Modelling and Simulation of Ecotoxicity of Ionic Liquids Using QSAR

by

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9109

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CERTIFICATION OF APPROVAL

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Approved by,

(Dr Mohanad El-Harbawi)

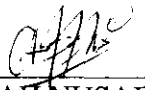
UNIVERSITI TEKNOLOGI PETRONAS

TRONOH, PERAK

July 2010

CERTIFICATION OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this project, that the original work is my own except as specified in the references and acknowledgements, and that the original work contained herein have not been undertaken or done by unspecified sources or persons.



ASIAH NUSAIBAH MASRI

ABSTRACT

Development of safer and environmentally friendly processes and products is required to achieve sustainable production and consumption patterns. Ionic liquids are compounds of high interest for industry because of their attractive properties as solvents, but the water solubility of these compounds may lead to aquatic pollution and related risks. Experimental toxicity evaluation (Daphnia magna EC_{50}) is a measurement of aquatic toxicity but there are theoretically over 1 trillion ionic liquids, which makes it necessary to estimate their properties by means of quantitative structure–activity relationships (QSARs). In this work, a novel QSAR based on multilinear regression analysis method is applied to estimate the ecotoxicity of ionic liquids. A data set of *Daphnia magna* EC_{50} was assembled to develop a novel group contribution method for estimating the EC_{50} of ionic liquids. The ionic liquids are the combination of different anion and cation which are bromide (Br^-), chloride (Cl^-), tetrafluoroborate (BF_4^-), hexafluorophosphate (PF_6^-) and bis((trifluoromethyl)sulfonyl)imide (TF_2N^-) as anions with imidazolium (im), pyridinium (py), dimethylamino pyridinium (DMApy), piperidino pyridinium (pipy), ammonium (N) and pyrrolidinium (pyr) as cations. However, due to complexicity of equations and time consuming to apply multilinear regression analysis by hand calculation, SPSS software 11.5 was used to apply the method. 44 data of ionic liquids were assembled and the results illustrated that the data range covered for log EC_{50} values in between 2.07 and -4.33. From the results, the contributions of anion, cation and alkyl substitutions has been established and found a good fitting value for predicting the EC_{50} with $r^2 = 0.934$, $r^2_{adj} = 0.910$ and variance = 0.022. From the results, it can be concluded that the toxicity contribution in increasing order for anions is hexafluorophosphate (PF_6^-) < chloride (Cl^-) < tetrafluoroborate (BF_4^-) < bis((trifluoromethyl)sulfonyl)imide (TF_2N^-), for cations is ammonium (N) < pyrrolidinium (pyr) < imidazolium (im) < pyridinium (py) < dimethylamino pyridinium (DMApy) < piperidino pyridinium (pipy), and while for alkyl is R < R_1 with R is long *n*-alkane chain and R_1 is an additional short chain (methyl). However, further investigations are necessary to increase the number of data in the training set in order to reduce the confidence range of some group contributions (e.g., pyrrolidinium based ionic liquids). In addition, other cations and anions need to be studied to increase the application of the novel group contribution method.

ACKNOWLEDGEMENT

First and foremost, I would like to acknowledge and extend my heartfelt gratitude to my supervisor of this project, Dr. Mohanad El-Harbawi for the valuable guidance and advice. He inspired me greatly to work in this project. His willingness to motivate me contributed tremendously to my project. I also would like to thank him for helping me in searching for books and journals during completion of this project. My grateful thanks also go to Mr. M. Ismail Hossain . A big contribution and hard worked from you during this years is very great indeed. My projects would be nothing without enthusiasm and imagination from both of you.

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CHAPTER 1
INTRODUCTION

1.1 BACKGROUND OF STUDY

Ionic liquids (ILs) are a class of material that are gamering much academic and industrial interest thanks to their unique properties (Chemistry Innovation KTN 2006). According to Cho *et al.*, (2007a), ionic liquids are non-volatile, non-flammable, have high thermal, chemical and electrochemical stabilities as well as favorable solvability behavior (many organic, organometallic and inorganic compounds can be dissolved in ionic liquids). In addition, ionic liquids have a large electrochemical window and excellent solvent properties for a wide range of inorganic and organic materials (Fuller *et al.*, 1997).

