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Research Article

Cytotoxicity of Ionic Liquids on Normal Human Dermal Fibroblasts in the Context of Their Present and Future Applications

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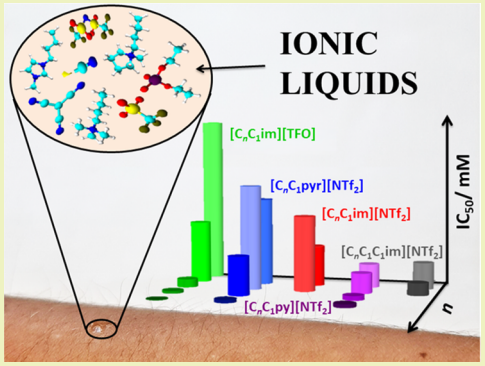
ABSTRACT: The skin is the part of the body that is the most exposed to toxic substances; therefore, the impact of chemicals on the skin should be thoroughly studied prior to their implementation in any industrial-scale application. Herein, we examined and analyzed the influence of the structure of both the cation and anion of 31 different ionic liquids (ILs) on their cytotoxicity against normal human dermal fibroblasts in the context of their present and future potential applications. We found that imidazolium-based ILs combined with dialkyl phosphate anions or with the ethyl sulfate anion are the least cytotoxic. Notably, 1,3-diethylimidazolium ethyl sulfate can be potentially used as a hydraulic fluid similar to the commercially available hydraulic medium based on 1-ethyl-3-methylimidazolium ethyl sulfate. Moreover, the dialkyl phosphate-based ILs are considered as an efficient solvent for the utilization of lignocellulose-based biomass and as an extractant in eco-friendly and cost-effective processes for the extraction of bioplastic. Pyrrolidinium-based and cyano-based ILs, often used as heat transfer media and base fluids for ionanofluids, were also identified herein as good candidates based on their relatively low toxicity compared to other ILs.



KEYWORDS: *toxicity, cytotoxicity, skin cells, ionic liquids, ionic liquids applications*

INTRODUCTION

Ionic liquids (ILs) have achieved great success due to their unique features such as high chemical and thermal stability and low vapor pressure, and also because they are non-explosive, nonflammable, and much more. This has resulted in a huge number of applications also on an industrial scale, e.g., biphasic acid scavenging utilizing ionic liquids (BASIL) and cellulose dissolution—BASF company; hydraulic ionic liquid compressor—Linde Group (world-leading gases and engineering company); TEGO1 Dispers (paint additives) and hydrosilylation process—Degussa company; batteries—Pionics, NantEnergy, NOHMs Technologies; and isomerization process—Eastman Chemical Company.[1,2] Because of the extensive use of ILs in academic research and the chemical industry, and due to their high stability and noticeable solubility (especially in water), their environmental impact cannot be avoided. Ionic liquids used to be called "green solvents"[3] due to their negligible vapor pressure. Consequently, they are usually less volatile than most classic organic solvents and do not pollute the air. However, ILs may be potential water and soil pollutants, especially during operational discharges or accidental leaks, and hence, their general toxicity plays a crucial role.[4−6] While many ILs have proven to be toxic, and in some cases, more toxic than common organic solvents, there has still not been enough attention paid to the effects of ILs on humans and the environment.[7−9]

To date, the toxicity of ILs has been studied, for example, against microorganisms (yeast, fungi, and bacteria), algae, plants, vertebrates, and invertebrates.[10] Note that the toxicity of ILs is dependent on the structure of the cation and anion, and study of the effect of each structural element on the toxicity of IL is required for designing low-toxic or even nontoxic ILs. This is especially important because the same IL may have various effects on different organisms and cell lines, and new experimental data are in fact needed. In some cases, the available data is sufficient to predict the toxicity of ILs only on the basis of the structure of cations and anions, e.g., Jafari et al.[11] derived and subsequently verified a chemical toxicity estimation model of *Vibrio fischeri* based on EC_{50} values (EC_{50} is the efficient concentration of the studied sample resulting in 50% of reduction on processes, such as growth or reproductive activity) for 187 ILs (250 experimental points) and,

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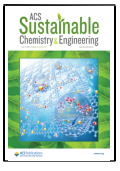

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Table 1. Full Names, Acronyms, Chemical Structures, Purities, and Water Contents of the Examined Materials[a,b,c,d,e]

