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Deep Eutectic Solvents: Are They Safe?

Laura Lomba *, Mª Pilar Ribate, Estela Sangüesa, Julia Concha, M ª Pilar Garralaga, Diego Errazquin, Cristina B. García and Beatriz Giner

Campus Facultad de Capital de la Salud, Farmacia, Universidad San Jorge, Autov A23 km 299, Villanueva de Gállego, 50830 Zaragoza, Spain; mpribate@usj.es (M.P.R.); esanguesa@usj.es (E.S.); jconcha@usj.es (J.C.); mpgarralaga@usj.es (M.P.G.); derrazquin@usj.es (D.E.); cbgarcia@usj.es (C.B.G.); bginer@usj.es (B.G.)

* Correspondence: llomba@usj.es; Tel.: +34-976-060-100; Fax: +34-976-077-584

Abstract: Deep eutectic solvents (DESs) are a relatively new type of solvent that have attracted the attention of the scientific community due to their environmentally friendly properties and their versatility in many applications. Many possible DESs have been described and, thus, it is not easy to unequivocally characterize and generalize their properties. This is especially important in the case of the (eco)toxicity information that can be found for these mixtures. In this review, we collect data on the human and environmental toxicity of DESs, with the aim of gathering and exploring the behavioral patterns of DESs. The toxicity data found were analyzed attending to different factors: hydrogen bond donors or acceptors that form part of the eutectic mixture, pH, and the presence of organic acids in the DES molar ratio of the components, or interactions with natural compounds. In the case of ecotoxicity, results generally depend on the biomodel studied, along with other factors that have been also revised. Finally, we also carried out a revision of the biodegradation of DESs.

Keywords: deep eutectic solvents (DESs); toxicity; ecotoxicity

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1. Deep Eutectic Solvents (DESs)

During recent decades, important efforts have been made to find new solvents that are safe, cheap, and safe for the environment and health, and that also have good properties for industrial applications. The principal features of solvents are related to solubility, recyclability, thermal and chemical stability, selectivity, flammability, corrosivity, viscosity, melting point, (eco)toxicity, physicochemical properties, availability, and cost [1,2].

Several groups of solvents have been studied in order to find competitive, safer, and greener substitutes for traditional solvents, including ionic liquids (ILs), supercritical solvents, and solvents from biomass [3,4].

ILs present interesting properties, such as low vapor pressure, high thermal, chemical, and electrochemical stability, being non-flammable [5–7], and the ability to dissolve or extract several chemicals (organics and inorganics; polar and nonpolar) [8], and can be used in separations [9] or as catalysts, among other important properties [10–12]. It should be noted that these characteristics are not common to all ionic liquids, and properties depend on the ions. Thus, it is possible to find different families of ILs based on the cation structure (pyridine ring, imidazolium, tetraalkylammonium), which can be linked to different anions (halides, tetrafluoroborate, dicyanaida, triflate, etc.). However, the main problem of these chemicals is their toxicity. There are some studies about their toxicity, biodegradability, and ecotoxicity, but there is not yet enough evidence, and more studies are needed in order to ensure the safety of ILs for the environment and health [13–17].

Supercritical fluids present the following characteristics: they can increase the transfer of mass and heat due to their high diffusion coefficient and low viscosity; their properties can be adjusted by changing pressure and temperature; and they can be easily removed and recycled by reversing conditions of temperature and pressure. Some supercritical fluids include supercritical carbon dioxide ($scCO_2$), supercritical water (scH_2O), and supercritical

ammonia ($scNH_3$). Most of their uses are related to organic synthesis, extractions, and solubilization, among others [18–22]. However, although $scCO_2$ might be considered a good alternative, it is a nonpolar solvent, and it cannot be used in many applications. In the case of scH_2O, it is important to note that the critical pressure and temperature are too high, so its uses are limited. Supercritical water behaves as a nonpolar solvent; therefore, it is not good for dissolving inorganic compounds. Finally, $scNH_3$ presents accessible critical pressure and temperature, but it is toxic and highly reactive and, therefore, it is dangerous to use in high-scale applications. Essentially, two problems have been found with this type of solvent: on the one hand, it is difficult and expensive to perform the installation of the equipment and, on the other hand, the polarity of these solvents is limited [23].

Solvents from biomass have been also widely studied, because their properties are based on green chemistry precepts [24]. These are compounds obtained via fermentation, enzymatic, or esterification processes. Agricultural and some industrial activities can produce large amounts of raw materials, used to produce commodity and fine chemicals, with lignocellulosic biomass being the production material with the largest potential volume and lowest cost. Some of these chemicals include fatty acid esters, furfural, levulinic acid, terpenes, glycols and their esters, low-molecular-weight alcohols, and lactates [25].

A new type of solvent—the so-called deep eutectic solvents (DESs)—was defined by Abbott et al. in 2003. DESs are liquid compounds that present a diminution of the melting point above 100 °C when it is compared to the forming substances [26,27]. These solvents are a mixture of two or more compounds that are generally solid at room temperature but, when they are combined in a specific molar ratio, form a liquid solution, i.e., a decrease in the melting point is observed for these mixtures because of the interaction between the molecules. In general, these mixtures present low vapor pressure and, therefore, low volatility, are non-flammable, and are compatible with water and, theoretically, environmentally friendly.

DESs have sometimes been confused with ILs, and it has even been considered that the two types of mixture can be interchangeable [28]. Certainly, DESs are natural competitors of ILs, since they share properties. However, both types of solvent are very different, due mainly to two reasons: on the one hand, the nature of the starting materials is quite different (ILs are mixtures of organic cations and organic or inorganic anions, while DESs are combinations of one or more hydrogen bond acceptors (HBAs) and hydrogen bond donors (HBDs); on the other hand, the methods used for their formation are also different: DESs can be prepared from single components via heating treatment, whereas ILs are normally synthesized using synthetic routes involving various reagents and solvents, consisting of two principal steps—cation formation, and anion exchange [29]. The nature of the components forming ILs and DESs also determines their behavior; for instance, ILs are dominated by ionic interactions, while DESs show strong hydrogen-bonding interactions.

DESs have attracted the attention of the scientific community because of their potential applications. These mixtures can be used in organic synthesis, electrochemistry, extraction media [30–34], biotechnology or biodiesel [35–37] synthesis and separation processes [38,39], as a solvent in biological assays or enzymatic reactions [40], in biocatalysis [41,42], or in biomedical applications [43–46]. Additionally, they have been used in the pharmaceutical industry as excipients for increasing the solubility of hydrophobic drugs, or in drug delivery formulations [47–51]. Thus, the number of scientific manuscripts related to DESs has been growing over the past several years (Figure 1).

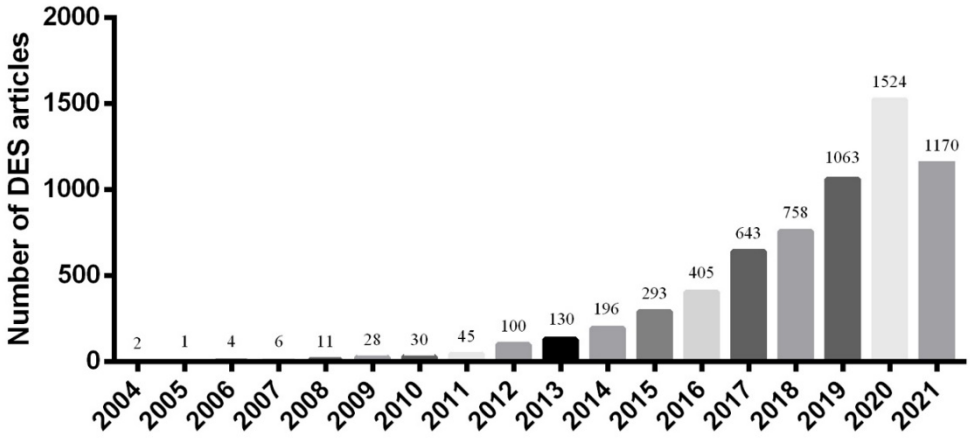

Figure 1. Evolution of the number of manuscripts about DESs in the period 2004–2021 (September). Source: Web of Science.

As mentioned previously, these mixtures are formed by a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD) [52]. Several substances can act as hydrogen bond acceptors; the more typical chemicals are quaternary salts such as choline chloride or betaine, but others—such as thymol, menthol, and decanoic or lauric acids—have also been used [53]. On the other hand, urea, amino acids (alanine, serine, glutamic acid, proline), sugars (glucose, mannose, xylose, fructose, galactose, sucrose, or lactose), alcohols (glycerol, xylitol, sorbitol, ethylene glycol, 1,2-propylene glycol), amides (acetamide, benzamide), amines, or carboxylic acids (malonic, lactic, maleic, oxalic, citric, malic, tartaric) can be used as hydrogen bond donors [43,46].

It is interesting to note that due to the massive amount of possible DESs, resulting from the abundance of possible combinations of raw materials (either hydrophilic or hydrophobic), it is possible to rationalize their use and select those that combine the best properties (chemical, toxicological, environmental, and safety) for a given application. However, this same versatility can present a problem, since it is not easy to unambiguously characterize and generalize their properties. It is worth mentioning that there are very few studies that have addressed the prediction of DESs' properties [54,55]. This lack of predictive models is even more pronounced in the case of (eco)toxic behavior [56]. Regarding this topic, in general, DESs are considered to be nontoxic compounds [57]. Nevertheless, there are no systematic studies from the health and environmental toxicity points of view that confirm this point. For this reason, an exhaustive revision of this information was carried out in this article. We want to focus on how DESs behave in the environment, and what studies exist related to their toxicity. This will shed light on and analyze recent articles to see what kind of information needs to be obtained in the future, and which DESs should be promoted due to their green and safety properties. In Figure 2, a scheme of DESs and their main properties is shown.

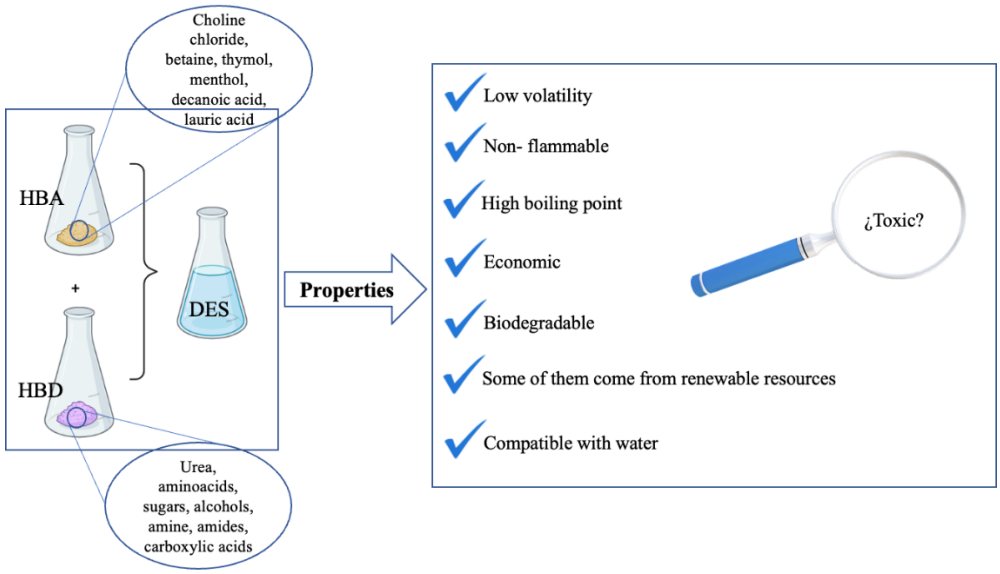

Figure 2. Properties of deep eutectic solvents.

