Review DOI: 10.1515/aiht-2017-68-2979

Toxicity mechanisms of ionic liquids

Marina Cvjetko Bubalo, Kristina Radošević, Ivana Radojčić Redovniković, Igor Slivac, and Višnja Gaurina Srček

Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia

[Received in April 2017; Similarity Check in April 2017; Accepted in August 2017]

Over the past three decades a growing awareness of environmental protection prompted the development of so-called green and sustainable technologies. Therefore, academic and wider community intensively explores new chemicals and safer, more energy efficient processes based on a rational compromise between economic, social, and environmental requirements. Due to low volatility and stability, ionic liquids emerged as a potential replacement for traditional volatile and harmful organic solvents. Various studies have been carried out to validate the green character of ionic liquids, whereby data published suggest that these compounds, due to their relatively high toxicity and poor biodegradability, could have an extremely negative impact on the environment. This paper presents the current knowledge on the toxicity of ionic liquids, with a special emphasis on the mechanisms by which this group of compounds causes changes in the morphology and physiology of organisms at different organisational levels of the ecosystem.

KEY WORDS: *green solvents; test-systems*

Almost two thirds of all industrial emissions are caused by volatile organic solvents which generate numerous adverse effects to the environment such as global climate changes and air pollution. Hence, the design of new environmentally friendly and harmless solvents is of great importance. According to the Green Chemistry concept, an ideal solvent should be of low volatility, chemically and physically stable, recyclable and reusable, and easy to use (1). Due to low volatility, non-flammability, high thermal, chemical and electrochemical stability, and in particular their capability of being recycled, ionic liquids gained considerable attention as potential green replacements for conventional organic solvents in different processes and technologies (1, 2). Great interest these solvents have aroused is reflected also in a considerable number of publications (> 50.000) developed over the last 15 years. These publications are related to ionic liquids' preparation; application (in chemistry, chemical engineering, and biotechnology); physical-chemical and biological characterisation; and impact on the environment (source: *Web of Science*). Since 2010, comprehensive studies on ionic liquids environmental impact including (bio) degradability and toxicity have been published (3-7). The purpose of this review is to present recent data on the biological effects of these compounds, with a special emphasis on their morphological and physiological changes in different biological systems, from bacteria to animal cells.

Correspondence to: Višnja Gaurina Srček, Full Professor, University of Zagreb, Faculty of Food Technology and Biotechnology, Pierottijeva 6, Zagreb, Croatia; E-mail: *vgaurinasrcek@pbf.hr*

Ionic liquids

Ionic liquids (ILs) are organic salts composed of ions that are in liquid state at standard conditions. In the literature, terms such as molten salts, salts of organic liquids, or fused salts are also used to describe this group of compounds. ILs consist of cations which are variously substituted organic compounds of low symmetry containing a positively charged nitrogen, sulfur, or phosphorus atom (*e.g.* N, N'-dialkylimidazolium, N-alkylpyridinium, alkylammonium, alkylphosphoniumand thiazolium cation) paired with different inorganic or organic anions such as halides (e.g. Br^-, Cl^-), tetrafluoroborate (BF_4^-), hexafluorophosphate (PF_6^-), bis (trifluoromethylsulfonyl) imide ($(CF_3SO_3)_2N^-$), acetate ($CH_3CO_2^-$), and dicyanamide ($N(CN)_2^-$). Typical structures of ILs are shown in Figure 1.

The unique properties of ILs, such as high solubility, high thermal, chemical and electrochemical stability, and non-flammability, make them suitable for use in different areas such as process technology, organic synthesis, electrochemistry and analytical chemistry. In the field of biotechnology, ILs can be applied as substitutes for hazardous volatile organic solvents in (bio)catalytic processes, as well as in the extraction and separation processes of biologically active compounds (8). The combinations of various cations and anions in ILs are tremendous, moreover, it is assumed that approximately 10^{18} ILs with different chemical and physical properties (melting point, solubility, acidity, hydrophobicity, density, viscosity, and refractive index) can be synthesised in order to obtain the ILs with optimal characteristics for a particular application (*e.g.* to increase their extraction capacity of certain compounds, influence (bio)catalyst's stability and

