevant safety precautions and instructions should be followed to reduce any possible dangers. This includes utilizing the appropriate personal protective equipment, making sure there is enough ventilation, and putting waste management plans in place for the disposal of DES. Following the rules and regulations established by the appropriate authorities is also crucial. Our review has shown that DES may exhibit a variety of degradation rates in terms of biodegradability. Under specific circumstances, some DES are easily biodegradable, while others may linger in the environment for a longer time. Further study is required to better understand the variables affecting DES biodegradation and create plans to boost their biodegradability.

CRediT authorship contribution statement

Anshu Sharma: Methodology, Visualization, Writing – original draft. **Bong-Seop Lee:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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References

Abranches, D.O., Coutinho, J.A.P., 2023. Everything you wanted to know about deep eutectic solvents but were afraid to Be told. Annu. Rev. Chem. Biomol. Eng. 14, 141–163. https://doi.org/10.1146/annurev-chembioeng-101121-085323.

Ahmadi, R., Hemmateenejad, B., Safavi, A., Shojaeifard, Z., Mohabbati, M., Firuzi, O., 2018. Assessment of cytotoxicity of choline chloride-based natural deep eutectic solvents against human HEK-293 cells: a QSAR analysis. Chemosphere 209, 831–838. https://doi.org/10.1016/j.chemosphere.2018.06.103.

Ali Redha, A., 2021. Review on extraction of phenolic compounds from natural sources using green deep eutectic solvents. J. Agric. Food Chem. 69, 878–912. https://doi.org/10.1021/acs.jafc.0c06641.

Alqarni, A.M., Mostafa, A., Shaaban, H., Gomaa, M.S., Albashrayi, D., Hasheeshi, B., Bakhashwain, N., Aseeri, A., Alqarni, A., Alamri, A.A., Alrofaidi, M.A., 2023. Development and optimization of natural deep eutectic solvent-based dispersive liquid-liquid microextraction coupled with UPLC-UV for simultaneous determination of parabens in personal care products: evaluation of the eco-friendliness level of the developed method. RSC Adv. 13, 13183–13194. https://doi.org/10.1039/d3ra00769c.

Amesho, K.T.T., Lin, Y.-C., Mohan, S.V., Halder, S., Ponnusamy, V.K., Jhang, S.-R., 2023. Deep eutectic solvents in the transformation of biomass into biofuels and fine chemicals: a review. Environ. Chem. Lett. 21, 183–230. https://doi.org/10.1007/s10311-022-01521-x.

Atilhan, M., Aparicio, S., 2021. Review and perspectives for effective solutions to grand challenges of energy and fuels technologies via novel deep eutectic solvents. Energy Fuel. 35, 6402–6419. https://doi.org/10.1021/acs.energyfuels.1c00303.

Azizi, N., Batebi, E., Bagherpour, S., Ghafuri, H., 2012. Natural deep eutectic salt promoted regioselective reduction of epoxides and carbonyl compounds. RSC Adv. 2, 2289–2293. https://doi.org/10.1039/c2ra01280d.

Azzouz, A., Hayyan, M., 2023a. Are deep eutectic solvents biodegradable? Process Saf. Environ. Protect. https://doi.org/10.1016/j.psep.2023.06.079.

Azzouz, A., Hayyan, M., 2023b. Are deep eutectic solvents biodegradable? Process Saf. Environ. Protect. 176, 1021–1025. https://doi.org/10.1016/j.psep.2023.06.079.

Benfenati Editor, E., 2016. In: Silico Methods for Predicting Drug Toxicity, Methods in Molecular Biology. Springer New York, New York, NY. https://doi.org/10.1007/978-1-4939-3609-0.

Benlebna, M., Ruesgas-Ramón, M., Bonafos, B., Fouret, G., Casas, F., Coudray, C., Durand, E., Cruzueroa-Espinoza, M., Feillet-Coudray, C., 2018. Toxicity of natural deep eutectic solvent betaine:glycerol in rats. J. Agric. Food Chem. 66, 6205–6212. https://doi.org/10.1021/acs.jafc.8b01746.

Biodegradability, R., 1992. OECD Guideline for testing of chemicals. OECD 71, 1–9.

Bryant, S.J., Awad, M.N., Elbourne, A., Christofferson, A.J., Martin, A.V., Meftahi, N., Drummond, C.J., Greaves, T.L., Bryant, G., 2022. Deep eutectic solvents as cryoprotective agents for mammalian cells. J. Mater. Chem. B 10, 4546–4560. https://doi.org/10.1039/D2TB00573E.