2. Cytotoxicity

It is known that DESs present several good properties for different applications. Although there are DESs with no natural HBD, in recent years, the trend has been to use raw materials of natural origin (as primary metabolites) that can act as solvents in living organisms, such as lipids or water. This implies cellular tolerance and a low cytotoxic profile. Since the first investigations, numerous studies—both in vitro and in vivo—have been carried out to test the cytotoxicity of DESs—mainly performed in immortalized cell lines, as well as other more complex organisms such as murine models, rats, or marine organisms [58]. No human toxicity studies have been carried out to date. In Figure 3, a summary of the revision made in this work regarding the toxicity of DESs is shown.

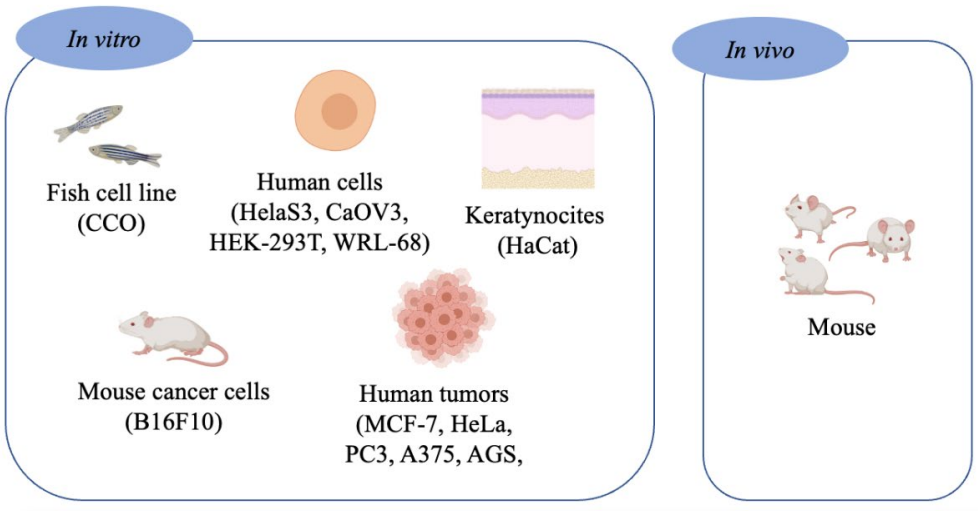

Figure 3. Most common in vitro and in vivo assays used in the determination of DES toxicity.

2.1. In Vitro Assays

Most of the studies carried out to date have been in vitro assays. They show that the toxicity of DESs depends on the cells or organisms tested, and may also be influenced by several parameters, which are described below. Several assays carried out by different authors related to cytotoxicity are listed in Table 1.

Table 1. Cytotoxicity assays of DESs in vivo.

Cell Line	DES	Result	Reference
Fibroblast-like cells (L929)	ChCl/glucose (1:1), ChCl/citric acid (1:1), ChCl/citric acid (2:1), ChCl/sucrose (4:1), ChCl/sucrose (1:1), ChCl/tartaric acid (2:1), ChCl/xylose (2:1), ChCl/xylose (3:1), Citric acid/sucrose (1:1), Citric acid/glucose (1:1), Glucose/tartaric acid (1:1)	HBD organic acids (tartaric and citric acids) are major enhancers of cytotoxicity	[58]
HelaS3, CaOV3, MCF-7 (human), B16F10 (murine cell line)	ChCl/malonic acid, ChCl/fructose/water, ChCl/glucose/water, ChCl/sucrose/water, ChCl/glycerol/water	HBD organic acids (e.g., malonic acid) increase toxicity. Glycerol (HBD) shows a lower toxicity profile.	[59]
MCF-7, A375, HT29, H413, PC3 (human cancer cells), HepG2 and OKF6 (human normal cells)	ChCl/glycerine, ChCl/glycerine/water ChCl/ethylene glycol, ChCl/ethylene glycol/water, ChCl/triethylene glycol, ChCl/triethylene glycol/water, ChCl/urea, ChCl/urea/water	DESs show greater toxicity than individual components. Molar ratio affects toxicity.	[60]
Fish cell lines (CCO), MCF-7 (human tumor cells)	(ChCl/glucose (1:2), ChCl/oxalic acid (1:1), ChCl/glycerol (2:1)	ClChl-based DESs show low or moderate (oxalic acid) toxicity.	[61]
Hela and MCF-7 (human)	ChCl/glucose, ChCl/fructose, ChCh/xylose, ChCl/malic acid	ClChl-based DESs show low toxicity (EC_{50} > 2000 mg/L)	[62]
HeLa, MCF-7 human tumor cells) and HEK-293T (human normal cells)	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).	Toxicity depends on cellular type and NADES nature. Tumor cells suffer great toxicity (higher energy demands). Oxalic acid ChCl-based NADES shows a lower EC_{50} in tumor cells. ChCl/urea increases pH and toxicity in MCF-7	[63]
HelaS3, PC3, A375, AGS, MCF-7, and WRL-68 hepatic cell lines	ChCl/glucose (2:1) and ChCl/fructose (2:1)	In vitro (cell lines) NADESs are less toxic than DESs (less acidity).	[64]
HEK-293 cell line (human)	28 selected ChCl-based NADESs	NADESs are more toxic than their individual components. HBA:HBD molar ratio and the number of HBD carbons have negative effects on toxicity, although dependent on the compounds.	[65]
Hacat and MNT-1	Combinations of HBA: ChCl, tetramethylammonium chloride (N_{1111}), and tetrabutylammonium chloride (N_{4444}); and HBD: hexanoic and butanoic acids, ethylene glycol, 1-propanol, and urea	ChCl- and N111-based DESs show great biocompatibility, while N_{4444} shows toxicity. Some compounds increased cell viability.	[66]

2.1.1. Choline Chloride (HBA)-HBD

In some studies of toxicity tested in several cell lines, HBDs were analyzed to select for less toxic mixtures. Choline chloride (ChCl) is probably the most common HBA in DESs. ChCl is a component of the vitamin B complex, so it intervenes in cellular metabolism [67]. In these cases, the HBD mainly determines the cytotoxicity of the mixture. A study carried out by Radošević et al. (2015) evaluated three different DESs with ChCl:ChCl/glucose, ChCl/oxalate, and ChCl/glycerol. In this study the cytotoxicity—measured by WST-1 test (similar to MTT test)—was determined towards fish cell lines (CCO) and human tumors (MCF-7). The results, expressed in EC_{50} values, revealed that ChCl/glucose and ChCl/glycerol showed low toxicity (EC_{50} > 2000 mg/L), while ChCl/oxalate possessed moderate cytotoxicity (EC_{50} = 218.7 mg/L (CCO) and 558.98 mg/L (MCF-7)). During the treatment of CCO cells with ChCl/oxalate, a formation of calcium oxalate crystals was observed, which can damage cells and, therefore, may partially explain the relatively higher cytotoxicity exhibited by this natural deep eutectic solvent (NADES) [61].

The same authors also compared ChCl/glucose, ChCl/fructose, ChCh/xylose, ChCl/glycerol, and ChCl/malic acid. The IC_{50} values for those compounds were higher than 2000 mg/L in two human tumor cell lines (HeLa and MCF-7), and these results were in concordance with their previous study; they conclude that these sugars and this organic acid possess low cytotoxicity in combination with ChCl [62].

Recently, Macario et al. (2019) tested the viability of HaCat (keratinocyte) and MNT-1 (melanoma) human cell lines against different combinations of DESs with ChCl, tetramethylammonium chloride (N_{1111}), or tetrabutylammonium chloride (N_{4444}) as HBAs; and hexanoic acid, butanoic acid, ethylene glycol, 1-propanol, or urea as HBDs. ChCl and N_{1111} showed great biocompatibility, while N_{4444}, with larger alkyl chains, showed cytotoxicity in the cell lines tested by MTT assay. The lowest IC_{50} was 34.1µg/mL for [N_{4444}] Cl:ethylene glycol in HaCat. ChCl:butanoic acid increased HaCat viability at low doses (100 µg/mL), demonstrating its potential for cosmetic uses; however, at higher doses (500 µg/mL), it turned out to be toxic in both cell lines (just up to 20% decreased in viability in MNT-1). MNT-1 increased its viability in a softer way when treated with ChCl:ethylene glycol and ChCl:1-propanol [66].

2.1.2. Presence of Organic Acids

As we have said, the most common organic acids used for the preparation of DESs are malonic, lactic, maleic, oxalic, citric, malic, and tartaric acids.

The presence of organic acids in DESs seems to decrease the viability of cells. Paiva et al. (2014) investigated the cytotoxicity of several NADESs using fibroblast-like cells (L929) [58]. The results pointed to the role of the organic acids (HBDs) as major enhancers of cytotoxicity. In agreement with this study, Hayyan et al. (2016) found, via the MTT assay, that NADESs prepared with organic acids as HBDs (malonic acid) were more cytotoxic to the human cell lines HelaS3 (IC_{50} = 20 ± 8.4 mM), CaOV3 (IC_{50} = 15 ± 8.2 mM), and MCF-7 (IC_{50} = 35 ± 8.3 mM), as well as the murine cancer cell line B16F10 (IC_{50} = 35 ± 8.8 mM), and should be used with caution. The cultured cells resulted in high tolerance to carbohydrate (lowest IC_{50} = 127 ± 9.22 mM for ChCl:fructose in MFC-7, and highest IC_{50} = 211 ± 8.0 mM for ChCl:glucose in B16F10) and glycerol DESs (lowest IC_{50} = 340 ± 10.3 mM in B16F10) [59].

A recent study by Mitar et al. (2019) that used a colorimetric assay (CellTiter 96® AQueous One Solution Assay), tested eight carboxylic acid NADESs against three human cell lines (HEK-293T, HeLa, and MCF-7). Most of the DESs included ChCl and malic acid. None of them showed inhibiting effects on the growth of human cells, even at the highest concentration tested (c = 2000 mg/L). All studied DESs with carboxylic acid could be classified as harmless regarding cell viability [68].

2.1.3. Influence of pH

The nature of the compounds forming the DES—especially the HBD—strongly affects the pH of the final mixture [69]. An excess of acidity can denature membrane proteins

and cause cell death. Zhao et al. (2015) proposed that the higher toxicity of the mixtures containing organic acids produces a modification in the cellular proliferation and metabolic pathways. Likewise, pH changes depend on the length of the carbonated chain as well as the presence of certain functional groups (benzene seems to be less toxic than carboxylic or alcohol groups) [70]. It seems that the pH of DESs decreases as temperature increases—at least in the 17 DESs tested by Skulcova et al. (2018). In general, organic acids present lower pH than alcohols from 25 °C to 60 °C [71]. Radošević et al. (2018) found that ChCl/urea was cytotoxic to MCF-7 cells, probably due to its high pH value (around 9.0) (being the optimal pH for the used culture around 7.0–7.4). HeLa and HEK293T did not show the same effect, which may indicate a cell-type-dependent cytotoxicity [63]. Hayyan et al. (2012) also discovered that in ChCl:fructose and derivatives, the pH decreased with HBD content. Furthermore, glucose presents neutral pH, while fructose shows greater acidity when combined with ChCl as an HBA [72].