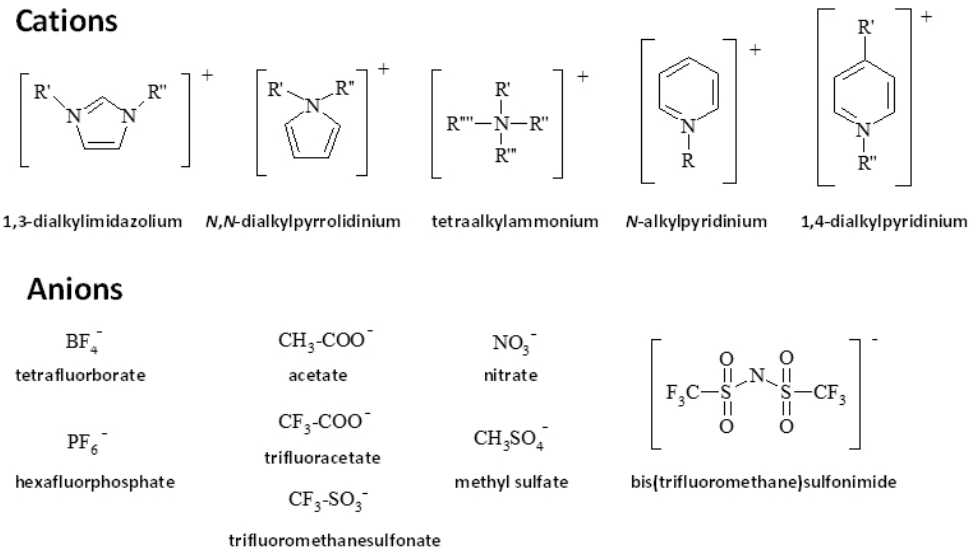

Figure 1 *Typical cations and anions in ionic liquids*

activity). This property of ILs to meet the requirement of their application makes them really unique solvents and for this reason they are often referred to as designer solvents (9).

Technological, environmental, and economic benefits of ILs are primarily based on their low volatility (Boiling Point (bp) at 250-450 °C), meaning that they can be easily recycled, enabling the amount of waste generated during the technological process to be reduced. Among the available technologies, regeneration of ILs can be performed by conventional operations such as distillation and extraction. Volatile products can be easily isolated from ILs by distillation under mild conditions, while low volatility products can be separated by extraction or membrane processes, such as nanofiltration and evaporation (10).

Although ILs are defined as environmentally friendly solvents due to the aforementioned properties, they can still reach soils, surface waters, and groundwater by accidental spills or effluents. Later, they accumulate in the environment and higher organisms and adversely affect homeostasis. Accordingly, sorption, biodegradability, and toxicity of ILs, as well as their degradation products, are of high importance for their impact and final fate in the environment (5, 6). Assessment of ILs toxicity is usually performed in various test organisms (*in vivo*) or tissues, animal cells and cell fractions (*in vitro*). Recently, toxicity of ILs was comprehensively investigated and assessed on different test organisms such as bacteria (*e.g., Vibrio fischeri, Escherichia coli, Staphylococcus aureus, Photobacterium phosphoreum, and Bacillus subtilis*); yeast (*Saccharomyces cerevisiae);* algae (*e.g. Oocystis submarine, Pseudokirchneriella subcapitata,* and *Cyclotella meneghiniana*); nematode (*Caenorhabditis elegans)*, water snail *(Phys acuta)*, water flea *(Daphnia magna)*, kelp *(Ulva lactuca),* invertebrates (*Folsomia candida*, *Lemna minor*), fish *(Danio rerio)*, plants (*Lepidium sativum* – cress, *Hordeum vulgare* - spring barley, *Triticum aestivum* L. – wheat, and *Raphanus sativus* - radish), as well as in different mammalian and fish cell cultures (3, 4). In general, different test systems have shown different degrees of sensitivity to ILs indicating that the trophic level of test systems determines its susceptibility to ILs (7). Furthermore, all of the abovementioned studies indicate a strong relationship between ILs' chemical structure and degree of toxicity. Toxicity was mainly dependent on the nature of the cation i.e. both the nature of the cation (*e.g.* type of heterocyclic ring) and the length of the alkyl side chain of ILs influenced the degree of toxic effects. For example, it was observed that the increase in the length of the alkyl chain on ILs heterocyclic ring significantly increased their toxicity in most test-systems (5). Also, ILs with the imidazolium heterocyclic ring were more toxic than ILs with the morpholinium or pyridinium ring. According to current studies, toxicity of ILs is less affected by the structure of the anion (4). However, a slight difference in ILs toxicity containing different anions was reported, implying that PF_6, BF_4, and $(CF_3SO_3)_2N^-$ anions were more toxic in comparison with Br^-, Cl^-, NO_3^-, and CH_3COO^-. Egorova et al. (8) reported that behaviour and impact of the ILs' anion is dependent on their interactions with water molecules. For example, small anions as hydrophilic Cl^- remained in solution, whereas a more hydrophobic anion like PF_6 formed a film at the lipid /water boundary. Furthermore, the hydrolysis of fluorinated anion and formation of fluorides, potent inhibitors of Na^+ K^+ ATPase (an enzyme which participates in the maintainance of the cell's static electric potential and which regulates cellular transport volume), also contribute to their higher toxicity (11).