Bystrzanowska, M., Tobiszewski, M., 2021a. Assessment and design of greener deep eutectic solvents – a multicriteria decision analysis. J. Mol. Liq. 321 https://doi.org/10.1016/j.molliq.2020.114878.

Bystrzanowska, M., Tobiszewski, M., 2021b. Assessment and design of greener deep eutectic solvents – a multicriteria decision analysis. J. Mol. Liq. 321, 114878 https://doi.org/10.1016/j.molliq.2020.114878.

Bystrzanowska, M., Tobiszewski, M., 2021c. Assessment and design of greener deep eutectic solvents – a multicriteria decision analysis. J. Mol. Liq. 321, 114878 https://doi.org/10.1016/j.molliq.2020.114878.

Cao, Y., Shan, S., Lu, Y., Luo, J., Li, Y., Hao, R., 2023a. Tuning toxic properties of polyethylene glycol-based deep eutectic solvents for achieving greener solvents. J. Mol. Liq. 369 https://doi.org/10.1016/j.molliq.2022.120879.

Cao, Y., Shan, S., Lu, Y., Luo, J., Li, Y., Hao, R., 2023b. Tuning toxic properties of polyethylene glycol-based deep eutectic solvents for achieving greener solvents. J. Mol. Liq. 369 https://doi.org/10.1016/j.molliq.2022.120879.

Cao, Y., Shan, S., Lu, Y., Luo, J., Li, Y., Hao, R., 2023c. Tuning toxic properties of polyethylene glycol-based deep eutectic solvents for achieving greener solvents. J. Mol. Liq. 369, 120879 https://doi.org/10.1016/j.molliq.2022.120879.

Cardellini, F., Germani, R., Cardinali, G., Corte, L., Roscini, L., Spreti, N., Tiecco, M., 2015. Room temperature deep eutectic solvents of (1S)-(+)-10-camphorsulfonic acid and sulfobetaines: hydrogen bond-based mixtures with low ionicity and structure-dependent toxicity. RSC Adv. 5, 31772–31786. https://doi.org/10.1039/C5RA03932K.

Cardellini, F., Tiecco, M., Germani, R., Cardinali, G., Corte, L., Roscini, L., Spreti, N., 2014. Novel zwitterionic deep eutectic solvents from trimethylglycine and carboxylic acids: characterization of their properties and their toxicity. RSC Adv. 4, 55990–56002. https://doi.org/10.1039/C4RA10628H.

Chandran, D., Khalid, M., Walvekar, R., Mubarak, N.M., Dharaskar, S., Wong, W.Y., Gupta, T.C.S.M., 2019. Deep eutectic solvents for extraction-desulphurization: a review. J. Mol. Liq. 275, 312–322. https://doi.org/10.1016/j.molliq.2018.11.051.

Chen, Y., Mu, T., 2021. Revisiting greenness of ionic liquids and deep eutectic solvents. Green Chemical Engineering 2, 174–186. https://doi.org/10.1016/j.gce.2021.01.004.

Chirico, N., Gramatica, P., 2011. Real external predictivity of QSAR models: how to evaluate It? Comparison of different validation criteria and proposal of using the concordance correlation coefficient. J. Chem. Inf. Model. 51, 2320–2335. https://doi.org/10.1021/ci200211n.

Coleman, D., Gathergood, N., 2010. Biodegradation studies of ionic liquids. Chem. Soc. Rev. 39, 600–637. https://doi.org/10.1039/b817717c.

Cornmell, R.J., Winder, C.L., Tiddy, G.J.T., Goodacre, R., Stephens, G., 2008. Accumulation of ionic liquids in Escherichia coli cells. Green Chem. 10, 836. https://doi.org/10.1039/b807214k.

Craveiro, R., Aroso, I., Flammia, V., Carvalho, T., Viciosa, M.T., Dionísio, M., Barreiros, S., Reis, R.L., Duarte, A.R.C., Paiva, A., 2016. Properties and thermal behavior of natural deep eutectic solvents. J. Mol. Liq. 215, 534–540. https://doi.org/10.1016/j.molliq.2016.01.038.

Cunha, S.C., Fernandes, J.O., 2018. Extraction techniques with deep eutectic solvents. TrAC, Trends Anal. Chem. https://doi.org/10.1016/j.trac.2018.05.001.

de Morais, P., Gonçalves, F., Coutinho, J.A.P., Ventura, S.P.M., 2015. Ecotoxicity of cholinium-based deep eutectic solvents. ACS Sustain. Chem. Eng. 3, 3398–3404. https://doi.org/10.1021/acssuschemeng.5b01124.

Emami, S., Shayanfar, A., 2020. Deep eutectic solvents for pharmaceutical formulation and drug delivery applications. Pharmaceutical Development and Technology 25, 779–796.