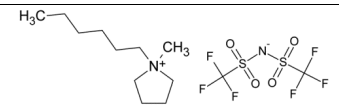
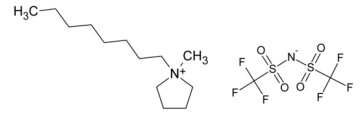
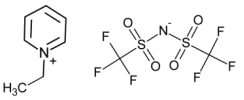
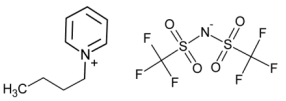
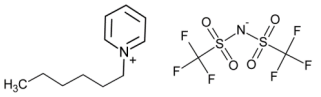
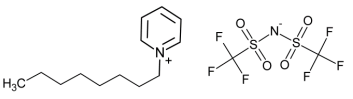
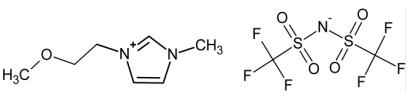
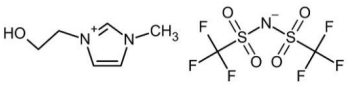
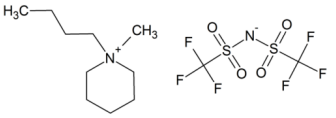
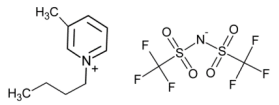
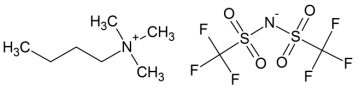
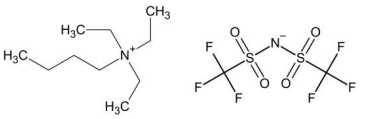
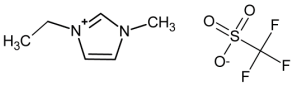
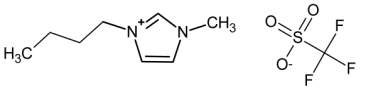
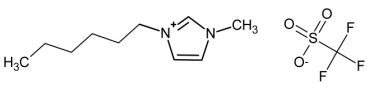
Full name/ Acronym	Structure	Purity[a]/ %; Water content[b]/ ppm
1-hexyl-1-methylpyrrolidinium bis(trifluoromethylsulfonyl)imide [C_6C_1pyr][NTf_2]		>99; 212 [c]
1-methyl-1-octylpyrrolidinium bis(trifluoromethylsulfonyl)imide [C_8C_1pyr][NTf_2]		>99; 250 [c]
N-ethylpyridinium bis(trifluoromethylsulfonyl)imide [C_2py][NTf_2]		>98; < 50 [d]
N-butylpyridinium bis(trifluoromethylsulfonyl)imide [C_4py][NTf_2]		>98; < 50 [d]
N-hexylpyridinium bis(trifluoromethylsulfonyl)imide [C_6py][NTf_2]		>98; < 50 [d]
N-octylpyridinium bis(trifluoromethylsulfonyl)imide [C_8py][NTf_2]		>98; < 50 [d]
1-(2-metoxyethyl)-3-methylimidazolium bis(trifluoromethylsulfonyl)imide [$C_1OC_2C_1$im][NTf_2]		>99; 75
1-(2-hydroxyethyl)-3-methylimidazolium bis(trifluoromethylsulfonyl)imide [HOC_2C_1im][NTf_2]		>99; 475
1-butyl-1-methylpiperidinium bis(trifluoromethylsulfonyl)imide [C_4C_1pip][NTf_2]		> 99; 57
1-butyl-3-methylpyridinium bis(trifluoromethylsulfonyl)imide [C_4C_1py][NTf_2]		>99; 84
N-butyl-*N*,*N*,*N*-trimethylammonium bis(trifluoromethylsulfonyl)imide [N_{4111}][NTf_2]		>99; 81
N-butyl-*N*,*N*,*N*-triethylammonium bis(trifluoromethylsulfonyl)imide [N_{4222}][NTf_2]		>99; 10
1-ethyl-3-methylimidazolium trifluoromethanesulfonate [C_2C_1im][TFO]		>99; 120 [c]
1-butyl-3-methylimidazolium trifluoromethanesulfonate [C_4C_1im][TFO]		>99; 154 [c]
1-hexyl-3-methylimidazolium trifluoromethanesulfonate [C_6C_1im][TFO]		>99; 240 [c]

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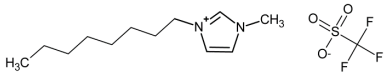
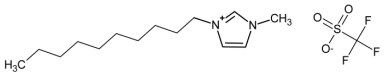
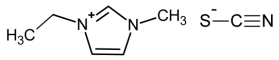
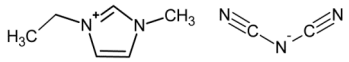
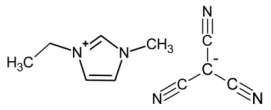
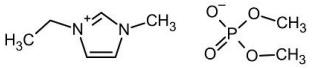
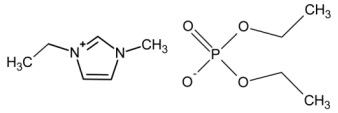
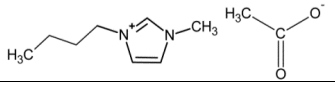
Table 1. continued

