University of Bremen

Investigations on the stability and ecotoxicity of selected ionic liquid cations and anions

by

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minilubes.net

MARIE CURIE ACTIONS

Reviewer: 1. Prof. Dr. Dr. h.c. mult. Bernd Jastorff

2. Prof. Dr. Piotr Stepnowski

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I was taught that the way of progress was neither swift nor easy

–Marie Skłodowska Curie–

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ABSTRACT

The field of ionic liquids (ILs) is rapidly growing and several applications have already been discussed or applied. For a long time, such substances were announced to be “green”. This was solely based on their low vapor pressure and the accompanying enhanced operational safety. This general term was refined when first evaluations on the ecotoxicity and biodegradability of ILs were published, indicating a certain environmental hazard for representatives. Within this thesis investigation on hydrolytic stability, biodegradation potential and ecotoxicological properties were extended to anions and cations which have not yet been studied. This includes three cyano based and three (per)fluorinated anions, five ammonium and several pyrrolidinium, morpholinium, piperidinium, imidazolium and pyridinium cations, as well as a first assessment for bivalent cations. An analytical method based on ion chromatography was developed for all of these compounds in order to monitor the degree of degradation. The studies presented here contribute to the hazard assessment of ILs and support their design with reduced hazard. The investigated anions showed considerable drawbacks since none of them was degraded via hydrolysis or microorganisms and the perfluorinated ones were especially toxic to aquatic organisms. The dicationic ILs seem to be an effective alternative. Their outstanding physico-chemical properties, specifically, the high thermal stability combined with a lower toxicity relative to monocationic analogues are already a step towards safer chemicals while fulfilling the application related profile. However, their biodegradability still needs to be improved. Here, ammonium and pyrrolidinium based ILs are preferable because they are readily biodegradable. This reveals the feasibility to design ILs with reduced hazard potential. However, this work represents an initial hazard assessment and further studies, *e.g.* long-term toxicity tests of ILs in order to determine chronic effects, are required for concluding evaluations.

ZUSAMMENFASSUNG

Die Substanzklasse der ionischen Flüssigkeiten (ILs, engl. ionic liquids) hat in den letzten Jahrzehnten einen rasenden Aufstieg erfahren. Vor allem der geringe Dampfdruck sowie die Möglichkeit Strukturen maßzuschneidern, die das gewünschte Anforderungsprofil erfüllen, machten ILs in vielen Bereichen zu einer vielversprechenden Alternative. Zunächst sollten ILs als „grüne Chemikalien“ eingesetzt werden um konventionelle, leicht flüchtige Lösungsmittel zu ersetzen. Im Laufe der Zeit wurden weitere Anwendungsbereiche erschlossen. Aber auch der Nachhaltigkeitsaspekt wurde differenzierter für die einzelnen Strukturen betrachtet, da Studien zur Ökotoxizität und Bioabbaubarkeit zeigten, dass Vertreter dieser Substanzklasse ein teilweise hohes Gefahrenpotential auswiesen.

Diese Arbeit soll das Wissen zum Umweltverhalten von ILs erweitern und damit zum Gestalten von Strukturen mit reduziertem Gefährdungspotenzial beitragen. Dabei lag ein besonderer Fokus auf der hydrolytischen Stabilität, Bioabbaubarkeit und Ökotoxizität von drei perfluorierten und drei cyanobasierten Anionen, sowie Kationen mit aliphatischen Kopfgruppen (Ammonium, Pyrrolidinium, Morpholinium oder Piperidinium) oder zwei ionischen Zentren. Zunächst wurde eine ionenchromatographische Methode zum quantitativen Nachweis dieser Ionen entwickelt. Dies diente in nachfolgenden Studien vor allem zur Verfolgung der Abbaurate. Die weiteren Ergebnisse zeigten, dass vor allem Cholin und andere kurzkettige, ammoniumbasierte Kationen eine geringe Ökotoxizität bei gleichzeitig rascher biologischer Abbaubarkeit aufwiesen. Auch die dikationischen ILs scheinen eine aussichtsreiche Alternative zu sein. Hier konnte gezeigt werden, dass die Toxizität im Vergleich zu monokationischen Homologen deutlich reduziert ist. Allerdings müssen deren Abbauraten noch deutlicher optimiert werden. Auch die getesteten Anionen haben den Nachteil des geringen biologischen und hydrolytischen Abbaus und sind, im Falle der perfluorierten Strukturen, teilweise äußerst schädlich für Wasserorganismen. Die präsentierten Ergebnisse zeigen, dass die Entwickelung von ILs mit vermindertem Gefährdungspotenzial möglich ist, stellen jedoch eine vorläufige Abschätzung dar. Weitere Studien, vor allem zum Langzeitverhalten in der Umwelt (z.B. chronische Toxizität, Sorption in Böden oder Bioakkumulation) werden benötigt um eine abschließende Risikobewertung zu ermöglichen.

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LIST OF ABBREVIATIONS

AchE	Acetylcholinesterase
AMP	Adenosine monophosphate
AOP	Advanced oxidation processes
BASIL	Biphasic Acid Scavenging utilising Ionic Liquids
CE	Capillary electrophoresis
CITP	Capillary isotachophoresis
CYP	Cytochrome P_{450}
EC_{50}	Half maximal effective concentration
ESI-MS	Electrospray ionization mass spectrometry
HILIC	Hydrophilic interaction liquid chromatography
IC	Ion chromatography
IL	Ionic liquid
IMP	inosine monophosphate
LC_{50}	half maximal lethal concentration
LOD	Limit of detection
LOQ	Limit of quantification
MINILUBES	Mechanisms of interactions in nano-scale of novel ionic lubricants with functional surfaces
OECD	Organisation for Economic Co-operation and Development
REACH	Registration, Evaluation, Authorization and Restriction of Chemicals
RP-HPLC	Reverse phase high performance liquid chromatography
SAR	Structure-activity relationships
ThOD	Theoretical oxygen demand
TOC	Total organic carbon
UFT	Center for Environmental Research and Sustainable Technology
UV	Ultraviolet
WST-1	2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt

The acronyms of IL cations and anions is identical with the ID code used in the UFT/Merck ionic liquids biological effects database.[1]

IL cations:

IM1-(1Ph-4Me)	1-Methyl-3-[(4-methylphenyl)methyl]- imidazolium
IM11	1,3-Dimethyl-imidazolium
IM1-10	1-Decyl-3-methyl-imidazolium
IM1-1Ph	1-Methyl-3-(phenylmethyl)-imidazolium
IM1-2Ph	1-Methyl-3-(2-phenylethyl)-imidazolium
IM12	1-Ethyl-3-methyl-imidazolium
IM13	1-Methyl-3-propyl-imidazolium
IM14	1-Butyl-3-methyl-imidazolium
IM15	1-Methyl-3-pentyl-imidazolium
IM16	1-Hexyl-3-methyl-imidazolium
IM17	1-Heptyl-3-methyl-imidazolium
IM18	1-Methyl-3-octyl-imidazolium
IM19	1-Methyl-3-nonyl-imidazolium
IM1i4	1-methyl-3-(2-methylpropyl)-imidazolium
IM22	1,3-Diethyl-imidazolium
IM23	1-Ethyl-3-propyl-imidazolium
IM26	1-Ethyl-3-hexyl-imidazolium
N1114	Butyltrimethylammonium
N2222	Tetraethylammonium
P1i4i4i4	Methyltris(2-methylpropyl)phosphonium
P4444	Tetrabutylphosphonium
Py4-4Me	1-Butyl-4-methylpyridinium
Py6-4NMe2	4-(Dimethylamino)-1-hexylpyridinium
Pyr14	1-Butyl-1-methylpyrrolidinium

IL anions:

$(C_2F_5)_3PF_3$	Tris(perfluoroethyl)trifluorophosphate
$(CF_3SO_2)_2N$	Bis(trfluoromethylsulfonyl)amide
$4MePhSO_3$	Tosylate
$B(CN)_4$	Tetracyanoborate
BF_4	Tetrafluoroborate
Br	Bromide
$C(CN)_3$	Tricyanomethanide
CF_3SO_3	Triflate
Cl	Chloride
$N(CN)_2$	Dicyanamide
PF_6	Hexafluorophosphate
SCN	Thiocyanate

Chapter I: INTRODUCTION

1. Ionic Liquids

1.1. General Information

The generic term “ionic liquids” (ILs) is used for salts with low melting points; usually 100 °C is named as a specific threshold. They consist of bulky organic cations and/or anions that lower the ability to form crystalline structures with strong ionic interactions. Thus, less energy, which equals lower temperatures, is necessary for melting these salts. The cations used to form such ILs are often phosphonium-, ammonium-, pyrrolidinium-, piperidinium-, morpholinium-, pyridinium- or imidazolium-based. They can be further altered by the length and functionalisation of the alkyl side chain or by linking two or more core structures to form multivalent ions. The anions differ from small inorganic ions like halides, sulphates, sulphonates and phosphates, to organic and more bulky ones such as tosylate, saccharinate or malonate. Also, perfluorinated forms (*e.g.* BF_4^-, PF_6^-, $CF_3SO_3^-$, $(CF_3SO_2)_2N^-$, $(C_2F_5)_3PF_3^-$) or cyano-based anions (*e.g.* SCN^-, $N(CN)_2^-$, $C(CN)_3^-$, $B(CN)_4^-$) can be found in the literature. Fig. 1 displays the structures of the most frequently used structural elements of ILs.

The very large structural variation and combination possibilities result in an enormous theoretical number of structures (up to several millions). By appropriate choice of cation and anion, the physico-chemical properties of the IL like density, viscosity, polarity, thermal stability or conductivity can be affected.[2–6] This comes along with the possibility to design ILs for their specific usage. Moreover, they mostly own a negligible vapour pressure which lead to diminished evaporation compared to conventional organic solvents. Hence, better handling and an increased operational safety is given due to the non-flammability and the reduced risk to be released to the atmosphere and being inhaled.

