IOP Conf. Series: Materials Science and Engineering 458 (2018) 012038 doi:10.1088/1757-899X/458/1/012038

Estimation of safe environmental concentrations of ionic liquids towards bacteria by chemical toxicity distribution method

Muhammad Ishaq Khan, Dzulkarnain Zaini*, Azmi Mohd Shariff, Muhammad Athar and Muhammad Yasir Shamim

Centre for Advanced Process Safety (CAPS), Chemical Engineering Department, Universiti Teknologi Petronas, 32610 Bandar Seri Iskandar, Perak Darul Ridzuan, Malaysia.

*dzulkarnain.zaini@utp.edu.my

Abstract. Ionic Liquids (ILs) are considered as the potential replacement of industrial organic solvents because of their salient physical and chemical properties, for example, ionic nature, low vapor pressure, and good solubility properties. These properties make ILs feasible for potential application. Industrialization of ILs has a potential to adversely affect and pose risk to the ecosystem because of their toxic nature. However, the conventional research on the ILs is mainly carried out by performing acute the toxicity assessment of individual species for individual ILs which is termed as effect assessment. Effect assessment for individual ILs is insufficient for the ecotoxicological risk assessment of ILs. Subsequently, effect assessment of single ILs on one species is not sufficient to evaluate risk. The main objective of the current research was to estimate the safe environmental concentrations (SECs) of ILs towards three bacterial strains *V fischeri*, *E coli* and *S vacuolatus* by Chemical Toxicity Distribution (CTD) method. CTDs provided the SECs for a group Imidazolium NTf2 ILs toward three bacteria. *E coli* was the most sensitive species amongst three species when exposed to the selected group of ILs with 2.976 x 10^{-4} mmol/L as SEC.

1. Introduction

Ionic liquids (ILs) are novel chemicals that gained a lot of interest in the industrial sector as well as in academic research because of their role as potential industrial and environmentally friendly solvents. ILs are salts at room temperature and usually have melting point below 100°C [1; 2]. ILs are the chemical composed of cations and anions. The cationic part is organic and usually anionic part is inorganic. Cationic part of ILs is considered as the most responsible part for the physical and chemical properties of the compound itself. The anionic part consists of inorganic anions. Most of the research on ILs have been published in the last two decades which shows that there is an extensive focus of academia and industries has shifted towards ILs in last recent years. Initially, ILs were thought to be green replacements for volatile organic compounds [3]. The literature reported that ILs are useful in enhancing the capability of chemical processes with no negative impact on the atmosphere compared to volatile organic solvents because of their very low or negligible vapor pressure. Due to negligible vapor pressure, ILs do not significantly contribute to air pollution [4].

The increasing use of ILs especially in the industrial sector which ultimately leads to increase their discharge into an environment. The possible hazards of ILs may be due to accidental spills, leaching of landfill sites or via the industrial discharge which heavily affects the aquatic and terrestrial life [5].

Therefore, the toxicological studies are conducted for individual ILs for individual species called effect assessment. Effect assessment for individual ILs is insufficient for the risk assessment because different types of metabolism and different routes of exposure and other factors affect environment differently. Subsequently, effect assessment of single ILs on one species is not sufficient to evaluate risk.

Potential hazards of ILs demand the assessment of the risk caused by the toxicity [6]. However, because of unavailability of exposure data of ILs, it is impossible to get the frequency or probability of the exposure concentrations. An extensive research is carried out to assess the acute toxicity of ILs at the laboratory level. Acute toxicity experiments are conducted to assess the toxicity of individual ILs towards individual test species.

2. Methodology

In ecotoxicology, there are two types of assessments needed to be done i.e. the effect and exposure assessment. Effect assessment can be done by acute toxicity testing and the effect concentration either can be taken from real exposure data. However, data on ILs exposure is not available yet [7]. In current work, we developed a methodology to model the safe environmental concentrations from effect data (toxicity). There are four steps involved in the estimation of SECs from toxicity data of ILs. These steps are described in a methodological framework which is presented in Figure1.

