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On the Freshwater Ecotoxicity and Biodegradation Properties of Some Common Ionic Liquids

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Abstract:

Ecotoxicological tests, using OECD standard methods on some ionic liquids, have revealed that several have very high toxicity towards freshwater algae and the freshwater invertebrate *Daphnia magna* (~10^4−10^6 times more ecotoxic than methanol for the worse cases). In addition these compounds show high levels of toxicity to the microorganisms responsible for biodegradation in the environment. These materials have a significant potential to damage the aquatic ecosystem if released into water. We conclude that considerable care should be exercised in the choice of ionic liquids to be used in chemical processes at design stage and that contamination of aqueous effluent streams by these materials should be avoided wherever practicable.

Introduction

Over the past 10 years or so, a great deal has been published in the area of green chemistry and engineering. A topic that has received much attention is that of ionic liquids.[1] These neoteric solvents have interesting properties, and examples of many diverse chemical reactions can be found, e.g., Friedel Crafts,[2] nucleophilic substitutions,[3] Palladium-catalysed reactions (Heck,[4] Suzuki,[5] and carbonylation[6]), metathesis,[7] hydrogenation,[8] fluorination,[9] and biocatatalysis.[10] Often ionic liquids will impart unusual characteristics to a reaction not found with conventional molecular solvents. This is especially true for metal- or enzyme-catalysed reactions.[11] Ionic liquids have negligible vapour pressure, are usually nonflammable, and (based on very limited data) have low mammalian acute toxicology.[12] Thus, these materials are often branded as "environmentally friendly," and ionic liquids have been suggested as ideal replacements for volatile organic solvents. However, the principles of green chemistry and engineering teach us that we should consider the whole process (life cycle analysis) rather than individual components of a reaction ("single issue sustainability"), and thus avoid replacement of materials with those that could turn out to be more damaging to the environment.[13,14]

With a number of possible processes under consideration (mainly bio- and metal-catalysed reactions),[15] the question arose: "on environmental grounds, which is the best ionic liquid for scale-up?" Unfortunately, to take a holistic view on this, certain data, especially ecotoxicity data, could not be readily located. Indeed, in October 2003 the Royal Society of Chemistry stated (about ionic liquids) that "The biggest barrier to implementation is the relative lack of fundamental physical property data and toxicity information compared to that available for conventional solvents."[16]

More data on the biological effects of ionic liquids is becoming available. Recent published literature include data on antimicrobial activity,[17] cytotoxicity,[18] toxicity to *Caenorhabditis elegans*,[19] resistance to biodegredation,[20] and effects on acetylcholinesterase [21] and zebra fish (*Danio rerio*).[22] Nevertheless, the lack of readily accessible ecotoxicity data is a big gap in our knowledge when considering the employment of ionic liquids on a pilot or manufacturing scale. Consequently, the AstraZeneca Environmental Science group set out to answer a number of these fundamental questions on the ecotoxicity of ionic liquids, and in this publication we wish to report our results.

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Table 1. **Ionic liquids examined**

substance number	type	R1	R2	MW cation	anion	trade name
I	imidazolium	C_4H_9	CH_3	139	PF_6^-	
II	imidazolium	C_4H_9	CH_3	139	Cl^-	
III	imidazolium	$C_{12}H_{25}$	CH_3	251	Cl^-	
IV	imidazolium	$C_{16}H_{33}$	CH_3	307	Cl^-	
V	imidazolium	$C_{18}H_{37}$	CH_3	335	Cl^-	
VI	pyridinium	C_4H_9	-	136	Cl^-	
VII	phosphonium	C_4H_9	C_2H_5	231	$(EtO)_2PO_2^-$	CY169
VIII	phosphonium	C_6H_{13}	$C_{14}H_{29}$	483	Cl^-	CY101
IX	ammonium	C_8H_{17}	CH_3	368	$N(SO_2CF_3)_2^-$	
X	ammonium[a]	$\{C_2H_4O(C_2H_4O)_4Me\}_2C_{14}H_{29}$	CH_3	696	$MeSO_4^-$	EcoEng500

[a] Contains two PEG5 groups plus C_{14} chain as R1.