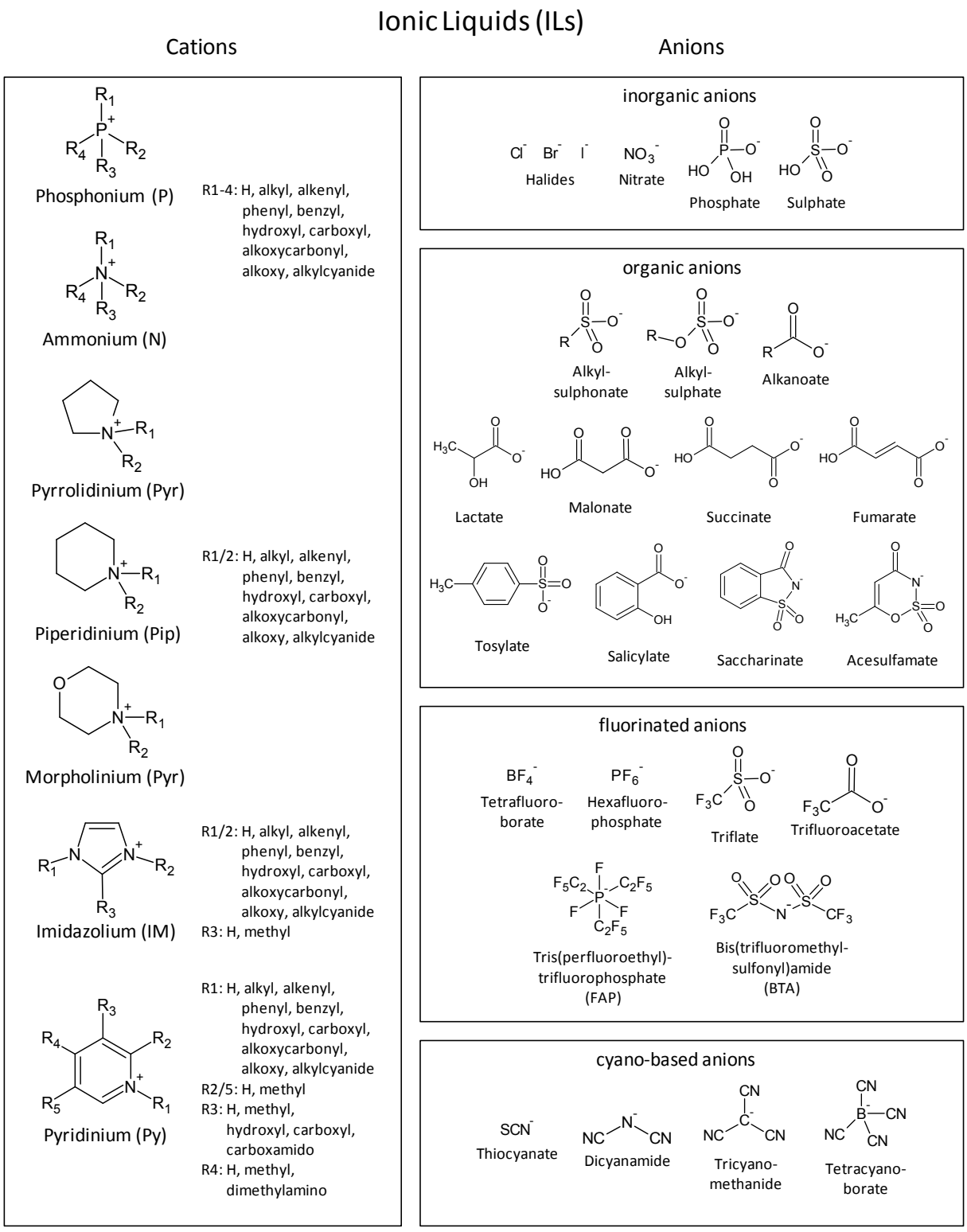


Fig. 1: Structures of cations and anions often used in ILs

Within the last 20 years, the potential to tune and tailor make ILs for a certain application caused rising research interests in this field. The number of publications and patents was increased from less than 100 in the early 1990ties to more than 5000 in 2012.[7] Likewise, the possible fields of applications were enlarged, including solvents, catalysts, batteries, dye-sensitized solar cells or additives in paints, fuels and lubricants (Fig. 2).[8] The BASIL™ (Biphasic Acid Scavenging utilising Ionic Liquids) technology from BASF is the first announced industrial usage of ILs. Since 2002, this process is applied in the production of alkoxyphenylphosphines, a raw material for the photoinitiator Lucirin®. During the reaction, an acid is formed that would decompose the desired product. Therefore, a scavenging agent is used to remove the formed by-product. In the conventional process, tertiary amines like triethylamine were used as scavenging agents. Their great disadvantage is the formation of a solid salt yielding a highly viscous suspension which is difficult to handle and purify. However, in the BASIL™ process the IL precursor 1-methylimidazole is utilized instead. This results in the formation of the IL methylimidazolium chloride, which can be easily separated from the reaction mixture. Furthermore, the methylimidazole acts as a catalyst and leads to higher yields of the product.[9]

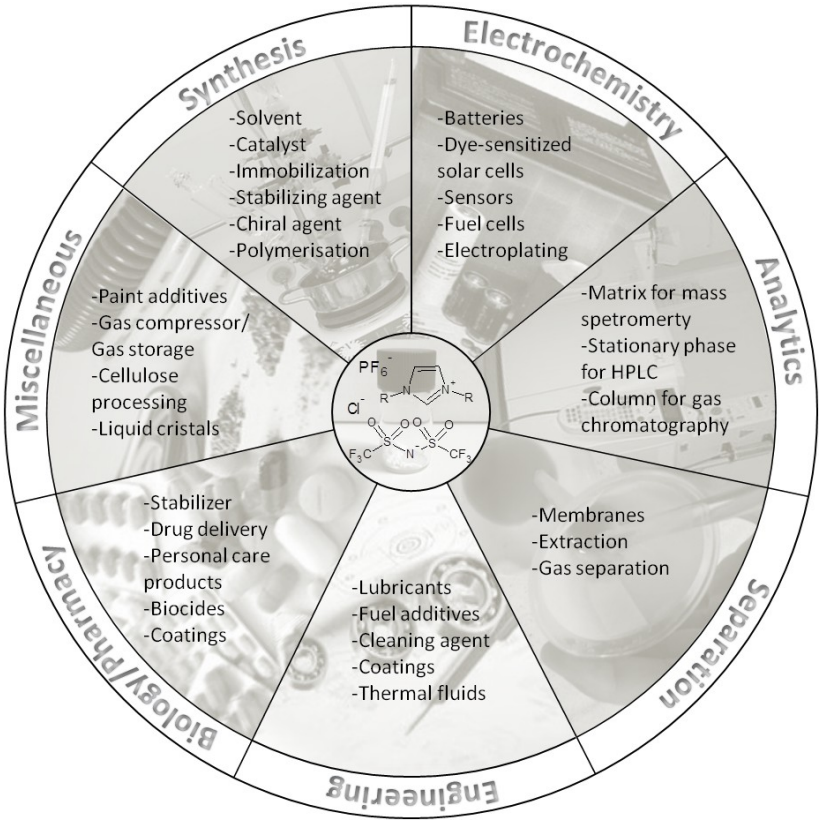


Fig. 2: Overview on reported applications of ILs (Pictures taken from [10–16])

1.2. Research on ionic liquids in the UFT

Within the Center for Environmental Research and Sustainable Technology (UFT), one of the main research fields is the development of sustainable chemicals. Such investigations aim to consider the technological needs of chemical substances while avoiding hazardous structures at the research and development stages of new industrial chemical products. ILs have aroused interest of many scientists because of their tunability, design possibilities, and broad range of application. In 2003, Jastorff et al. stated the lack of knowledge concerning the toxicity and ecotoxicity of ILs and presented a strategy for systematic hazard assessment using the "test-kit-concept" to identify structure-activity relationships (SAR).[17] This research was conducted in an interdisciplinary team and with the cooperation of the University of Gdańsk. It included, not only the hazard assessment of ILs[18,19], but also their sustainable synthesis[20–22], recovery[23,24] and degradation via advanced oxidation processes[25,26]. Furthermore, several application-related projects (e.g. ILs as lubrication additive, thermofluid or catalyst) were attended to select the ILs with optimized physicochemical, economical and environmental properties in an early stage of development.

Within the last 10 years, about 250 studies on the ecotoxicity and environmental fate of ILs were published. Out of these studies, about 20 % were based on the results achieved by the UFT and cooperation partners. However, some data are still missing; primarily for the biodegradation of ILs owing head groups other than imidazolium and pyridinium, the stability and ecotoxicity of "novel" anions, as well as the analysis of both mentioned substructures. The following sections summarize the research state at the beginning of this work and the perceived knowledge gaps aimed to be filled by the research of this thesis.

1.3. State of research at the beginning of this work

1.3.1. Analytics

In order to understand the manner and fate of ILs in the environment, the development of simple and reliable analytical methods is required and can be used to monitor their purity, degradation, sorption to soils, stability, or the nominal and set concentration in toxicity tests. Depending on the desired information, the method should fulfil different requirements: a sensitive quantitative detection of IL traces (cation and/or anion), the determination of ILs in

the presence of high amounts of other organic or inorganic ions (*e.g.* in mineral media used in toxicity tests), the qualitative and/or quantitative detection of impurities, and the analysis of a single IL or mixtures of them. Different types of stationary phases, mobile phases and detection methods are described in the literature. A summary is given in Tab. 1. The technique of choice to analyse IL cations is usually reverse phase high performance liquid chromatography (RP-HPLC) using C8 or C18-columns and electrospray ionization mass spectrometry (ESI-MS) or ultraviolet (UV) detection.[27–31] The retention mechanism is mainly influenced by hydrophobic interactions between the alkyl side chains of the cation and the apolar moieties of the stationary phase. The electrostatic interactions between the cation and charged silanol-residuals are also influential. In stationary phases modified with phenyl-groups, π-π interactions are dominant for cations owing short alkyl chains, whereas longer chained cations are mainly retarded due to hydrophobic interaction.[32] The addition of salts in the mobile phase has a high influence on the retention behaviour of the cation. The retention is increased with higher ion-pairing salt content. The use of chaotropic anions is favoured since it improves the peak shape and leads to reproducible retention factors for the same IL cation coupled with different anions.[30] The limits of detection and quantification are usually in the range of ppb; however, the usage of UV-detection is limited to cations with absorption maxima in the specific wavelength range. The utilisation of a conductivity detector does not lead to these limitations and enables the monitoring of cations and anions.[33,34] Through tandem ion exchange columns, Stepnowski et al. were able to separate and analyze alkyl-methyl-imidazolium cations (alkyl chain length from four to ten carbon atoms) and 5 anions (Cl^-, Br^-, BF_4^-, PF_6^-, $(CF_3SO_2)_2N^-$) simultaneously in a single chromatographic run.[35] Within these ion chromatography (IC) procedures, ionic interactions are primarily responsible for the retention of the ions. However, reversed-phase behaviour was also observed depending on the content of the organic modifier in the mobile phase.

Capillary electrophoresis (CE) is a suitable alternative for analysing ions. The separation is based on the migration of the analytes in an electric field and is usually detected via UV or fluorescence. This technique requires only small sample amounts and is also applicable for complex samples, *e.g.* in environmental matrices. A similar method, also based on electrophoresis, is the capillary isotachophoresis (CITP). With this procedure, the sample is induced between electrolytes of different ion mobility and separated in an electric field. The

leading and terminal electrolyte induces a gradient in the field force where the analyte ions are separated due to their ion mobilities. Both techniques have been shown to be suitable alternatives to determine ILs with low limits of detection and quantification[36–39] or for IL impurities[40].

Sample pre-treatment steps can improve the quantification of the analytes, particularly in the case of very low concentrations or in the presence of a high matrix load. Cation exchange solid phase extraction has proven to be able to concentrate the amount of 1-alkyl- or 1-aryl-3-methylimidazolium cations by a factor of 100-120 with a satisfactory recovery of >90 % in tap-, fresh- and seawater samples.[41] The recovery of 1-alkyl-3-methylimidazolium via ion-pair solid phase extraction depended on the chain length of the cation, the ion-pair reagent and their concentrations.[42] They ranged from 70-100 % in freshwater and from 75-100 in seawater. The best recovery rates were achieved for longer chained cations in high concentrations (50 mM) and sodium 1-heptanesulfonate as an ion-pair reagent. The extraction of ILs from soils, potatoes, rye grain and grass was successful by means of liquid-solid extraction with different (in)organic acids.[43] Whereas in soil samples the extraction of the shorter chained 1-butyl-3-methyl-imidazolium (IM14) was much better compared to the hexyl-derivate (98 % and 40 % in kaolinite respectively), the opposite trend was observed in the plant samples.