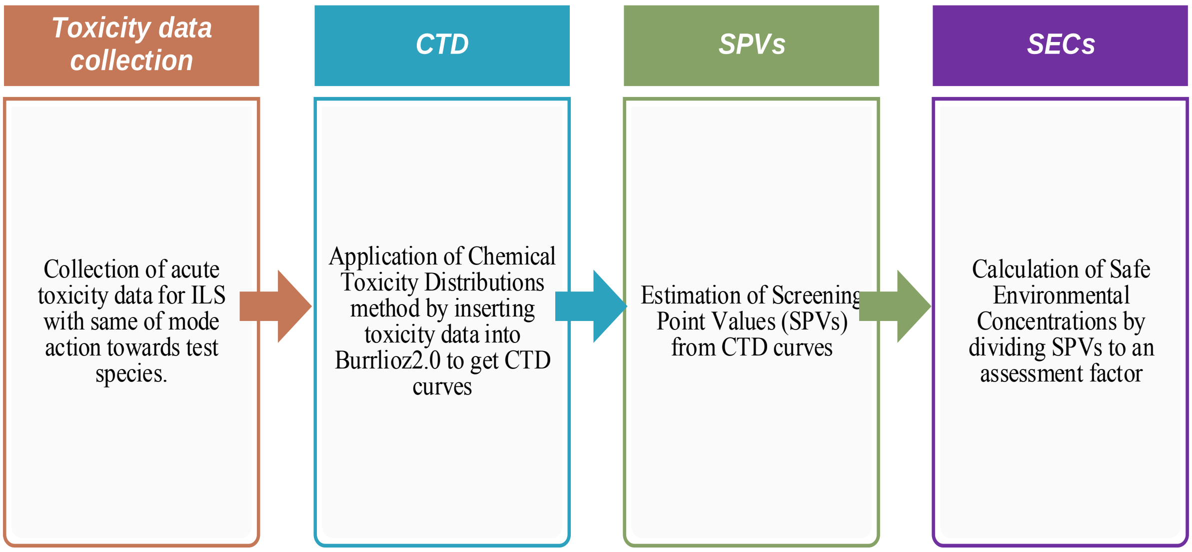

Figure 1. Methodological Framework

2.1. Toxicity data collection

The solubility of imidazolium ILs in water is very high[8] which makes these ILs highly toxic towards aquatic species including. We have collected toxicity data of imidazolium ILs with NTf_2 anions towards three bacteria *E coli*, *V fischeri* and *S vacuolatus* from literature. Toxicity data of [EMIM]NTf_2 [9; 10], [BMIM]NTf2[9; 11-13], [HMIM]NTf2[9; 11; 14] and [OMIM]NTf_2 is tabulated in Table 1. The trend of increasing toxicity with enlargement of the alkyl chain is observed in the data tabulated above. It is noticeable that same cation and the same anion show different toxicities when alkyl chain length is varied.

Table 1. Toxicity of Imidazolium NTf_2 ILS towards different Bacterial species

Ionic Liquids	Ec50(mmol/L)		
	E coli	*V fischeris*	*S vacuolatus*
[EMIM]NTf_2	-	0.844[9]	0.10[10]
[BMIM]NTf_2	2.0[11]	0.339[9]	0.057[12] [13]
[HMIM]NTf_2	1.0 [14]	0.051[9]	0.0011[13]
[OMIM]NT_2	0.7[14]	0.016[15]	-

2.2. Chemical toxicity Distributions

Chemical Toxicity Distribution method was used by William et al. [16] to assess the effect of a group of same chemicals with the same mode of action. In the current research, we used the CTD method to assess the effect of a group of ILs to single species to find safe environmental concentration (SECs). For each species, the EC50 of ILs with the same mode of action are used to calculate CTDs according to the methods outlined by Williams et al. [9]. The EC50 data for all ILs towards target species were ranked. The ranks were converted to % ranks (j) using the Weibull formula [17]

$$j = 1000 * (i)/(n + 1) \tag{1}$$

where *i* is the rank and n is the total number of compounds. The CTDs curves were plotted using log EC50 against the probit of the % rank, calculated as =NORMINV((j),5,1).

3. Results and Discussions

Toxicity data (EC50 concentrations) of selected ILs towards three bacteria *E coli*, *V fischeri* and *S vacuolatus* were selected. EC50 of the Imidazolium ILs with the same mode of action for each species was statistically analyzed by CTD method. The resulting CTD curves provided the concentrations called screening point values. Toxicity data taken from literature was arranged according to increasing alkyl chain length. The selected data was good enough as the toxicity of the imidazolium ILs to three of the bacterial strain followed the accepted trends of increasing alkyl chain length. "Probit of % effect" against the Log EC50 was plotted in Figures 2-4. Input data was taken from the CTDs spreadsheet of ILs towards algae.

A graph was plotted from CTD spreadsheets of *E coli* and presented in Figure 2. The equation of graph was used to calculate the concentrations on 1^{st} and 5^{th} percentiles. These concentrations were Termed as Screening point values (SPV). These SPVs are then divided by an assessment factor of 1000 to convert these concentrations to SECs[7].