Toxicity mechanisms of ionic liquids

Most authors pointed out that the toxic effects of ILs were directly related to disruption of cell membrane. Long alkyl chains, often presented in the cationic part of ILs, increase the lipophilic character of the IL molecule, meaning that their ability to interact with both cell membrane phospholipid bilayer and hydrophobic domains of

membrane proteins also increases. These interactions lead to disruption of membrane physiological functions, causing the leakage of cellular content and, consequently to cell death. A strong correlation between ILs toxicity and the length of the alkyl chain was found in various cell lines such as IPC-81, CCO, and HeLa (12-14), as well as in other unicellular and multicellular test organisms such as *Caenorhabditis elegans* (15), *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Saccharomyces cerevisiae* (16), *Scenedesmus quadricauda,* and *Chlamydomonas reinhardtii* (17), *Vibrio fischeri,* and *Daphnia magna* (18). In general, the relationship between lipophilicity and toxicity is a common phenomenon called narcosis (in mammals) or basic toxicity (in aquatic organisms). This effect is based on the fact that an increased transport of a substance across the membrane increases its concentration in the cell and thus triggers excessive toxic effects (12). In plants, chloroplasts also possess a phospholipid bilayer which can be affected by ILs, leading to inhibition of photosynthesis (18).

To confirm the relationship between ILs toxicity and membrane damage, Petkovic et al. (19) examined the impact of alkyltributylphosphonium chlorides on membrane integrity in conidia of the filamentous fungus *Aspergillus nidulans* by fluorescence microscopy and propidium iodide staining. Conidia were incubated with different concentrations of ILs (0.01-100 mM) during 1 h and stained with propidium iodide. The highest tested concentration (100 mM) of ILs with long alkyl chain of cation (n=8 and 12) caused membrane damage in almost 90 % cells. At the same time, only 25 % of damaged membranes were detected when conidia were exposed to the same concentration of ILs with a shorter alkyl chain on the cationic part (n=1 and 4) (Figure 2). This result clearly demonstrated the relationship between cell membrane damage and ILs alkyl chain length.

To determine if ILs are incorporated into the cell membrane or accumulated in the cell, Cornmell et al. (20) performed chemical analysis of *Escherichia coli* cells by infrared spectroscopy with Fourier transformation (FT-IR) after exposure of cells to trihexyltetradecylphosphonium bis(trifluoromethylsulfonyl)imide. In general, FT-IR analysis is a readily applicable analytical method that provides information on the composition and amount of individual chemicals in the analysed sample by using interferometric methods on the resulting infrared spectra and mathematical processing by Fourier transformation algorithms. This technique can determine the metabolic fingerprint of a sample, which is very useful for identifying microorganisms or monitoring their physiological state. ILs have characteristic IR spectra that differ significantly from the spectra of cellular components, allowing their simple identification in cell content. In this work, *E. coli* was cultivated in the presence of trihexyltetradecylphosphonium bis(trifluoromethylsulfonyl) imide (23 %, v/v), and after cultivation, cytoplasmic and membrane fractions of cells were separated. IL was detected only in a membrane fraction of the cells (Figure 3), which is the first direct evidence that IL is really settled between lipid membranes. Again, it has been confirmed that the toxicity of ILs is associated with their lipophilic character and membrane damage.

Once pollutants, such as ILs, enter the human or animal bodies, they can interact with various enzymes and affect their activities. Consequently, this causes pathological changes, where enzymatic inhibition assays can provide essential data to predict the impact of ILs on humans. Therefore, to explain the mechanisms of ILs' toxicity to living organisms, several authors have studied the effects of ILs on the enzymes that are essential for a normal function of metabolism (21-23). The impact of (dimethylamino)pyridinium, pyridinium, imidazolium, morpholinium, piperidinium, pyrrolidinium, and quaternary