2.1.4. Molar Ratio

The cytotoxicity of DESs seems to be affected by the molar ratio of their components. Some studies have shown that ChCl/urea at a 1:3 molar ratio produces acute toxicity via in vitro MTT assay, while in vivo studies showed greater toxicity at a 1:2 molar ratio [60].

Ahmadi et al. (2018) confirmed, via MTT assay, the importance of the molar ratio of HBA to HBD compounds in the toxicity of DESs to the HEK-293 cell line; ChCl/glycerol and ChCl/glucose decreased the toxicity as the glycerol (IC_{50} = 39.34 ± 1.90 mM for 1:2 ratio and IC_{50} = 61.48 ± 1.90 mM for 1:4 ratio) or glucose proportion increased (IC_{50} = 32.99 ± 2.32 mM for 1:1 ratio and IC_{50} = 23.38 ± 4.10 mM for 2.5:1 ratio), while ChCl/fructose showed lower toxicity at an intermediate molar ratio of 1:1.5 (IC_{50} = 40.84 ± 3.54 mM for 1:1.5 ratio compared to IC_{50} =26.95 ± 5.24 mM for 1:1 ratio (lowest) and IC_{50} = 29.36 ± 5.20 mM for 1:1.25 (highest)). Conversely, 1,2-propanediol enhanced the toxicity as the proportion increased (IC_{50} = 75.46 ± 17.40 mM for 1:1 ratio and IC_{50} = 48.18 ± 16.36 mM for 1:3 ratio). These results indicate that toxicity based on molar ratio depends on the specific compounds. The DESs' cytotoxicity was dependent on their chemical structure, and the molar ratio of HBA to HBD plays an important role in the cytotoxicity of these compounds against human HEK-293 cells [65].

2.1.5. Synergistic Effects

Hayyan et al. examined the cytotoxicity of some phosphonium-based DESs (2013) and ammonium-based DESs (2015) compared with their individual components. For ammonium-based DESs, mixtures showed lower LD_{50} in mice (5.31–6.39 g/kg for mixtures vs. 9.71 - > 20 g/kg compared with the toxicity of the individual components) as well as lower IC_{50} in immortalized cell lines (from 18.07 ± 1.62 μg/mL in A375 cells to 54.67 ± 8.33 μg/mL in H413 cells), indicating a synergistic effect for the toxicity of components [60,73].

Zhao et al. (2015) confirmed that DESs were more toxic than their individual components due to the delocalization of charges on hydrogen bonding. This effect is especially greater for DESs containing tartaric and citric acids as HBDs (more compact structure) [70].

Mbous et al. (2017) studied the anticancer potential and cytotoxicity of DESs (ChCl/glucose and ChCl/fructose) using HelaS3, PC3, A375, AGS, MCF-7, and WRL-68 hepatic cell lines, and compared the results with the individual components of the DESs. The EC_{50} values of the DESs measured by MTT assay were significantly higher than those of the aqueous solutions of their individual components, but were similar to those of the aqueous solutions of combinations of their chief elements; the EC_{50} of the DESs' components ranged from 98 to 516 mM, while that of the DESs was higher (34–120 mM) [64].

Ahmadi et al. (2018) investigated the cytotoxicity (MTT assay) of 28 selected ChCl-based DESs and their raw materials at different concentrations against human HEK-293 cells. Once more, DESs showed more toxicity than their individual components (IC_{50} = 3.52 ± 0.67–75.46 ± 17.40 mM) [65].

2.1.6. Natural Cellular Intermediaries

Hayyan et al. (2016) proposed that the cytotoxic mechanisms of DESs could be based on the aggregation and interaction of solvents with cellular membranes, by altering cell viability. In this case, components required for natural metabolism (hydrocarbonated compounds, glycerol, or choline) seemed to present better cell tolerance. Conversely, those components that inhibit metabolic pathways generated high cytotoxicity (e.g., malonic acid in Krebs cycle). These aspects depended on the cell type [59]. Radošević et al. (2018) evaluated the cytotoxicity of 10 NADESs (ChCl, betaine, and citric acid as HBAs) via a colorimetric method (CellTiter 96® AQueous One Solution Cell Proliferation assay) in HeLa, MCF-7, and HEK-293T cells, at concentrations of 500–2000 g/L. Carbohydrates as HBDs were used as energy sources for cells, allowing metabolism and proliferation and, thus, minimizing possible toxic effects. All of them showed no toxicity, independently of the HBA type (EC_{50} > 2000 mg/L). The same was found for other intermediaries of metabolism (polyols, glycerol). Malonic acid (organic acid) DESs had cytotoxic potential, as seen previously; however, when combined with betaine or proline, they showed a curious stimulatory proliferative effect (both are osmoprotectants), attenuating the cytotoxicity [63].

2.1.7. Viscosity

The chemical nature of DESs affects the viscosity, water content, and melting point of synthesized DESs. [74]. Zhao et al. (2015) evaluated the viscosity (in Pa·s, at 30 °C) of 20 different DESs formed using ChCl as an HBA and urea, acetamide, ethylene glycol, glycerol, 1,4-butenediol, triethylene glycol, xylitol, p-toluenesulfonic acid, oxalic acid, sorbitol, malic acid, citric acid, levulinic acid, malonic acid, tartaric acid, xylose/water, sucrose/water, fructose/water, glucose/water, and maltose/water as HBDs. It was observed that the viscosity was higher in ChCl/tartaric acid (2:1), ChCl/citric acid (1:1), ChCl/malic acid (1:1), and ChCl/D-sorbitol (1:1), with values > 10 Pa·s. These compounds are more viscous because the presence of extra hydroxyl groups creates more hydrogen bonds and increases the attractive forces between molecules (H-bonding network, van der Waals forces, and electrostatic interplays) [43,70]. Dai et al. showed that the viscosity of DESs is affected by their water content, and if more than 50% water is present, the hydrogen bond framework of the DES components is destroyed [75].

It could be considered that high viscosity produces an increase in toxicity. Hayyan et al. indicated that high viscosity was related to higher lethality rates (lowest IC_{50} values in MTT test). The DES ChCl/sucrose/water (4:1:4) and the ChCl/malonic acid (1:1) had the highest viscosity values (853.3 mPa·s and 616.0 mPa·s, respectively) and the lowest IC_{50} in HelaS3 (166.58 ± 5.8 and 20 ± 8.4, respectively), CaOV3, B16F10, and MCF-7 cell lines. They concluded that the introduction of substances with high viscosity (above 500 mPa·s) in a cell medium can result in a major variation in cytoplasmic viscosity (homeostatic range from 1 to 400 mPa·s) [76], and could cause cell death [59].

Mbous et al. (2017) studied the cytotoxicity of two NADESs composed of ChCl and glucose or fructose, and N,N-diethylethanol ammonium chloride–triethylene glycol on the HelaS3, A375, AGs, WRL-68, MCF-7, and PC3 cancer cell lines. In this case, they found that both ChCl:glucose and ChCl:fructose were less toxic compared to DESs. However, ChCl:glucose and ChCl:fructose NADESs showed higher toxicity in the in vivo tests [64], since viscosity seemed to affect more complex organisms differently.

2.2. In Vivo Assays

In vitro assays present some limitations, since they cannot not completely predict in vivo results; they are generally specific to an organism or organ and, therefore, the effects are not comparable to whole animals or humans. Taking this into account, it is necessary to carry out in vivo studies, since they are the optimal approach to evaluate the toxic potential of compounds—for instance, in the case of immune systems, whose responses have a multifunctional and complex character. These kinds of studies should combine identification, effect measurement, and assessment of the immune system [77].

There have been few cytotoxicity studies carried out using in vivo assays to evaluate the toxicity of DESs; most have been in murine models or rats, while no human studies have been performed to date. This information is shown in Table 2.

Table 2. Cytotoxicity assays of DESs in vivo.

Cell Line	DES	Result	Reference
Mice	ChCl/glycerine, ChCl/ethylene glycol, ChCl/triethylene glycol, ChCl/urea	DESs show greater toxicity than their individual components. Molar ratio affects toxicity.	[60]
Mice	ChCl/glucose (2:1) and ChCl/fructose (2:1)	In vivo (mice) NADESs are more toxic than DESs (more viscosity)	[64]
Male Wistar rats	Betaine:glycerol (1:2)	Short-term oral administration toxicity in rats.	[78]
Mice	ChCl-glycerol (1:2)	No toxicity in mice (LD_{50} = 7733 mg/kg)	[79]

In 2015, Hayyan et al. tested a variety of ammonium-based DESs in vivo in a murine model using six imprinting control regions. They found that eutectic mixtures are more toxic than their individual components, as in vitro tests had shown previously. The DES-treated group suffered an alteration of hepatic enzymes (AST:ALT ratio) and a slight increase in markers of renal function (urea, Na^+, K^+, Cl^-). These results seemed to indicate hepatocellular and renal patterns of injury. In the same study, they corroborated the importance of molar ratio, as ChCl/urea 1:3 was lethal, while a 1:2 ratio was not (LD_{50} = 5.64 g/kg ± 0.36) [60].

Mbous et al. (2017) performed another study on mice using six imprinting control regions and comparing the toxicity of DESs and NADESs. They found that NADESs (LD_{50} = 1.84 g/mL and 1.24 g/mL) showed higher toxicity than DESs (LD_{50} = 4.46 g/mL), probably because of their higher viscosity, leading to a more difficult circulation of the mixture in mice as well as a higher threshold concentration, which induces liver failure [64].

Chen et al. (2017) tested ChCl/glycerol (1:2) in mice at different doses, from 5000 mg/kg (no toxicity) to 12,000 mg/kg (death), estimating LD_{50} via the Karber method. They concluded that mortality is dose-dependent, but that ChCl/glycerol is safe, with an estimated LD_{50} of 7733 mg/kg of oral administration (7130–8387 mg/kg, 95% confidence interval) [79].

Recently, Benlebna et al. (2018) administered 3 mL of oral betaine:glycerol (1:2 + 10% (*v*/*v* of water) NADES to Wistar rats, finding a variety of short-term adverse effects, including death in some of the specimens. The treatment induced oxidative stress, hyperlipaemia, weight loss, and excessive water consumption. This could be due to the excessive oral dose administered to the rats [78].

In conclusion, complementary studies are needed in order to complete these studies, since few DESs have been tested previously.

3. Ecotoxicity

In addition to studying how DESs can affect humans, it is also important to pay attention to the environment, since the widespread use of DESs and their introduction in the market could be an important source of pollution, with a consequent impact on ecosystems and, ultimately, on humans. Thus, the effect of DESs in natural aquatic, terrestrial, and aerial media, along with trophic chains, should be studied. However, as previously mentioned, DESs present very low vapor pressure; therefore, it is not expected that they can be released in the air. Finally, it is necessary to analyze the available biodegradability studies to determine how long DESs can remain in the environment. In this section, we focus on aquatic biomodels and biodegradability. As far as we know, there are no terrestrial

assays for evaluating the ecotoxicity of DESs. Figure 4 shows a summary of the biomodels normally used in the evaluation of the ecotoxicity of DESs.