Tab. 1: Overview on published analytical methods for ILs[a)]

ILs[b)]	stationary phase	mobile phase	detector	comment	Ref.
HPLC					
IM13 Br, IM14 Cl, IM15 Cl, IM16 Cl, IM17 Cl, IM22 Br, IM23 Br, IM26 Cl, IM1-1Ph BF4, IM1-2Ph Cl, IM1-(1Ph-4Me) Cl	C8 MetaSil Basic, 250x4.6 mm, 5 µm, Varian	acetonitrile/ 20 mM NH_4 CH_3COO, 1 % CH_3COOH	ESI-MS	Gradient	27
IM12, IM14, IM16, IM18 with BF4 and PF6	Kromasil C8, 150x4 mm, 5 µm, Cluzeau Infolabo	acetonitrile/ 0.01 M $NaPF_6$	UV (230 nm)	LOD[c)]: 9-60 mg L^{-1}	30
IM12 Cl, IM13 Cl, IM14 Cl, IM16 Cl, IM1-1Ph Cl, IM1-2Ph Cl, Py4-4Me Cl	Synergi Polar-RP, 150×4.6 mm, 4 µm, Phenomenex	acetonitrile/ 5 mM KH_2PO4, H_3PO_4, pH 3	UV (218 nm)	LOD: 0.2-0.8 mg L^{-1}	32
20 IM14-based ILs with amino acid anions	Ultimate ODS, 200×4.6 mm, 5µm, Welch Materials	Acetonitrile/water, both containing 0.5 mM C_3F_7COOH, CH_3COOH, pH 3.0)	ESI-MS	gradient, LOD: 1-50 µg L^{-1}	44

IM12 Cl, IM22 Cl, IM13 Cl, IM14 Cl, IM16 Cl, IM18 Cl, IM1-1Ph Cl, IM1-2Ph Cl, Py4-4Me Cl	Discovery HS F5, 150×4.6 mm, Bellefonte	acetonitrile/ 10 mM KH_2PO_4, H_3PO_4, pH 3	UV (218 nm)	LOD: 3-5 ng L^{-1}	[45]
IC					
IM14 Cl, IM14 Br, IM14 BF4, IM14 PF6, IM14 (CF3SO2)2N	SphereClone SAX, 250x4.6 mm, 5 µm, Phenomenex	acetonitrile/ 8 mM phthalic acid, tris-buffer, pH 4	conductivity	LOD: 1-1.6 mg L^{-1}	[34]
IM14 Cl, IM14 Br, IM14 BF4, IM14 N(CN)2, IM14 (CF3SO2)2N, IM14 4MePhSO3	MetrosepA Supp 5, 250x4 mm, 5 µm, Metrohm	acetonitrile/ 3.2 mM Na_2CO_3, 1 mM $NaHCO_3$)	conductivity	LOD: 1.2 mg L^{-1}	[34]
CE					
IM12 Cl, IM14 Cl, IM1i4 Cl	Fused-silica capillary, 50 µm i.d.[d], 53.6 cm length (43.5 cm effective length), Polymicro Technologies	5.0 mM triethylamine, 2.0 mM α-cyclodextrin, CH_3COOH, pH 4.5	UV (210 nm)	LOD: 0.9-1.4 mg L^{-1}	[36]
IM11 Br, IM12 Br, IM22 Br, IM13 Cl, IM14 BF4, IM16 BF4, IM18 BF4, IM19 BF4, IM1-10 Cl, IM1-1Ph BF4, IM1-(1Ph-4Me) Cl	Fused-silica capillary, 50 µm i.d., 45 cm length (41.5 cm effective length) Polymicro Technologies	200 mM citrate buffer, pH 4	UV (214 nm)	LOD: 10 µg L^{-1} (IM12)	[37]
CITP					
IM13 BF4, IM14 Cl, IM14 BF4, IM15 BF4, IM16 BF4, IM18 BF4	2 fluoroethylene-propylene polymer columns, 0.8×90 mm and 0.3×160 mm	cation detection LE[e]: 10 mM NH_4 CH_3COO, 0,1 % HEC[f] TE[g]: 5 mM $N(C_4H_9)_4$ ClO_4	conductivity	LOD: 25 ng/L	[38]
		anion detection: LE: β-alanine chloride, 3 mM BTP[h], 0,1 % HEC TE: 2 mM citric acid		LOD: 10-15 ng/L	
IM16 (CF3SO2)2N, IM14 PF6, IM14 BF4, IM14 CF3SO3, IM14 N(CN)2, IM12 Cl, IM13 Cl. N2222 Cl, Pyr14 Cl, Py6-4NMe2 Cl, P1i4i4i4 4MePhSO3, P4444 Cerl	2 fluoroethylene-propylene polymer columns, 0.8×90 mm and 0.3×160 mm	cation detection LE: 10 mM KOH, 10 mM CH_3COOH TE: 10 mM β-alanine, 10 mM CH_3COOH	conductivity	LOD: 0.3-0.8 mg L^{-1}	[39]
		anion detection: LE: 10 mM L-histidine, 10 mM L-histidine monohydrochloride TE: 5 mM L-histidine, 5 mM glutamic acid		LOD: 0.1-0.6 mg L^{-1}	

[a] this is not a full list, [b] please refer to the list of abbreviations for full IL names, [c] limit of detection, [d] internal diameter, [e] leading electrolytes, [f] hydroxyethylcellulose, [g] terminating electrolytes, [h] 1,3-bis[tris(hydroxymethyl)-methylamino]propane

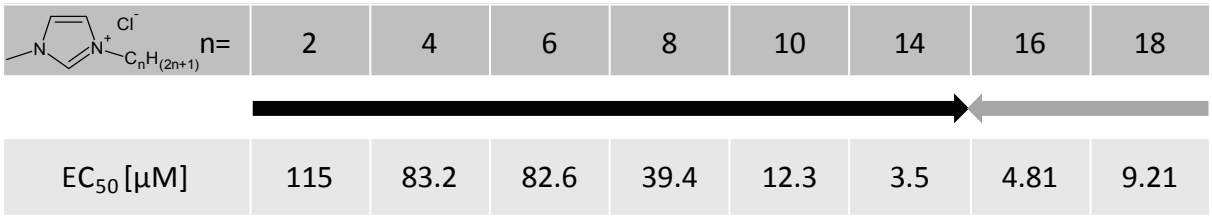
1.3.2. Toxicity

One key aspect in the hazard assessment of chemicals is the toxicity of the compound to different model organisms. Under REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) legislation, the amount of tests required for a substance depends on its production volume. The standard toxicity information needed for chemicals produced in quantities >1 t/a is their skin irritation or corrosion (*in vitro* testing), eye irritation (*in vitro* testing), skin sensitisation (*in vivo* testing), mutagenicity (tested *in vitro* in bacteria), acute toxicity after oral uptake, the short term toxicity on invertebrates (preferable *Daphnia* species) and growth inhibition tests on aquatic plants (preferable algae) as well as any other information that is already available.[46] This is going to be enlarged whenever a new tonnage band is reached (>10 t/a, >100 t/a or >1000 t/a). Since only a few ILs are produced in such high quantities, the toxicological studies are usually based on *in vitro* tests in order to identify possible modes of toxic action and structure-activity-relationships investigating the influence of the cations' side chain and head group and the anion of an IL to its overall toxicity. However, for the ecotoxicological hazard assessment of ILs, algae and higher plants and organisms, such as invertebrates or fish, were used.

Tests on the molecular level are ideal models to study the interaction potential of chemicals and are able to indicate a certain mode of toxic actions. The test systems enquiring ILs were the enzyme inhibition of acetylcholinesterase (AchE)[47,48], adenosine monophosphate (AMP) deaminase[49] and cytochrome P_{450} (CYP)[50,51]. AchE is an important enzyme in the signal transmission of neutrons and muscles. By inhibition of this enzyme the neurotransmitter acetylcholine cannot be hydrolysed and is enriched in the synaptic cleft. This leads to a continuous stimulus transmission and therefore to seizures, respiratory paralysis and, finally, to death.[52] A detailed description is given in chapter II, section 1.1. AMP deaminase converts AMP to inosine monophosphate (IMP), which is important for the regulation of the adenine nucleotide concentrations. The group of enzymes united under the generic term cytochrom P450 (more than 500 isoenzymes are identified) are present in nearly all organs, but are in high concentrations especially in the liver. These monooxygenases play an important role in the metabolization of xenobiotics and are therefore of high interest in ecotoxicology. Moreover, they are involved in the biosynthesis of steroid hormones, bile acid and eicosanoids. CYP are characterized by a broad substrate specificity, which is defined by the

lipophilic binding site. Out of these three molecular test systems, AchE was the most intensively studied.[47,48,53,54] The results concerning the influence of the head group and side chain of the cation to the overall toxicity could have also been found in nearly all higher test systems. First of all, the side chain seems to play the major role for defining the inhibition potential. With increasing number of carbon atoms in the alkyl chain, the enzyme activity (for AchE as well as CYP) was reduced.[47,50] This could be mainly related to the raising lipophilicity of the IL and the better interaction potential it has with the binding site of the enzyme. However, for very long side chains (C>14) it was found that the inhibition is no longer increased, but diminished by enlarging the alkyl chain.[55] The dependency of the hydrophobicity and the AchE inhibition potential of the IL was further confirmed as the introduction of polar functional groups in the side chain, e.g. hydroxyl or ether, reduced the adverse effect.[55] In addition, the hydrophobic head groups quinolinium or dimethylamino-pyridinium showed a 100 times higher effect than the non-aromatic morpholinium and phosphonium based ILs.[55] For several methyl-butylpyridinium cations, an influence of the position of the methyl group in the ring on the enzyme activity was demonstrated.[55] In general, the methylated derivatives had a higher inhibition potential than the native butylpyridinium. The symmetrical 4-methyl-butylpyridinium cation exhibited the lowest decrease in enzyme activity of all isomers, whereas the methylation in the 2-position led to the highest loss. This may be related to the greater similarity of the 2-methyl-1-butylpyridinium and the natural substrate, thus better binding possibilities or orientation to the active centre. An illustration of the discussed results is shown in Fig. 3. The anions' inhibition potential is expected to be low for AchE. This is due to the fact that the active centre, as well as its periphery, is negatively charged. For most of the tested anions, the enzyme activity was not affected.[54,55] However, fluorinated anions like PF_6 and SbF_6 (tested as alkali salt) owned an increased inhibition potential, but their hydrolytically instability (see section 1.3.4) has to be taken into account since fluoride was also shown to have an influence.[55] Likewise, the AMP deaminase was stronger inhibited by ILs consisting of IM14 and BF_4^- and PF_6^- compared to Cl^- and tosylate.[49]

Influence of the side chain length



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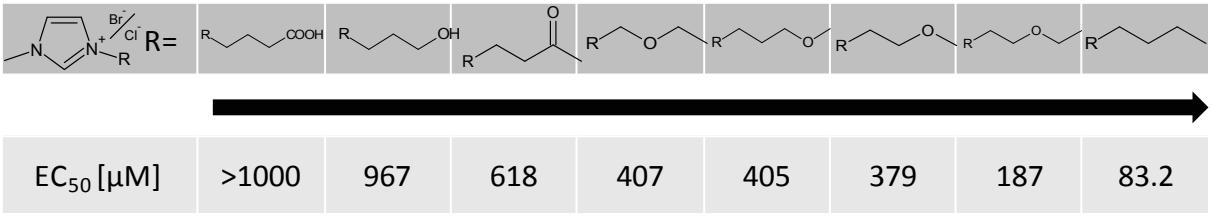
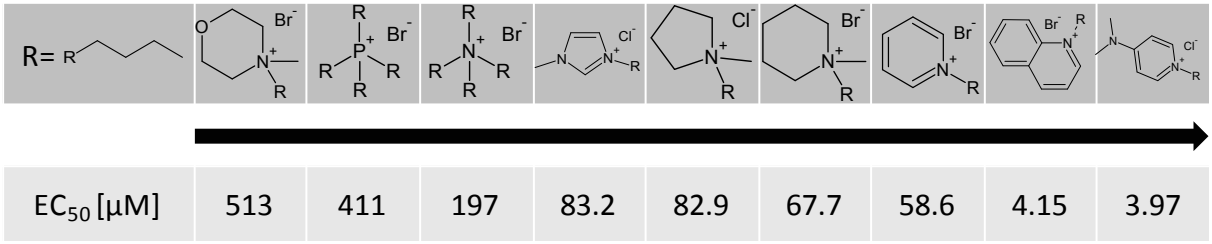


Influence of the head group



Influence of the head group methylation

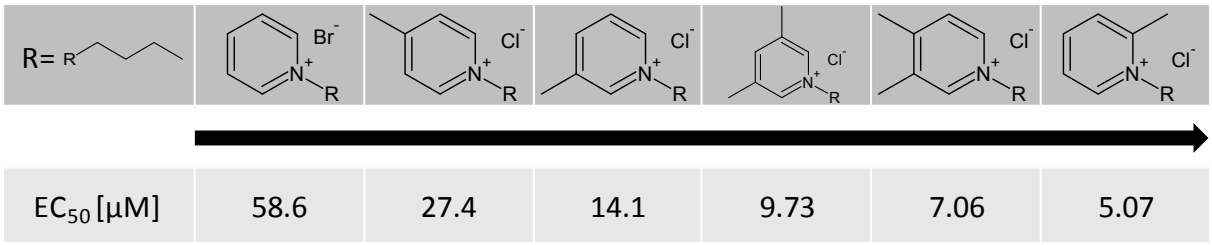


Fig. 3: Influence of structural elements of the IL cation on AchE inhibition (Data taken from [1])