Full name/ Acronym	Structure	Purity[a]/ %; Water content[b]/ ppm
1-methyl-3-octylimidazolium trifluoromethanesulfonate [C_8C_1im][TFO]		>99; 168 [c]
1-decyl-3-methylimidazolium trifluoromethanesulfonate [$C_{10}C_1$im][TFO]		>99; 92 [c]
1-ethyl-3-methylimidazolium thiocyanate [C_2C_1im][SCN]		>98; 200 [e]
1-ethyl-3-methyl-imidazolium dicyanamide [C_2C_1im][N(CN)$_2$]		>98; 250
1-ethyl-3-methylimidazolium tricyanomethanide [C_2C_1im][C(CN)$_3$]		>98; 156
1-ethyl-3-methylimidazolium dimethylphosphate [C_2C_1im][DMP]		>98; 70
1-ethyl-3-methylimidazolium diethylphosphate [C_2C_1im][DEP]		>98; 627 [e]
1-butyl-3-methylimidazolium dimethylphosphate [C_4C_1im][OAc]		>98; 1512 [e]

[a]Reported by Iolitec. [b]Determined using the Karl Fischer method. [c]Ref 28. [d]Ref 23. [e]Ref 22.

importantly, the authors obtained a satisfactory compliance. Nevertheless, the database necessary to achieve acceptable results must be very large.

Recently, increasing amounts of cytotoxic data were obtained for mammalian cell lines, namely normal murine fibroblasts,[12] normal human keratinocytes and fibroblasts,[13] as well as human cancer cell lines.[14−16] In the studies mentioned above, mainly IC_{50} values (IC_{50} is the inhibitory concentration of the examined material causing 50% inhibition of the activity of biochemical or biological systems) were applied to estimate the IL cytotoxicity. In most cases, the authors examined ionic systems with halide anions (for example, with bromide $[Br]^-$ or chloride $[Cl]^-$ anions). Generally, in all tested cell lines, it was found that ILs with short alkyl chains attached to the cation have weaker biological effectiveness compared to those with long apolar alkyl chains. Łuczak et al.[17] postulated that the alkyl chain length in the cation plays a crucial role in the biocidal activity of ILs and the type of anion has a significantly smaller impact. It should be mentioned that the effects of anion on toxicity have been less frequently studied and, additionally, the current studies are not wholly consistent with each other on this topic. Concomitantly, some studies involving ILs with a large variety of anions showed a relatively strong effect of anions on the IL toxicity,[18,19] i.e., ILs containing $[Cl]^-$, $[Br]^-$, tetrafluoroborate $[BF_4]^-$, and hexafluorophosphate $[PF_6]^-$ anions usually yield EC_{50} values of similar magnitude for the same cation, whereas bis-(trifluoromethylsulfonyl)imide $[NTf_2]$-based ILs often are more toxic than their halide analogues.[18]

The purpose of the present work is to investigate the influence of the structure of the anion, cation, and alkyl chain length on IL cytotoxicity. To this end, tests of 23 ILs were performed on normal human skin cells, and the results were reported in the form of IC_{50} values. Additionally, the values obtained in this work were compared with our previous data for different ILs[20] and analyzed in the context of their present and future applications. In total, the cytotoxicity of 31 ILs was compared therein. Notably, the skin is directly exposed to toxic compounds in our everyday life and working environment; thus, the effects of chemical substances on the skin should be carefully examined, in particular for materials classified as present or potential industrial chemicals. Environmental agencies also require to do skin irritation testing for compounds that will be used in amounts >1 tonne/year.[13] However, the cytotoxicity of ILs on the skin has not been fully established, and the information obtained from this study can complement the currently insufficient knowledge.

We have found that ILs with ethyl sulfate anion (1-ethyl-3-methylimidazolium ethyl sulfate, $[C_2C_1im][C_2SO_4]$, and 1,3-diethylimidazolium ethyl sulfate, $[C_2C_2im][C_2SO_4]$[20]) and dialkyl phosphate anions (1-ethyl-3-methylimidazolium dimethyl phosphate, $[C_2C_1im][DMP]$, and 1-ethyl-3-methylimidazolium diethyl phosphate, $[C_2C_1im][DEP]$) are the least cytotoxic. Additionally, cyano-based ILs also are characterized by their relatively low cytotoxicity. Among ILs with the most popular $[NTf_2]^-$ anion, the pyrrolidinium ones with a non-aromatic five-membered ring have the lowest cytotoxicity. In contrast, 1-butyl-1-methylpiperidinium bis-(trifluoromethylsulfonyl)imide $[C_4C_1pip][NTf_2]$ has the highest cytotoxicity, over 30 times greater than that of $[C_2C_1im]$-[DMP].

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EXPERIMENTAL SECTION

Materials. The supplier of the 19 ILs was Iolitec (Germany), whereas 4-*N*-alkylpyridinium bis(trifluoromethylsulfonyl)imides, $[C_npy][NTf_2]$ (n = 2, 4, 6, 8), were prepared in the QUILL Research Centre (U.K.). Note that in some cases, we have used the same batch of materials as in previous studies. Thus, the detailed specification was presented in refs 21, 22, while details about the synthesis, purification, and storage of the pyridinium-based ILs can be found in ref 23. The specification of the tested samples is reported in Table 1. All examined ILs were dried under a pressure of ~1 kPa at temperatures ≤373.15 K.