Espino, M., de los Ángeles Fernández, M., Gomez, F.J.V., Silva, M.F., 2016. Natural designer solvents for greening analytical chemistry. TrAC, Trends Anal. Chem. 76, 126–136. https://doi.org/10.1016/j.trac.2015.11.006.

Ferreira, I.J., Meneses, L., Paiva, A., Diniz, M., Duarte, A.R.C., 2022a. Assessment of deep eutectic solvents toxicity in zebrafish (Danio rerio). Chemosphere 299, 134415. https://doi.org/10.1016/j.chemosphere.2022.134415.

Ferreira, I.J., Oliveira, F., Jesus, A.R., Paiva, A., Duarte, A.R.C., 2022b. Current methodologies for the assessment of deep eutectic systems toxicology: challenges and perspectives. J. Mol. Liq. https://doi.org/10.1016/j.molliq.2022.119675.

Garralaga, M.P., Lomba, L., Leal-Duaso, A., Gracia-Barberán, S., Pires, E., Giner, B., 2022. Ecotoxicological study of bio-based deep eutectic solvents formed by glycerol derivatives in two aquatic biomodels. Green Chem. 24, 5228–5241. https://doi.org/10.1039/D2GC01293F.

Ge, X., Gu, C., Wang, X., Tu, J., 2017. Deep eutectic solvents (DESs)-derived advanced functional materials for energy and environmental applications: challenges, opportunities, and future vision. J Mater Chem A Mater. https://doi.org/10.1039/c7ta01659j.

Goss, M., Li, Z., McLachlan, M.S., 2020. A simple field-based biodegradation test shows pH to be an inadequately controlled parameter in laboratory biodegradation testing. Environ Sci Process Impacts 22, 1006–1013. https://doi.org/10.1039/c9em00491b.

Halder, A.K., Cordeiro, M.N.D.S., 2019a. Development of predictive linear and non-linear QSTR models for Aliivibrio fischeri toxicity of deep eutectic solvents. International Journal of Quantitative Structure-Property Relationships 4, 50–69. https://doi.org/10.4018/IJQSPR.2019100104.

Halder, A.K., Cordeiro, M.N.D.S., 2019b. Probing the environmental toxicity of deep eutectic solvents and their components: an in silico modeling approach. ACS Sustain. Chem. Eng. 7, 10649–10660. https://doi.org/10.1021/acssuschemeng.9b01306.

Hammond, O.S., Bowron, D.T., Edler, K.J., 2017. The effect of water upon deep eutectic solvent nanostructure: an unusual transition from ionic mixture to aqueous solution. Angew. Chem. Int. Ed. 56, 9782–9785. https://doi.org/10.1002/anie.201702486.

Hayyan, A., Mjalli, F.S., Alnashef, I.M., Al-Wahaibi, T., Al-Wahaibi, Y.M., Hashim, M.A., 2012. Fruit sugar-based deep eutectic solvents and their physical properties. Thermochim. Acta 541, 70–75. https://doi.org/10.1016/j.tca.2012.04.030.

Hayyan, M., Hashim, M.A., Al-Saadi, M.A., Hayyan, A., AlNashef, I.M., Mirghani, M.E.S., 2013a. Assessment of cytotoxicity and toxicity for phosphonium-based deep eutectic solvents. Chemosphere 93, 455–459. https://doi.org/10.1016/j.chemosphere.2013.05.013.

Hayyan, M., Hashim, M.A., Al-Saadi, M.A., Hayyan, A., AlNashef, I.M., Mirghani, M.E.S., 2013b. Assessment of cytotoxicity and toxicity for phosphonium-based deep eutectic solvents. Chemosphere 93, 455–459. https://doi.org/10.1016/j.chemosphere.2013.05.013.

Hayyan, M., Hashim, M.A., Hayyan, A., Al-Saadi, M.A., AlNashef, I.M., Mirghani, M.E.S., Saheed, O.K., 2013c. Are deep eutectic solvents benign or toxic? Chemosphere 90, 2193–2195. https://doi.org/10.1016/j.chemosphere.2012.11.004.

Hayyan, M., Looi, C.Y., Hayyan, A., Wong, W.F., Hashim, M.A., 2015a. In Vitro and in Vivo toxicity profiling of ammonium-based deep eutectic solvents. PLoS One 10. https://doi.org/10.1371/journal.pone.0117934.

Hayyan, M., Looi, C.Y., Hayyan, A., Wong, W.F., Hashim, M.A., 2015b. In Vitro and in Vivo toxicity profiling of ammonium-based deep eutectic solvents. PLoS One 10. https://doi.org/10.1371/journal.pone.0117934.