In-vitro cytotoxicity assays are a suitable alternative for a preliminary assessment of chemicals since they offer, compared to *in-vivo* studies, a higher throughput combined with increased reproducibility and lower costs. Furthermore, investigations for modes of toxic actions, *e.g.* membrane disruption, are possible. On the other hand, *in-vitro* testing is limited to the acute effect on specific cell cultures. Even though, in some cases, a correlation of *in-vitro* tests with the fish cell line PLHC-1 and *in-vivo* fish test was found[56,57], the transmission of the observed cytotoxicity to higher organism is not easily possible. As the group of ILs

include several thousand substances, this screening method is a rapid tool for a first examination. Different cell lines have already been investigated, *i.a.* IPC-81 (leukaemia rat cells)[53,58–60], C6 (rat glioma cells)[60], MCF7 (human breast cancer cells)[61], CaCo-2 (human colon carcinoma cells)[62,63] and HeLa (human tumor cell line)[64,65] as the most used. The cell lines from different tissues and organism showed variations in their sensitivity. Leukaemia, melanoma and lung carcinoma cells tend to show the highest responsiveness.[60,66–68] As it was discussed for the enzyme inhibition test, the cytotoxicity is mainly influenced by the lipophilicity of the cation.[59,69] In nearly all research studies, an increasing side chain length led to higher cytotoxicity.[54,60–63,65] An exception was found by Stepnowski *et al.*[64] where the HeLa cell showed a discontinuous side chain length dependency. However, Wang and co-workers[65] could not confirm this observation within their study using the same cell line, but with slightly different testing methods instead. For different 1-alkyl-3-methyl-imidazolium ILs (C=4, 6 and 8), it was shown that the uptake of an IL in the cells is higher for longer chains[70], which is a possible explanation of the dependency of lipophilicity and cytotoxicity. If polar groups are present in the side chain, the cytotoxicity, compared to the aliphatic side chains, is decreased.[59,62,65] Though, for ether groups, the position in the side chain influences the cytotoxicity drastically. It was found that an ethoxymethyl group showed, regardless of the cationic head group, a similar or an even higher decrease in cell viability of IPC-81 cells than a butyl chain, whereas the methoxyethyl group led to lower cytotoxic effects.[59] A similar, but not that pronounced effect, was found for ethoxyethyl compared to methoxypropyl.[59] The aliphatic head groups (morpholinium, piperidinium, pyrrolidinium and ammonium) showed, by trend, a higher half maximal effective concentration (EC_{50}), correlating with a lower cytotoxicity, than the aromatic structures imidazolium and pyridinium.[59,61,65] Methylation of the pyridinium ring can also have an impact on the cytotoxicity; however, the trend found is thereby different for a different length of the N-alkyl chain. Whereas the cytotoxicity of butyl-pyridinium derivates to the leukaemia rat cell line decreased from the un-methylated form to the meta-, ortho- and para-isomers[1], the octyl-pyridinium based ILs showed a nearly opposite behaviour for both IPC-81 and MCF7 cells (4-methyl-octylpyridinium less toxic than 3-methyl-octylpyridinium, octylpyridinium and 2-methyl-octylpyridinium; all tested as halides[1,61]). A significant decrease in cell viability was obtained when a dimethylamino group was substituted into the *p*-position of the pyridinium ring (EC_{50} 42 times lower compared to imidazolium).[59] For cations consisting of this structural element, the dependency between

cytotoxicity and lipophilicity (expressed as log k and determined via HPLC retention time) is no longer valid.[59] Thus, additional or different modes of toxic actions can be assumed. After looking at the anion moiety, most of them owned only marginal cytotoxic effects.[58] PF_6^- was found to lower the adverse effect of ILs *vs.* ILs containing halides or Na PF_6, which can possibly be related to higher hydrolysis rates for the alkali salt and/or ion pair formations in the IL.[60,64] The effect of acesulfamate or saccharinate in 3-hydroxy-1-(propoxymethyl)pyridinium ILs was similar.[54] Some of the tested anions, usually highly fluorinated, like SbF_6^-, $N(CF_3)_2^-$, $CF_3SO_3^-$, $(CF_3SO_2)_2N^-$, $(C_2F_5)_3PF_3^-$ or $(C_2F_5)_2PO_2^-$ as well as bis-oxalato-borate, bis-(1,2-benzenediolato)-borate and long and/or branched chained phosphates, had a noticeable impact on the cytotoxicity of the IL.[58,61,65,71] However, these observations were less pronounced than the side chain effect. For an application of ILs as tumour-therapeutics, they should own a high growth inhibition rate but low cytotoxicity. Studies indicated that phosphonium based ILs were more active and less toxic than ammonium analogues.[67] Also, an increasing alkyl chain led to higher anti-cancer activity and cytotoxicity. In a series of alkyl-methylimidazolium ILs, the best results were obtained for the C-12 chain. Whereas shorter alkyl chains induced significant loss in the anti-tumour activity, chains consisting of more carbon atoms increased not only the growth inhibition, but also cytotoxicity.[68]

One of the first investigations on toxicological properties of ILs was conducted with bacteria. The antimicrobial activity of ILs was intensively studied, particularly by the group of Prof. Pernak (Poznan, Poland).[72–83] Usually the Gram-negative strains were more resistant than the Gram-positve ones.[73,74,84] This can be related to their differences in the cell wall. Whereas Gram-positive bacteria own a thick murein layer, in Gram-negative bacteria this layer is smaller, but reinforced by a second outer membrane of porine and lipopolysaccharides. One exception was the methicillin-resistant *S. Aureus* strain which showed similar sensitivity to Gram-negative bacteria.[76] However, this might be due to their thicker cell wall with a modified peptidogylcan.[85] Also, the studies from Docherty et al. demonstrated the resistance of the Gram-positive strain *S. Aureus*.[84] The results from ILs with varying alkyl or alkoxymethyl chains illustrate, once more, the influence of their length on the overall toxicity. Again, the adverse effects were enhanced when the chain length was increased.[73–76,78–80,86–88] However, for very long chains (C>10-14, depending on the headgroup and further

substituents in the ring) the antimicrobial activity may have dropped due to steric aspects.[74–76,86] Also for this test organism, a higher toxicity of dimethylamino-substituded pyridinium[75] and quinolinium[89] ILs was found. When testing 10 different fungal strains, Petkovic *et al.* observed a high resistance (up to 0.05 M), especially for choline based ILs showing the high biocompatibility of this IL cation.[90] Contrary to the results observed in cytotoxicity test[18] and towards the marine bacteria *V. fischeri*[84], the precursors imidazole and pyridine were more toxic than the ILs[90]. The reason for this is still unclear, but may be associated to different test methodologies. Likewise, the results for test systems discussed before the anion effect were found to be secondary and less predictive. For phosphonium ILs, an antimicrobial activity loss was found when BF_4^- PF_6^-, NO_3^- or $(CF_3SO_2)_2N^-$ was present instead of chloride, which can be traced back to the faster adsorption of the halide.[81,91] Though, for several imidazolium based ILs combined with $(CF_3SO_2)_2N^-$ or octylsulfate, an increasing growth inhibition to bacteria has been shown.[92] Among the tested alkonates, a trend similar to the side chain effect of the cation was found. With the increasing number of carbon atoms, the tendency to be effective against fungal strains is higher.[93] Moreover, when comparing the anions with the same number of carbons, the linear chained are slightly more toxic than the branched isomers.[93]

As described above, tests on higher organisms are still rare. However, studies with rodents demonstrated an acute toxicity after oral uptake (LD_{50} 500 mg per kg body weight) and the tendency for eye and skin irritation for IM14 Cl.[94] At sub-lethal doses, IM14 Cl and 1-Decyl-3-methyl-imidazolium chloride (IM1-10 Cl) pose a loss of average maternal weight, mortality, lower fetus weight or an increased number of malformations in mice, indicating a teratogenic potential.[95,96] Tests for the genotoxicity (Sister Chromatid Exchange test) of IM14 BF4 and mutagenicity (Ames Test) of several 1-alkyl-3-methyl-imidazolium bromide (n=4, 6 or 8), 1-alkyl-3-methylpyridinium bromide (n=4, 6 or 8) and tetraalkylammonium bromide (n=1, 2, 4 or 6) were negative in the tested concentration range[18,97], yet IM1-10 BF4 showed a dose dependent, but not statistically significant, trend for higher frequencies of sister chromatic exchange between 0 and 10 µM[18].

Since ILs are barely volatile, the possibility for them to end up as an air pollutant is unlikely. However, increasing industrial usage can easily result in water and soil contamination. Thus, the investigation of the aquatic and terrestrial ecotoxicity of ILs is of high importance. Different model organisms were used, most of all *V. fischeri*[60,84,98–100] (a gram-negative

marine bacteria, see chapter II, section 1.2), algae (several green algae and diatomas, among them *O. submarina*[101,102], *P. subcapitata*[103–108], *S. vacuolatus*[53,100], *S. quadricauda*[109], *C. reinhardtii*[109,110] and *C. meneghiniana*[101,102], *B. paxillifer*[111], *S. marinoi*[102], respectively), and *D. magna*[99,105,108,112–114] (a crustacean, see chapter II, section 1.3). For all these test systems, the higher toxicity for longer side chains in the cations[60,84,98,99,113], the reduced toxicity for aliphatic cation structures compared to aromatic ones[100], and the secondary effects for the anionic moiety[60] were, once again, found in several studies. The determined EC_{50} values depended strongly on the incubation time and species. The values were usually the lowest for *D. magna*. For the different green algae and diatom species, it was suggested that the higher resistance of the latter[101,102,111] is due to its silica cell wall[115]. Also, the media composition was found to influence the sensitivity of the algae species, *viz.* higher nutrient or salt content possibly reduces the toxicity due to ion-pairing or complexation of the cation, thus lowering bioavailability. In the test with *V. fischeri,* the octylsulfonate and bis(1,2-benzenediolato)borate anion (tested as alkali salt) had a significantly lower EC_{50} value *vs.* BF_4^- and $(CF_3SO_2)_2N^-$.[53] The latter increased the toxicity of IM14 compared to the chloride IL, indicating synergistic effects. This was further verified by a mixture of IM14 Cl and Li $(CF_3SO_2)_2N$, which showed a greater luminescence inhibition as predicted from their single EC_{50} values via the concentration addition model. However, when IM14 was replaced by 1-methyl-3-octyl-imidazolium (IM18), any mixture effects were masked due to the dominant influence of the cation on the overall toxicity.[53] The high toxicity of $(CF_3SO_2)_2N^-$ was reinforced by several other studies on *V. fisheri*, algae (*S. vacuolatus*), wheat (*T. aestivum*), cress (*L. sativum*) and springtail (*F. candida*).[53] Surprisingly, among the tested anions (Cl^-, Br^-, BF_4^- and PF_6^-), the IM14 Br showed the highest toxicity towards *D. magna*.[116] Within this test species, oxidative stress seems likely to be part of the toxicity mechanism since the activity of superoxide dismutase, catalase, glutathione peroxidase and glutathione S-transferase (enzymes in the antioxidant machinery) was found to be increased at IL concentrations around the EC_{50} value.[114] At sub-lethal concentrations of IM14 Br or BF_4, chronic effects as a reduced number of first-brood neonates, total number of neonates and average brood size were observed.[116] Also, the freshwater snail (*P. acuta)* demonstrated sub-lethal effects, *i.e.* lower feeding rates.[117] For tests on higher plants such as duckweed (*L. minor*)[53], wheat (*T. aestivum*)[53] or cress (*L. sativum*)[53] and higher organism, for instance *P. acuta* (freshwater snail)[117], *D. polymorpha* (zebra mussel)[118] and *F. candida* (springtail)[53], the dependency of

longer alkyl chains and higher toxicity for imidazolium- and pyrrolidinium-based ILs was, once again, reported. However, in the terrestrial tests, the sorption mechanism seems to influence the toxicity. 1-ethyl-3-methyl-imidazolium (IM12), which has a lower tendency to adsorb on soils, was similarly, or slightly more toxic than IM14.[119,120] Furthermore, the addition of organic matter, which is known to increase the sorption of IL cations, reduced the toxicity due to lower bioavailability.[119] For *D. rerio* (zebra fish), a median lethal dose (LD_{50}) could only be determined for two ammonium ILs owning very long side chains (AmmoEng 100™ and AmmoEng 130™), whereas the other tested ILs did not show lethal effects to more than 50 % up to 100 mg L^{-1}.[108,121] The tendency to be teratogenic, as described above for mice, was fortified by the results for IM18 Br, which causes increased embryonic mortality and morphological malformations in *R. nigromaculata* (frog)[122] and *C. auratus* (goldfish)[123].