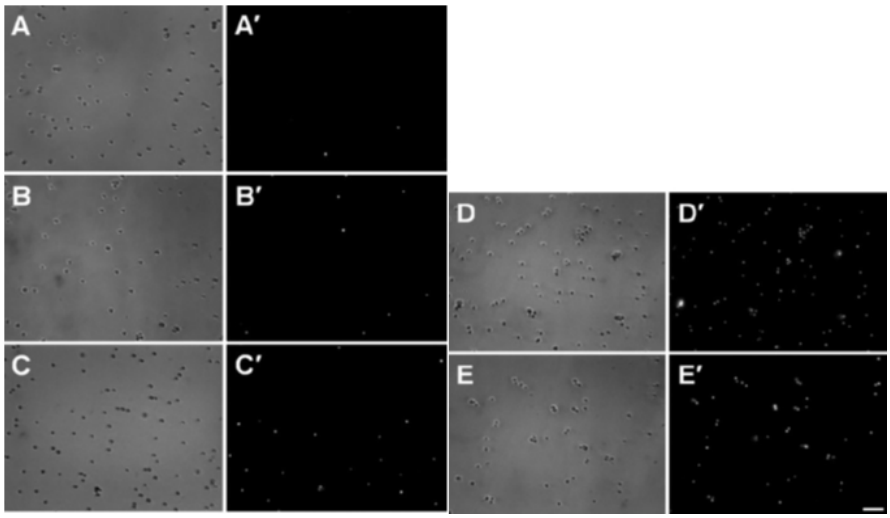

Figure 2 *Effects of alkyltributylphosphonium chlorides (100 mM) on membrane integrity in conidia of the filamentous fungus* Aspergillus nidulans *by microscopy. Left columns (A–E) show conidia population (phase-contrast microscopy); right columns (A'–E') show conidia stained by propidium-iodide (fluorescent microscpy): (A, A') control; (B, B') n=1; (C, C') n=4; (D, D') n = 8, and (E, E') n = 12. Scale (E'): 20 mm (19). Reproduced from Ref 19 with permission of The Royal Society of Chemistry*

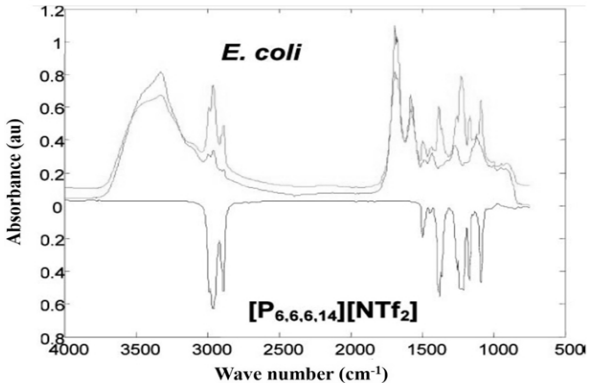

Figure 3 *FT-IR spectra (red) of* E. coli *membrane fraction after exposure to trihexyltetradecylphosphonium bis(trifluoromethy lsulfonyl) imide (23 % v/v) and membrane fractions of non-exposed cells (blue). Trihexyltetradecylphosphonium bis(trifluoromethyl sulfonyl) imide spectrum is shown reversed for better results interpretation (20). Reproduced from Ref 20 with permission of The Royal Society of Chemistry*

ammonium ILs containing inorganic, organic, and complex borate anions on enzyme acetylcholinesterase (enzyme involved in neurotransmission in nearly all higher organisms) was studied by Arning et al. (21). It was noticed that the majority of ILs tested exhibited no effect on the enzyme; only the fluoride and fluoride containing anion species (which readily undergo hydrolytic cleavage) could be identified to act as enzyme inhibitors. Dong et al. (22) investigated the effects of six typical *N*-methylimidazolium-based ILs (n = 4, 6, 8; anions= Br^-, Cl^-, BF_4^-, $CF_3SO_3^-$) on the activity of lactic dehydrogenase, the major oxidative enzyme in carbohydrate metabolism. Experimental results showed that the enzyme activity was inhibited in the presence of ILs, whereby *N*-methylimidazolium derivatives incorporating longer alkyl chain length demonstrated a stronger inhibitory effect on the enzyme compared to those with short alkyl chain. The molecular interaction mechanism of ILs and the enzyme were also investigated with the aid of spectroscopic techniques, which indicated that hydrophobicity was the major driven force underlying the interactions of ILs and the enzyme with no remarkable changes in the secondary structures of the latter. The effects of various imidazolium- and pyridinium-based ILs on the lipase activity (an essential role in the digestion and transformation of fats in most living organisms) and the IL-lipase interaction mechanism at the molecular level were studied by Fan et al. (23). Results also revealed the inhibitory ability of the Cl^-- and Br^--based ILs to be increased with the increase in the alkyl chain length in the IL cation with hydrophobicity and hydrogen bonding ability of ILs being the major driven force underlying the interactions of the ILs tested lipase.