Hayyan, M., Looi, C.Y., Hayyan, A., Wong, W.F., Hashim, M.A., 2015c. In vitro and in vivo toxicity profiling of ammonium-based deep eutectic solvents. PLoS One 10, e0117934. https://doi.org/10.1371/journal.pone.0117934.

Hayyan, M., Mbous, Y.P., Looi, C.Y., Wong, W.F., Hayyan, A., Salleh, Z., Mohd-Ali, O., 2016. Natural deep eutectic solvents: cytotoxic profile. SpringerPlus 5. https://doi.org/10.1186/s40064-016-2575-9.

Hou, X.-D., Liu, Q.-P., Smith, T.J., Li, N., Zong, M.-H., 2013. Evaluation of toxicity and biodegradability of cholinium amino acids ionic liquids. PLoS One 8, e59145. https://doi.org/10.1371/journal.pone.0059145.

Ijardar, S.P., Singh, V., Gardas, R.L., 2022. Revisiting the physicochemical properties and applications of deep eutectic solvents. Molecules. https://doi.org/10.3390/molecules27041368.

Inayat, S., Ahmad, S.R., Awan, S.J., Nawshad, M., Ali, Q., 2023. In vivo and in vitro toxicity profile of tetrabutylammonium bromide and alcohol-based deep eutectic solvents. Sci. Rep. 13, 1777. https://doi.org/10.1038/s41598-023-28928-y.

Jangir, A.K., Lad, B., Dani, U., Shah, N., Kuperkar, K., 2020. In vitro toxicity assessment and enhanced drug solubility profile of green deep eutectic solvent derivatives (DESDs) combined with theoretical validation. RSC Adv. 10, 24063–24072. https://doi.org/10.1039/c9ra10320a.

Janjhi, F.A., Castro-Muñoz, R., Boczkaj, G., 2023. Deep eutectic solvents – ideal solution for clean air or hidden danger? Sep. Purif. Technol. 314, 123590 https://doi.org/10.1016/j.seppur.2023.123590.

Jiang, W., Jia, H., Li, Hongping, Zhu, L., Tao, R., Zhu, W., Li, Huaming, Dai, S., 2019. Boric acid-based ternary deep eutectic solvent for extraction and oxidative desulfurization of diesel fuel. Green Chem. 21, 3074–3080. https://doi.org/10.1039/C9GC01004A.

Juneidi, I., Hayyan, M., Hashim, M.A., 2015a. Evaluation of toxicity and biodegradability for cholinium-based deep eutectic solvents. RSC Adv. 5, 83636–83647. https://doi.org/10.1039/C5RA12425E.

Juneidi, I., Hayyan, M., Hashim, M.A., 2015b. Evaluation of toxicity and biodegradability for cholinium-based deep eutectic solvents. RSC Adv. 5, 83636–83647. https://doi.org/10.1039/C5RA12425E.

Kareem, M.A., Mjalli, F.S., Hashim, M.A., AlNashef, I.M., 2010. Phosphonium-based ionic liquids analogues and their physical properties. J. Chem. Eng. Data 55, 4632–4637. https://doi.org/10.1021/je100104v.

Kowtharapu, L.P., Katari, N.K., Muchakayala, S.K., Marisetti, V.M., 2023. Green metric tools for analytical methods assessment critical review, case studies and crucify. TrAC, Trends Anal. Chem. https://doi.org/10.1016/j.trac.2023.117196.

Krishnan, A., Gopinath, K.P., Vo, D.-V.N., Malolan, R., Nagarajan, V.M., Arun, J., 2020. Ionic liquids, deep eutectic solvents and liquid polymers as green solvents in carbon capture technologies: a review. Environmental Chemistry Letters 18, 2031–2054.

Kudłak, B., Owczarek, K., Namieśnik, J., 2015. Selected issues related to the toxicity of ionic liquids and deep eutectic solvents—a review. Environ. Sci. Pollut. Control Ser. 22, 11975–11992. https://doi.org/10.1007/s11356-015-4794-y.

Kumari, P., Shobhna, Kaur, S., Kashyap, H.K., 2018. Influence of hydration on the structure of reline deep eutectic solvent: a molecular dynamics study. ACS Omega 3, 15246–15255. https://doi.org/10.1021/acsomega.8b02447.

Lapeña, D., Errazquin, D., Lomba, L., Lafuente, C., Giner, B., 2021a. Ecotoxicity and biodegradability of pure and aqueous mixtures of deep eutectic solvents: glyceline, ethaline, and reline. Environ. Sci. Pollut. Control Ser. 28, 8812–8821. https://doi.org/10.1007/s11356-020-11144-w.