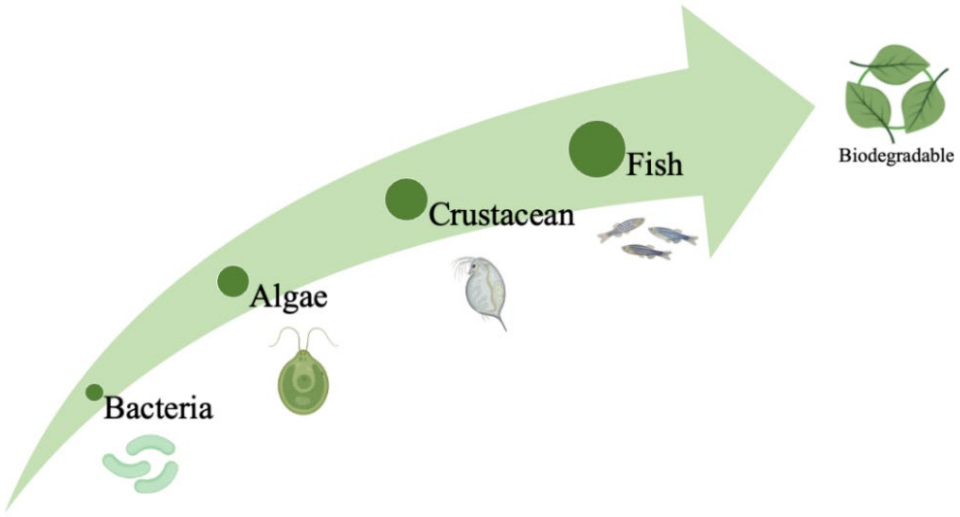

Figure 4. Biomodels used in the ecotoxicity evaluation of DESs, and the relationship with the trophic chain.

3.1. Aquatic Biomodels

For a correct assessment of toxicity, tests in different biomodels are required in order to ensure a complete representation of the trophic chain in the environment. For this reason, bacteria, algae, crustaceans, plants, and fish should be studied [80]. It has been seen that, depending on the used organism, there is different sensitivity to the tested compounds [81]. Despite the lack of previous comprehensive ecotoxicological studies on eutectic mixtures, there are some data available concerning the ecotoxicological effects of these moieties in bacteria, algae, crustaceans, and fish. In all cases, toxicity depended on the biomodel used and the components of the deep eutectic mixtures. Furthermore, there are also several additional factors that directly affect the behavior, which are detailed in the next sections.

Ability to cross biological barriers: The ability to enter organisms is strongly related to lipophilicity. Thus, it can be affected by many factors, such as the pH [82] or the length of the alkyl chain. Thus, the toxicity can be modified depending on the charge, and on the number of carbons in the chain [56].

Presence of water: The presence of water in these compounds modifies their toxicity. It has been seen that the formation of hydrogen bonds between water (can act both as donor and acceptor) and DES components leads to the formation of a complex, of which water molecules form a part [83]. A disruption of the structure of these compounds may occur at a certain limit of the water content. This interaction can increase or decrease the toxicity, depending on the used biomodel, the water content, and the composition of the mixture [84].

Starting materials: Although a clear trend has not been observed, the starting materials of DESs present different toxicity than the eutectic mixtures [82,84–86]. The physicochemical properties of the eutectic mixtures are different from those of the starting materials, mainly due to the extent of the hydrogen bonds. Any different molar proportion may give different properties to the DESs composed of the same starting materials. The interaction of these compounds with the bacterial membrane seems to cause the toxic effect. However, the full mechanism of action is still unknown. On the one hand, it is known that compounds that exhibit charge delocalization are more toxic [87]; thus, it is suspected that eutectic mixtures that present a delocalized charge in their structure (HBA) interact more with the membrane, causing its disruption. Depending on the type of HBD of these mixtures, the charge will be delocalized in the HBA and, therefore, the toxicity will vary. On the other hand, the formation of hydrogen bonds between the components of the eutectic mixtures

seems to prevent the formation of these bonds with elements of the plasma membrane [88]. However, other theories maintain that HBDs cause denaturation of the membrane proteins, activating enzymatic mechanisms that cause bacterial death [89].

3.1.1. Bacteria Biomodel

Most of the studies on the ecotoxicity of DESs were carried out in bacteria—particularly in *Aliivibrio fischeri* (*A. fischeri*). This information was compiled from the existing bibliography and is shown in Table 3.

Table 3. Ecotoxicological data of some DESs in *A. fischeri*.

DES (Molar Ratio)	EC_{50} (mg/L) 15 min	EC_{50} (mg/L) 30 min	Reference
ChCl:cetic acid (1:2)	125 ± 15	130 ± 9	[82]
ChCl:acetic acid	200 ± 14	197 ± 17	[82]
ChCl:acetic acid (2:1)	343 ± 32	337 ± 34	[82]
ChCl:lactic acid (1:2)	34.2 ± 2.6	33.6 ± 0.8	[82]
ChCl:lactic acid	63.0 ± 6.0	61.8 ± 11.6	[82]
ChCl:lactic acid (2:1)	68.6 ± 12.3	67.0 ± 36.0	[82]
ChCl:glycolic acid (1:2)	30.4 ± 1.6	30.2 ± 0.8	[82]
ChCl:glycolic acid	33.1 ± 6.1	32.9 ± 5.6	[82]
ChCl:glycolic acid (2:1)	63.0 ± 12.2	62.2 ± 12.4	[82]
ChCl:citric acid (1:2)	15.6 ± 0.9	15.6 ± 0.6	[82]
ChCl:citric acid	22.5 ± 2.6	22.4 ± 2.4	[82]
ChCl:citric acid (2:1)	31.1 ± 2.6	31.9 ± 4.6	[82]
$[N_{1111}]$Cl:1-propanol (1:1)	19,930 ± 2310	20,870 ± 3950	[89]
$[N_{1111}]$Cl:1-propanol (2:1)	15,420 ± 3680	16,150 ± 4050	[89]
$[N_{1111}]$Cl:1-propanol (4:1)	16,430 ± 1720	15,360 ± 1360	[89]
$[N_{2222}]$Cl:1-propanol (1:2)	20,710 ± 3200	22,260 ± 3110	[89]
$[N_{2222}]$Cl:1-propanol (1:1)	17,590 ± 5140	18,090 ± 4650	[89]
$[N_{2222}]$Cl:1-propanol (2:1)	13,860 ± 2250	15,550 ± 2750	[89]
$[N_{2222}]$Cl:1-propanol (4:1)	8571 ± 898	9500 ± 945	[89]
$[N_{3333}]$Cl:1-propanol (1:2)	1467 ± 328	1555 ± 374	[89]
$[N_{3333}]$Cl:1-propanol (1:1)	6106 ± 2350	4981 ± 2199	[89]
$[N_{3333}]$Cl:1-propanol (2:1)	1700 ± 210	1845 ± 227	[89]
$[N_{3333}]$Cl:1-propanol (4:1)	989.6 ± 184.6	1120 ± 214.5	[89]
$[N_{1111}]$Cl:EG (1:2)	29,010 ± 2630	30,200 ± 9610	[89]
$[N_{1111}]$Cl:EG (1:1)	52,110 ± 5320	53,990 ± 6110	[89]
$[N_{1111}]$Cl:EG (2:1)	42,410 ± 3600	49,250 ± 2780	[89]
$[N_{1111}]$Cl:EG (4:1)	54,520 ± 16,970	65,620 ± 18,260	[89]
$[N_{2222}]$Cl:EG (1:2)	18,980 ± 3620	18,930 ± 3660	[89]
$[N_{2222}]$Cl:EG (1:1)	24,500 ± 3310	23,940 ± 3420	[89]
$[N_{2222}]$Cl:EG (2:1)	9069 ± 187	18,610 ± 4860	[89]
$[N_{2222}]$Cl:EG (4:1)	37,180 ± 9530	36,390 ± 8600	[89]
$[N_{3333}]$Cl:EG (1:2)	778.6 ± 68.6	971.3 ± 237.7	[89]
$[N_{3333}]$Cl:EG (1:1)	3074 ± 125	3665 ± 224	[89]
$[N_{3333}]$Cl:EG (2:1)	908.7 ± 104.5	945.3 ± 119.4	[89]
$[N_{3333}]$Cl:EG (4:1)	1273 ± 110	1285 ± 143	[89]
ChCl:glycerol (1:2)		86,726 ± 8727	[84]
ChCl:glycerol:water (1:2:1)		143,686 ± 8885	[84]
ChCl:urea (1:2)		26,346 ± 24	[84]
ChCl:urea:water (1:2:1.7)		98,409 ± 9598	[84]
ChCl:EG (1:2)		108,526 ± 19,101	[84]
ChCl:EG:water (1:2:1)		115,450 ± 17,943	[84]

This biomodel is a marine bacterium widely used in ecotoxicity tests due to its ability to emit a blue-green luminescence. This process is related to the cellular metabolism of the bacteria, and serves as a sensitive indicator of toxicity [90]. Inhibition of luminescence tests are carried out on this organism, and offer quick, cost-effective, reproducible, and accurate information about toxicity [90,91].

De Morais et al. investigated the ecotoxicity of different mixtures of choline chloride (HBA) and carboxylic acids (acetic, lactic, glycolic, and citric acids) as HBD. In this case, all the of EC_{50} results showed the same ascending pattern of toxicity—ChCl << ChCl:acid (2:1) << ChCl:acid << ChCl:acid (1:2) << acid—indicating that the toxicity increases with the acid content. These results in *A. fischeri* indicate that the HBD composition plays an important role in the toxicity of the eutectic mixture. The mechanism of action of these compounds in *A. fischeri* seems to be related to their ability to cross the cell membrane. It appears that the toxic effect occurs in the cytoplasm, altering the pH and affecting cell viability (hence, the toxic effect when increasing the pH of the acid) [73,92]. Even if the physicochemical properties of the eutectic mixtures were different from those of the starting materials, the toxicities between DESs and the reference organic acids were still similar. However, in this case, the predominant effect of the acids showed similar toxicities between DESs and their reference organic acids. The DESs tested were also found to be more toxic than the respective ionic liquids. This could be related to the presence of the delocalized charge of choline chloride because, as mentioned before, compounds with delocalized charges seem to be more toxic [87].

In the study carried out by Macario et al., the toxicity of some DESs was tested in *A. fischeri*. These compounds were made up of combinations of ethylene glycol and 1-propanol as HBDs, and ammonium quaternary salts as HBAs, at different molar ratios [89]. In this study, DESs were tested as aqueous solutions, so the components of the mixture were dissociated. Thus, the positively charged quaternary ammonium salt could have interacted with polysaccharides or peptide chains of the bacterial membrane thanks to the formation of hydrogen bonds or electrostatic interactions, producing a cellular disruption and generating the toxic effect. In this experiment, the trend already reported in other trials was also observed: the length of the alkyl chain affects toxicity. If the number of carbons in the alkyl chains increases, the lipophilicity leads to a higher permeability of the compound in the membrane [93,94]. In this case, the change in HBA structure caused the variation in toxicity. Other tests also considered the role of HBDs as a determinant of the toxicity of the compounds [59,61,85].