Cell wall is essential for the maintenance of cell morphology but it also acts as a barrier against mechanical stress and environmental changes. Thus, the differences in the sensitivity of different organisms to xenobiotics are often associated with the presence of the cell wall and its native structure. Kulacki and Lamberti (17) and Latala et al. (24) showed that due to the functional groups containing silica in the cell wall, diatoms were very good biosorbents for charged chemicals such as ILs. Latala et al. (24) have also suggested a stronger sensitivity of diatoms in comparison with green algae. In fact, these organisms have specific proteins with acidic amino acid residues on the surface of cells, which can increase the ion connections to ILs. This allows a better interaction between cell surface cells and IL, resulting in a stronger toxic effect. Also, the authors pointed out that among green algae, cellulose cell wall (*e.g.* in *Scenedesmus quadricauda*) was less resistant in comparison with glycoprotein cell wall (*e.g.* in *Chlamydomonas reinhardtii*). To clarify whether ILs cause changes in the cell wall, Petkovic et al. (19) studied cell wall integrity of *Aspergillus nidulans* conidia exposed to dodecyltributylphosphonium chloride by fluorescence microscopy (Figure 4). A suspension of conidia was exposed to ILs (100 mM) and incubated during four hours and

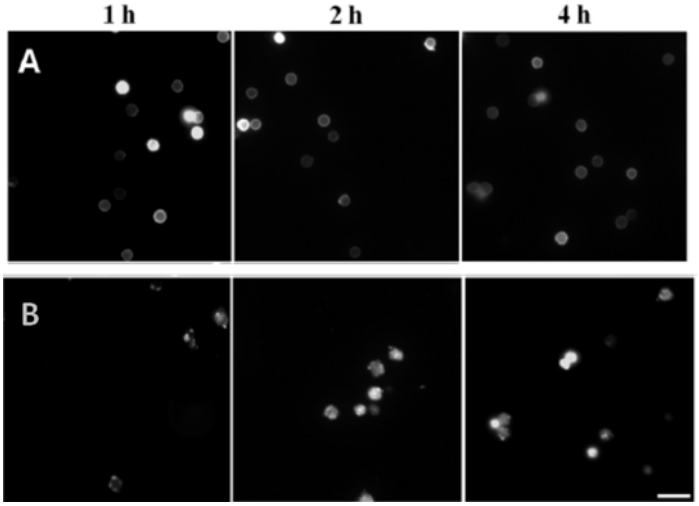

Figure 4 *Effects of alkyltributylphosphonium chlorides (100 mM) on cell wall integrity of the filamentous fungus* Aspergillus nidulans *by light microscopy (conidia are stained by Calcluor White). (A) control; (B) treated cells. Scale (B, 4 h): 10 mm (19). Reproduced from Ref 19 with permission of The Royal Society of Chemistry*

stained by Calcofluor White (CFW), which binds to chitin and glucan. Disrupted cell wall as a consequence of improper distribution of fluorescence was observed, suggesting that, along with membrane damage, cell wall damage plays an important role in the toxicity of phosphonium ILs (Figure 4). Since it was expected that the damage of the cell wall was caused by the changes in the overall morphology of conidia, the authors studied morphology changes by electron microscopy (Figure 5). While control conidia of *Aspegillus nidulans* were spherical with regular wrinkled surface, the treated conidia were shrived and of irregular shape. These results also indicate that toxicity of ILs depends on the presence and composition of the cell wall.

Kumar et al. (25) studied the oxidative damage in macroscopic algae *Ulva lactuca* caused by IL 1-dodecyl-3-methylimidazium bromide. It is well known that the stress caused by exposure to toxic compounds can lead to excessive accumulation of reactive oxygen species (ROSs). ROSs can induce lipid peroxidation and oxidation of DNA or proteins, which directly affect their function, and consequently cause cell death. Exposure of algae to the concentrations of ILs corresponding to half of LC_{50}, LC_{50}, and twice the LC_{50} during four days induced oxidative stress, with a significant increase in membrane lipid peroxidation and changes in the concentration of antioxidant enzymes. Histochemical localisation of ROS within the cells showed a significant accumulation of O_2• and H_2O_2 in cells (Figure 6). Since oxidative stress can lead to DNA damage, they also studied DNA damage caused by 1-dodecyl-3-methylimidazolium bromide (25). Changes in the genome were detected by gel electrophoresis of individual cells (Comet assay). In general, in the presence of an electric field, DNA fragments travel to the anode at different speeds, depending on their size, forming a pattern of a comet. According to the length of the tail and the percentage of DNA in the tail, tail moment is calculated. It was noticed that an increase in the concentration of ILs to twice the LC_{50} caused a significant induction of DNA damage (about 70 % increase in % tail DNA over control) (Figure 7). Cvjetko Bubalo et al. (26) studied the effects of 1-alkyl-3-methylimidazolium (n=4, 7, 10) bromide, acetate, and tetrafluoroborate on germination and growth of barley seedlings. The tested ILs showed toxic effects on the early development stages of barley seedlings, wherein at higher concentrations of ILs the antioxidant system could not effectively remove reactive oxidative species, leading to lipid peroxidation and damage of the photosynthetic system. This was manifested as a reduced amount of chlorophyll and increased quantities of malondialdehyde, an indicator of lipid peroxidation, as well as formation of H_2O_2. Furthermore, increased activities of antioxidant enzymes superoxide dismutase, non-specific peroxidase, catalase and ascorbate peroxidase, as well as an increased content of non-protein thiole were observed. The influence of ILs on the mammalian cell antioxidant system was studied by Jing et al. (27). They observed that 1-octyl-3-methylimidazolium chloride could cause changes in antioxidant enzymes and glutathione S-transferase activities inducing oxidative damage in QGY-7701 cells. In addition, DNA damage was also noticed showing that 1-octyl-3-methylimidazolium chloride caused biochemical and genetic toxicity in the tested cells.