Lapeña, D., Errazquin, D., Lomba, L., Lafuente, C., Giner, B., 2021b. Ecotoxicity and biodegradability of pure and aqueous mixtures of deep eutectic solvents: glyceline, ethaline, and reline. Environ. Sci. Pollut. Control Ser. 28, 8812–8821. https://doi.org/10.1007/s11356-020-11144-w.

Lee, B.-S., 2022. Ion-dipole complex effect on activity coefficient of L-methionine in aqueous electrolyte solutions. SSRN Electron. J. https://doi.org/10.2139/ssrn.4065781.

Li, Y., Luo, J., Shan, S., Cao, Y., 2023. High toxicity of amino acid-based deep eutectic solvents. J. Mol. Liq. 370 https://doi.org/10.1016/j.molliq.2022.121044.

Li, Z., Liu, D., Men, Z., Song, L., Lv, Y., Wu, P., Lou, B., Zhang, Y., Shi, N., Chen, Q., 2018. Insight into effective denitrification and desulfurization of liquid fuel with deep eutectic solvents: an innovative evaluation criterion to filtrate extractants using the compatibility index. Green Chem. 20, 3112–3120. https://doi.org/10.1039/C8GC00828K.

Ma, C., Laaksonen, A., Liu, C., Lu, X., Ji, X., 2018a. The peculiar effect of water on ionic liquids and deep eutectic solvents. Chem. Soc. Rev. https://doi.org/10.1039/c8cs00325d.

Ma, C., Liu, C., Lu, X., Ji, X., 2018b. Techno-economic analysis and performance comparison of aqueous deep eutectic solvent and other physical absorbents for biogas upgrading. Appl. Energy 225, 437–447. https://doi.org/10.1016/j.apenergy.2018.04.112.

Macário, I.P.E., Jesus, F., Pereira, J.L., Ventura, S.P.M., Gonçalves, A.M.M., Coutinho, J.A.P., Gonçalves, F.J.M., 2018a. Unraveling the ecotoxicity of deep eutectic solvents using the mixture toxicity theory. Chemosphere 212, 890–897. https://doi.org/10.1016/j.chemosphere.2018.08.153.

Macário, I.P.E., Oliveira, H., Menezes, A.C., Ventura, S.P.M., Pereira, J.L., Gonçalves, A.M.M., Coutinho, J.A.P., Gonçalves, F.J.M., 2019. Cytotoxicity profiling of deep eutectic solvents to human skin cells. Sci. Rep. 9, 3932. https://doi.org/10.1038/s41598-019-39910-y.

Macário, I.P.E., Ventura, S.P.M., Pereira, J.L., Gonçalves, A.M.M., Coutinho, J.A.P., Gonçalves, F.J.M., 2018b. The antagonist and synergist potential of cholinium-based deep eutectic solvents. Ecotoxicol. Environ. Saf. 165, 597–602. https://doi.org/10.1016/j.ecoenv.2018.09.027.

Mahdi, T., Ahmad, A., Nasef, M.M., Ripin, A., 2015. State-of-the-art technologies for separation of azeotropic mixtures. Separ. Purif. Rev. https://doi.org/10.1080/15422119.2014.963607.

Makoś, P., Słupek, E., Gębicki, J., 2020. Extractive detoxification of feedstocks for the production of biofuels using new hydrophobic deep eutectic solvents – experimental and theoretical studies. J. Mol. Liq. 308 https://doi.org/10.1016/j.molliq.2020.113101.

Malolan, R., Gopinath, K.P., Vo, D.-V.N., Jayaraman, R.S., Adithya, S., Ajay, P.S., Arun, J., 2021. Green ionic liquids and deep eutectic solvents for desulphurization, denitrification, biomass, biodiesel, bioethanol and hydrogen fuels: a review. Environ. Chem. Lett. 19, 1001–1023. https://doi.org/10.1007/s10311-020-01113-7.

Mao, S., Li, K., Hou, Y., Liu, Y., Ji, S., Qin, H., Lu, F., 2018a. Synergistic effects of components in deep eutectic solvents relieve toxicity and improve the performance of steroid biotransformation catalyzed by Arthrobacter simplex. J. Chem. Technol. Biotechnol. 93, 2729–2736. https://doi.org/10.1002/jctb.5629.

Mao, S., Li, K., Hou, Y., Liu, Y., Ji, S., Qin, H., Lu, F., 2018b. Synergistic effects of components in deep eutectic solvents relieve toxicity and improve the performance of steroid biotransformation catalyzed by Arthrobacter simplex. J. Chem. Technol. Biotechnol. 93, 2729–2736. https://doi.org/10.1002/jctb.5629.