Table 2. **Ecotoxicity of ionic liquids to *Daphnia* and algae**

	Daphnia magna			*Selenastrum capricornutum*		
substance number	48 h EC_{50} (mg L^{-1})	95% CI (mg L^{-1})	end-point analysis[a]	48 h EC_{50} (mg L^{-1})	95% CI (mg L^{-1})	end-point analysis[a]
I	24	21−27	101	45	42−49	92
II	6.5	5.4−7.8	93	38.5	38.1−38.8	96
III	0.0043	0.0034−0.0056	45−106[b]	0.0011	0.001−0.0013	65−87[b]
IV	0.0034	0.0027−0.004	47−124	0.0041	0.0034−0.0052	<10%
V	0.0017	0.0013−0.002	52	0.0129	0.0129	<10%
VI	20	17−24	104	63	55−73	103
VII	11	8.7−14	102	6.2	5.6−6.8	84
VIII	0.072	0.054−0.093	0[b,c]	0.042	0.037−0.048	<10
IX	0.2	0.14−0.27	28	0.058	0.052−0.066	<10
X	1	0.60 − 1.6	N/A[d]	0.088	0.079−0.097	N/A
XINaPF$_6$	>100	−	N/A	42	35−51	N/A
XII NaMeSO$_4$	>100	−	97	>100	−	73
XIII HN(SO$_2$CF$_3$)$_2$	76	69−85	94	61	56−66	95

[a] % analysis of substrate at termination of experiment compared to initial assay result. [b] See text. [c] No residual substrate could be detected. [d] N/A = no analysis.

Table 3. **Ecotoxicity of some common molecular organic solvents to *Daphnia* and algae[a]**

solvent	type	*Daphnia* EC_{50} mg L^{-1}	algal EC_{50} mg L^{-1}
toluene	hydrocarbon	11.5	>100
dichloromethane	chlorohydrocarbon	135	>660
methanol	polar protic	>10000	>10000
t-butyl methyl ether	ether	650	>800
xylenes	hydrocarbon	1−10	~5
acetonitrile	dipolar aprotic	>100	>500

[a] Data from ref 25.

Table 4. **Biodegradation of ionic liquids**

	% inhibition of glucose/glutamate biodegradation at substance concentration of:			
substance	100 mg L^{-1}	10 mg L^{-1}	1 mg L^{-1}	measured biodegradation[a]
I	9	8	28	0
II	0	18	15	0
III	97	59	3	not tested[b]
IV	100	100	16	not tested[b]
V	100	100	100	not tested[b]
VI	4	13	21	0
VII	19	15	16	9
VIII	100	100	78	not tested[b]
IX	23	37	26	0
X	100	100	47	not tested[b]
XI (NaPF$_6$)	−	−	−	inorganic
XII (NaMeSO$_4$)	−	−	−	not tested[c]
XIII (HN(SO$_2$CF$_3$)$_2$)	−	−	−	0[d]

[a] % oxygen uptake (biodegradation) after 28 days = BOD/measured COD. [b] Inhibitory at test concentration. [c] BOD5 result shows material is readily biodegradable. [d] Note low carbon content of substrate.

We chose to look at a range of ionic liquids (identified as **I** to **X**) to explore molecular weight and size effects on their potential fate and ecotoxicological properties. We investigated ionic liquids that, at the start of this work, we considered were regularly used as a basis for organic synthesis (as reported in the literature), were already used on a small scale within the Process Chemistry group at AstraZeneca, and could be obtained in sufficient quantities for pilot-plant scale work (Table 1). Also examined were some simple compounds (**XI** to **XIII**, Tables2 and 4) of the appropriate anions to check for any anion effects.

Results and Discussion

The ecotoxicological test data from the experiments using the invertebrate *Daphnia magna* and the green alga *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*) are shown in Table 2. The results are reported as EC_{50} values (concentration at which 50% of the exposed organisms are either immobilized or killed) together with the statistical 95% confidence interval (CI) values appropriate to these types of test. All of the statistical

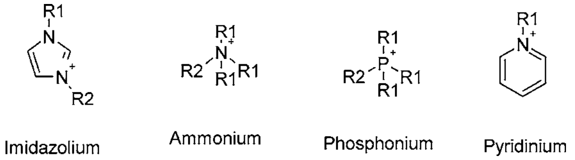

Figure 1. **Structures of ionic liquids tested.**

calculations on the data were carried out on nominal concentrations. Typically, chemical analysis of the test solutions was carried out at the beginning and end of the test to ensure that organism exposure was consistent with that expected from the nominal concentrations used. In cases where the measured substance concentrations at the end of the test were significantly lower than the nominal concentrations ($<$80%), the EC_{50} values should be viewed with care, as the actual EC_{50} values could be lower than the values quoted.