Cell Culture. The normal human dermal fibroblasts (NHDF) (supplier-PromoCell) were cultured in Dulbecco's modified Eagle medium (DMEM), with 15% fetal bovine serum (Gibco) and 100 mg·L^{-1} of gentamycin (Gibco). The cells were grown as an adherent monolayer culture in standard conditions (95% humidity, 5% CO_2, 310.15 K).

Cytotoxicity Assay. Twenty-four hours before adding the tested ILs, the cells (4.0×10^3 cells/well) were seeded onto 96-well plates (Nunc). The next day, solutions of ILs were prepared in the culture medium (in a concentration range from 0.01 to 30 mM) and then added to the cells. The cytotoxicity assay was performed after 72 h by exchanging the medium with testes ILs with fresh DMEM (100 μL) containing 20 μL of CellTiter 96 AQueous One Solution Cell Proliferation Assay (MTS) (Promega). The cells were incubated with MTS for 1 h, and then the absorbance of red formazan was measured on the microplate reader (Synergy4 from BioTek). For calculation of the IC_{50} values, the absorbance of cells incubated with tested ILs was compared to the absorbance of untreated cells. IC_{50} values along with standard deviations (confidence level 0.95) were determined using the GraphPad Prism 8 software. Each experiment was repeated three times in triplicate (for each IL).

Density Measurements. The density of the tested ILs needed for the IC_{50} calculations was recorded at 310.15 K by means of a DMA 5000 M vibrating-tube densimeter (Anton Paar, Austria). The apparatus was calibrated (an extended-temperature calibration procedure was used) with redistilled water and dried air. Importantly, viscosity correction was made automatically. The uncertainty of the listed density values in Table 2, in each case, did not exceed ±0.1 kg·m^{-3}. The uncertainty in the density was evaluated using the following ref 24, in which the impact of the impurities on the uncertainty was taken into account.

RESULTS AND DISCUSSION

Structure vs Cytotoxicity. The NHDF cell line was employed to investigate the cytotoxicity of ILs because it is a common and reliable model for measuring the toxicity in *in vitro* initial studies. The results obtained are gathered in Table 2 and depicted in Figures 1 and 2, along with the values presented by our group recently.[20,25,26] The density of $[C_2C_1im][SCN]$, $[C_2C_1im][N(CN)_2]$, $[C_2C_1im][C(CN)_3]$, $[C_2C_1im][DMP]$, and $[N_{4222}][NTf_2]$, $[C_4C_1py][NTf_2]$, $[C_1OC_2C_1im][NTf_2]$, $[C_4C_1pip][NTf_2]$, and $[N_{4111}][NTf_2]$ needed to calculate the IC_{50} parameter in the reported unit (mM) was measured at 310.15 K and are listed in Table 2. For the other analyzed ILs, we used the density data reported in the previous studies.[22,27,28]

Influence of the Alkyl Chain of the Cation. Thus far, many studies have analyzed the toxicity of ILs on human cell lines.[16,29−33] Nevertheless, there are few existing studies on the influence of the anion structure in ILs on the cytotoxicity in contrast to the numerous studies that have investigated the impact of the alkyl chain length in the cation. As can be observed by a review of Table 2 and Figure 1, an increase of the alkyl chain length in the cation leads to an increase in the IL cytotoxicity. In each homologous series, the change in cytotoxicity when the chain is lengthened by two $-CH_2-$ groups is higher than 50%. Hence, the observed behavior is consistent with the observations made in numerous studies.[6,14,15] Generally, ILs with longer alkyl chains (n > 4) are more lipophilic than those with shorter alkyl chains. It can be assumed that the former tends to incorporate into the phospholipid bilayers of biological membranes.[10,33−36] According to Wu et al.,[10] the stronger the lipophilicity, the greater the possibility of contact with the hydrophobic proteins and lipid

Table 2. Density and Cytotoxicity of the Examined ILs at 310.15 K along with the Cytotoxicity Values Reported Previously