Marchel, M., Cieśliński, H., Boczkaj, G., 2022a. Deep eutectic solvents microbial toxicity: current state of art and critical evaluation of testing methods. J. Hazard Mater. 425, 127963 https://doi.org/10.1016/j.jhazmat.2021.127963.

Marchel, M., Cieśliński, H., Boczkaj, G., 2022b. Thermal instability of choline chloride-based deep eutectic solvents and its influence on their Toxicity—Important limitations of DESs as sustainable materials. Ind. Eng. Chem. Res. 61, 11288–11300. https://doi.org/10.1021/acs.iecr.2c01898.

Maugeri, Z., Domínguez de María, P., 2012. Novel choline-chloride-based deep-eutectic-solvents with renewable hydrogen bond donors: levulinic acid and sugar-based polyols. RSC Adv. 2, 421–425. https://doi.org/10.1039/C1RA00630D.

Mbous, Y.P., Hayyan, M., Hayyan, A., Wong, W.F., Hashim, M.A., Looi, C.Y., 2017. Applications of deep eutectic solvents in biotechnology and bioengineering—promises and challenges. Biotechnol. Adv. 35, 105–134. https://doi.org/10.1016/j.biotechadv.2016.11.006.

Mbous, Y.P., Hayyan, M., Wong, W.F., Hayyan, A., Looi, C.Y., Hashim, M.A., 2020. Simulation of deep eutectic solvents' interaction with membranes of cancer cells using COSMO-RS. J. Phys. Chem. B 124, 9086–9094. https://doi.org/10.1021/acs.jpcb.0c04801.

Modica-Napolitano, J.S., Aprille, J.R., 2001. Delocalized lipophilic cations selectively target the mitochondria of carcinoma cells. Adv. Drug Deliv. Rev. 49, 63–70. https://doi.org/10.1016/S0169-409X(01)00125-9.

Nguyen, C.-H., Augis, L., Fourmentin, S., Barratt, G., Legrand, F.-X., 2021. Deep Eutectic Solvents for Innovative Pharmaceutical Formulations, pp. 41–102. https://doi.org/10.1007/978-3-030-53069-3_2.

Oliveira, F.S., Pereiro, A.B., Rebelo, L.P.N., Marrucho, I.M., 2013. Deep eutectic solvents as extraction media for azeotropic mixtures. Green Chem. 15, 1326–1330. https://doi.org/10.1039/c3gc37030e.

Othman, Z.S., Hassan, N.H., Zubairi, S.I., 2015. Alcohol based-deep eutectic solvent (DES) as an alternative green additive to increase rotenone yield. In: AIP Conference Proceedings. American Institute of Physics Inc., 050004 https://doi.org/10.1063/1.4931283

Paiva, A., Craveiro, R., Aroso, I., Martins, M., Reis, R.L., Duarte, A.R.C., 2014. Natural deep eutectic solvents - solvents for the 21st century. ACS Sustain. Chem. Eng. https://doi.org/10.1021/sc500096j.

Petkovic, M., Hartmann, D.O., Adamová, G., Seddon, K.R., Rebelo, L.P.N., Pereira, C.S., 2012. Unravelling the mechanism of toxicity of alkyltributylphosphonium chlorides in Aspergillus nidulans conidia. New J. Chem. 36, 56–63. https://doi.org/10.1039/C1NJ20470J.

Petkovic, M., Seddon, K.R., Rebelo, L.P.N., Pereira, C.S., 2011. ChemInform abstract: ionic liquids: a pathway to environmental acceptability. ChemInform 42. https://doi.org/10.1002/chin.201127270 no-no.

Pollet, P., Davey, E.A., Ureña-Benavides, E.E., Eckert, C.A., Liotta, C.L., 2014. Solvents for sustainable chemical processes. Green Chem. 16, 1034–1055. https://doi.org/10.1039/C3GC42302F.

Radošević, K., Čanak, I., Panić, M., Markov, K., Bubalo, M.C., Frece, J., Srček, V.G., Redovniković, I.R., 2018a. Antimicrobial, cytotoxic and antioxidative evaluation of natural deep eutectic solvents. Environ. Sci. Pollut. Control Ser. 25, 14188–14196. https://doi.org/10.1007/s11356-018-1669-z.

Radošević, K., Čanak, I., Panić, M., Markov, K., Bubalo, M.C., Frece, J., Srček, V.G., Redovniković, I.R., 2018b. Antimicrobial, cytotoxic and antioxidative evaluation of natural deep eutectic solvents. Environ. Sci. Pollut. Control Ser. 25, 14188–14196. https://doi.org/10.1007/s11356-018-1669-z.