The ecotoxicological data for algae and invertebrates showed some striking comparisons. In general the toxicity of a particular substance to algae and *Daphnia* was surprisingly similar, and toxicity values for the most and least toxic ionic liquids were separated by more than 4 orders of magnitude for both trophic levels. There appeared to be a strong correlation of toxicity with alkyl side-chain length for the alkyl methylimidazolium species with the C_4 species tested being only moderately toxic, whilst the C_{12}, C_{16}, and C_{18} species were very highly toxic to both organisms tested (see Table 2).

This correlation appeared also to hold well for the pyridinium, phosphonium, and ammonium species tested where C_4 side-chain constituents conferred only moderate toxicity on the ionic liquid in question, whereas C_6 and longer side chains conferred significant increases in toxicity (Table 2) (Figure 1). In no instance did it appear, from the data available, that the counteranion contributed significantly to the toxicity of the ionic liquid. From the alkyl methylimidazolium data, it appeared that increases in ecotoxicity due to an extension in chain length from C_{12} to C_{18} were insignificant when compared to the increase between C_4 and C_{12}. Indeed, the toxicity to algae actually fell slightly as the chain length increased. However, this observation should be tempered by the low final measured test substance concentrations in the algal tests for longer-chain length imidazolium species, which may well significantly affect the EC_{50} values. It was not possible with the current data to estimate how the toxicity increases as chain length increases from C_4 to C_{12}; however, further testing on homologues between these two chain lengths is planned.

The measured concentrations for each of the test substances at the end of each test are also shown in Table 2. Generally, measured concentrations compared well to nominal values, particularly in the *Daphnia magna* tests; however, there were exceptions. The longer-chain alkyl methylimidazolium compounds proved difficult to detect at the lower concentrations used. Typically, recoveries of test substance at the lowest concentrations tested were below the level of detection of the analytical methods employed. At higher concentrations the mass balance improved until at the highest concentrations recoveries reached approximately 100%.

In general, analytical recovery of test substances during the algal tests was poorer than for the invertebrate tests, presumably due to the influence of the components (absorption on- or into the biomass) of the algal medium compared to those in the *Daphnia magna* medium. Poor recoveries appeared to be linked to increased side-chain length, and this may be due to an increase in the surfactant properties of the materials as side-chain length increases.

Our findings agree well with EC_{50} values of 8.03−19.91 mg L^{-1} reported by Bernot et al.,[23] 4−90 μM L^{-1} reported by Garcia et al,[24] and −1.15 to −2.19 log mM L^{-1} published by Couling et al.[25] Compared with the data on *Daphnia magna* reported by Couling et al.[25] for substances of similar molecular weight, our results for **VI** and **VII** fall closely within the expected range for pyridinium and phosphonium ionic liquids of these molecular weights.

To place the ecotoxicity results for ionic liquids in context, the ecotoxicity of a range of common molecular organic solvents is shown in Table 3.[26] The least toxic ionic liquids showed comparable ecotoxicity to hydrocarbons such as toluene and xylene. The most toxic ionic liquids are many orders of magnitude more acutely ecotoxic than organic solvents such as methanol, *tert*-butyl methyl ether, acetonitrile, and dichloromethane. It should, of course, be recognised that simple acute ecotoxicity measurements do not fully characterize the full impact of a solvent released to the environment but are only part of the environmental impact assessment.

The ecotoxicology results from the algal and invertebrate testing showed good correlation with the possible exception of the polyethoxylated quaternary ammonium species Ecoeng 500 (compound **X**) which was at least an order of magnitude less toxic to *Daphnia magna* than to the algal species *Pseudokirchneriella subcapitata*. However, it should be noted that we were unable to develop a suitable analytical technique for this compound. The results for Ecoeng 500 suggest that the ecotoxicity of the compound may not be caused by action on particular species-specific receptors but by a more general mechanism. This toxicity correlation was also observed in the microbial tests where biochemical oxygen demand tests could not be carried out on the five most ecotoxic ionic liquids (**III**, **IV**, **V**, **VIII**, and **X**) since they all showed significant bacterial inhibition at concentrations of 10 mg L^{-1} or below (see Table 4).

The findings are in line with the work of Stock et al.[21] who used an acetylcholinesterase enzyme assay to show that toxicity of alkyl methylimidazolium-based ionic liquids ranged from 189 to 13 μM (40−4.0 mg L^{-1}) as side-chain length increased from C_3 to C_{10}. The EC_{50} value reported for the C_4 BF_4^- salt was 105 μM L^{-1} ($\sim$24 mg L^{-1}), which

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is similar to the EC_{50} values being reported here for the PF_6^- and Cl^- salts (**I** and **II**) to *Daphnia magna* and *Pseudokirchneriella subcapitata* (6.5−45 mg L^{-1}): see Table 2.