In another study conducted by the same authors, an estimation of the toxicity of some DESs was performed by obtaining the EC_{50} values of their raw materials [95]. The objective of the test was to observe the synergistic or antagonistic behavior of choline chloride and ethylene glycol, glycerol, propionic acid, 1,2-propanediol, and urea mixtures at different molar ratios in *A. fischeri*. It was seen that most of the compounds showed an antagonistic effect, being less toxic in combination than their components separately. However, in some cases, a synergistic effect was observed, showing that sometimes the starting compounds had less toxicity than the DES. Even so, this was a predictive approach, and it is always preferable to physically carry out the tests on the corresponding biomodel [89,95]. Nevertheless, it was found that the toxicity of DESs cannot be based on predictions of the behavior of their starting materials.

3.1.2. Crustacean

Daphnia magna (*D. magna*) is a planktonic crustacean highly sensitive to environmental changes. It is used in toxicity studies due to its short life cycle and standardized protocols, with the mobility of the *Daphnia* being a quantifiable toxicity parameter [96,97]. Bibliographic information on the toxicity of DESs towards *D. magna* assays is presented in Table 4.

Table 4. Ecotoxicological data of some DESs in *D. magna*.

DES (Molar Ratio)	EC_{50} (mg/L)	Reference
ChCl:glycerol (1:2)	2528 ± 151	[84]
ChCl:glycerol:water (1:2:1)	1762 ± 83	[84]
ChCl:urea (1:2)	1099 ±25	[84]
ChCl:urea:water (1:2:1.7)	1533 ± 37	[84]
ChCl:EG (1:2)	1868 ± 175	[84]
ChCl:EG:water (1:2:1)	2353 ± 46	[84]

As far as we know, there has only been one previous study, carried out by Lapeña et al., in which this biomodel was used to test the ecotoxicity of DESs [84]; They studied some DESs and their mixtures with water:glycerine (Gly) (1:2 choline chloride:glycerol), reline (Rel) (1:2 choline chloride:urea), ethaline (Etha) (1:2 choline chloride:ethylenglicol), glycerine and water (GlyW) (1:2:1 choline chloride:glycerol:water), reline and water (RelW) (1:2:1.7 choline chloride:urea:water), and ethaline and water (EthaW) (1:2:1 choline chloride:ethylenglicol:water). Results obtained with *D. magna* were compared to those obtained for other biomodels, such as *A. fischeri* and *Raphidocelis subcapitata* (*R. subcapitata*). The ranges of concentrations tested for *D. magna* studies were 150–4500 mg/L for Rel, 250–8200 mg/L for RelW, 300–1000 mg/L for Gly, 350–12,000 mg/L for GlyW, 190–6000 mg/L for Etha, and 250–8000 mg/L for EthaW. The results of this study showed the dependence of the biomodel used. In this case, *D. magna* turned out to be the most sensitive biomodel. None of the mixtures were considered toxic, since the EC_{50} values found were higher than 1000 mg/L. The addition of water to the compound modified its toxicological properties; however, the reason for this phenomenon could not be ascertained.

On the other hand, there are studies on phosphonium-type DESs [73] and ChCl-DESs [88] tested in *Artemia salina* (*A. salina*). Tests performed with this bioindicator are widely used for the detection of many toxic substances (heavy metals, pesticides, detection of mycotoxins, etc.), and are very useful for a preliminary study of toxicity [98].

It seems that *A. salina* was very susceptible to phosphonium-based DESs and ChCl-based compounds. In both assays, to determine the toxicity, the mortality was quantified, counting the number of dead brine shrimp over time. The time it took for 2, 4, 6, and 10 specimens to die was recorded, in minutes. The phosphonium-based DESs were formed from methyltriphenylphosphonium bromide (MTPB) combined with glycerine (Gly), ethylene glycol (EG), and triethylene glycol (TEG) as HBDs. It was observed that the highest survival time for 10 specimens was only 3.58 min for MTPB:TEG, followed by MTPB:EG (3.27 min) and MTPB:Gly (2.32 min) [73]. For the ChCl-based compounds, the tested DESs were based on ChCl as an HBA and glycerine (Gl), ethylene glycol (EG), triethylene glycol (TEG), and urea (U) as HBDs. In this case, the survival times were higher, but still not enough: 5.32 min for ChCl:Gl, 10.13 min for ChCl:EG, 2.58 min for ChCl:TEG, and 4.05 min in ChCl:U DESs. In both assays, the toxicity of the primary materials was lower than the DESs' toxicity [88].

3.1.3. Algae

There are not many studies of DESs in algae. Lapeña et al. [84] studied the ecotoxicity of pure DES in *Raphidocelis subcapitata* (*R. subcapitata*) (algae), which is a unicellular green alga commonly used in this kind of study due to its easy cultivation, good sensitivity, and involvement in biomagnification processes [99,100]. This information is shown in Table 5.

Table 5. Ecotoxicological data of some DESs in *R. subcapitata*.

DES (Molar Ratio)	EC_{50} (mg/L)	Reference
ChCl:glycerol (1:2)	7080 ± 65	[84]
ChCl:glycerol:water (1:2:1)	6617 ± 159	[84]
ChCl:urea (1:2)	8532 ± 157	[84]
ChCl:urea:water (1:2:1.7)	2896 ± 103	[84]
ChCl:EG (1:2)	9196 ± 795	[84]
ChCl:EG:water (1:2:1)	3536 ± 77	[84]

The range concentrations tested in algae were 1000–40,000 mg/L for Rel, 100–50,000 mg/L for RelW, 500–60,000 mg/L for Gly, 1000–45,000 mg/L for GlyW, 200–45,000 mg/L for Etha, and 100–45,000 mg/L for EthaW. Results for the algae biomodel followed a decreasing pattern, as follows: RelW > EthaW > GlyW > GLy > Rel > Etha.

3.1.4. Fish

Juneidi et al. [85] tested ChCl mixed with different alcohol group compounds (glycerine, EG, diethylene glycol, and TEG), sugar group compounds (fructose and glucose), acid group compounds (*p*-toluene sulfonic acid, malic acid), $ZnCl_2$, and U in the biomodel *Cyprinus carpio* fish. The test was performed at 10, 30, 50, 80, and 100 mg/L concentrations, and the mortality was recorded after 96 h and employed to calculate the LC_{50}. DESs were classified according to the Passino and Smith classification [101], from practically harmless to highly toxic. In this test, only DESs formed by $ZnCl_2$ showed toxicity; the other DESs were considered practically harmless, low-toxicity compounds (LC_{50} > 100 mg/L). This information was compiled from the existing bibliography and is shown in Table 6.

Table 6. Ecotoxicological data of some DESs in *Cyprinus carpio*.

DES (Molar Ratio)	LC_{50} (mg/L)	Reference
ChCl:$ZnCl_2$ (1:2)	4099	[85]
ChCl:urea (1:2)	>100	[85]
ChCl:glycerol (1:3)	>100	[85]
ChCl:EG (1:3)	>100	[85]
ChCl:diethylene glycol (1:2)	>100	[85]
ChCl:TEG (1:3)	>100	[85]
ChCl:fructose (2:1)	>100	[85]
ChCl:glucose (2:1)	>100	[85]
ChCl:malonic acid (1:1)	>100	[85]
ChCl:*p*-toluene sulfonic acid (1:3)	>100	[85]

3.2. Terrestrial Biomodels

Terrestrial environments have the greatest diversity of organisms contributing to decomposition processes; however, these environments can be affected by chemicals that have been deposited in soil. In order to predict how substances impact on soil-dwelling organisms, and the potential damage to soil ecosystems [102], special toxicity studies on representative terrestrial organisms can be carried out. Edaphic organisms are the most widely used biomodel to evaluate the quality of soil; they are important because of their functions in decomposition, cycling, and regularization of nutrients in biological systems [103,104].

Several papers show the toxicity of organic pollutants [103,104], domestic effluents [105], hydrocarbons [106], solvents from biomass [25], and heavy metals [107] in terrestrial environments. However, no existing literature on terrestrial toxicity has been found. It seems that ecotoxicity essays of DESs have not yet been carried out on these types of biomodels. More systematic studies should be carried out in order to ascertain the real environmental impact of chemicals.

3.3. Biodegradability

Biodegradation tests have been widely performed to determine the impact of these substances in the environment. Most of the tests used methodologies in accordance with the OECD 301 D guidelines for the evaluation of aerobic biodegradability [101].

Cholinium-based solvents have been determined to be readily biodegradable [108], showing more than 60% biodegradability in a time lapse between 7 and 14 days. Compounds tested by Kristina Radošević et al. [61] reached 96% biodegradability for ChCl:oxalic acid, 84% for ChCl:glyceline, and 66% for ChCl:glucose. The individual components of mixtures were also classified as readily biodegradable according to the OECD criteria. In the case of reported DESs, it was found that low toxicity is related to higher biodegradability [61,87].

For ILs, the alkyl chain and the lipophilicity of the compound influence its biodegradability, although it has been seen that some compounds show worse biodegradability profiles due to the presence of a larger number of carbons [109]. The effect of hydroxyl groups also improves biodegradability.

Wen et al. determined the biodegradability of eight DESs combining two cholinium salts with four HBDs. It was found that ChCl-based DESs presented a higher biodegradability than ChAc-based ones. However, in this test, only ChCl/urea and ChCl/acetamide compounds could be considered to be readily biodegradable [87].

In other works, the pure DES, glycerine (Gly), reline (Rel), and ethaline (Etha) were considered to be readily biodegradable, but when these mixtures were combined with water, the biodegradability of Gly and Etha decreased, while Rel reached a final percentage of biodegradation notably higher than that of the pure mixture [84]. Since there is not yet any proven explanation as to how the introduction of water into the system affects these compounds' biodegradability, only assumptions can be made. To obtain a full understanding of the influence of water on biodegradability, it is necessary to know how water affects the physicochemical and, therefore, the toxicological and biodegradable properties of these chemicals. DESs are formed via intermolecular hydrogen bonding between the individual components of the mixture. When water is introduced, these intermolecular bonds are broken, and new ones are formed with water [110]. Higher concentrations cause a reduction in inter- and intramolecular interactions, so dissociation can be more easily observed [111]. The dissociation of the mixture compounds could be one of the causes of variation in biodegradability. In general, no clear trend has been reported to date.

4. Conclusions and Future Perspective

The toxicity of a wide variety of DESs has been evaluated. Different biomodels have been used for this purpose, and in vitro studies have been carried out to a higher extent than in vivo studies. In general, several key factors have been identified as being relevant to the cytotoxic effects of some DESs, such as the selection of different HBAs or HBDs, the presence of organic acids as HBDs (pH alteration), the molar ratios of the components, the occurrence of synergistic toxicity, and the incorporation of components that alter metabolic pathways or viscosity levels. Fewer studies have been conducted using in vivo models, and more such studies would be an important step for the future development of DESs and their possible applications in the health field.

Moreover, different sensitivities of diverse biomodels have been found in ecotoxicity studies of DESs. The composition of DESs (e.g., HBA/HBD components or the ability to cross cell barriers, among others) also influences ecotoxicity results. It can be highlighted that the individual toxicity of the initial components cannot be used as a predictive factor of DES ecotoxicity. Nevertheless, the starting materials are indicative of the biodegradability properties of the final DES.

DESs are still promising solvents with many applications, but their safety should be deeply checked prior to their generalized use. A lack of predictive methods for (eco)toxicity behavior has also been identified. As mentioned, the large number of possible combinations

of DES components makes it difficult to characterize and generalize their properties. Future investigations should address this problem. A comprehensive database for the (eco)toxicological properties of DESs, covering several endpoints and as many HBA/HBD combinations as possible, is needed as a first step. Secondly, computational predictive methods must be developed, supporting the variability of components of the mixtures and different prediction conditions.