Radošević, K., Cvjetko Bubalo, M., Gaurina Srček, V., Grgas, D., Landeka Dragičević, T., Radojčić Redovniković, I., 2015. Evaluation of toxicity and biodegradability of choline chloride based deep eutectic solvents. Ecotoxicol. Environ. Saf. 112, 46–53. https://doi.org/10.1016/j.ecoenv.2014.09.034.

Radošević, K., Železnjak, J., Cvjetko Bubalo, M., Radojčić Redovniković, I., Slivac, I., Gaurina Srček, V., 2016. Comparative in vitro study of cholinium-based ionic liquids and deep eutectic solvents toward fish cell line. Ecotoxicol. Environ. Saf. 131, 30–36. https://doi.org/10.1016/j.ecoenv.2016.05.005.

Rodrigues, L.A., Radojčić Redovniković, I., Duarte, A.R.C., Matias, A.A., Paiva, A., 2021. Low-phytotoxic deep eutectic systems as alternative extraction media for the recovery of chitin from Brown crab shells. ACS Omega 6, 28729–28741. https://doi.org/10.1021/acsomega.1c03402.

Rodríguez-Juan, E., López, S., Abia, R., Muriana, J.G.F., Fernández-Bolaños, J., García-Borrego, A., 2021. Antimicrobial activity on phytopathogenic bacteria and yeast, cytotoxicity and solubilizing capacity of deep eutectic solvents. J. Mol. Liq. 337, 116343 https://doi.org/10.1016/j.molliq.2021.116343.

Russell, A.D., 2003. Similarities and differences in the responses of microorganisms to biocides. J. Antimicrob. Chemother. 52, 750–763. https://doi.org/10.1093/jac/dkg422.

Sajid, M., Płotka-Wasylka, J., 2022. Green Analytical Chemistry Metrics: A Review. Talanta. https://doi.org/10.1016/j.talanta.2021.123046.

Samorì, C., 2011. Ionic liquids and their biological effects towards microorganisms. Curr. Org. Chem. 15, 1888–1904. https://doi.org/10.2174/138527211795703658.

Scholz, M., Frank, B., Stockmeier, F., Falß, S., Wessling, M., 2013. Techno-economic analysis of hybrid processes for biogas upgrading. Ind. Eng. Chem. Res. 52, 16929–16938. https://doi.org/10.1021/ie402660s.

Serhan, M., Sprowls, M., Jackemeyer, D., Long, M., Perez, I.D., Maret, W., Tao, N., Forzani, E., 2019. Total iron measurement in human serum with a smartphone. In: AIChE Annual Meeting, Conference Proceedings. American Institute of Chemical Engineers. https://doi.org/10.1039/x0xx00000x.

Singh, M.B., Kumar, V.S., Chaudhary, M., Singh, P., 2021. A mini review on synthesis, properties and applications of deep eutectic solvents. J. Indian Chem. Soc. 98, 100210 https://doi.org/10.1016/j.jics.2021.100210.

Skarpalezos, D., Detsi, A., 2019. Deep eutectic solvents as extraction media for valuable flavonoids from natural sources. Appl. Sci. 9, 4169. https://doi.org/10.3390/app9194169.

Słupek, E., Makoś, P., Gębicki, J., 2020. Theoretical and economic evaluation of low-cost deep eutectic solvents for effective biogas upgrading to bio-methane. Energies 13. https://doi.org/10.3390/en13133379.

Smith, E.L., Abbott, A.P., Ryder, K.S., 2014. Deep eutectic solvents (DESs) and their applications. Chem. Rev. 114, 11060–11082. https://doi.org/10.1021/cr300162p.

Tiecco, M., Cappellini, F., Nicoletti, F., Del Giacco, T., Germani, R., Di Profio, P., 2019. Role of the hydrogen bond donor component for a proper development of novel hydrophobic deep eutectic solvents. J. Mol. Liq. 281, 423–430. https://doi.org/10.1016/j.molliq.2019.02.107.

Tomé, L.I.N., Baião, V., da Silva, W., Brett, C.M.A., 2018. Deep eutectic solvents for the production and application of new materials. Appl. Mater. Today. https://doi.org/10.1016/j.apmt.2017.11.005.

Torregrosa-Crespo, J., Marset, X., Guillena, G., Ramón, D.J., María Martínez-Espinosa, R., 2020a. New guidelines for testing "Deep eutectic solvents" toxicity and their effects on the environment and living beings. Sci. Total Environ. 704 https://doi.org/10.1016/j.scitotenv.2019.135382.

Torregrosa-Crespo, J., Marset, X., Guillena, G., Ramón, D.J., María Martínez-Espinosa, R., 2020b. New guidelines for testing "Deep eutectic solvents" toxicity and their effects on the environment and living beings. Sci. Total Environ. 704, 135382 https://doi.org/10.1016/j.scitotenv.2019.135382.