Imidazolium ionic liquids become the most popular ones among the whole group of this type of substance (Studzinska and Buszewski, 2009). They typically consist of nitrogen-containing organic cations and inorganic or organic anions (Welton, 1999). Due to their favorable properties, ionic liquids have been used in drugs (Macfarlane *et al.*, 2007), double-layer capacitors (Ue, 2007), solid-state dye-sensitized solar cells (Yanagida *et al.*, 2007) and biocatalysis (Lozano *et al.*, 2007).

As ILs are nonvolatile, they are comparatively harmless to the atmosphere, but due to their high solubilities in water, it must be considered that a release of ILs from industrial processes into aquatic environments may lead to water pollution and related potential risks (Luis *et al.*, 2006, Asiah *et al.*, 2010). The effects of ionic liquids on aquatic organisms have recently been reported for the marine bacterium *vibrio fischeri* (Ranke *et al.*, 2004, Docherty and Kulpa, 2005), algae (Latala *et al.*, 2005, Cho *et al.*, 2008), the freshwater crustacean *daphnia magna* (Bernot *et al.*, 2005b), the freshwater snail *physa acuta* (Bernot *et al.*, 2005a), and the zebra fish *danio rerio* (Pretti *et al.*, 2006). Some of these studies also revealed that ILs toxicity increased with increasing alkyl chain length (Ranke *et al.*, 2004, Bernot *et al.*, 2005a). In view to the applications and current interest of ILs, it is important to develop new procedures for the estimations of the toxicity of ILs.

1.2 PROBLEM STATEMENT

1.2.1 Problem Identification

From recent finding, it can be hypothesized that ionic liquids may pose environmental risks to aquatic ecosystems and their accurate data on toxicities are likely to be foremost important. However, there are still trillions of ILs which has not been tested. Calculating by experiment would rather be time consuming and require investment of money. Therefore, it is necessary to estimate their properties by means of quantitative structure-activity relationship, QSAR. Among all method performed, a novel group contribution method would be a good opportunity to predict toxicity of ionic liquid for QSAR development.

1.2.2 Significance of Project

This project will focus on predicting toxicity of ionic liquids on *Daphnia magna* using group contribution method. By group contribution method, the effect of anion, cation and substitution on toxicity can be calculated. *Daphnia magna* is one of the most widely used water crustacean around the world, it is also an important link in the food web of freshwater communities and has been the international standard model animal for toxicity testing studies. Therefore, *Daphnia magna* was used as a model organism to evaluate the toxicity of Ionic liquids, and the related biological responses induced by ionic liquids. Hence, predicting toxicity of ionic liquids on *Daphnia magna* would help industry on estimating the effect of specific ionic liquids on environment before using it.

1.3 OBJECTIVES AND SCOPE OF STUDY

The objectives of this project are:

1) To analyze the effect of anion, cation and substitution to toxicity of ionic liquids.
2) To establish a quantitative structure-activity relationship, QSAR model to predict toxicity for *Daphnia magna* by using a novel group contribution method.
3) To apply multilinear regression analysis method using SPSS Software 11.5.

Scope of the study:

The scope of the project include collecting data from literature review, analyzing structure of ionic liquid, calculating log EC_{50} from data obtained, studying on multilinear regression analysis, and develop quantitative structure-activity relationship, QSAR.As multilinear regression analysis method is very complicated and time consuming, SPSS software 11.5 was used to apply the method. SPSS software 11.5 is a mathematical software which has a function of multilinear regression analysis.

1.4 FEASIBILITY OF PROJECT WITHIN SCOPE AND TIME FRAME

Based on the draft methodology and scheduling for modeling, the project's objectives are considered achievable within the given time frame (refer to Appendix I).