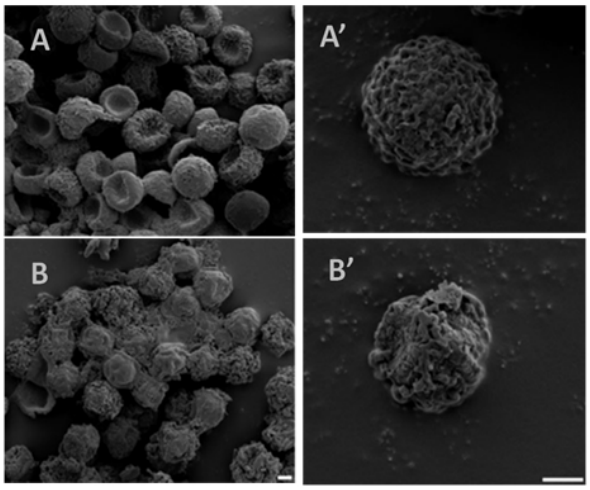

Figure 5 *Effects of alkyltributylphosphonium chlorides (100 mM) on cell wall integrity of the filamentous fungus* Aspergillus nidulans *by electron microscopy. (A) control cells; (B) treated cells. Scale (B, B'): 1 μm (19). Reproduced from Ref 19 with permission of The Royal Society of Chemistry*

In order to determine the level of ILs toxicity in animal cell cultures and cell death pattern (apoptosis or necrosis), Radošević et al. (14) performed a study of imidazolium-based ILs with bis(trifluoromethylsulfonyl) imide anion toxicity in the Channel Catfish Ovary (CCO) cells. Briefly, according to the classification of cell death there are two basic processes: apoptosis and necrosis. Apoptosis is a highly organised and genetically regulated cell death mechanism characterised by specific regulatory signals. It plays an important role in the maintenance of cellular homeostasis. In turn, necrosis is accidental cell death caused by direct chemical, mechanical, or physical and chemical damage that leads to a series of non-regulated and non-repayable morphologic changes. In the cases where the integrity of the cell is seriously damaged, cells can start the process of self-destruction (apoptosis). When cell damage is severe and irreparable, the cell will die by necrosis (28). In the work of Radošević et al. (14), during the initial experiment, CCO cells were cultivated in the culture medium with 1 mM of 1-pentyl-3-methylimidazolium bis (trifluoromethylsulfonyl) imide and 1-heptyl-3-methylimidazolium bis (trifluoromethylsulfonyl) imide, and morphological changes in CCO cells after exposure to ILs were observed by light microscopy and crystal violet staining, as well as fluorescence microscopy by acridine orange (AO) and ethidium bromide (EB) staining (Figure 7). In general, AO enters viable and non-viable cells and interferes with DNA, resulting in green fluorescence. EB does not enter viable cells, leaving intact chromatin in the