Warrag, S.E.E., Peters, C.J., Kroon, M.C., 2017. Deep eutectic solvents for highly efficient separations in oil and gas industries. Curr. Opin. Green Sustainable Chem. https://doi.org/10.1016/j.cogsc.2017.03.013.

Wazeer, I., Hadj-Kali, M.K., Al-Nashef, I.M., 2021. Utilization of deep eutectic solvents to reduce the release of hazardous gases to the atmosphere: a critical review. Molecules. https://doi.org/10.3390/MOLECULES26010075.

Wazeer, I., Hadj-Kali, M.K., Al-Nashef, I.M., 2020. Utilization of deep eutectic solvents to reduce the release of hazardous gases to the atmosphere: a critical review. Molecules 26, 75. https://doi.org/10.3390/molecules26010075.

Weaver, K.D., Kim, H.J., Sun, J., MacFarlane, D.R., Elliott, G.D., 2010. Cyto-toxicity and biocompatibility of a family of choline phosphate ionic liquids designed for pharmaceutical applications. Green Chem. 12, 507. https://doi.org/10.1039/b918726j.

Wen, Q., Chen, J.-X., Tang, Y.-L., Wang, J., Yang, Z., 2015a. Assessing the toxicity and biodegradability of deep eutectic solvents. Chemosphere 132, 63–69. https://doi.org/10.1016/j.chemosphere.2015.02.061.

Wen, Q., Chen, J.-X., Tang, Y.-L., Wang, J., Yang, Z., 2015b. Assessing the toxicity and biodegradability of deep eutectic solvents. Chemosphere 132, 63–69. https://doi.org/10.1016/j.chemosphere.2015.02.061.

Wen, Q., Chen, J.X., Tang, Y.L., Wang, J., Yang, Z., 2015c. Assessing the toxicity and biodegradability of deep eutectic solvents. Chemosphere 132, 63–69. https://doi.org/10.1016/j.chemosphere.2015.02.061.

Wikene, K.O., Rukke, H.V., Bruzell, E., Tønnesen, H.H., 2017. Investigation of the antimicrobial effect of natural deep eutectic solvents (NADES) as solvents in antimicrobial photodynamic therapy. J. Photochem. Photobiol., B 171, 27–33. https://doi.org/10.1016/j.jphotobiol.2017.04.030.

Xu, P., Zheng, G.-W., Zong, M.-H., Li, N., Lou, W.-Y., 2017a. Recent progress on deep eutectic solvents in biocatalysis. Bioresour Bioprocess 4, 34. https://doi.org/10.1186/s40643-017-0165-5.

Xu, P., Zheng, G.W., Zong, M.H., Li, N., Lou, W.Y., 2017b. Recent progress on deep eutectic solvents in biocatalysis. Bioresour Bioprocess. https://doi.org/10.1186/s40643-017-0165-5.

Yang, T.-X., Zhao, L.-Q., Wang, J., Song, G.-L., Liu, H.-M., Cheng, H., Yang, Z., 2017. Improving whole-cell biocatalysis by addition of deep eutectic solvents and natural deep eutectic solvents. ACS Sustain. Chem. Eng. 5, 5713–5722. https://doi.org/10.1021/acssuschemeng.7b00285.

Yang, Z., 2019. Toxicity and biodegradability of deep eutectic solvents and natural deep eutectic solvents. In: Deep Eutectic Solvents. Wiley, pp. 43–60. https://doi.org/10.1002/9783527818488.ch3.

Yang, Z., 2018. Natural Deep Eutectic Solvents and Their Applications in Biotechnology, pp. 31–59. https://doi.org/10.1007/10_2018_67.

Zaib, Q., Eckelman, M.J., Yang, Y., Kyung, D., 2022. Are deep eutectic solvents really green?: a life-cycle perspective. Green Chem. 24, 7924–7930. https://doi.org/10.1039/d2gc01752k.

Zdanowicz, M., Wilpiszewska, K., Spychaj, T., 2018. Deep eutectic solvents for polysaccharides processing. A review. Carbohydr. Polym. 200, 361–380. https://doi.org/10.1016/j.carbpol.2018.07.078.

Zhao, B.-Y., Xu, P., Yang, F.-X., Wu, H., Zong, M.-H., Lou, W.-Y., 2015. Biocompatible deep eutectic solvents based on choline chloride: characterization and application to the extraction of rutin from Sophora japonica. ACS Sustain. Chem. Eng. 3, 2746–2755. https://doi.org/10.1021/acssuschemeng.5b00619.