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Abbreviations

A. fischeri	*Aliivibrio fischeri*
A375	Human malignant melanoma cell line
ALT	Alanine transaminase
AST	Aspartate transaminase
B16F10	Murine melanoma cell line
CaOV3	Human adenocarcinoma ovary cell line
CCO	Channel catfish ovary
ChCl	Choline chloride
D. magna	*Daphnia magna*
DESs	Deep eutectic solvents
EC_{50}	Effective concentration at 50%
IC_{50}	Inhibitory concentration at 50%
EG	Ethylene glycol
Etha	Ethaline
EthaW	Ethaline and water
Gly	Glycerine
GlyW	Glycerine and water
GlyW	Glycerine and water
H413	Human squamous-cell carcinoma, buccal mucosa
HaCat	Human keratinocyte cell line
HBA	Hydrogen bond acceptor
HBD	Hydrogen bond donor

HEK-293	Human embryonic kidney
HelaS3	Human cervical cancer cell line
ILs	Ionic liquids
LC_{50}	Lethal concentration at 50%
MCF-7	Human breast cancer cell line
MNT-1	Human malignant melanoma cell line
MTPB	Methyltriphenylphosphonium bromide
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
N_{1111}	Tetramethylammonium chloride
N_{4444}	Tetrabutylammonium chloride
NADESs	Natural deep eutectic solvents
PC3	Human prostate cancer cell line
R. subcapitata	*Raphidocelis subcapitata*
Rel	Reline
RelW	Reline and water
$scCO_2$	Supercritical carbon dioxide
scH_2O	Supercritical water
$scNH_3$	Supercritical ammonia
TEG	Triethylene glycol
U	Urea
WRL-68	Human cervical cancer cell line
WST-1	Water-soluble tetrazolium salt

References

1. Bart, H.-J. *Extraction of Natural Products from Plants—An Introduction*; John Wiley & Sons, Ltd: Hoboken, NJ, USA, 2011.
2. Mbous, Y.P.; Hayyan, M.; Hayyan, A.; Wong, W.F.; Hashim, M.A.; Looi, C.Y. Applications of deep eutectic solvents in biotechnology and bioengineering—Promises and challenges. *Biotechnol. Adv.* **2017**, *35*, 105–134. [CrossRef] [PubMed]
3. Devi, B.K.; Naraparaju, S.; Soujanya, C.; Gupta, S.D. Green Chemistry and Green Solvents: An Overview. *Curr. Green Chem.* **2020**, *7*, 314–325. [CrossRef]
4. Abdussalam-Mohammed, W.; Qasem Ali, A.; O Errayes, A. Green Chemistry: Principles, Applications, and Disadvantages. *Chem. Methodol.* **2020**, *4*, 408–423. [CrossRef]
5. Khalil, R.; Chaabene, N.; Azar, M.; Malham, I.B.; Turmine, M. Effect of the chain lengthening on transport properties of imidazolium-based ionic liquids. *Fluid Phase Equilib.* **2020**, *503*, 112316. [CrossRef]
6. Shoja, S.M.R.; Abdouss, M.; Beigi, A.A.M. Synthesis and characterization of physicochemical properties of imidazolium-based ionic liquids and their application for simultaneous determination of sulfur compounds. *J. Mol. Struct.* **2021**, *1230*. [CrossRef]
7. Bounsiar, R.; Gascón, I.; Amireche, F.; Lafuente, C. Volumetric properties of three pyridinium-based ionic liquids with a common cation or anion. *Fluid Phase Equilib.* **2020**, *521*, 112732. [CrossRef]
8. Fang, J.; Wu, J.; Li, C.; Li, H.; Yang, Q. Molecular design and comprehensive evaluation of solvents capable of simultaneously extracting multiple active substances. *Sep. Purif. Technol.* **2020**, *245*, 116882. [CrossRef]
9. Hussain, A.; AlAjmi, M.F.; Hussain, I.; Ali, I. Future of Ionic Liquids for Chiral Separations in High-Performance Liquid Chromatography and Capillary Electrophoresis. *Crit. Rev. Anal. Chem.* **2019**, *49*, 289–305. [CrossRef] [PubMed]
10. Kang, M.; Jin, F.; Li, Z.; Song, H.; Chen, J. Research and Application of Supported Ionic Liquids. *Prog. Chem.* **2020**, *32*, 1274. [CrossRef]
11. Cheng, K.; Wu, Q.; Jiang, L.; Liu, M.; Li, C. Protein stability analysis in ionic liquids by 19F NMR. *Anal. Bioanal. Chem.* **2019**, *411*, 4929–4935. [CrossRef] [PubMed]
12. Javaherian, M.; Saghanezhad, S.J. Synthesis, Characterization and Applications of Dicationic Ionic Liquids in Organic Synthesis. *Mini. Rev. Org. Chem.* **2019**, *17*, 450–464. [CrossRef]
13. Liu, R.; Shi, Y.; Zhang, R.; Dai, F. Progress in environmental behaviors and safety of ionic liquids. *Chinese Sci. Bull.* **2019**, *64*, 3158–3164. [CrossRef]
14. Errazquin, D.; Mohamadou, A.; Dupont, L.; De Gaetano, Y.; García, C.B.; Lomba, L.; Giner, B. Ecotoxicity interspecies study of ionic liquids based on phosphonium and ammonium cations. *Environ. Sci. Pollut. Res.* **2021**, 1–11. [CrossRef]
15. Delgado-Mellado, N.; Ayuso, M.; Villar-Chavero, M.M.; García, J.; Rodríguez, F. Ecotoxicity evaluation towards Vibrio fischeri of imidazolium- and pyridinium-based ionic liquids for their use in separation processes. *SN Appl. Sci.* **2019**, *1*, 1–9. [CrossRef]
16. Kaur, N.; Kumar, S.; Shiksha; Gahlay, G.K.; Mithu, V.S. Cytotoxicity and Membrane Permeability of Double-Chained 1,3-Dialkylimidazolium Cations in Ionic Liquids. *J. Phys. Chem. B* **2021**, *125*, 3613–3621. [CrossRef] [PubMed]
17. Leitch, A.C.; Abdelghany, T.M.; Probert, P.M.; Dunn, M.P.; Meyer, S.K.; Palmer, J.M.; Cooke, M.P.; Blake, L.I.; Morse, K.; Rosenmai, A.K.; et al. The toxicity of the methylimidazolium ionic liquids, with a focus on M8OI and hepatic effects. *Food Chem. Toxicol.* **2020**, *136*, 111069. [CrossRef] [PubMed]

18. Shirai, M.; Hiyoshi, N.; Rode, C.V. Stereoselective Aromatic Ring Hydrogenation over Supported Rhodium Catalysts in Supercritical Carbon Dioxide Solvent. *Chem. Rec.* **2019**, *19*, 1926–1934. [CrossRef]
19. Huang, X.; Li, T.; Zhao, J.; Wang, C. Rigorous modeling of solubility of normal alkanes in supercritical CO_2. *Pet. Sci. Technol.* **2019**, *37*, 1008–1015. [CrossRef]
20. Ahmad Jelani, N.A.; Azlan, A.; Khoo, H.E.; Razman, M.R. Fatty acid profile and antioxidant properties of oils extracted from dabai pulp using supercritical carbon dioxide extraction. *Int. Food Res. J.* **2019**, *26*, 1587–1598.
21. Park, S.I.S.H.J.M.S. Extraction of Active Compounds from Angelica gigas using Supercritical Carbon Dioxide and its Physiological Activity. *J. Converg. Inf. Technol.* **2021**, *11*, 206–212. [CrossRef]
22. Zhang, Y.; Zhang, X.; Chi, S.; Li, Z. Pulping of bamboo using supercritical ammonia with recovery and reuse of the ammonia. *BioRes* **2019**, *14*, 5586–5594.
23. Zhou, F.; Hearne, Z.; Li, C.J. Water—the greenest solvent overall. *Curr. Opin. Green Sustain. Chem.* **2019**, *18*, 118–123. [CrossRef]
24. García, J.I.; Pires, E.; Aldea, L.; Lomba, L.; Perales, E.; Giner, B. Ecotoxicity studies of glycerol ethers in Vibrio fischeri: Checking the environmental impact of glycerol-derived solvents. *Green Chem.* **2015**, *17*, 4326–4333. [CrossRef]
25. Lomba, L.; Muñiz, S.; Pino, M.R.; Navarro, E.; Giner, B. Ecotoxicity studies of the levulinate ester series. *Ecotoxicology* **2014**, *23*, 1484–1493. [CrossRef]
26. Pandey, A.; Dhingra, D.; Pandey, S. Hydrogen Bond Donor/Acceptor Cosolvent-Modified Choline Chloride Based Deep Eutectic Solvents. *J. Phys. Chem. B.* **2017**, *121*, 4202–4212. [CrossRef] [PubMed]
27. Abbott, A.P.; Capper, G.; Davies, D.L.; Rasheed, R.K.; Tambyrajah, V. Novel solvent properties of choline chloride/urea mixtures. *Chem. Commun.* **2003**, *1*, 70–71. [CrossRef] [PubMed]
28. Płotka-Wasylka, J.; de la Guardia, M.; Andruch, V.; Vilková, M. Deep eutectic solvents vs ionic liquids: Similarities and differences. *Microchem. J.* **2020**, *159*, 105539. [CrossRef]
29. Ratti, R. Ionic Liquids: Synthesis and Applications in Catalysis. *Adv. Chem.* **2014**, 1–16. [CrossRef]
30. Nakhle, L.; Kfoury, M.; Mallard, I.; Landy, D.; Greige-Gerges, H. Microextraction of bioactive compounds using deep eutectic solvents: A review. *Environ. Chem. Lett.* **2021**, *19*, 3747–3759. [CrossRef]
31. Ruesgas-Ramón, M.; Figueroa-Espinoza, M.C.; Durand, E. Application of Deep Eutectic Solvents (DES) for Phenolic Compounds Extraction: Overview, Challenges, and Opportunities. *J. Agric. Food Chem.* **2017**, *65*, 3591–3601. [CrossRef]
32. Lin, Z.; Zhang, Y.; Zhao, Q.; Chen, A.; Jiao, B. Ultrasound-assisted dispersive liquid-phase microextraction by solidifying L-menthol-decanoic acid hydrophobic deep eutectic solvents for detection of five fungicides in fruit juices and tea drinks. *J. Sep. Sci.* 2021. [CrossRef]
33. Habila, M.A.; Alabdullkarem, E.A.; Alothman, Z.A.; Yilmaz, E.; Soylak, M. Thiomalic acid/ferric chloride-based deep eutectic solvent for microextraction of chromium in natural water samples prior to FAAS analysis. *Int. J. Environ. Anal. Chem.* **2020**, 1–9. [CrossRef]
34. Kurtulbaş, E.; Pekel, A.G.; Toprakçı, İ.; Özçelik, G.; Bilgin, M.; Şahin, S. Hydrophobic carboxylic acid based deep eutectic solvent for the removal of diclofenac. *Biomass Convers. Biorefinery* **2020**, 1–9. [CrossRef]
35. Troter, D.Z.; Todorović, Z.B.; Dokić-Stojanović, D.R.; Stamenković, O.S.; Veljković, V.B. Application of ionic liquids and deep eutectic solvents in biodiesel production: A review. *Renew. Sustain. Energy Rev.* **2016**, *61*, 473–500. [CrossRef]
36. Malolan, R.; Gopinath, K.P.; Vo, D.-V.N.; Jayaraman, R.S.; Adithya, S.; Ajay, P.S.; Arun, J. Green ionic liquids and deep eutectic solvents for desulphurization, denitrification, biomass, biodiesel, bioethanol and hydrogen fuels: A review. *Environ. Chem. Lett.* **2020**, *19*, 1001–1023. [CrossRef]
37. Lima, F.; Branco, L.C.; Silvestre, A.J.D.; Marrucho, I.M. Deep desulfurization of fuels: Are deep eutectic solvents the alternative for ionic liquids? *Fuel* **2021**, *293*, 120297. [CrossRef]
38. Zhang, T.; Doert, T.; Wang, H.; Zhang, S.; Ruck, M. Inorganic Synthesis Based on Reactions of Ionic Liquids and Deep Eutectic Solvents. *Angew. Chemie Int. Ed.* **2021**, *60*, 22148–22165. [CrossRef]
39. Wang, Y.; Yao, Q.-X.; He, J.-R.; Liang, Z.-H.; Li, X.; Cheng, H.; Li, L.-L. L-proline-catalyzed Knoevenagel reaction promoted by choline chloride-based deep eutectic solvents. *Biomass Convers. Biorefinery* **2021**, 1–7. [CrossRef]
40. Liu, Y.; Friesen, J.B.; McAlpine, J.B.; Lankin, D.C.; Chen, S.-N.; Pauli, G.F. Natural Deep Eutectic Solvents: Properties, Applications, and Perspectives. *J. Nat. Prod.* **2018**, *81*, 679–690. [CrossRef]
41. Ghobadi, R.; Divsalar, A. Enzymatic behavior of bovine liver catalase in aqueous medium of sugar based deep eutectic solvents. *J. Mol. Liq.* **2020**, *310*. [CrossRef]
42. Wang, Y.; Cheng, H.; He, J.-R.; Yao, Q.-X.; Li, L.-L.; Liang, Z.-H.; Li, X. Enzymes-Catalyzed Knoevenagel Condensation Promoted by Ionic Liquid and Deep Eutectic Solvent. *Catal. Lett.* **2021**, 1–9. [CrossRef]
43. Yang, Z. Natural Deep Eutectic Solvents and Their Applications in Biotechnology. *Adv. Biochem. Eng. Biotechnol.* **2018**, *168*, 31–59. [CrossRef]
44. Silva, J.M.; Pereira, C.V.; Mano, F.; Silva, E.; Castro, V.I.B.; Sá-Nogueira, I.; Reis, R.L.; Paiva, A.; Matias, A.A.; Duarte, A.R.C. Therapeutic Role of Deep Eutectic Solvents Based on Menthol and Saturated Fatty Acids on Wound Healing. *ACS Appl. Bio Mater.* **2019**, *2*, 4346–4355. [CrossRef] [PubMed]
45. Pradeepkumar, P.; Rajan, M.; Almoallim, H.S.; Alharbi, S.A. Targeted Delivery of Doxorubicin in HeLa Cells Using Self-Assembled Polymeric Nanocarriers Guided by Deep Eutectic Solvents. *ChemistrySelect* **2021**, *6*, 7232–7241. [CrossRef]