IL	ρ (kg·m^{-3})	IC_{50} (mM)
$[C_3C_1pyr][NTf_2]$		6.24 ± 0.22[a]
$[C_4C_1pyr][NTf_2]$		7.29 ± 0.35[a]
$[C_6C_1pyr][NTf_2]$	1325.87[b]	2.68 ± 0.35
$[C_8C_1pyr][NTf_2]$	1278.42[b]	0.21 ± 0.03
$[C_2py][NTf_2]$	1525.70[c]	1.65 ± 0.35
$[C_4py][NTf_2]$	1479.62[c]	1.39 ± 0.30
$[C_6py][NTf_2]$	1437.29[c]	0.29 ± 0.10
$[C_8py][NTf_2]$	1372.34[c]	0.23 ± 0.04
$[C_2C_1C_1im][NTf_2]$		1.87 ± 0.39[d]
$[C_4C_1C_1im][NTf_2]$		0.85 ± 0.34[d]
$[C_3C_1im][NTf_2]$		2.85 ± 0.15[a]
$[C_4C_1im][NTf_2]$		5.25 ± 1.03[a]
$[C_1OC_2C_1im][NTf_2]$	1494.97	1.64 ± 0.46
$[HOC_2C_1im][NTf_2]$	1563.03	5.18 ± 0.19
$[C_4C_1pip][NTf_2]$	1370.02	0.32 ± 0.02
$[C_4C_1py][NTf_2]$	1403.64	2.81 ± 0.28
$[N_{4111}][NTf_2]$	1382.23	2.22 ± 0.36
$[N_{4222}][NTf_2]$	1328.70	1.51 ± 0.09
$[C_2C_1im][TFO]$	1372.34[b]	11.8 ± 1.2
$[C_4C_1im][TFO]$	1287.90[b]	4.42 ± 0.68
$[C_6C_1im][TFO]$	1229.37[b]	0.56 ± 0.10
$[C_8C_1im][TFO]$	1179.89[b]	0.17 ± 0.02
$[C_{10}C_1im][TFO]$	1144.85[b]	0.025 ± 0.001
$[C_2C_1im][C_2SO_4]$		20.82 ± 0.90[e]
$[C_2C_2im][C_2SO_4]$		20.4 ± 1.5[e]
$[C_2C_1im][SCN]$	1108.85	11.27 ± 0.28
$[C_2C_1im][N(CN)_2]$	1101.91	14.08 ± 0.62
$[C_2C_1im][C(CN)_3]$	1072.97	8.01 ± 0.32
$[C_2C_1im][DMP]$	1214.00	30.5 ± 2.5
$[C_2C_1im][DEP]$	1136.02[f]	25.6 ± 1.4
$[C_4C_1im][OAc]$	1044.18[f]	5.2 ± 1.5

[a]Ref 25. [b]Calculated from the polynomial reported in ref 28. [c]Calculated from the polynomial reported in ref 23. [d]Ref 26. [e]Ref 20. [f]Calculated from the polynomial reported in ref 22.

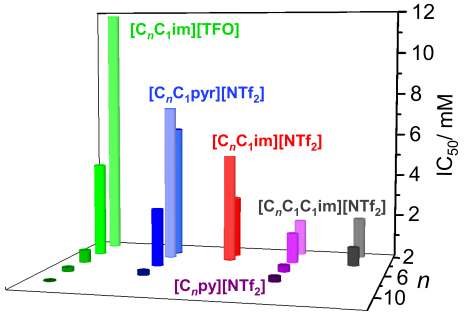

Figure 1. Cytotoxicity as a function of the alkyl chain length in the cation.

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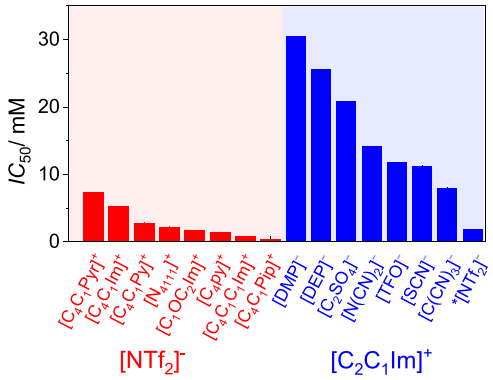

Figure 2. Cytotoxicity for ILs with the $[NTf_2]^-$ anion and different cations (red left side) and for ILs with $[C_2C_1im]^+$ cation and various anions (blue right side). * $[C_2C_1C_1im][NTf_2]$.

bilayer of the membrane. This increase in lipophilic character, as well as the presence of delocalized charges, affects the normal physiological function of the cell membrane and, unfortunately, promotes its disruption and increases the internal acidity. Ranke et al.[37] have shown that the lipophilicity of ILs predominates their in vitro cytotoxicity over a broad range of structural variations. Notably, it is also presented in the literature that the toxicities do not increase after a certain number of $-CH_2-$ groups (for example, $n = 12$), which is generally called as a "cutoff effect".[18,38] According to literature,[38] this may be attributed to the kinetic aspects (related to steric hindrance for substances that have a big molecular size) or inadequate solubility (the nominal concentration differs from the actual test concentration).

Interestingly, Pham et al.[6] reported that ILs containing a cation with polar hydroxyl, nitrile, or ether functional groups in the side chain (instead of a nonpolar simple alkyl chain) have lower cytotoxicity. Stolte et al.[18] postulated that the groups mentioned above hinder cellular uptake by membrane diffusion and reduce lipophilic-based interactions with the cell membrane. However, as clearly seen in Table 2, the difference between the cytotoxicities of $[C_2C_1C_1im][NTf_2]$ and $[C_2OC_1im][NTf_2]$ is negligible and does not exceed the experimental error (1.87 ± 0.39 vs 1.64 ± 0.46 mM).