CHAPTER 2
LITERATURE REVIEW

2.1 IONIC LIQUIDS

2.1.1 Terminology

Many people are familiar with ionic solutions from classroom chemistry lessons, but ionic liquids are chemically a very different breed. According to writer in Frontiers (2006), when an ionic salts (such as table salt – sodium chloride or NaCl) is dissolved in a polar solvent (such as water), the salt's crystal lattice is broken up to form an ionic solution – separate positive (Na^+) and negative (Cl^-) ions which are surrounded by the solvent water molecules. In contrast, ionic liquids (ILs) consist solely of ions, without any additional solvent. They are liquids over a wide range of temperatures, are not flammable and release no fumes. They can also act as catalysts, making chemical reactions faster and cheaper.

The term ionic liquids was introduced to cover systems below 100°C, one reason being to avoid the words 'molten salts' in phrases such as 'ambient temperature molten salts,' another to create an impression of freshness and a third, perhaps, for patent purposes (Keith, 2007). Neoretic solvents is a term applied to ionic liquids and supercritical fluids, another type of solvent that shows a huge promise for clean synthesis. The word neoretic is well established in the English Language. It is used for ionic liquids to indicate a class of novel solvents that have remarkable new properties, that "break new ground", and that offer a huge potential for industrial application (Fitzwater *et al.*, 2005).

Ionic liquids are also considered as "green solvent" because their negligible vapor pressure, volatility and flammability (Cho *et al.*, 2007b). This property decrease the risk of exposure and loss of solvent due to evaporation; thereby, reducing air pollution (Cho *et al.*, 2007a). In a simplest explanation, ILs consist of anion and cation pairs that are liquid around room temperature (Chemistry Innovation, 2006).

2.1.2 Properties

The properties of a modern ionic liquid are summarized in Table 2.1 (Fitzwater *et al.*, 2005, Thomas, 2005, Keith, 2007, Romero *et al.*, 2008, and Pretti *et al.*, 2009).

Table 2.1: Properties of Ionic Liquids

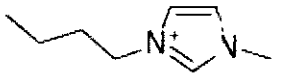
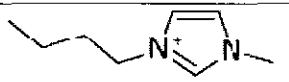
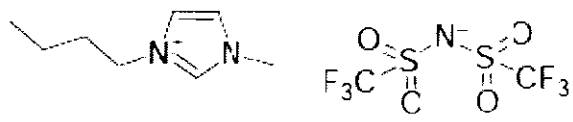
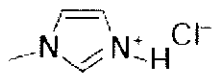
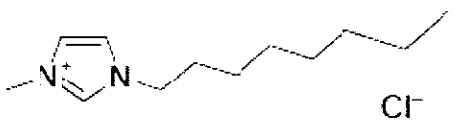
Properties	
Freezing point	Preferably below 100°C.
Liquidus range	Often > 200°C
Thermal stability	Usually high
Viscosity	Normally < 100 cP, workable
Dielectric constant	Implied ≤ 30
Polarity	Moderate
Specific Conductivity	Usually < 10 $mScm^{-1}$, "Good"
Molar conductivity	< 10 $Scm^2 mol^{-1}$
Electrochemical window	2V, even 4.5 V, except for Brønsted acidic systems
Solvent and/or catalyst	Excellent for many organic reactions, inorganic and polymeric materials.
Vapor pressure	Usually negligible
Flammability	Non – flammable