Biodegradation

The results obtained from biodegradation studies are presented in Table 4.

BOD5 [27] measurements on the organic compounds studied showed that only one compound, **XII** (sodium methyl sulfate), showed any detectable BOD over this time scale. This result was confirmed by the 28-day ready biodegradability tests (see Table 4), which indicated some oxygen uptake for this compound during the test and effectively complete organic carbon removal from the system. Total organic carbon measurements of the test solutions after the 28-day biodegradation study showed there was no detectable removal of organic carbon for any of the other test compounds apart from **IX** (methyltrioctylammonium bistriflamide) (although there was no measurable oxygen uptake) and partial removal (ca 17%) for the test substance **VII** (ethyl tributylphosphonium diethyl phosphate) where oxygen uptake was equivalent to 9% of the measured COD for the compound.

Where total organic carbon removal was observed, there are a number of possible explanations:

1. The test substance may not have fully dissolved (if direct weigh techniques were used) or may have been removed from solution by adsorption either to the equipment or the biomass.

2. The carbon in the test substance may have been oxidized to CO_2 together with incorporation into the biomass due to biological growth.

3. The material may be volatile enough to escape the liquid into the enclosed headspace.

In the case of sodium methyl sulfate, its low volatility together with the evidence of oxygen uptake and the complete removal of organic carbon at the end of the test suggests biodegradative breakdown. In the case of test substance **VII** (ethyl tributylphosphonium diethyl phosphate) the level of organic carbon removal (17%) corresponds quite closely to the proportion of the organic carbon content of the material present in the diethyl phosphate counterion (22%). It is therefore possible that the counterion degraded, leaving the phosphonium moiety intact. Confirmation of this was not possible without detailed parent compound analysis, which was not carried out in this study. The complete absence of organic carbon at the end of the study on test substance **IX** (methyltrioctylammonium bistriflamide) together with the complete absence of detectable oxygen uptake suggests either that the material did not dissolve (this was the only material where direct weigh technique was used) or that adsorption of the test substance to either equipment or biomass was a strong possibility.

Biodegradation tests on 3-butyl-methylimidazolium hexafluorophosphate have been reported by Gathergood et al.[20,28] using both a closed bottle test (OECD 301D), where no biodegradation was observed, and modified Sturm tests (OECD 301B), where 60% mineralisation to carbon dioxide was seen. It was suggested that innoculum density was responsible for the difference in these results, innoculum density being significantly greater in the modified Sturm test than in the closed bottle test. Interestingly, the Hach (OECD 301F) tests, used in our investigations, had a similar innoculum density to that used in the modified Sturm test, but no evidence of biodegradation was observed.

Pernak et al.[17] tested the antimicrobial effects of a range of alkoxymethyl-1-methylimidazolium salts ($R = 3-16$, counteranions = Cl^-, BF_4^-, PF_6^-) to a range of microorganisms and presented data that showed for the $R = 3$ and $R = 4$ alkoxymethyl homologues the minimum inhibitory concentration (MIC) values were $>5850\ \mu M\ L^{-1}$ in all cases ($\gtrsim 1100$ mg L^{-1} for the $R = 3$ Cl^- salt) but that MIC values fell significantly as chain length increased. Further work by Pernak et al.[17] on the antimicrobial properties of alkyl methylimidazolium analogues again highlighted a significant increase in antimicrobial toxicity (by more than 2 orders of magnitude) as the alkyl chain length increased. Interestingly the toxicity to bacteria as measured by the MIC, reached a maximum when $R = 10-11$, decreasing again for $R = 12$. This observation mirrors the trend we found when using *Pseudokirchneriella subcapitata* to test the ecotoxicity of alkyl methylimidazolium chlorides in that the ecotoxicity had reached a peak by at least $R = 12$ and was declining up to $R = 18$. However, this observation was not noted when using *Daphnia magna* as the test organism where toxicity continued to increase as chain length increased. Again these trends should be treated with caution due to the low compound mass balances noted by specific compound analyses at the end of each test (see Table 2). In conclusion, as well as displaying high ecotoxicity, most of the ionic liquids studied here were resistant to ready biodegradation.