Notably, we observed that homologues with three carbons in a chain in a substituent, i.e., $[C_3C_1pyr][NTf_2]$ and $[C_3C_1im][NTf_2]$, have a higher cytotoxicity than the others, i.e., $[C_4C_1pyr][NTf_2]$ and $[C_4C_1im][NTf_2]$, respectively (Table 2). Analogous results were presented by Ventura et al.[39] Specifically, the authors reported that after 300 s of exposure to *V. fischeri* (luminescent marine bacteria), $[C_4C_1im][NTf_2]$ has a lower toxicity than $[C_3C_1im][NTf_2]$.

Influence of the Head Groups of the Cation. In the case of $[NTf_2]$-anion-based ILs with cations containing different head groups and the same alkyl chain length attached to the cation, the cytotoxicity increases in the following order: $[C_4C_1pyr]^+ < [C_4C_1im]^+ < [C_4C_1py]^+ < [N_{4111}]^+ < [C_4py]^+ < [C_4C_1pip]^+$ (Figure 2). Piperidinium-based IL is ca. 20 times more toxic than the pyrrolidinium one (0.32 ± 0.02 vs 7.29 ± 0.35), demonstrating that the head group has also a significant impact on the IL cytotoxicity. On the other hand, Wang et al.[30] observed that the imidazolium-based ILs showed a higher inhibition of HeLa cells (an immortal cell line) than pyridinium and ammonium-based ILs with the bromide anion ($[N_{4222}]^+ < [C_4py]^+ < [C_4C_1im]^+$—Wang et al. for Br^--based ILs vs $[C_4C_1im]^+ < [N_{4222}]^+ \approx [C_4py]^+$—this work for $[NTf_2]^-$-based ILs). Nevertheless, the authors also observed that for each class of cation (imidazolium, pyridinium, ammonium, choline-based ILs with Br^-, $[NTf_2]^-$, and $[BF_4]^-$ anions) an increase of the side-chain length of the cation of the homologues (n = 2, 4, 6, 8) leads to a decrease in the cytotoxicity as claimed in the present investigation.

Influence of the Anion. As mentioned in the introduction, several authors have indicated that anion change has only a minimal effect on the toxicity of ILs.[17,34,40] Consequently, the toxicity of ILs seems to be related to the alkyl chain branching and the hydrophobicity of the cation but not to the various anions. However, Stolte et al.[41] stated that anionic compartments with lipophilic and hydrolyzable structural elements are important concerning the IL toxicity. In this work, the contribution of the anion moiety in ILs on their cytotoxicity is evaluated by comparing the IC_{50} values obtained for imidazolium ILs with one headgroup with one specific side-chain length (ethyl), i.e. $[C_2C_1im]^+$ with various anions, i.e., $[C_2SO_4]^-$, $[DMP]^-$, $[DEP]^-$, $[SCN]^-$, $[N(CN)_2]^-$, $[C(CN)_3]^-$, and $[TFO]^-$. The strongest toxic effect toward normal human dermal fibroblasts was detected for $[C_2C_1im][C(CN)_3]$, and the least cytotoxic was found to be $[C_2C_1im][DMP]$. We found that ethyl sulfate ILs are relatively less cytotoxic and, in addition, nontoxic toward, e.g., *Escherichia coli*,[7] anaerobic bacteria,[42] and luminescent bacteria[5] in comparison to other ILs and are not harmful to the eyes (no irritating impact).[5] Taking into account the uncertainty of the IC_{50}, the cytotoxicities of $[C_4C_1im]^+$-based ILs, namely $[C_4C_1im][OAc]$, $[C_4C_1im][TFO]$, and $[C_4C_1im][NTf_2]$, are comparable. On the other hand, the $[NTf_2]^-$ anion contains fluorinated alkyl side chains with lipophilic interaction potential. This facilitates the interaction with hydrophobic protein domains and cell membranes, potentially disrupting fundamental physiological functions. Since $[NTf_2]^-$ is a stable anion under physiological conditions, the increase in cytotoxicity, in this case, cannot be related to hydrolysis and the formation of HF (hydrofluoric acid) like in the case of $[BF_4]^-$- and $[PF_6]^-$-based ILs, but results from the increased lipophilicity of the anion.