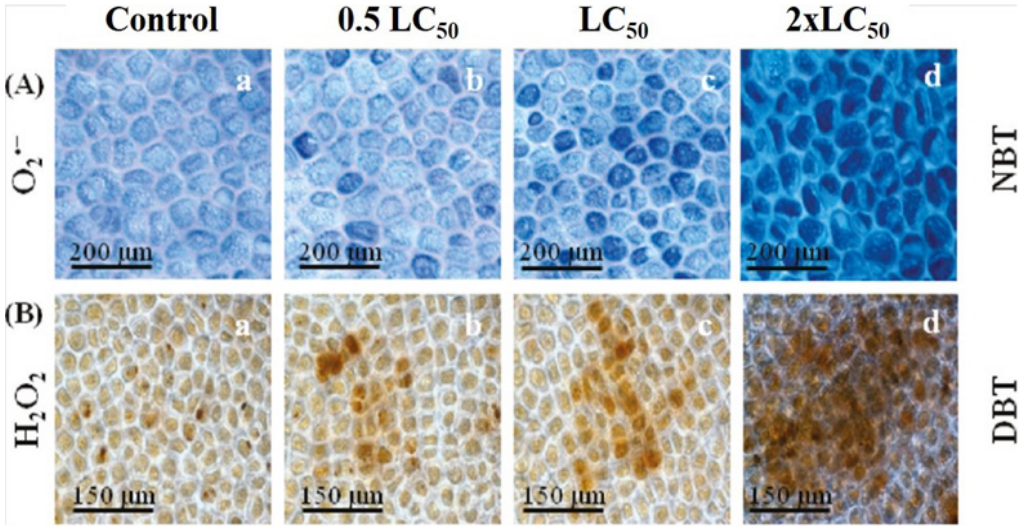

Figure 6 *ROS generation in seaweed* Ulva lactuca *following exposure to 1-dodecyl-3-methylimidazium bromide (A)* $O_2^{\bullet -}$ *and (B)* H_2O_2. *Radicals are detected after nitroblue tetrazolium (NBT) and 3,3-diamino benzidine (DAB) staining (25). Reprinted with permission from Ref 25. Copyright 2011 American Chemical Society*

nuclei of cells and fluorescence green. Therefore, necrotic cells with seriously disrupted membrane have bright orange coloured chromatin with an organised structure, while apoptotic cells show bright green fluorescence. On the other hand, apoptotic cells with damaged cell membrane have bright orange chromatin that is highly condensed or fragmented. It has been observed that cytotoxicity of tested ILs for CCO cells occurred mainly due to necrosis (Figure 8). This implies that the tested ILs caused severe cell damage through the necrosis process since there was no time to activate the mechanisms of survival, *i.e.* cells died almost instantly after being exposed to ILs. This study is in agreement to the assumptions that the toxicity of IL is primarily related to the impairment of the cell membrane. Li et al. (29), who studied the cytological alterations and damages of 1-octyl-3-methylimidazolium chloride in the rat pheochromocytoma (PC12) cells, also concluded that apoptosis was the main cell death pattern after cell exposure to 1-octyl-3-methylimidazolium chloride. Experimental results revealed that 1-octyl-3-methylimidazolium chloride exposure induced DNA damage, an increase in intracellular Ca^{2+}, overproduction of reactive oxygen species, and it gradually exhausted cellular ATP and mitochondrial permeability transition in PC12 cells. Therefore, the authors supposed that mitochondrial permeability transition and mitochondrial dysfunction might be the major cytotoxicity mechanism of the ILs tested on PC12 cells.

Perspectives for the development of environmentally friendly ionic liquids

Previous studies indicate that conventional ILs, based on various heterocyclic cations and halide anions, as discussed above, are moderately to highly toxic. In some cases, their toxicity resembles that of common organic solvents (*e.g.* methanol, dimethylformamide, propan-2-ol), or they are even 2-4 orders of magnitude more toxic (14). Furthermore, poor biocompatibility of ILs, their decomposition and deposition after they have been used, together with their relatively high price (5-20 times higher than common organic solvents) are some of the issues that are not in accordance with the principles of green chemistry. This prompted scientists to search for ILs with a different chemical structure that would maintain the technological properties of conventional ILs but would be of reasonable price and harmless to the environment (4, 30). Thus, based on the known ILs structure-toxicity dependency, scientists have actively designed and synthesised a variety of ILs (and still are), wherein the toxicity is often estimated using the quantitative methods of structure-activity relationships (QSAR). This method represents a widely accepted set of statistical methods by which the structural property of molecules (ILs) leads to the quantitative relationship (correlation) with their biological activity (toxicity) (31-32). In general, most of the related QSARs for predicting toxicity of ILs are based on toxicity testing and *in vitro* systems such as cell lines or primary cultures. These approaches have led to the development of so-called natural ILs where synthetic quaternary cations (*e.g.* ammonium, imidazolium, and pirydinium) are replaced by naturally occurring cations such as choline chloride. Accordingly, harmful fluorinated anions (*e.g.* tetrafluoroborate and hexafluorophosphate) are replaced by anions from natural sources, such as amino acids or organic acids. Another important characteristic of these new ILs is their relatively low price compared to conventional ILs. To confirm the favourable environmental profile of cholinium-based ILs, Hou et al. (33) studied the effects of ILs containing different amino acids as the anions to the acetylcholinesterase enzyme activity and to different bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Salmonella enteritidis* and *Lysteria monocytogenes*). Obtained results showed low toxicity profile in tested biological systems. Recently, Radošević et al. (34)

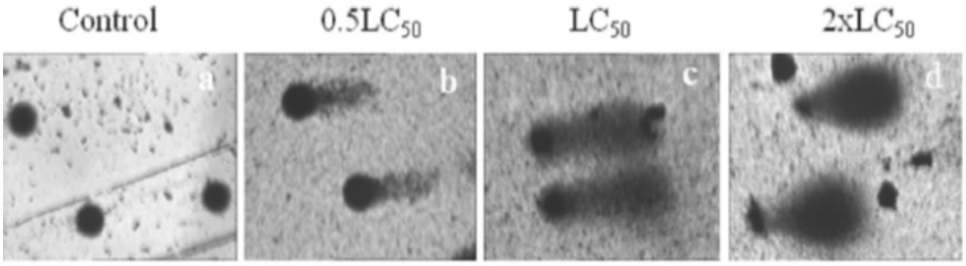

Figure 7 *Comet assay of 1-dodecyl-3-methylimidazium bromide effects in seaweed* Ulva lactuca*. (a) control; (b) 0.5 LC_{50}; (c) LC_{50}; and (d) 2xLC_{50} (25). Reprinted with permission from Ref 25. Copyright 2011 American Chemical Society*