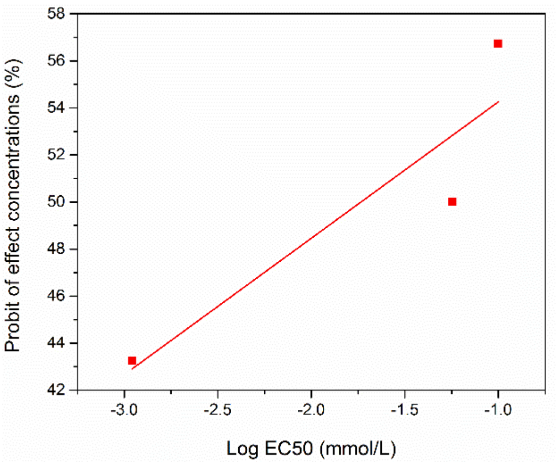

Figure 2. CTD curve of Imidazolium NTf2 ILs toward *E coli*

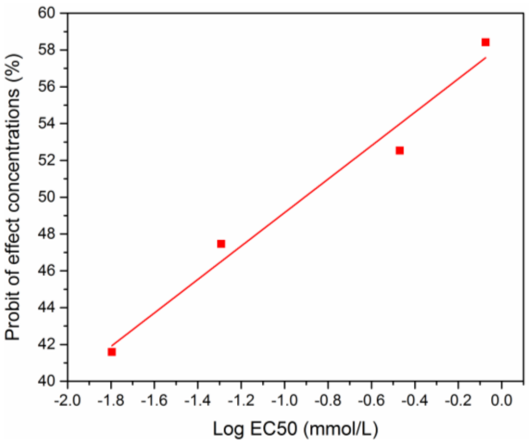

Figure 3. CTD curve of Imidazolium NTf2 ILs toward *V fischeris*

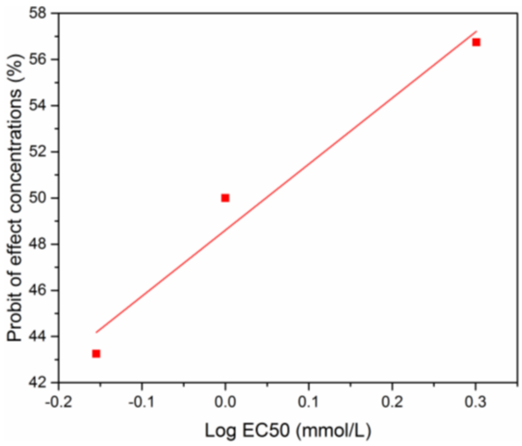

Figure 4. CTD curve of Imidazolium NTf2 ILs toward *S vacuolatus*

The Figure 3 and Figure 4 were plotted from CTD spreadsheets of *V fischeris* and *S vacuolatus* respectively. In each figure, the probit of % ranking was plotted against the log of toxicity values to form an equation which was used to calculate percentiles. The concentration at the 5th percentile was taken as Screening Point values. SPVs were then divided by an assessment factor of 1000 to convert these concentrations to SECs. The results are presented in Table 2.

Table 2: SECs of Imidazolium NTf_2 ILs Based on CTDs

Organism	Centile	SPV(mmol/L)	SECs (mmol/L)
E coli	5th	0.297674309	2.976×10^{-4}
V fischeris	5th	0.001915	1.915×10^{-6}
S vacuolatus	5th	2.68487×10^{-5}	2.68487×10^{-8}

The 5th percentile was calculated from the equation of the above graph and taken as screening point values. SPVs were divided by an assessment factor of 1000 to get Safe Environmental Concentration (SEC). SEC for *E coli* was 2.976×10^{-4} mmol/L. Similarly, the SECs of imidazolium ILs towards *V* *fischeris* and *S vacuolatus* were 1.915 x 10^{-6} mmol/L and 2.68487 x 10^{-8} mmol/L. SECs indicated that *E coli* are the most sensitive species amongst three species when exposed to the selected group of ILs. 2.976 x 10^{-4} mmol/L is the concentration which could be considered as a safe concentration for aquatic ecosystem containing *E coli*, *V fischeris*, and *S vacuolatus.*

4. Conclusion

Chemical Toxicity Distribution method is used to assess the toxicological effect the ILs which do not have any major industrial accident data so for because of lack of industrial applications. The outcome of the CTD method is the safe environmental Concentrations. SECs are the concentrations at which the 95 % species are protected from the hazards of toxic chemicals. In current work, *E coli* (having 0.0002967 mmol/L as SEC) was proved to be more sensitive species amongst *V fischeris* and *S vacuolatus*. It means that even a small amount of imidazolium NTf2 ILs can affect 5 % of the *E coli* in an aquatic ecosystem containing *E coli, V fischeris* and *S vacuolatus*. SECs could be used as the standard value to compare with real environmental concentrations to quantify the risk. If the ratio of measured environmental concentration to SECs is less than 1, the environment will be considered as safe. If this ratio exceeds 1, there is a need to control ECs. Risk assessment of ILs requires leakage data and CTDs is the best alternative techniques because of the unavailability of risk assessment data of ILs. In future, a huge number of data may be used to effectively assess the risk posed by ILs to different organisms of ecosystems. Furthermore, new modeling techniques on the toxicity of ILs can be adopted to reduce the impact of a group of ILs towards different compartment s of the ecosystems.