Cytotoxicity vs Applications. As mentioned before, the exceptional properties of ILs compared to molecular solvents have allowed the use of this group in many areas of the chemical industry, such as extraction, electrochemistry, biocatalysis, catalysis, separation, biotechnology, as well as in the food and pharmaceutical industry.[1,2] Furthermore, ILs may be used as working fluids, i.e., lubricants, hydraulic, and heat transfer fluids, while the search for more favorable, noncorrosive, easy-to-supercool working fluids with excellent thermal stability (wide liquidus range) and low toxicity still is the subject of many studies.[20,25,26,43,44] Among the examined samples, $[C_2C_1im][DMP]$, $[C_2C_1im][DEP]$, and $[C_2C_1im][C_2SO_4]$, $[C_2C_2im][C_2SO_4]$ have the lowest cytotoxicity (see Table 2). $[C_2C_1im][C_2SO_4]$ is imported and/or manufactured in the European Economic Area in an amount greater than 100 tonnes/year. This information is publicly available within the C&L Inventory held by the European Chemicals Agency (ECHA).[45] According to the aforementioned website, this substance is used by consumers, in formulation, in articles, or re-packing, at industrial sites, and in manufacturing commercially available hydraulic fluid.[45] Interestingly, in our previous work, we observed that $[C_2C_2im][C_2SO_4]$ has analogous features to $[C_2C_1im][C_2SO_4]$, namely, the coefficients of

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isobaric thermal expansion and isothermal compressibility are low, which is important for its potential use as a hydraulic fluid.[20] Both ethyl sulfate ILs are thermally stable, relatively cheap (can be efficiently and easily prepared), can be synthesized without chloride contamination (which has a great influence on the corrosion of metals; namely, corrosion progresses much faster in the presence of halides), and have a broad temperature range in their liquid state (from glass transition temperature to decomposition temperature; no tendency for crystallization).[46] Importantly, lower surface tension and viscosity, as well as better wettability features than $[C_2C_1im][C_2SO_4]$ were recorded for the $[C_2C_2im]$-$[C_2SO_4]$ homologue. $[C_2C_1im][DMP]$, $[C_2C_1im][DEP]$, and $[C_4C_1im][OAc]$ can be considered as efficient solvents for the utilization of lignocellulose-based biomass.[47−51] Additionally, Dubey et al.[52] derived an eco-friendly and cost-effective process for the extraction of a potentially biodegradable plastic material (polyhydroxybutyrate) from *Halomonas hydrothermalis* (marine bacteria) by employing $[C_2C_1im][DEP]$ as an extractant.

On the other hand, undeniably, ILs with the $[NTf_2]^-$ anion are more toxic than the others tested herein. However, they are one of the most popular IL families due to their unique properties, so it is worth taking a closer look at the obtained results. To date, $[NTf_2]^-$ is still widely used as it provides hydrophobic ILs with larger operational liquid-range temperatures, CO_2 solubility, and is more stable chemically and thermically than those containing fluor, such as $[BF_4]^-$ and $[PF_6]^-$.[5353] $[NTf_2]^-$-based ILs were also recognized as a good heat-transfer medium,[25,26,43,44] explaining also why they are widely used for thermo-electrochemical applications.[1−3,54] To further depict their potential as a heat transfer medium, their main key properties are plotted in Figure 3 and compared with those collected for industrial benchmarks, such as Dowcal ™ 200[55] (based on propylene glycol), PES-4[43] and PMS-100[43] (organosilicon fluids), Therminol 66,[56] Therminol VP-3,[57] Therminol VP-1,[58] and Marlotherm SH[59] (based on aromatic hydrocarbons). One can assume that most of the investigated ILs have high and near-constant volumic heat capacity, i.e., ratio of molar isobaric heat capacity to molar volume C_p/V_m (Figure 3c). Except for $[C_2C_1im][DEP]$ and $[C_4C_1im][OAc]$, all presented ILs have a similar viscosity at 313.15 K (Figure 3b). However, ILs with $[NTf_2]^-$ anion show exceptional thermal stability and optimal thermal conductivity characteristic of high-temperature heat transfer media (Therminol 66,[56] Therminol VP-1,[58] Therminol VP-3,[57] and Marlotherm SH[59]). Among $[NTf_2]^-$-based ILs, the thermal stability decreases in the following order: $[C_nC_1C_1im]^+ > [C_nC_1pyr]^+ > [C_nC_1pip]^{+60} \approx [N_{1224}]^{+61} > [C_nC_1py]^+ > [C_npy]^+$ (see Figure 3a). The length of the alkyl substituent present in the cation structure does not significantly influence the IL thermal stability. Furthermore, by taking into account also their cytotoxicity, the most promising ILs are those based on the pyrrolidinium cation, which have the least harmful effects (see Figure 2), explaining why $[C_nC_1pyr][NTf_2]$ (n = 3, 4) could be promising as a heat transfer medium.