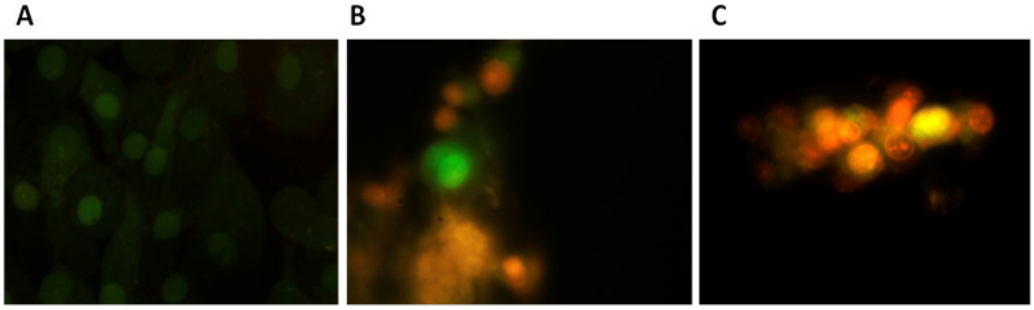

Figure 8 *Fluorescent microscopy of CCO cells stained with acridine orange and ethidium bromide after exposure to ionic liquids: (A) control cells, (B) cells treated by 1 mM 1-pentyl-3-methylimidazolium bis (trifluoromethylsulfonyl) imide, (C) cells treated by 1 mM1-heptyl-3-methylimidazolium bis (trifluoromethylsulfonyl) imide. Magnification 400 x (14). Reprinted from Ref 14, Copyright 2013, with permission from Elsevier*

examined *in vitro* toxicity of cholinium-based ILs with amino/organic acids as anions on CCO cells. The obtained results also revealed low toxicity of these solvents. The performed studies showed that this new class of ILs certainly have favourable ecotoxicological effects. It is not possible, nor necessary, to conduct toxicity tests for each newly synthesised IL, due to their large chemical diversity and the number of possible combinations but it would be necessary to carefully and critically examine their environmental impact if their broader technological applications were expected.

In conclusion, although ILs are considered to be green replacements for harmful organic solvents, numerous studies indicate that this group of solvents, depending on their chemical structure, can be equally or even more toxic than organic solvents. Toxicological studies on ILs mechanism of toxicity have shown that conventional ILs cause cell membrane desintegration followed by increased production of ROS and DNA damage. In mammalian cells, the damage is so strong that it caused cell death by necrosis. Therefore, searching for the structures of ILs with lower toxicity (such as cholinim-based ILs), as well as creating a database of chemical structures of environmentally friendly ILs based on their toxicity, is necessary to obtain guidance and define legislation before production and use of ILs on industrial scale.

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Mehanizmi toksičnosti ionskih kapljevina

U protekla tri desetljeća sve više raste svijest ljudi o potrebi zaštite okoliša, pa se velika pozornost pridaje tzv. *zelenim* i održivim tehnologijama. Stoga se u akademskim sredinama, a i u široj zajednici, intenzivno istražuju nove kemikalije te sigurniji i energetski učinkovitiji procesi koji se zasnivaju na prihvatljivom kompromisu između ekonomskih, socijalnih i ekoloških zahtjeva. Ionske se kapljevine zbog neznatne hlapljivosti i stabilnosti smatraju potencijalnom *zelenom* zamjenom za tradicionalna i škodljiva organska otapala. Kako bi se potvrdio *zeleni* karakter ionskih kapljevina, posljednjih godina provode se različita istraživanja njihova učinka na okoliš. Do sada objavljeni podaci upućuju na to da bi ova skupina spojeva, zbog relativno visoke toksičnosti i slabe biorazgradljivosti, ipak mogla imati izrazito negativan utjecaj na okoliš. U ovom radu prikazana su dosadašnja saznanja o toksičnosti ionskih kapljevina, s naglaskom na mehanizme kojima ova skupina spojeva uzrokuje promjene u morfologiji i fiziologiji organizama koji se nalaze na različitim organizacijskim razinama ekosustava.

KLJUČNE RIJEČI: *okoliš; test-sustavi; zelena otapala*