In summary, according to these published results, the structural design of ILs with reduced hazard potential is already straight forward. In the best case, cations and anions of low lipophilicity, *i.e.* aliphatic head groups substituted with short and functionalized side chains, and halide or biocompartible anions should be used. Through all of this, some noticeable knowledge gaps are still present and further discussed in section 2 (missing data).

1.3.3. Biodegradation

Biodegradation is also of special interest within the hazard assessment of chemicals. It is not only important with regards to if and how fast the chemicals are degraded, but also to what extent. A non-biodegradable substance that is also resistant to other degradation pathways, like hydrolysis or photolysis, can be persistent and abundant in the environment. The permanent exposure might become relevant in terms of chronic toxicity at sub-acute concentrations or, due to increasing concentrations, with respect to acute effects. Furthermore, bioaccumulation (enrichment of the chemical within an organism at higher concentrations than in the environment) or -magnification (increase of the chemicals' concentration within the food chain) could occur. Examples for persistent and bioaccumulative chemicals are Musk xylene and several long-chained perfluorinated carboxylic acids (11-14 carbon atoms) that are used in cosmetics and as plasticizers. In accordance with Article 59(10) of the REACH regulation, these chemicals are included in the Candidate List of Substances of Very High Concern for Authorisation.[124] The importance of

identifying degradation products should also be considered as they might be toxic, whereas the parent compound was classified to be harmless. According to REACH legislation, these investigations are required depending on the production volume of the chemical.[46]

Most of the published studies on the biodegradability of ILs were investigating the ready biodegradation potential using methods suggested by the Organisation for Economic Co-operation and Development (OECD) (see chapter II, section 2.3).[125] Thereby, parameter like oxygen consumption or carbon dioxide evolution are investigated and related to the theoretical oxygen demand (ThOD) and total organic carbon (TOC) of the compound, respectively. According to this, the biodegradation rate can be calculated and should exceed 60 % within 28 d to classify a compound as readily biodegradable. Furthermore, primary degradation studies are available which examine the breakdown of the parent compound (cation or anion), but not its total mineralization.

The inorganic IL anions like halides, BF_4^-, PF_6^-, which have no carbon source, need to be considered as not biodegradable. Thus, for those anions, abiotic degradation pathways such as hydrolysis are more relevant (see section 1.3.4). In numerous research examinations, the biodegradation rate for ILs containing a non-biodegradable cation and alkylsulphates owning different alkyl chain length (hexyl to decyl, dodecyl) was enhanced, indicating the biodegradability of the anion.[92,113,126–128] The same is expected by combining ILs with anions from biomaterials (*e.g.* acetate, fumarate, lactate, tartrate or succinate).[126,129] Contrary results were found for dialkylphosphates; whereas diethylphosphate is assumed to be mineralized,[105] the dibutylphosphate showed no biodegradability[126]. The trend for acesulfamate and saccharinate is similar. Harjani et al. were able to enhance the biodegradation rate of a IM14 based IL when it was combined with saccharinate.[126] However, Stepnowski and co-workers were not able to significantly raise the degree of biodegradation for various 1-alkoxymethyl-3-hydroxy-pyridinium ILs except for 1-undecoxymethyl-3-hydroxy-pyridinium saccharinate.[54] Perfluorinated anions like triflate, trifluoracetate or $(CF_3SO_2)_2N^-$ were found to be recalcitrant to microbial degradation.[105,130]

Many more studies were performed with regards to the cationic moiety of the IL, primarily for imidazolium based ILs. The results are illustrated in Fig. 4.

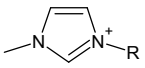
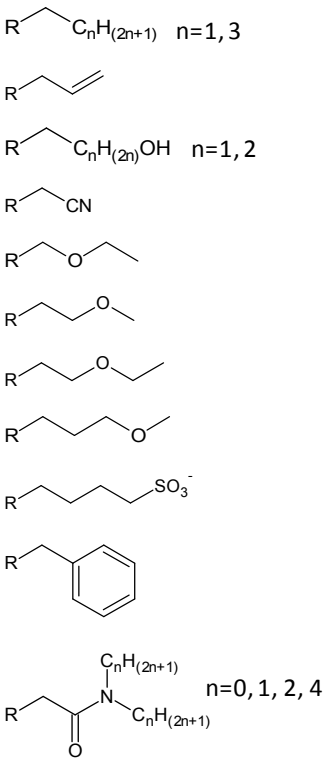
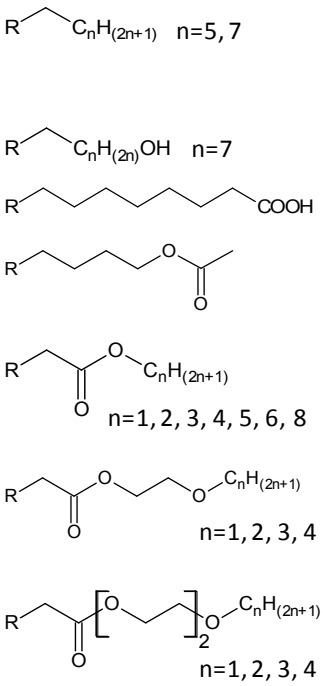
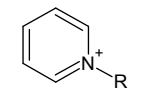
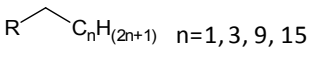
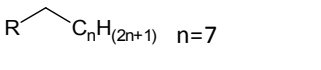
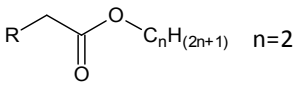
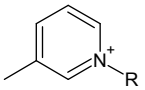
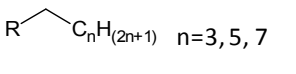
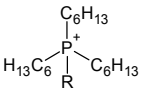
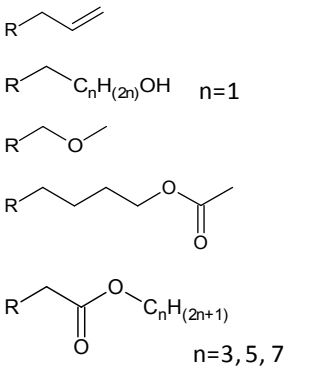
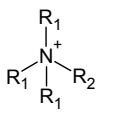
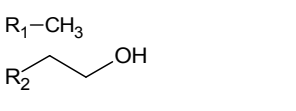
	not biodegradable	partially biodegradable	readily biodegradable
			
			
			
			
			

Fig. 4: Overview on biodegradation results of IL cations

Whereas the precursor imidazole and C-substituted derivates can be fully mineralized, the N-substituted compounds are known to be poorly biodegradable.[131] Thus, it was expected and proven in various studies that the core structure of imidazolium based cations is stable to microbial breakdown.[132,133] However, the alkyl side chain is, when owing a certain length (6<C<12), degraded.[105,132,133] This can be further improved when combined with functionalisations, *e.g.* by hydroxyl, carboxyl or ester groups.[92,113,126,128,132,134,135] On the other hand, hydroxylation in short chains (C<6) or the introduction of ether, amides, phenyl or allyl groups, did not enhance the biodegradation rate of the cation.[126,132,136] These trends have also been found for pyridinium based ILs. Though, the pyridinium core was also attacked in the case of a biodegradable side chain.[137] Studies on phosphonium and ammonium based ILs indicate that an elongation of the side chain easily led to a diminished biodegradation rate due to an inhibitory effect of the inoculum.[105,127] Therefore, especially short chained and cations from natural sources, *e.g.* choline, showed increased biodegradation potential.[129]

This state of knowledge was summarized at the beginning of this work in a review and is included in this thesis (appendix, paper No. 6, page 107).

1.3.4. Hydrolysis

Hydrolysis represents the most important abiotic degradation pathway for environmental chemicals. On the other hand, hydrolysis during technical usage can cause not only reduced applicability or diminished performance, but also serious problems in case corrosive and/or toxic hydrolysis products are formed. For the IL cations, only few data concerning their stability in an aqueous environment are available. Gorman-Lewis et al. showed that the 1-butyl-3-methyl-imidazolium is stable in the pH range from 6 to 10, whereas at lower and higher pH values the stability is doubtful.[138] Both, the UV-spectrum, as well as the thin-layer chromatogram, showed obvious differences compared to the parent compound and solutions of pH values between 6 and 10. An identification of hydrolysis products has not been done and other studies could not confirm this result.[139]

Considering the IL anions, the development of highly stable moieties has been successful. In early years the ILs mainly contained $AlCl_4^-$ as the counter ion. These ILs were extremely instable in the presence of moisture as the anion hydrolyses rapidly. The stability in an aqueous solution was still unsatisfactory after substituting the anion with BF_4^-. Even at room

temperature the formation of degradation products could be observed within 24 h.[139] The hydrolysis rate was further enhanced when the alkyl chain in the cation was elongated.[139] A similar cation dependent observation was done for the PF_6^- anion. Whereas K PF_6 was found to be stable in acidic, neutral and alkaline solutions[140], the hydrolysis of Li PF_6 is catalysed by acids and, due to the formation of *i.a.* HF, an autocatalytic process[141]. ILs containing the PF_6^- anion showed an increased stability compared to BF_4^- analogues. However, Freire et al. were able to show the presence of $PO_2F_2^-$ when the IL was exposed to high temperature or acids.[139] Another study demonstrated the formation of white crystals, identified by x-ray analysis as IM14 $F \cdot H_2O$, during the purification process of IM14 PF_6.[142] An effective advancement in the stability of IL anions was the introduction of perfluorinated alkyl chains (C_2F_5) to PF_6^- yielding in $(C_2F_5)_3PF_3^-$. No HF formation was detected for ILs consisting of this anion after 5 h in boiling water.[143] A detailed study on the hydrolytic stability of IM14 BF_4, IM14 PF_6, IM14 $(CF_3SO_2)_2N$ and 1-hexyl-3-methyl-imidazolium (IM16) $(C_2F_5)_3PF_3$ has been performed using a rapid colorimetric assay based on pH changes.[144] BF_4^- showed a significant pH decrease (to pH 4) at 25 °C within 1 h and at 50 °C in 15 min. The other three anions were stable at ambient conditions. However, for all of them, a decrease to lower pH values was detected at 50 °C after 24 h (for PF_6^-) and one week (for $(CF_3SO_2)_2N^-$ and $(C_2F_5)_3PF_3^-$), respectively. For non-halogenated anions, an alkylsulfate anion with a long chain length (C>8) seems to be a suitable alternative, whereas the smaller analogues showed hydrolysis at 80 °C.[145]