Moreover, the conclusions reported recently by Jóźwiak et al.[21,62] highlight that $[C_2C_1im][SCN]$-based ionanofluids containing 1 wt % multiwalled carbon nanotubes (MWCNTs) or 1 wt % carboxylic group-functionalized multiwalled carbon nanotubes (oMWCNTs) show unique transport properties including high thermal conductivity and low viscosity. With respect to these findings, the amalgamation of MWCNTs with

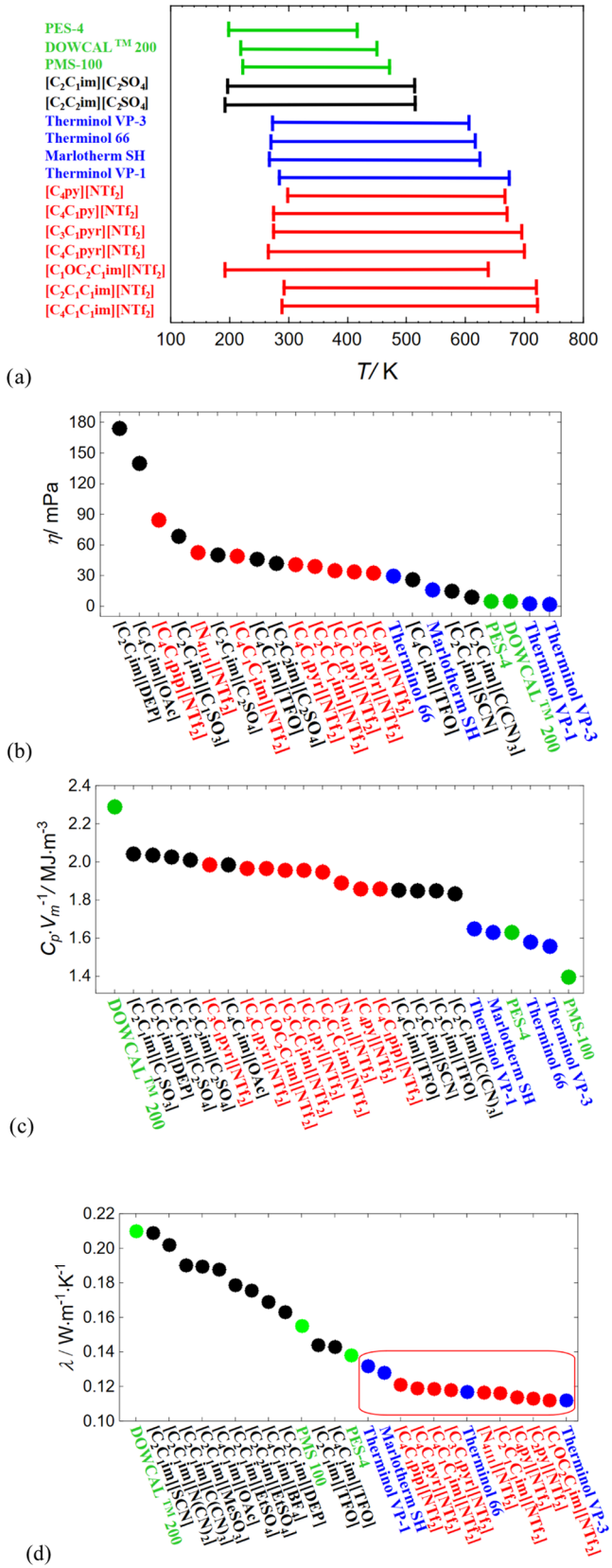

Figure 3. Comparison of the characteristics of ILs with those of commercial heat transfer media: (a) thermal stability, (b) viscosity, (c) volumic heat capacity, and (d) estimated thermal conductivity at 313.15 K [25, 26, 43, 55−59].

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$[C_2C_1im][SCN]$ was in fact justified from both an economic and an engineering point of view.[63] In this context, the relatively low cytotoxicity of $[C_2C_1im][SCN]$, shown herein, seems to be even more interesting to further justify their selection for future energy storage applications.

CONCLUSIONS

The cytotoxicity of 31 ILs toward normal human skin cells was studied and analyzed in the concept of their application. The influence of alkyl chain length and the structure of cation and anion was analyzed. The obtained results show that each element of the structure is important, but two main factors that influence the cytotoxicity are the type of anion and the length of the alkyl chain substituent to the ion. We found that ILs with ethyl sulfate anions ($[C_2C_1im][C_2SO_4]$, commercially available as the hydraulic medium, and $[C_2C_2im][C_2SO_4]$) and dialkyl phosphate anions ($[C_2C_1im][DMP]$ and $[C_2C_1im]$-$[DEP]$) are the least cytotoxic.

$[C_nC_1pyr][NTf_2]$ (n = 3, 4) have the most promising features (mainly exceptional thermal stability) for application as a heat transfer medium as they also have the least harmful effects of all tested $[NTf_2]^-$-based ILs. Unfortunately, it should also be noted that ILs based on the $[NTf_2]^-$ anion have the highest cytotoxicity among all of the ILs investigated herein and $[NTf_2]^-$-based ILs are also well known to be the most toxic to aquatic organisms.[64,65] In other words, prior to designing any industrial application using this particular IL family, further investigations are needed. Moreover, it should be remembered that as known, the different models to test toxicity respond differently to the same ILs.[64,65] Finally, according to their relatively low cytotoxicity and based on their unique properties, $[C_2C_1im][SCN]$ seems to be a good candidate to formulate alternative ionanofluids.[21,62,63]

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Notes

The authors declare no competing financial interest.

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