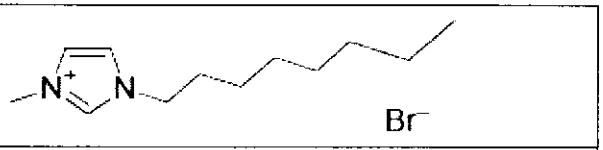
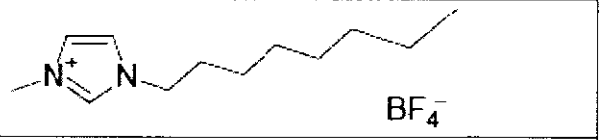
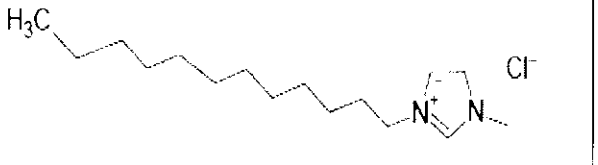
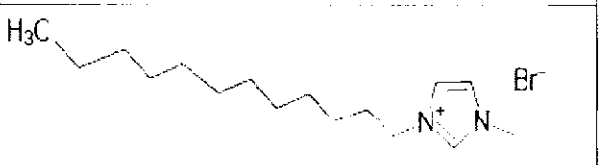
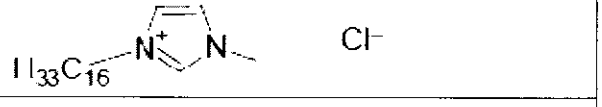
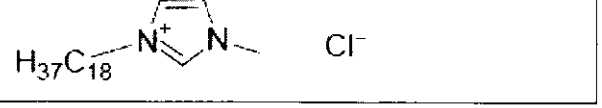
Particularly significant properties are (*i.*) the low vapor pressures in most instances which contrast the environmental problems of volatile organic solvents and (*ii.*) moderate specific conductivities, usually in the same range as those aqueous electrolytes (Keith, 2007). In addition, the nonflammability of ionic liquids adds a valuable safety bonus; noncombustible solvents (Fitzwater *et al.*, 2005). However, it has shown that a large group of ILs are combustible (Pretti *et al.*, 2009).

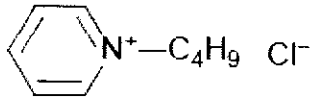
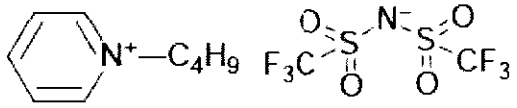
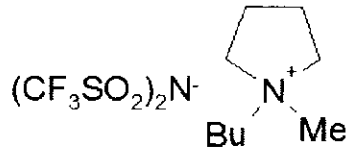
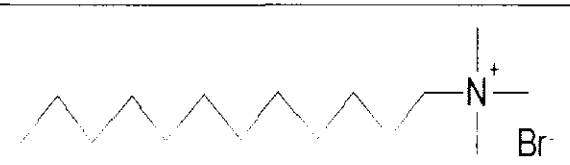
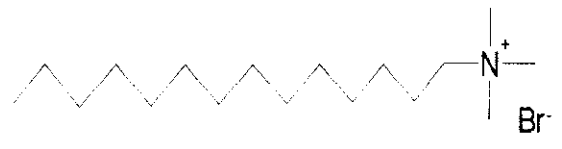
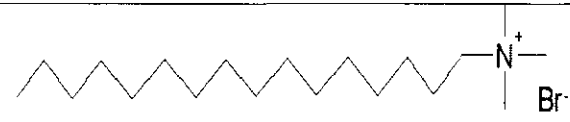
2.1.3 Structure