2. Missing data

The previous section summarized the enormous data set published regarding the hazard assessment of ILs. Nevertheless, in such a variable and huge substance class, some knowledge gaps can still be found. The instrumental analysis is often of fundamental need for a comprehensive investigation of the toxicity (determination of nominal concentrations in different test media) and biodegradation and hydrolysis (determination of degree of degradation or identification of resulting products). The methods developed so far were mainly demonstrated for UV-active cations (imidazolium and pyridinium), and only few anions (halides, BF_4^-, PF_6^- and $(CF_3SO_2)_2N^-$). Possibly, also due to this, most studies on the toxicity or environmental fate have been focusing on these structural elements. The first focus of this thesis was the design of an universal method for UV-inactive cations (*e.g.*

containing ammonium, phosphonium or pyrrolidinium head groups) and further anions (*e.g.* $N(CN)_2^-$, $C(CN)_3^-$, $B(CN)_4^-$, $(C_2F_5)_3PF_3^-$). Since ILs are only composed of ions, IC with conductometric detection seemed to be a suitable principle. The major objectives of this work were not the separation of a mixture of different IL cations or anions, but the quantification of traces of the single compound in presence of a large amount of inorganic ions, usually present in test media. Furthermore, by altering the eluent (organic modifier and/or acid content), the applicability to analytes owning a wide range of polarity should be ensured. The results can be found in paper No. 1 (page 29)

The anionic moiety of the IL is usually the most important component defining its physico-chemical properties. The cyano-based anions $N(CN)_2^-$, $C(CN)_3^-$ and $B(CN)_4^-$ and the fluoroorganic anions $(CF_3SO_2)_2N^-$ and $(C_2F_5)_3PF_3^-$ are frequently discussed in the literature.[146–148] The main advantages are their improved electrochemical properties and an increased hydrophobicity, and, consequently, a lower water miscibility of the IL containing such anions (with the exception of $N(CN)_2^-$).[4,143,149–152] However, their stability to hydrolysis and microbial degradation, as well as the influence on the toxicological properties of the IL, are still missing. The investigations of these characteristics were the next goals within this thesis. The hydrolysis does not only represent the most important abiotic degradation way for chemicals, but also an undesirable effect during application. Studies considering the stability in aqueous solutions should not only comprise pH values present in environmental surroundings, but also harsh conditions that may occur during technical usage. Furthermore, the identification of hydrolysis products completes such a study since toxic or corrosive compounds can be formed. Paper No. 2 (page 39) summarizes the results of the experiments. The biodegradation potential of these anions was not the main focus within this thesis. However, my contributions are included in paper No 7 (appendix, page 137). Fluorinated anions have already been identified as a potentially toxic component of an IL.[53,58] Compounds containing the cyano-based anions usually showed comparable physico-chemical properties. In terms of sustainable product design, substances with lower hazard potential are, in this case, preferred. A study on their toxicological behavior towards test systems of different biological complexity can be found in paper No. 3 (page 51).

The studies on the hazard potential of imidazolium based ILs are already straight forward. Alternatives fulfilling both technical needs and low hazard to man and environment are

desired since the imidazolium core tends to persist and accumulate in the environment. The investigation of possible substitutes was the third aim within this thesis. Cations from natural sources, *e.g.* choline, are suitable candidates because of their biocompatibility including low toxicities and high biodegradation potential. The investigations of these parameters, in combination with the possible applicability in the field of lubrication, were the purposes of an international and interdisciplinary project within Marie-Curie-actions called MINILUBES (Mechanisms of interactions in nano-scale of novel ionic lubricants with functional surfaces). The results are summarized in Paper No. 4 (page 65) and No. 8 (appendix, page 149). Since 2003, multivalent cations consisting of several (different) head groups linked by an alkyl chain of different length are also described in the literature.[153] These ILs own the advantage of being even more thermally stable and thus have a wider liquid range.[154,155] However, no research on their toxicity and biodegradability has been published until now. A first evaluation, including simple dicationic ILs of different side and linkage chain length, investigating if known structure-activity-relationships of monocationic IL are also applicable to dicationic homologues is shown in paper No 5 (page 75). Furthermore, to fill the knowledge gap for the biodegradability of other head groups (like morpholinium, piperidinium, pyrrolidinium), a study was performed by Neumann et al. and Pernak et al., which include some of my experiments (Paper No. 9 and 10, appendix, page 171 and page 189).

Chapter II: EXPERIMENTAL PART

1. Test systems to investigate the toxicity of ILs

1.1. Enzyme inhibition

Isolated enzymes are ideal test systems to investigate the toxicity on a molecular level. This includes the study of the mechanism and molecular interactions that cause toxicity. The enzyme AchE is present in almost all higher organisms, including humans. There, it catalyses the degradation of acetylcholine, which is important for the signal transmission between neurons and muscles and among neurons themselves. AchE is the main target of many insecticides (organophosphates or carbamates) and used to examine the pesticide burden in non-target organisms[156]. The enzyme is well studied in terms of the structure of the active centre and the substrate binding process.[157] The active centre is located in a narrow cleft, which consists of negatively charged amino acid residuals at the entry and hydrophobic aromatic once along the gorge. The negatively charged entrance is responsible for the binding and orientation of the substrate towards the active centre. This has an additional negatively charged moiety to bind the quaternary ammonium of the substrate, whereas the acetyl group is located at the catalytic esteratic site. After the hydrolysis of the ester bond and the formation of choline, the enzyme is regenerated in the presence of water, releasing the acetate anion. Due to the shape of the AchE, two possible inhibition mechanisms are conceivable: 1) by binding directly to the active centre or 2) by blocking the cleft and thus inhibiting the transport to or from the active centre. As stated in chapter I, section 1.3.2, this enzyme was already studied for several ILs with the aim to identify the influence of the head group, side chain and anion of ILs on the inhibition potential. Within the research summarized in Paper No. 3, 4, 5 and 8, the enzyme was used as a model test system to study further IL components that were not described in the previous literature. A detailed description of the test procedure can be found therein.

1.2. Cytotoxicity

In vitro cytotoxicity assays, respectively tests using unicellular species, are a useful alternative to *in vivo* testing since the procedure is usually easy, fast, cheap and has high reproducibility. The amount of chemicals needed for the test is typically lower compared to

others and the throughput is increased. The possibility to identify effects on the cellular level, including membrane disruption, interference of the metabolism, protein biosynthesis or the signal transduction pathway, is helpful to investigate the mode of toxic action of the toxicant. On the other hand, this means that organ- or tissue specific interactions are not captured and kinetic and metabolic aspects cannot be considered. Furthermore, for toxicological classification of chemicals, such tests are not accepted.

The leukaemia rat cell line IPC-81 is well established in our laboratory. Several studies using this cell type are published which showed the sensitivity compared to other cells, e.g. glioma C_6.[60] The cells were isolated from the brown Norway rat and resembled human leukaemia cells with regard to their histological and cytochemistry.[158] The assay is based on the metabolic conversion of the dye 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt (WST-1, slightly red) to formazan (deep red) which can be observed photometrically. The detailed protocol can be found in paper No. 2-5, 8 and 10 where this assay was applied.

The marine bacteria *Vibrio fischeri* can be found in oceans in many parts of the world, typically living in symbiosis with higher organisms, *e.g.* in the light organs of squids. It is Gram-negative, heterotrophic and moves assisted by flagella. *V. fischeri* is a widely used and standardized test organism for determining the water quality or assessing ecotoxicological effects of chemicals.[159] The luminescence intensity of these bacteria is directly related to its metabolism and a sensitive endpoint to determine toxic effects. This easily feasible and rapid assay (only 5 to 30 min of incubation is necessary) has the further advantage of being commercially available with lyophilised bacteria leading to a high repeatability. Moreover, due to the standardized and intensive utilisation, loads of literature data for reference compounds are available and can be compared with the newly gained data. A detailed operation procedure can be found in the papers 3, 4 and 8, where this test system was investigated.

Algae represent an important ecological and aquatic test species since they are primary producers, important for the oxygen production and regulation of mass transport. Furthermore, they serve as a food source for higher organisms. Thus, toxicological effects can impact the whole aquatic ecosystem.

The green algae *Scenedesmus vacuolatus* belongs to the class of Chlorophyceae and is found in freshwater and moistly soil. It is spherically shaped and of 10 µm diameter. These algae own a thin, but stable cell wall and a well-developed photosynthesis system, comparable to higher plants. Their high reproduction rate (approximately by a factor of 10 within 24 h) represents a sensitive endpoint to determine ecotoxicological effects of chemicals. The species *Selenastrum capricornutum*, also known as *Pseudokirchneriella subcapitata* and recently again renamed to *Raphidocelis subcapitata*, is also a member of the class Chlorophyceae. The cells appear in sickle shape with 8 to 14 µm length and between 2 and 3 µm in width. Both species are recommended for ecotoxicity testing from OECD[160] and a modified test protocol, according to the suggested procedure, was used in papers No. 3, 5 (*S. vacuolatus*) and 4 (*R. subcapitata*). Within these publications one can find the detailed description.

1.3. Toxicity to higher organism

Lemna minor, popularly known as duckweed, is a monocotyledon aquatic plant widespread in slow-moving streams and freshwater lakes. Like algae, this primary producer is important for the ecological balance in aquatic environments. The leaves of this plant, the so-called fronds, contain small air spaces which enables the plant to float on or near the water surface. They are rich in proteins and fats making them an imported food source for fishes and birds. The bloom is degenerated and the reproduction generally occurs agamous and rapid. This test organism, proposed by OECD guideline[161], was used in paper No. 3 to investigate the influence of the anionic moiety to the ecotoxicity of ionic liquids. Therein the assay is described in detail.

The crustacean *Daphnia magna*, also named water flea, is a cladocera ubiquitous present in freshwater lakes and rivers. The body of the animals, excluding the head with the compound eye and the second antennae, is covered by a carapace. The females are of up to 5 mm in length and the males only approximately 2 mm. However, the males are produced only under harsher environmental conditions, e.g. in winter times. Otherwise the reproduction is parthenogenetic. The life span is about two month, but depends strongly on the temperature. This organism is a frequently used model, since the handling is easy and the nearly transparent body enables an effortless study of their organs. Several standardized

assays are described[162,163] and allow the classification of chemicals according to their hazard to the aquatic environment. The test with *D. magna* is included and illustrated in the research study in papers 3, 4, 5 and 8.

2. Test systems to investigate the stability of ILs

In general, as described in chapter I, section 1.3.3 and 1.3.4, the stability of a compound is of high concern since persistence or partial metabolism can lead to enrichment in the environment and affect the aquatic or terrestrial ecosystem. The following testing methods are, among others, suitable and (to some extent) recommended strategies for the investigation of the stability of chemicals and were used within this work.

2.1. Sludge inhibition test

The Activated Sludge - Respiration Inhibition Test (OECD guideline 209)[164] is a toxicity test on a multiple microbial community. The inoculum, used for biodegradation experiments, is studied for its sensitivity to the particular compound. Therefore, the sludge is exposed to different concentrations of the substance in the presence of synthetic feed. Then the activity of the microorganisms is determined via their oxygen respiration rate with and without test substance. A lower respiration is related to an inhibition of the sludge community by the test compound. Such inactivation of the microorganism in biodegradation experiments would lead to the non-biodegradability of the test substance and probably to false-negative results. Thus, this test procedure can have an impact on the interpretation of the results obtained from the biodegradation experiments. Paper No. 5 includes a detailed test procedure.

2.2. Primary biodegradation

The primary biodegradation is used in our laboratory as a simple preliminary test. The procedure is related to OECD guidelines[125], but does not allow a classification of the compound as readily biodegradable. The concentration of the parent compound in presence of a microbial sludge community from a domestic waste water treatment plant is monitored via chromatographic methods (HPLC, LC-MS, IC) for at least 28 days. Thus, only primary degradation can be observed, but allows for a fast and simple screening for compounds that are able to undergo biodegradation and should be further tested as suggested by regulations. The utilisation of MS techniques enables the possibility of identifying

metabolites and can help to identify the degradation mechanism. However, it is necessary that a reliable analytical method is available and paper No. 1 should, therefore, be considered as the basis for the research investigating the primary degradation of ILs (paper No. 4, 5, 7, 8, 9, 10).