From the results of these studies it is clear that some ionic liquids are very far away from displaying the green image that is often cited in the literature. Four different classes of ionic liquid have been examined, and none of them would be described as having low ecotoxicity (EC_{50} values > 100 mg L^{-1}). Whilst some of the ionic liquids such as the shorter-side-chain 3-alkyl-1-methyl imidazolium and pyridinium salts exhibit only moderate ecotoxicity to bacteria, algae, and invertebrates, it is clear that, in each case where compounds were tested containing side chains of C_8 and longer, the ecotoxicity profile of these compounds deteriorated dramatically. This also holds for higher-molecular weight phosphonium- and ammonium-based ionic liquids.

Ionic liquids have received much attention in the literature and will undoubtedly receive much more. Their unique properties open up new and exciting chemistries of significant potential benefit. This excitement however should be tempered by the knowledge that based on the studies presented here, for some, possibly many, ionic liquids there are also significant potential environmental risks associated with their use in manufacturing which should not be ignored. It should be pointed out that the substances studied here could be

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(27) BOD5 Ref HMSO Methods for Examination of Waters and Associated Materials: 5 Day Biochemical Oxygen Demand (BOD5) 2nd ed., 1988 (with Dissolved Oxygen in Waters, Amendment 1988).

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considered as very much the "first-generation" ionic liquids. The "second generation" of ionic liquids is currently being developed. The use of natural products,[29] biodegradable materials such as choline,[30] task-specific ionic liquids,[31] and consideration for the environment at the design stage will undoubtedly bring materials with a much more acceptable environmental profile.[32]

Experimental Section

The ionic liquids were obtained commercially at the highest grade available (≥95%). No attempt was made to ultrapurify any particular ionic liquid since it was deemed highly improbable that any potential user would do this prior to use in a large-scale manufacturing process.

The ecotoxicity of each of the ionic liquids was tested to the freshwater invertebrate *Daphnia magna* according to the procedures set out in OECD guideline 202.[33] Twenty organisms were exposed for 48 h to various concentrations of the test material dissolved in *Daphnia magna* test media, and the EC_{50} value (concentration at which 50% of the exposed organisms are either immobilized or killed) was calculated. The counteranions of the hexafluorophosphate-, methyl sulfate-, and bistriflamide-based ionic liquids were also tested (as their sodium salts or free acids) to determine the likely role that these counterions play in the observed toxicity of the materials.

The ecotoxicity of each of the ionic liquids was also tested to the freshwater green alga *Pseudokirchneriella subcapitata* according to the procedures set out in OECD guideline 201.[34] Algal cultures in exponential growth phase were exposed to various concentrations of the test chemicals for 72 h at which point the growth of the culture was examined by electronic particle counting techniques. As in the *Daphnia magna* case, the counteranions of the hexafluorophosphate-, methyl sulfate-, and bistriflamide-based ionic liquids were also tested as their sodium salts or free acids to determine the likely role that these counterions play in the observed toxicity of the materials. Negative controls, carried out with each test, were all within limits of mortality/growth as specified by the relevant guidelines. Organism population health was also confirmed (carried out on a biannual basis, also as recommended within the relevant guidelines).

The BOD5 value of each of the test substances (except sodium hexafluorophosphate) was measured according to procedures recommended by the Standing Committee of Analysts of the UK Department of the Environment together with the effect of each of the test substances on the degree of biodegradation of two easily biodegradable substrates, glucose and glutamic acid, according to the guideline.[27] Where the results from the glucose/glutamic acid biodegradation inhibition tests indicated little or no inhibition at a test substance concentration of 100 mg L^{-1}, a 28-day biochemical oxygen demand test was carried out based on OECD Test Method 301F[35] and EC Test Method C.4-D.[36] Organic carbon analysis was carried out on a Dohrman DC-190 analyzer according to the guidelines in the Instrumental Determination of Total Organic Carbon and Related Determinands: Methods for Examination of Waters and Associated Materials.[37] Substance specific analytical methods for test substance **X** could not be developed, and analysis was not carried out for the test substance **XI** ($NaPF_6$). Samples from the exposure vessels were analysed using a ThermoFinnigan Surveyor liquid chromatograph (LC) coupled to a Finnigan TSQ Quantum Ultra mass spectrometer (MS) with electrospray ionization (ESI). Separations for the cations were achieved using a Waters Hypersil H5BDS-C_8 LC column (10 cm × 2.1 mm) with an acetonitrile/water mobile phase containing 20 mM ammonium acetate and 1% acetic acid. The mass spectrometer was operated in positive ESI mode, and the samples were quantified against standards of known concentration in 50:50 acetonitrile/water.

The anions were analysed by direct flow injection LC−MS with no chromatographic separation. The mass spectrometer was operated in negative ESI mode, and the samples were quantified against standards of known concentration in water.

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