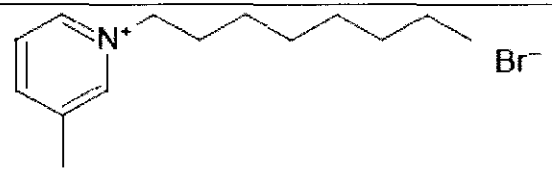
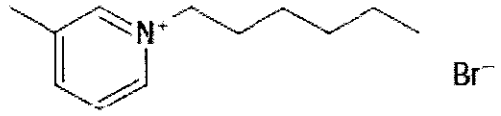
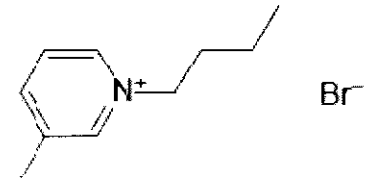
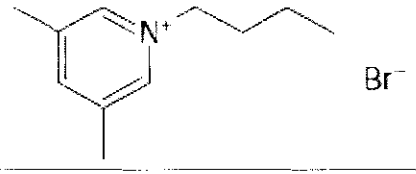
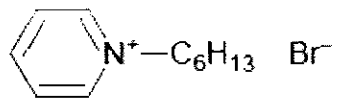
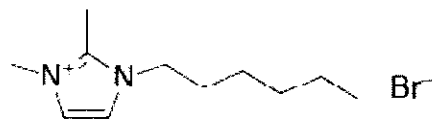
Typically ILs consist of an organic cation with delocalized charges and a small inorganic anion, most often halogen anions weakly coordinating (Romero *et al.*, 2008). An ionic liquid can be thought of as "designer" solvent so it should be possible to design, or tailor, a solvent for a certain reaction (Romero *et al.*, 2008 and Stefan *et al.*, 2006). The possibility to modify structural elements in order to optimize technological features like solvation properties, viscosity, conductivity and thermal as well as electrochemical stability is ideal in terms of technical applicability (Stefan *et al.*, 2006). Structure for ILs that will be used for this research can be referred to Table 2.2.

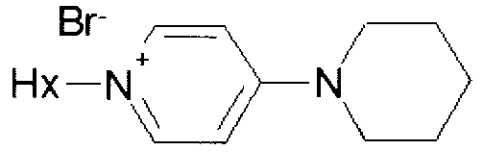
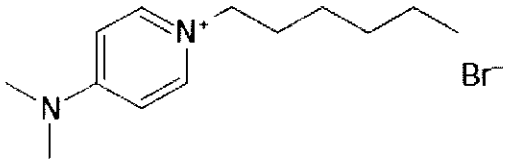
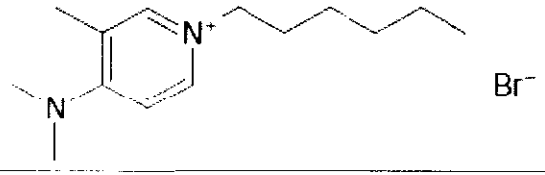
Table 2.2 Structure of ionic liquids (Zhang *et al.*, 2009)

No	Name	IUPAC name	M.F	MW	Structure
1	bmimCl	1-Butyl-3-methylimidazolium chloride	$C_8H_{15}ClN_2$	174.67	
2	bmimBr	1-Butyl-3-methylimidazolium bromide	$C_8H_{15}BrN_2$	219.12	
3	$bmimBF_4$	1-Butyl-3-methylimidazolium tetrafluoroborate	$C_8H_{15}BF_4N_2$	226.02	
4	$bmimPF_6$	1-Butyl-3-methylimidazolium hexafluorophosphate	$C_8H_{15}F_6N_2P$	284.18	
5	$bmimTF_2N$	1-Butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide	$C_{10}H_{15}F_6N_3O_4S_2$	419.36	
6	hmimCl	1-Methylimidazolium chloride	$C_4H_7ClN_2$	118.56	
7	hmimBr	1-Hexyl-3-methylimidazolium bromide	$C_{10}H_{19}BrN_2$	247.18	
8	$hmimBF_4$	1-Hexyl-3-methylimidazolium tetrafluoroborate	$C_{10}H_{19}BF_4N_2$	254.08	
9	omimCl	1-Methyl-3-octylimidazolium chloride	$C_{12}H_{23}ClN_2$	230.78	

10	omimBr	1-Methyl-3-octylimidazolium bromide	$C_{12}H_{23}BrN_2$	275.23	
11	omimBF_4	1-Methyl-3-octylimidazolium tetrafluoroborate	$C_{12}H_{23}BF_4N_2$	282.13	
12	C_{10}mimBr	1-Decyl-3-methylimidazolium bromide	$C_{14}H_{27}BrN_2$	303.28	
13	C_{12}mimCl	1-Dodecyl-3-methylimidazolium chloride	$C_{16}H_{31}ClN_2$	286.88	
14	C_{12}mimBr	1-Dodecyl-3-methylimidazolium bromide	$C_{16}H_{31}BrN_2$	331.34	
15	C_{16}mimCl	1-Hexadecyl-3-methylimidazolium chloride	$C_{20}H_{39}ClN_2$	342.99	
16	C_{18}mimCl	1-Octadecyl-3-methylimidazolium chloride	$C_{22}H_{43}ClN_2$	371.04	