2.3. Ready biodegradation

To study the ready biodegradation of compounds, the OECD recommends six different methods that consider sum parameters that correlate with the ultimate metabolization of chemicals, in the best case to only CO_2, H_2O or NO_3.[125] For passing the most stringent test protocol, a substance should be biodegraded to at least 60 % within 28 days, whereas the time frame between 10 % and 60 % of biodegradation should not exceed 10 days. Within the manometric respiration test, the oxygen consumption of the inoculum is monitored via the decreasing of the pressure in a closed vessel. By correlating the measures of O_2 uptake and the theoretical oxygen demand (ThOD) of the substance (calculated based on the elemental composition), the degree of biodegradation can be calculated. Another test procedure involves using the amount of evolved CO_2 to determine the biodegradation rate. This means that a compound has to be fully mineralized to observe any result, which ensures no or very little uncertainty for remaining metabolites, but could also end in false negative results. However, the O_2 consumption test has some drawbacks: O_2 consumption close to the ThOD does not necessarily mean a fully mineralization since oxidized, but stable biodegradation productions could still be present. This has to be kept in mind when performing and interpreting such tests. By combining the measurement of the sum parameters with analytical techniques like MS, such uncertainties could be diminished. A study including all these aspects has been performed and is depicted in paper No. 4 and was part of the research in paper No. 5, 8, 9 and 10.

2.4. Hydrolytic stability

The determination of the hydrolytic stability is of interest for both the technical applicability and the hazard assessment. Hydrolysis, as an important abiotic degradation pathway, can result in the same advantages and drawbacks as described for biodegradation, *i.e.* alternative route for the breakdown of xenobiotics and formation of toxic and/or persistent degradation products, respectively. The hydrolysis and its kinetic (time and temperature depended)

properties are determined in environments of different pH values (ranging from acidic to alkaline and including environmental conditions).[165] The degree of hydrolysis is monitored by chromatographic methods (HPLC, LC-MS, IC). Paper No. 1 represented the basis for the study regarding the hydrolysis of anions often used in ILs (paper No. 2).

Chapter III: PUBLICATIONS

1. Paper No. 1: Ion chromatographic determination of structurally varied ionic liquid cations and anions-a reliable analytical methodology applicable to technical and natural matrices

Authors: Stefan Stolte, Stephanie Steudte, Aleksandra Markowska, Jürgen Arning, Jennifer Neumann, Piotr Stepnowski
Journal: ANALYTICAL METHODS, Volume 3; Issue: 4; Pages: 919-926
DOI: 10.1039/c1ay05029j

Own contributions:
Apart from the determination of the basic validation parameter, as well as the case study with biodegradation samples, the experimental work of this research, as well as the evaluation of the results, was fully conducted by me. I contributed text modules, figures and tables for the preparation of the publication. In discussion with the other co-authors, Dr. Stefan Stolte was supervising the experimental work and was mainly responsible for writing the publication.

Due to copyright reasons pages 31-38 are not shown. The publication is available online:

http://pubs.rsc.org/en/Content/ArticleLanding/2011/AY/C1AY05029J#!divAbstract

2. Paper No. 2: Hydrolysis study of fluoroorganic and cyano-based ionic liquid anions - consequences for operational safety and environmental stability

Authors: Stephanie Steudte, Jennifer Neumann, Ulrike Bottin-Weber, Michael Diedenhofen, Jürgen Arning, Piotr Stepnowski, Stefan Stolte
Journal: GREEN CHEMISTRY, Volume: 14, Issue: 9, Pages: 2474-2483
DOI: 10.1039/c2gc35855g

Own contributions:
Apart from the cytotoxicity and the mass-spectrometry experiments and the calculations of the pKa-values, all experimental work was performed or coordinated by me. The evaluation and interpretation of the results was also my task. In discussion with the other co-authors, I summarized the results for this publication, including text, figures and tables.

3. Paper No. 3: (Eco)toxicity of fluoro-organic and cyano-based ionic liquid anions

Authors: Stephanie Steudte, Piotr Stepnowski, Chul-Woong Cho, Jorg Thöming, Stefan Stolte
Journal: CHEMICAL COMMUNICATIONS, Volume: 48, Issue: 75, Pages: 9382-9384
DOI: 10.1039/c2cc34955h

Own contributions:

Besides the theoretical calculations for the lipophilicity parameters, all experimental work and the evaluation and interpretation of the results was performed by me. In cooperation with the other Co-authors, I was responsible for the preparation of the publication, including text, figures and tables.

Due to copyright reasons pages 53-64 are not shown. The publication is available online:

http://pubs.rsc.org/en/content/articlelanding/2012/CC/c2cc34955h#!divAbstract

4. Paper No. 4: Ionic liquids as lubricants or lubrication additives: An ecotoxicity and biodegradability assessment

Authors: Stefan Stolte, Stephanie Steudte, Olatz Areitioaurtena, Francesco Pagano, Jorg Thöming, Piotr Stepnowski, Amaya Igartua
Journal: CHEMOSPHERE, Volume: 89, Issue: 9, Pages: 1135-1141
DOI: 10.1016/j.chemosphere.2012.05.102

Own contributions:
Under the coordination and supervision of Dr. Stefan Stolte, I conducted all experiments, except the acute ecotoxicity test with the green algae, for this publication and evaluated the results. The preparation of the publication text, figures and tables was carried out by Dr. Stefan Stolte with contribution of text modules by me and the other co-authors.

5. Paper No. 5: Toxicity and biodegradability of dicationic ionic liquids

Authors: Stephanie Steudte, Steve Bemowsky, Maria Mahrova, Ulrike Bottin-Weber, Emilia Tojo-Suarez, Piotr Stepnowski, Stefan Stolte

Journal: RSC ADVANCED, Volume: 4, Issue: 10, Pages: 5198-5205

Own contributions:

For this publication, all experimental work, apart from hereinafter referred exceptions, was performed by me. Steve Bemowsky conducted the primary biodegradation experiments and a first estimation of the toxicity tests as part of his bachelor thesis under my supervision. Maria Mahrova synthesised two of the investigated ionic liquids and Ulrike Bottin-Weber was responsible for the cytotoxicity assay. The evaluation and interpretation of the results was my task. In discussion with the other co-authors, I prepared the text, figures and tables for the publication.

Chapter IV: RESULTS AND DISCUSSION

1. Analytics

At the beginning of this work analytical methods were available mainly for simple IL anions (halides, BF_4^- and PF_6^-) via ion chromatography and conductivity detection, as well as for UV-active IL cations (imidazolium or pyridinium-based) by HPLC-UV or LC-MS (Tab. 1). The results published in Paper No. 1 extend the detection for a huge variety of both substructures.

Six typical IL anions ($N(CN)_2^-$, $C(CN)_3^-$, $B(CN)_4^-$, $(CF_3SO_2)_2N^-$, $(C_2F_5)_3PF_3^-$ and $H(C_2F_4)SO_3^-$) were baseline separated from both each other (except for $B(CN)_4^-$ and $(CF_3SO_2)_2N^-$) and inorganic anions (Cl^-, Br^- HPO_4^{2-}, SO_4^{2-}) which are usually present in biological and technical matrices, *e.g.* in test media or biodegradation samples. The influence of the organic modifier content on the retention behaviour of the anions was also investigated and retention times were decreasing when the eluent contained more acetonitrile. Basic validation parameters were determined and repeatability (0.6-3.1 %), linearity ($0.9986<R<0.9999$) and low limits of detection (<0.10 µM) and quantification (<0.30 µM) were achieved.

For 20 different cationic moieties, the influence of the eluent composition (HNO_3 concentration and CH_3CN content) was evaluated. A higher concentration led to smaller retention times of the cation. All cations could be baseline separated by proper mobile phase composition. The validation parameters were determined for four representatives and gave slightly poorer results than this obtained for the anions. However, all values are still satisfactory.

The validated anions and cations were used in a case study with biodegradation samples and showed recovery rates of 76-107 % indicating the applicability of this method to samples from complex matrices. The established analytical method represents an important basis for almost all further publications, e.g. to determine the degree of biodegradation and hydrolysis.

In Paper No. 5, the application of IC was expanded to dicationic ILs. Due to the dicationic character, high concentrations of HNO_3 and organic modifier had to be used to receive reasonable retention times. The high salt content in the mobile phase increased its

conductivity and therefore raised the limits of detection and quantification to 1-16 µM and 4-50 µM, respectively.

It has been shown that by changing the composition of the mobile phase, salt or acid concentration and content of organic modifier, this method can be universally applied to IL ions independent of the presence of UV-active elements or limitations to the hydrophobicity. When injected as single compounds, the polar ions are favored due to lower acetonitrile content. However, the more hydrophobic ions are easier to separate from inorganic matrices, whereas the hydrophilic ones are challenging in this specific task. Here, hydrophilic interaction liquid chromatography (HILIC) has already proven to be an ideal alternative.[29,132] Moreover, compared to IC, HPLC is preferred by higher throughput, smaller sample volume and lower prices for column and sample-preparation material, e.g. filter and pre-column. On the other hand, IC can be equipped with modules for inline sample preparation like filtration, dialysis, matrix elimination, preconcentration or heavy metal removal. This could be advantageous for specific industrial applications where such pretreatment is essential and would reduce time and costs. The usage of a suppressor for the analysis of anions results in a salt free mobile phase and would allow the linking to a MS detector. This enables an efficient and reliable identification of the analytes if unknown components are present (*e.g.* degradation products or contaminants).

2. Cyano- and fluoro-based IL anions

The cyano-based IL anions $N(CN)_2^-$, $C(CN)_3^-$ and $B(CN)_4^-$, as well as the fluorinated anions $(CF_3SO_2)_2N^-$, $(C_2F_5)_3PF_3^-$ and $H(C_2F_4)SO_3^-$, are frequently mentioned in the literature as suitable counter ions to design ILs with excellent electrochemical properties to be used in dye-sensitized solar cells or batteries.[166] However, only little was known for their ecotoxicity (apart from $(CF_3SO_2)_2N^-$) and stability. Paper No. 7 summarized the results from biodegradation experiments and it has to be concluded that none of the anions are biodegradable under aerobic or nitrify conditions. Furthermore, the hydrolysis studies in Paper No. 2 showed the stability of these anions in environmental media (pH value of 7 and 9). On the other hand, harsh conditions (pH 1 and 13), as could maybe be found in technical applications, led to complete hydrolysis of $N(CN)_2^-$ and $C(CN)_3^-$, whereas all other anions can be assumed to be stable at ambient temperatures for at least one year. Kinetic studies for the

hydrolysis rates at different temperatures have been performed for aforementioned unstable anions. By MS measurements, some hydrolysis products could be identified and a degradation mechanism was predicted. Theoretical calculations of pKa values support this pathway and could help to explain observed contrary hydrolysis velocities for both anions in the investigated pH environments. The ecotoxicity studies summarized in paper No. 3 showed an evident acute effect for $(C_2F_5)_3PF_3^-$ in all aquatic test systems (*V. fischeri*, *S. vacuolatus*, *D. magna* and *L. minor*), whereas $N(CN)_2^-$ was similarly toxic compared to the IL containing chloride as counter ion. The other three anions had various influences, highly depending on the test organism.

Until now, ILs have not been determined in the environment and, unless they are used only in small scale, their entry in large amount is unexpected. Though, raising commercialization would require an assured waste management in order to minimize the risk of being discharged into the environment (*e.g.* by using the compound in closed systems or recycling them). Within REACH, an exposure analysis is required for the registration of chemicals produced in amounts of $> 1\ t\ a^{-1}$. This is of especially high concern for $(C_2F_5)PF_3^-$ and $(CF_3SO_2)_2N^-$ since they showed severe ecotoxicities in aquatic test systems. Furthermore, highly halogenated compounds (*e.g.* perfluorooctane sulfonic acid, polychlorinated biphenyls or dichlordiphenyltrichlorethan) are already known to be hardly degradable in the environment and therefore restricted or eliminated by the Stockholm Convention.[167] Also, the results within this thesis illustrated that, most likely, none of these anions is degraded when they are released into the environment. This might lead to persistence, enrichment and/or, for the more hydrophobic anions, to bioaccumulation. However, for such statements, further testing is necessary. Investigations with *e.g.* inoculum pre-adopted to the test substance or biodegradation under realistic conditions in a waste water treatment plant are needed for a sound hazard assessment. Furthermore, alternative degradation pathways, like UV-treatment and/or advanced oxidation processes (AOP), should be considered. Considering toxicity testing, long term effects at sub-acute concentrations or mixture effects of these anions among each other or with other environmental chemicals, still need to be investigated.