Acknowledgment

The authors would like to gratitude Universiti Teknologi PETRONAS, Malaysia for providing research facilities and YUTP funding (0153AA-E19) to make this research feasible.

References

[1] Araque, J.C., Hettige, J.J., and Margulis, C.J., Modern room temperature ionic liquids, a simple guide to understanding their structure and how it may relate to dynamics, The Journal of Physical Chemistry B 119 2015, 12727-12740.

[2] Moniruzzaman, M. and Goto, M., Ionic liquids: future solvents and reagents for pharmaceuticals, *J. Chem. Eng. Jpn.* **44** 2011, 370-381.

[3] Gutowski, K.E., Broker, G.A., Willauer, H.D., Huddleston, J.G., Swatloski, R.P., Holbrey, J.D., and Rogers, R.D., Controlling the aqueous miscibility of ionic liquids: aqueous biphasic systems of water-miscible ionic liquids and water-structuring salts for recycle, metathesis, and separations, *J. Am. Chem. Soc.* **125** 2003, 6632-6633.

[4] Bier, M. and Dietrich, S., Vapour pressure of ionic liquids, *Mol. Phys.* **108** 2010, 211-214.

[5] Ventura, S.P.M., e Silva, F.A., Gonçalves, A.M.M., Pereira, J.L., Gonçalves, F., and Coutinho, J.A.P., Ecotoxicity analysis of cholinium-based ionic liquids to Vibrio fischeri marine bacteria, *Ecotoxicol. Environ. Saf.* **102** 2014, 48-54.

[6] Bubalo, M.C., Radošević, K., Redovniković, I.R., Halambek, J., and Srček, V.G., A brief overview of the potential environmental hazards of ionic liquids, *Ecotoxicol. Environ. Saf.* **99** 2014, 1-12.

[7] Khan, M.I., Zaini, D., Shariff, A.M., and Moniruzzaman, M., Framework for Ecotoxicological Risk Assessment of Ionic Liquids, *Proc. Engg.* **148** 2016, 1141-1148.

[8] Freire, M.G., Carvalho, P.J., Gardas, R.L., Marrucho, I.M., Santos, L.M., and Coutinho, J.A., Mutual solubilities of water and the [C n mim][Tf2N] hydrophobic ionic liquids, *J. Phys. Chem. B* **112** 2008, 1604-1610.

[9] Ventura, S.P., Gonçalves, A.M., Sintra, T., Pereira, J.L., Gonçalves, F., and Coutinho, J.A., Designing ionic liquids: the chemical structure role in the toxicity, *Ecotoxicology* **22** 2013, 1-12.
[10] Steudte, S., Stepnowski, P., Cho, C.-W., Thöming, J., and Stolte, S., (Eco) toxicity of fluoro-organic and cyano-based ionic liquid anions, Chem. Commun. 48 2012, 9382-9384.
[11] Łuczak, J., Jungnickel, C., Łącka, I., Stolte, S., and Hupka, J., Antimicrobial and surface activity of 1-alkyl-3-methylimidazolium derivatives, *Green Chemistry* **12** 2010, 593-601.
[12] Matzke, M., Stolte, S., Thiele, K., Juffernholz, T., Arning, J., Ranke, J., Welz-Biermann, U., and Jastorff, B., The influence of anion species on the toxicity of 1-alkyl-3-methylimidazolium ionic liquids observed in an (eco)toxicological test battery, *Green Chemistry* **9** 2007, 1198-1207.
[13] Izadiyan, P., Fatemi, M., and Izadiyan, M., Elicitation of the most important structural properties of ionic liquids affecting ecotoxicity in limnic green algae; a QSAR approach, *Ecotoxicol. Environ. Saf.* **87** 2013, 42-48.
[14] Deng, Y., *Physico-chemical properties and environmental impact of ionic liquids*, PhD Thesis, Université Blaise Pascal-Clermont-Ferrand II, 2011.
[15] Mutalib, M.I.A. and Ghanem, O.B., Ecotoxicity of Ionic Liquids Towards Vibrio fischeri: Experimental and QSAR Studies, in: *Progress and Developments in Ionic Liquids,* S. Handy, ed., InTech, 2017, pp. 429-449.
[16] Williams, E.S., Berninger, J.P., and Brooks, B.W., Application of chemical toxicity distributions to ecotoxicology data requirements under REACH, *Environ. Toxicol. Chem.* **30** 2011, 1943-1954.
[17] Dobbins, L.L., Usenko, S., Brain, R.A., and Brooks, B.W., Probabilistic ecological hazard assessment of parabens using Daphnia magna and Pimephales promelas, *Environ. Toxicol. Chem.* **28** 2009, 2744-2753.