17	bpyCl	N-Butylpyridinium chloride	$C_9H_{14}ClN$	171.67	
18	bpyTF_2N	N-Butylpyridinium bis(trifluoromethylsulfonyl)imide	$C_{11}H_{14}F_6N_2O_4S_2$	416.37	
19	bmpyrTF_2N	N-butyl-N-methyl-pyrrolidinium bis (trifluoromethanesulfonyl) imide	$C_{11}H_{20}N_2S_2O_4F_6$	422	
20	C_{12} C_3NBr	dodecyltrimethylammonium bromide	$C_{15}H_{34}NBr$	307.9	
21	C_{14} C_3NBr	tetradecyltrimethylammonium bromide	$C_{17}H_{38}NBr$	335.9	
22	C_{16} C_3NBr	hexadecyltrimethylammonium bromide	$C_{19}H_{42}NBr$	363.9	

23	ompyBr	1-n-Octyl-3-methylpyridinium bromide	$C_{14}H_{24}BrN$	286.25	
24	hmpyBr	1-n-Hexyl-3-methylpyridinium bromide	$C_{12}H_{20}BrN$	252.20	
25	bmpyBr	1-n-Butyl-3-methylpyridinium bromide	$C_{10}H_{16}BrN$	230.14	
26	bmmpyBr	1-n-Butyl-3,5-dimethylpyridinium bromide	$C_{11}H_{18}NBr$	244.17	
27	hpyBr	1-n-Hexylpyridinium bromide	$C_{11}H_{18}BrN$	244.17	
28	hmmimBr	1-n-Hexyl-2,3-dimethylimidazolium bromide	$C_{11}H_{21}F_3N_2O_3S$	330.37	

29	hPiPyBr	1-n-Hexyl-4-piperidinopyridinium bromide	$C_{16}H_{27}N_2Br$	326.9	
30	HDMAPyBr	1-n-Hexyl-4-dimethylaminopyridinium bromide	$C_{13}H_{23}N_2Br$	287.24	
31	HMDMAPyBr	1-n-Hexyl-3-methyl-4-dimethylaminopyridinium bromide	$C_{14}H_{25}BrN_2$	301.27	

2.1.4 Application

Room-temperature ionic liquids have been developed over the past decade as green solvents for industrial applications, ranging from the petrochemical industry, via heavy chemicals, fine chemicals, agrochemicals, and pharmaceuticals to the nuclear industry (Atkins *et al.*, 2004 and Fitzwater *et al.*, 2005). Ionic liquids are also used in BASIL process, Difasol process, battery electrolytes, methanol carbonylation and Headspace GC (Chemistry Innovation KTN 2006).

According to other writers and journalists, in the field of designing chemicals, ionic liquids represent an excellent model substance class because of their broad applicability, e.g. in synthesis, electrochemistry, extraction, separation techniques, liquid crystal, and biocatalysis and their high structural diversity leading to enormous number of possible compound (Stefan *et al.*, 2007, Cho *et al.*, 2007a, Studzinska and Buszewski, 2009, and Ioana Stan, 2009).

2.1.5 State of Art

The potential of ionic liquids to act as solvents for a broad spectrum of chemical processes is attracting increasing attention from industry because they promise significant environmental benefits (Fitzwater *et al.*, 2005). Figure 2.1 below illustrates the rise in publications concerning ionic liquids over the past few decades (and these are just the papers using the term "ionic liquids") (Seddon, 2002).