46. Arriaga, S.; Aitor, A. Advances and Applications of Partitioning Bioreactors. In *Advances in Chemical Engineering*; Elsevier: Amsterdam, The Netherlands, 2019.
47. Álvarez, M.S.; Zhang, Y. Sketching neoteric solvents for boosting drugs bioavailability. *J. Control. Release* **2019**, *311–312*, 225–232. [CrossRef]
48. Kim, J.; Shi, Y.; Kwon, C.J.; Gao, Y.; Mitragotri, S. A Deep Eutectic Solvent-Based Approach to Intravenous Formulation. *Adv. Healthc. Mater.* **2021**, *10*, 2100585. [CrossRef]
49. Gutiérrez, A.; Atilhan, M.; Aparicio, S. Theoretical Study on Deep Eutectic Solvents as Vehicles for the Delivery of Anesthetics. *J. Phys. Chem. B* **2020**, *124*, 1794–1805. [CrossRef]
50. Golgoun, S.; Mokhtarpour, M.; Shekaari, H. Solubility Enhancement of Betamethasone, Meloxicam and Piroxicam by Use of Choline-Based Deep Eutectic Solvents. *Pharm. Sci.* **2020**, *27*, 86–101. [CrossRef]
51. Mustafa, N.R.; Spelbos, V.S.; Witkamp, G.-J.; Verpoorte, R.; Choi, Y.H. Solubility and Stability of Some Pharmaceuticals in Natural Deep Eutectic Solvents-Based Formulations. *Molecules* **2021**, *26*, 2645. [CrossRef]
52. Atilhan, M.; Costa, L.T.; Aparicio, S. On the behaviour of aqueous solutions of deep eutectic solvents at lipid biomembranes. *J. Mol. Liq.* **2017**, *247*, 116–125. [CrossRef]
53. Florindo, C.; Branco, L.C.; Marrucho, I.M. Quest for Green-Solvent Design: From Hydrophilic to Hydrophobic (Deep) Eutectic Solvents. *ChemSusChem* **2019**, *12*, 1549–1559. [CrossRef] [PubMed]
54. Kovács, A.; Neyts, E.C.; Cornet, I.; Wijnants, M.; Billen, P. Modeling the Physicochemical Properties of Natural Deep Eutectic Solvents. *ChemSusChem* **2020**, *13*, 3789–3804. [CrossRef] [PubMed]
55. Alkhatib, I.I.I.; Bahamon, D.; Llovell, F.; Abu-Zahra, M.R.M.; Vega, L.F. Perspectives and guidelines on thermodynamic modelling of deep eutectic solvents. *J. Mol. Liq.* **2020**, *298*, 112183. [CrossRef]
56. Giner, B.; Lafuente, C.; Lapeña, D.; Errazquin, D.; Lomba, L. QSAR study for predicting the ecotoxicity of NADES towards Aliivibrio fischeri. Exploring the use of mixing rules. *Ecotoxicol. Environ. Saf.* **2020**, *191*, 110004. [CrossRef] [PubMed]
57. Sekharan, T.R.; Chandira, R.M.; Tamilvanan, S.; Rajesh, S.C.; Venkateswarlu, B.S. Deep Eutectic Solvents as an Alternate to Other Harmful Solvents. *Review* **2022**, *12*, 847–860. [CrossRef]
58. Paiva, A.; Craveiro, R.; Aroso, I.; Martins, M.; Reis, R.L.; Duarte, A.R.C. Natural Deep Eutectic Solvents – Solvents for the 21st Century. *ACS Sustain. Chem. Eng.* **2014**, *2*, 1063–1071. [CrossRef]
59. Hayyan, M.; Mbous, Y.P.; Looi, C.Y.; Wong, W.F.; Hayyan, A.; Salleh, Z.; Mohd-Ali, O. Natural deep eutectic solvents: Cytotoxic profile. *Springerplus* **2016**, *5*, 1–12. [CrossRef] [PubMed]
60. Hayyan, M.; Looi, C.Y.; Hayyan, A.; Wong, W.F.; Hashim, M.A. In Vitro and In Vivo Toxicity Profiling of Ammonium-Based Deep Eutectic Solvents. *PLoS ONE* **2015**, *10*, e0117934. [CrossRef]
61. Radošević, K.; Bubalo, M.C.; Srček, V.G.; Grgas, D.; Landeka Dragičević, T.; Redovniković, I.R. Evaluation of toxicity and biodegradability of choline chloride based deep eutectic solvents. *Ecotoxicol. Environ. Saf.* **2014**, *112*, 46–53. [CrossRef] [PubMed]
62. Radošević, K.; Ćurko, N.; Gaurina Srček, V.; Cvjetko Bubalo, M.; Tomašević, M.; Kovačević Ganić, K.; Radojčić Redovniković, I. Natural deep eutectic solvents as beneficial extractants for enhancement of plant extracts bioactivity. *LWT* **2016**, *73*, 45–51. [CrossRef]
63. Radošević, K.; Čanak, I.; Panić, M.; Markov, K.; Bubalo, M.C.; Frece, J.; Srček, V.G.; Redovniković, I.R. Antimicrobial, cytotoxic and antioxidative evaluation of natural deep eutectic solvents. *Environ. Sci. Pollut. Res.* **2018**, *25*, 14188–14196. [CrossRef] [PubMed]
64. Mbous, Y.P.; Hayyan, M.; Wong, W.F.; Looi, C.Y.; Hashim, M.A. Unraveling the cytotoxicity and metabolic pathways of binary natural deep eutectic solvent systems. *Sci. Rep.* **2017**, *7*, 1–14. [CrossRef] [PubMed]
65. Ahmadi, R.; Hemmateenejad, B.; Safavi, A.; Shojaeifard, Z.; Mohabbati, M.; Firuzi, O. Assessment of cytotoxicity of choline chloride-based natural deep eutectic solvents against human HEK-293 cells: A QSAR analysis. *Chemosphere* **2018**, *209*, 831–838. [CrossRef] [PubMed]
66. 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. Cytotoxicity profiling of deep eutectic solvents to human skin cells. *Sci. Rep.* **2019**, *9*, 1–9. [CrossRef] [PubMed]
67. Florindo, C.; Oliveira, F.S.; Rebelo, L.P.N.; Fernandes, A.M.; Marrucho, I.M. Insights into the Synthesis and Properties of Deep Eutectic Solvents Based on Cholinium Chloride and Carboxylic Acids. *ACS Sustain. Chem. Eng.* **2014**, *2*, 2416–2425. [CrossRef]
68. Mitar, A.; Panić, M.; Kardum, J.P.; Halambek, J.; Sander, A.; Kučan, K.Z.; Redovniković, I.R.; Radošević, K. Physicochemical Properties, Cytotoxicity, and Antioxidative Activity of Natural Deep Eutectic Solvents Containing Organic Acid. *Chem. Biochem. Eng. Q.* **2019**, *33*, 1–18. [CrossRef]
69. Kareem, M.A.; Mjalli, F.S.; Hashim, M.A.; AlNashef, I.M. Phosphonium-Based Ionic Liquids Analogues and Their Physical Properties. *J. Chem. Eng. Data* **2010**, *55*, 4632–4637. [CrossRef]
70. Zhao, B.-Y.; Xu, P.; Yang, F.-X.; Wu, H.; Zong, M.-H.; Lou, W.-Y. Biocompatible Deep Eutectic Solvents Based on Choline Chloride: Characterization and Application to the Extraction of Rutin from Sophora japonica. *ACS Sustain. Chem. Eng.* **2015**, *3*, 2746–2755. [CrossRef]
71. Skulcova, A.; Russ, A.; Jablonsky, M.; Sima, J. The pH behavior of seventeen deep eutectic solvents. *BioResources* **2019**, *13*, 5042–5051. [CrossRef]
72. Hayyan, A.; Mjalli, F.S.; Alnashef, I.M.; Al-Wahaibi, T.; Al-Wahaibi, Y.M.; Hashim, M.A. Fruit sugar-based deep eutectic solvents and their physical properties. *Thermochim. Acta* **2012**, *541*, 70–75. [CrossRef]