The results of the ecotoxicity studies presented in paper No. 3 indicate a lower ecotoxicological hazard potential for the cyano based anions $N(CN)_2^-$, $C(CN)_3^-$ and $B(CN_4)^-$.

However, the technical application of $N(CN)_2^-$ and $C(CN)_3^-$ in strongly acidic or basic conditions might pose problems through hydrolysis. Even though the hydrolysed solutions showed similar or less cytotoxicity compared to the non-hydrolysed one, adverse effects cannot be excluded since some of the identified/predicted hydrolysis products are classified as toxic. Additional, the reduced lifetime might lead to loss in performance or machine damage and, thus to more waste and costs. The hydrolysis and biodegradation studies presented here hypothesize a persistence of these anions in the environment. Recently it was shown that enzymatic hydrolysis by nitrile hydratase led to the corresponding amides.[168] This indicates degradation in the environment when microorganisms containing this enzyme are present or opens the possibility for effluent treatment.

Slight modifications in the chemical structure of these anions are an option to design alternatives. The partial replacement of the perfluorinated chains with alkyl chains or the introduction of functional groups could be a possibility to design structures with reduced hazard. Hydroxyl groups, for instance, can serve as a contact point for biological breakdown and will reduce the lipophilicity of the anion, frequently coming along with less ecotoxicity. Also for the cyano based anions, substitution of single cyano groups with other functional groups, e.g. carboxylic acids, is possible.[169] However, it is questionable if such modification will lead to anions that can fulfil a similar property profile and are suitable for applications.

3. IL cations

As summarized in Chapter I, the toxicity and biodegradability of the cationic moiety has already been extensively studied. However, there is a huge knowledge gap for the biodegradation potential of IL cations owning head groups different from imidazolium and pyridinium. This was intended to be filled with the study summarized in paper No 4, 8 and 9.

Ready biodegradation was found in paper No. 4 for the ammonium based cations choline and its unsubstituted derivative (butyltrimethylammonium, N1114), whereas the methoxy-choline and triethylmethylammonium cation was not degraded at all. Contrary to this, in paper No. 8, N1114 and tributylmethylammonium were not readily biodegradable. Such differences for the same substance within different biodegradation experiments were already reported by other groups.[133,137] Variations in the microbial composition of the inoculum are the most plausible explanation for this. However, this might lead to high

uncertainties and false negative results when evaluating the studies. OECD standards seem not to be enough to guarantee reproducible results here and need to be further improved. The usage of a homogeneous and commercially available mixture of a freeze-dried microorganism sounds ideal, but was not successful in previous studies.[132] Techniques available to identify the microbial composition, *e.g.* gene sequence analysis, is another option, although this is usually very time-consuming and cost-intensive.

The toxicity studies of the investigated ammonium based cations underlined the biocompatibility of choline. In all test systems, ILs containing this cation showed no adverse effects up to 100 mg L^{-1}. The other ammonium based cations usually had similar ecotoxicological properties. Solely *D. magna* was affected in the presence of ILs with methoxy-choline and N1114 cations. The choice of compounds originating from natural sources is therefore a good opportunity to design sustainable products. However, their hydrophilic character limits their application potential.

In paper No. 9, it could be shown that some of the investigated cations are readily or inherently biodegradable. Above all, the pyrrolidinium-based IL cations showed enhanced biodegradation potential compared to imidazolium. Here, the compounds with shorter side chains (C=4) were fully mineralized in a prolonged test duration. In combination with the toxicological properties (similar to imidazolium based ILs), this type of head group should be intensively considered when designing sustainable ILs. The other way around is the behaviour of ILs containing a morpholinium core. Here, the biodegradation is similar to imidazolium based cations. In both cations, only the side chain is degraded, whereas the ring structure remains intact. However, it is already known that morpholinium ILs gave lower toxicities and should therefore be preferred towards imidazolium or piperidinium.

Diionic cations were shown in paper No. 5 to be, in some cases, significantly less toxic than monoionic cations with short side chains. The structure-activity-relationships known for monocations, meaning a higher toxicity for longer alkyl chains, could also be found for the dications. However, differences between the test systems were observed in the sensitivity for prolonged side or linkage chains of the dication. The biodegradation of the tested dicationic ILs was poor: none of them were primarily degraded, even if linked by long and functionalised chains. Here, the ring structure might impede a bacterial attack. The idea for

further design should therefore be the prolongation or functionalisation of the side chains. Combining this with the usage of pyrrolidinium rings, found to be degraded in monocationic ILs, could enhance the biodegradation potential of dicationic ILs.

Chapter V: CONCLUSION

This thesis intended to fill knowledge gaps in the hazard assessment of ILs and to gain data for their structural design. With the results presented here, promising IL substructures could be identified that fulfil the criteria for reduced hazard to humans and the environment.

Choline and the other ammonium based cations, for instance, showed low toxicity and some of them were readily biodegradable. This makes them excellent candidates to form ILs with low environmental hazard, especially for applications where such hydrophilic compounds are desired. Also, the dicationic structures seem to be a capable alternative, in particular, due to their powerful physico-chemical properties and reduced toxicity compared to monocationic analogues. However, their biodegradability still needs to be improved. This may be possible considering the data available for monocationic ILs.

For the anions, both the cyano-based and perfluorinated ones showed considerable drawbacks. All of them were not hydrolytically degraded under environmental conditions nor readily biodegradable. Furthermore, $N(CN)_2^-$ and $C(CN)_3^-$ can be used to a limited degree for applications with extreme acidic or alkaline solutions and $B(CN)_4^-$ is already known to be toxic to higher organisms (rats)[170]. Also, the perfluorinated anions are worrisome since they showed the highest ecotoxicity among the tested compounds and are not readily biodegradable or hydrolytically degraded. The (eco)toxicological profile of the investigated hydrophobic anions show that a profound waste management will be necessary in order to minimize the risk of ILs to be released in the environment. The design of hydrophobic, but environmentally benign alternatives still appears to be challenging and research efforts in this direction need to be done.

Even though a lot of work has already been done in the hazard assessment of ILs, one can still find connection points for future projects. This would include long term studies for both toxicity and biodegradation with the intention to simulate more realistic conditions. Moreover, the environmental fate, *e.g.* adsorption of the compound to soil, or the potential for biomagnifications is not yet fully investigated. However, unless the main usage of ILs is within a laboratory scale, such investigations will be too intricate and excessive.

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APPENDIX

1. Further Publications

1.1. Paper No. 6: The Biodegradation of Ionic Liquids - the View from a Chemical Structure Perspective

Authors: Stefan Stolte, Stephanie Steudte, Amaya Igartua, Piotr Stepnowski

Journal: CURRENT ORGANIC CHEMISTRY, Volume: 15, Issue: 12, Pages: 1946-1973

DOI: 10.2174/138527211795703603

Due to copyright reasons pages 109-136 are not shown. The publication is available online:

http://benthamscience.com/journal/abstracts.php?journalID=coc&articleID=74193

1.2. Paper No. 7: Biodegradability of fluoroorganic and cyano-based ionic liquid anions under aerobic and anaerobic conditions

Authors: Jennifer Neumann, Chul-Woong Cho, Stephanie Steudte, Jan Köser, Marc Uerdingen, Jorg Thöming, Stefan Stolte

Journal: GREEN CHEMISTRY, Volume: 14, Issue: 2, Pages: 410-418

DOI: 10.1039/c1gc16170a

Due to copyright reasons pages 139-148 are not shown. The publication is available online:

http://pubs.rsc.org/en/content/articlelanding/2012/gc/c1gc16170a#!divAbstract

1.3. Paper No. 8: Ionic liquid long-term stability assessment and its contribution to toxicity and biodegradation study of untreated and altered ionic liquids

Authors: Lucia Pisarova, Stephanie Steudte, Nicole Doerr, Ernst Pittenauer, Günter Allmaier, Piotr Stepnowski, Stefan Stolte

Journal: PROCEEDINGS OF THE INSTITUTION OF MECHANICAL ENGINEERS PART J-JOURNAL OF ENGINEERING TRIBOLOGY, Volume: 226, Issue: J11, Special Issue: SI, Pages: 903-922

DOI: 10.1177/1350650112451696

Due to copyright reasons pages 151-170 are not shown. The publication is available online:

http://pij.sagepub.com/content/226/11/903

1.4. Paper No. 9: Biodegradability of 32 pyrrolidinium, morpholinium, piperidinium, imidazolium and pyridinium ionic liquid cations under aerobic conditions

Authors: Jennifer Neumann, Stephanie Steudte, Chul-Wong Cho, Jorg Thöming and Stefan Stolte

Journal: GREEN CHEMISTRY, accepted

Due to copyright reasons pages 173-188 are not shown. The publication is available online:

http://pubs.rsc.org/en/content/articlelanding/2014/gc/c3gc41997e#!divAbstract

1.5. Paper No. 10: Synthesis, toxicity, biodegradability and physicochemical properties of 4-benzyl-4-methylmorpholinium-based ionic liquids

Authors: Juliusz Pernak, Nina Borucka, Filip Walkiewicz, Bartosz Markiewicz, Przemysław Fochtman, Stefan Stolte, Stephanie Steudte, Piotr Stepnowski

Journal: GREEN CHEMISTRY, Volume: 13, Issue: 10, Pages: 2901-2910

DOI: 10.1039/c1gc15468k

Due to copyright reasons pages 191-200 are not shown. The publication is available online:

http://pubs.rsc.org/en/content/articlelanding/2011/GC/c1gc15468k#!divAbstract

2. Curriculum vitae

06.07.1985	Born in Hoyerswerda, Germany
Education	
1991-1995	Primary school, Pablo-Neruda-Grundschule, Hoyerswerda, Germany
1995-2001	Secondary school, Konrad-Zuse-Gymnasium, Hoyerswerda, Germany
2001-2003	Secondary school, Leon-Foucault-Gymnasium, Hoyerswerda, Germany; degree: Abitur
10/2003-09/2008	Studies of Chemistry (Diplom), University of Bremen, Bremen, Germany; degree: Dipl. Chem.
Work experience	
10/2008-09/2009	Scholarship holder, University of Bremen, Center for Environmental Research and Sustainable Technology (UFT), Department Chemical Engineering-Recovery and Recycling, Bremen, Germany
10/2009-09/2012	Marie Curie Fellow, Department of Environmental Analytics, University of Gdańsk, Gdańsk, Poland
04/2010-06/2010	Exchange student, Fundación Tekniker, Eibar, Spain
05/2011-06/2011 and 09/2011-12/2011	Exchange student, Universida de Vigo, Vigo, Spain
10/2012-12/2012	DAAD scholarship holder, University of Bremen, Center for Environmental Research and Sustainable Technology (UFT), Department Sustainable Chemistry, Bremen, Germany
since 01/2013	Research assistant, University of Bremen, Center for Environmental Research and Sustainable Technology (UFT), Department Sustainable Chemistry, Bremen, Germany

3. Erklärung

Hiermit erkläre ich, dass ich die Doktorarbeit mit dem Titel

„Investigations on the stability and ecotoxicity of selected ionic liquid cations and anions"

selbständig verfasst und geschrieben habe und außer den angegebenen Quellen keine weiteren Hilfsmittel verwendet habe.

Ebenfalls erkläre ich hiermit, dass es sich bei den von mir abgegebenen Arbeiten um drei identische Exemplare handelt.

_____

(Unterschrift)