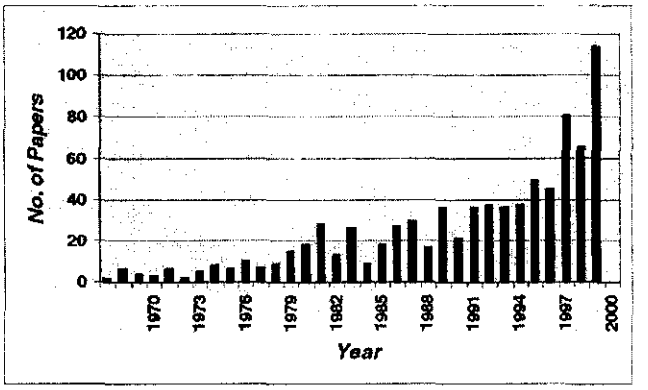


Figure 2.1: The rise in publications concerning ionic liquids as a function of time, as determined using SciFinder.

Professor Paul T. Anastas, White House Office of Science and Technology Policy in Washington D.C., and advisor to both President Clinton and President Bush (as reported in *Chemical & Engineering News*) convinced: "Green chemistry aims to design in the hazards out of chemical products and processes, including solvents. With ionic liquids, you do not have the same concerns as you have with, for example, volatile organic solvents, which can contribute to air pollution. Ionic liquid chemistry is a very new area that is not only extremely interesting from a fundamental chemistry point of view, but could also have a very large impact on industry"(Seddon, 2002 and Fitzwater *et al.*, 2005).

However, it is hypothesized that ionic liquids may pose environmental risks to aquatic ecosystems as it is poorly decomposed by microorganisms (Cho *et al.*, 2007a). In order to inform decision-makers during the development of chemical products and processes as well as for regulators concerned with the global environmental risk of the release of chemicals, Ranke generates ecotoxicological risk profiles while Pitner *et al.* (2007) developed a toxicological screening and assessment program.

2.1.6 Toxicity of Ionic Liquids

Although ILs can lessen the risk of air pollution do to their insignificant vapor pressure, they do have significant solubility in water (Anthony *et al.*, 2001, McFarlane *et al.*, 2005, Wong *et al.*, 2002). As a result, this is the most likely medium through which ILs will be released into the environment. Figure 2.2 below shows the ecotoxicological test battery to test toxicity of ionic liquids.