73. Hayyan, M.; Hashim, M.A.; Al-Saadi, M.A.; Hayyan, A.; Alnashef, I.M.; Mirghani, M.E.S. Assessment of cytotoxicity and toxicity for phosphonium-based deep eutectic solvents. *Chemosphere* **2013**, *93*, 455–459. [CrossRef] [PubMed]
74. Zhang, Q.; Vigier, K.D.O.; Royer, S.; Jérôme, F. Deep eutectic solvents: Syntheses, properties and applications. *Chem. Soc. Rev.* **2012**, *41*, 7108–7146. [CrossRef] [PubMed]
75. Dai, Y.; Van Spronsen, J.; Witkamp, G.-J.; Verpoorte, R.; Choi, Y.H. Natural deep eutectic solvents as new potential media for green technology. *Anal. Chim. Acta* **2013**, *766*, 61–68. [CrossRef]
76. Liu, T.; Liu, X.; Spring, D.R.; Qian, X.; Cui, J.; Xu, Z. Quantitatively mapping cellular viscosity with detailed organelle information via a designed PET fluorescent probe. *Sci. Rep.* **2014**, *4*, 5418. [CrossRef] [PubMed]
77. Barile, F.A. *Clinical Toxicology: Principles and Mechanisms*; CRC Press: Boca Raton, FL, USA, 2010; ISBN 9781420092264.
78. Benlebna, M.; Ruesgas-Ramón, M.; Bonafos, B.; Fouret, G.; Casas, F.; Coudray, C.; Durand, E.; Cruz Figueroa-Espinoza, M.; Feillet-Coudray, C. Toxicity of Natural Deep Eutectic Solvent Betaine:Glycerol in Rats. *J. Agric. Food Chem.* **2018**, *66*, 6205–6212. [CrossRef] [PubMed]
79. Chen, J.; Wang, Q.; Liu, M.; Zhang, L. The effect of deep eutectic solvent on the pharmacokinetics of salvianolic acid B in rats and its acute toxicity test. *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.* **2017**, *1063*, 60–66. [CrossRef] [PubMed]
80. United States Environmental Protection Agency. *Using Toxicity Tests in Ecological Risk Assessment. ECO Update*; United States Environmental Protection Agency: Washington, DC, USA, 1994; Volume 2, number 1.
81. Lagesson, A.; Fahlman, J.; Brodin, T.; Fick, J.; Jonsson, M.; Byström, P.; Klaminder, J. Bioaccumulation of five pharmaceuticals at multiple trophic levels in an aquatic food web - Insights from a field experiment. *Sci. Total Environ.* **2016**, *568*, 208–215. [CrossRef]
82. de Morais, P.; Gonçalves, F.; Coutinho, J.A.P.; Ventura, S.P.M. Ecotoxicity of Cholinium-Based Deep Eutectic Solvents. *ACS Sustain. Chem. Eng.* **2015**, *3*, 3398–3404. [CrossRef]
83. El Achkar, T.; Fourmentin, S.; Greige-Gerges, H. Deep eutectic solvents: An overview on their interactions with water and biochemical compounds. *J. Mol. Liq.* **2019**, *288*, 111028. [CrossRef]
84. Lapeña, D.; Errazquin, D.; Lomba, L.; Lafuente, C.; Giner, B. Ecotoxicity and biodegradability of pure and aqueous mixtures of deep eutectic solvents: Glyceline, ethaline, and reline. *Environ. Sci. Pollut. Res.* **2020**, *28*, 8812–8821. [CrossRef]
85. Juneidi, I.; Hayyan, M.; Mohd Ali, O. Toxicity profile of choline chloride-based deep eutectic solvents for fungi and Cyprinus carpio fish. *Environ. Sci. Pollut. Res.* **2016**, *23*, 7648–7659. [CrossRef]
86. Spasiano, D.; Siciliano, A.; Race, M.; Marotta, R.; Guida, M.; Andreozzi, R.; Pirozzi, F. Biodegradation, ecotoxicity and UV254/H2O2 treatment of imidazole, 1-methyl-imidazole and N,N′-alkyl-imidazolium chlorides in water. *Water Res.* **2016**, *106*, 450–460. [CrossRef] [PubMed]
87. Wen, Q.; Chen, J.X.; Tang, Y.L.; Wang, J.; Yang, Z. Assessing the toxicity and biodegradability of deep eutectic solvents. *Chemosphere* **2015**, *132*, 63–69. [CrossRef] [PubMed]
88. Hayyan, M.; Hashim, M.A.; Hayyan, A.; Al-Saadi, M.A.; AlNashef, I.M.; Mirghani, M.E.S.; Saheed, O.K. Are deep eutectic solvents benign or toxic? *Chemosphere* **2013**, *90*, 2193–2195. [CrossRef]
89. 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. Unraveling the ecotoxicity of deep eutectic solvents using the mixture toxicity theory. *Chemosphere* **2018**, *212*, 890–897. [CrossRef]
90. Abbas, M.; Adil, M.; Ehtisham-Ul-Haque, S.; Munir, B.; Yameen, M.; Ghaffar, A.; Shar, G.A.; Asif Tahir, M. Vibrio fischeri bioluminescence inhibition assay for ecotoxicity assessment: A review. *Sci. Total Environ.* **2018**, *626*, 1295–1309. [CrossRef]
91. Jafari, M.; Keshavarz, M.H.; Salek, H. A simple method for assessing chemical toxicity of ionic liquids on Vibrio fischeri through the structure of cations with specific anions. *Ecotoxicol. Environ. Saf.* **2019**, *182*, 109429. [CrossRef] [PubMed]
92. Domínguez De María, P.; Maugeri, Z.; Flitsch, S.; Gotor, V. Ionic liquids in biotransformations: From proof-of-concept to emerging deep-eutectic-solvents This review comes from a themed issue on Biocatalysis and Biotransformation Edited Ionic liquids: Context and development. *Curr. Opin. Chem. Biol.* **2010**, *15*, 220–225. [CrossRef]
93. Egorova, K.S.; Ananikov, V.P. Toxicity of Ionic Liquids: Eco(cyto)activity as Complicated, but Unavoidable Parameter for Task-Specific Optimization. *ChemSusChem* **2014**, *7*, 336–360. [CrossRef] [PubMed]
94. Matzke, M.; Arning, J.; Ranke, J.; Jastorff, B.; Stolte, S. Design of Inherently Safer Ionic Liquids: Toxicology and Biodegradation. *Handb. Green Chem.* **2010**, *6*, 233–298. [CrossRef]
95. Macário, I.P.E.; Ventura, S.P.M.; Pereira, J.L.; Gonçalves, A.M.M.; Coutinho, J.A.P.; Gonçalves, F.J.M. The antagonist and synergist potential of cholinium-based deep eutectic solvents. *Ecotoxicol. Environ. Saf.* **2018**, *165*, 597–602. [CrossRef] [PubMed]
96. Villegas-Navarro, A.; Romero González, M.C.; Rosas López, E.; Domínguez Aguilar, R.; Sachetin Marçal, W. Evaluation of Daphnia magna as an indicator of toxicity and treatment efficacy of textile wastewaters. *Environ. Int.* **1999**, *25*, 619–624. [CrossRef]
97. Lal, H.; Misra, V.; Viswanathan, P.N.; Murti, A.R.K. The Water Flea (Daphnia magna) as a Sensitive Indicator for the Assessment of Toxicity of Synthetic Detergents. *Ecotoxicol. Environ. Saf.* **1984**, *8*, 447–450. [CrossRef]
98. Hartl, M.; Humpf, H.U. Toxicity assessment of fumonisins using the brine shrimp (Artemia salina) bioassay. *Food Chem. Toxicol.* **2000**, *38*, 1097–1102. [CrossRef]
99. Erzinger, G.S.; Schmoeller, F.; Pinto, L.H.; Américo, L.; Hemmersbach, R.; Hauslage, J.; Häder, D.P. Bioluminescence systems in environmental biosensors. *Bioassays Adv. Methods Appl.* **2018**, 241–262. [CrossRef]
100. McCormick, P.V.; Cairns, J. Algae as indicators of environmental change. *J. Appl. Phycol.* **1994**, *6*, 509–526. [CrossRef]
101. Passino, D.R.M.; Smith, S.B. Acute bioassays and hazard evaluation of representative contaminants detected in great lakes fish. *Environ. Toxicol. Chem.* **1987**, *6*, 901–907. [CrossRef]

102. Fantke, P.; Bijster, M.; Guignard, C.; Hauschild, M.Z.; Huijbregts, M.A.J.; Jolliet, O.; Kounina, A.; Magaud, V.; Margni, M.; McKone, T.E.; et al. USEtox 2.0 Documentation (Version 1). USEtox International Center. 2017, p. 208. Available online: https://orbit.dtu.dk/en/publications/usetox-20-documentation-version-100 (accessed on 11 August 2021). [CrossRef]
103. Clasen, B.; de Moura Lisbôa, R. Ecotoxicological Tests as a Tool to Assess the Quality of the Soil. *Soil Contam. Altern. Sustain. Dev.* **2019**, 13–33. [CrossRef]
104. Baretta, D.; JCP JCPS; Segat, J.C.; Geremia, E.V.; LCI OF; Alves, M.V. Edafic fauna and soil quality. In *Topics in Soil Science*; SBCS: Viçosa, MG, Brazil, 2011; pp. 119–170; ISBN 1519-3934.
105. Natal-Da-Luz, T.; Ojeda, G.; Pratas, J.; Van Gestel, C.A.M.; Sousa, J.P. Toxicity to Eisenia andrei and Folsomia candida of a metal mixture applied to soil directly or via an organic matrix. *Ecotoxicol. Environ. Saf.* **2011**, *74*, 1715–1720. [CrossRef] [PubMed]
106. Gainer, A.; Hogan, N.; Siciliano, S.D. Soil invertebrate avoidance behavior identifies petroleum hydrocarbon contaminated soils toxic to sensitive plant species. *J. Hazard. Mater.* **2019**, *361*, 338–347. [CrossRef] [PubMed]
107. Wan, Y.; Jiang, B.; Wei, D.; Ma, Y. Ecological criteria for zinc in Chinese soil as affected by soil properties. *Ecotoxicol. Environ. Saf.* **2020**, *194*. [CrossRef]
108. Hou, X.-D.; Liu, Q.-P.; Smith, T.J.; Li, N.; Zong, M.-H. Evaluation of Toxicity and Biodegradability of Cholinium Amino Acids Ionic Liquids. *PLoS ONE* **2013**, *8*, e59145. [CrossRef] [PubMed]
109. Juneidi, I.; Hayyan, M.; Hashim, M.A. Evaluation of toxicity and biodegradability for cholinium-based deep eutectic solvents. *RSC Adv.* **2015**, *5*, 83636–83647. [CrossRef]
110. Gabriele, F.; Chiarini, M.; Germani, R.; Tiecco, M.; Spreti, N. Effect of water addition on choline chloride/glycol deep eutectic solvents: Characterization of their structural and physicochemical properties. *J. Mol. Liq.* **2019**, *291*, 111301. [CrossRef]
111. Hammershøj, R.; Birch, H.; Redman, A.D.; Mayer, P. Mixture Effects on Biodegradation Kinetics of Hydrocarbons in Surface Water: Increasing Concentrations Inhibited Degradation whereas Multiple Substrates Did Not. *Environ. Sci. Technol.* **2019**, *53*, 3087–3094. [CrossRef] [PubMed]