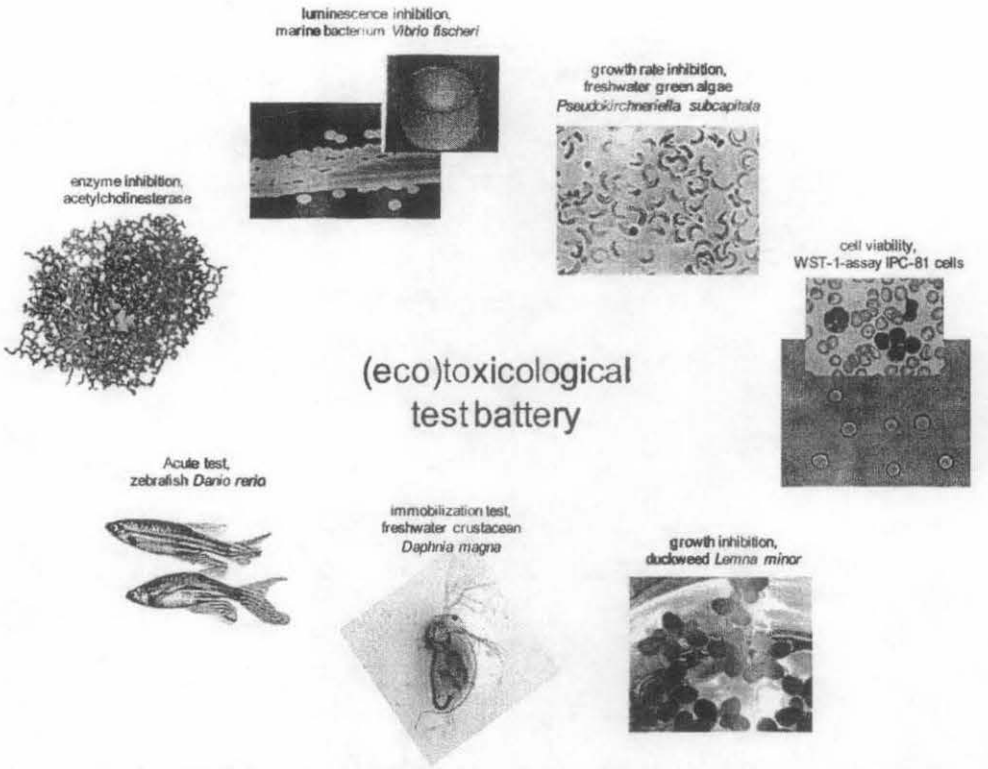


Figure 2.2 Ecotoxicological test battery to test toxicity of ionic liquids

According to Pham *et al.* (2010), for effect of ILs in an enzyme level, enzyme inhibition assays to acetylcholinesterase suggest that the trend in which cationic moiety is the dominating factor influencing the toxicity of ILs, especially when substituted with a long alkyl chain. In particular, the IL with pyridinium as cationic core structure inhibited the enzyme slightly stronger than imidazolium analogue whereas the compound based on phosphonium was less inhibitory. Regarding anions types, perfluoronated ions are toxicological interest due to hydrolysis resulting in HF formation, while the others cause less prominent effect.

Bacteria serve as an ideal starting point for ILs toxicity estimations as they have short generation times. Preliminary toxicological investigations have shown quaternary ammonium and pyridinium compounds have critical inhibitory effects on a variety of bacteria and fungi (Babalola, 1998, Li *et al.*, 1998,Kelman *et al.*, 2001). In the studies of Pernak's group (Pernak *et al.*, 2001a, Pernak *et al.*, 2001b; Pernak and Chwala, 2003, Pernak *et al.*, 2003, Pernak *et al.*, 2004a, Cieniecka-Roslonkiewicz *et al.*, 2005), they observed a trend of increasing toxicity with an increase in the alkyl chain

length substituent in the pyridinium, imidazolium and quaternary ammonium salts to various bacteria including rods, cocci and fungi.

Several groups have focused their attention on the use of algal primary producers to assess the effects of ILs to aquatic environments (Latala *et al.*, 2005; Grabinska-Sota and Kalka, 2006; Wells and Coombe, 2006; Matzke *et al.*, 2007;Cho *et al.*, 2007; Stolte *et al.*, 2007a; Cho *et al.*, 2008a,b,c; Kulacki and Lamberti, 2008; Matzke *et al.*, 2008; Pham *et al.*, 2008a,b; Pretti *et al.*, 2009;). Cho and co-workers used Pseudokirchneriella subcapitata (formerly known as Selenastrum capricornutum) to study the effect of different head groups, side chains and anions of ILs on algal growth rate and photosynthetic activity. The data revealed that the toxic influence of ILs on growth rates were more significant than those of photosynthetic performance (Pham *et al.*, 2008b). Once again, the trend of increasing toxicity with increasing alkyl chain length was observed in their reports (Cho *et al.*, 2007; Pham *et al.*, 2008b). Regarding the anionic effects, P. subcapitata was sensitive to the anion moieties in the order: $[SbF_6]^-$> $[PF_6]^-$<$[BF_4]^-$> $[CF_3SO_3]^-$> $[C_8H_{17}OSO_3]^-$> $[Br]^- \approx [Cl]^-$.

As a cellular test system, promyelotic leukemia rat cell line IPC-81 has been frequently used in cytotoxicity assays of ILs, with the reduction of the WST-1 dye as an indicator of cell viability (Matzke *et al.*, 2007; Ranke *et al.*, 2004; Ranke *et al.*, 2007a; Stasiewicz *et al.*, 2008; Stolte *et al.*, 2006; Stolte *et al.*, 2007b; Torrecilla *et al.*, 2009). It was observed that ILs with polar ether, hydroxyl and nitrile functional groups within the side chains exhibited low cytotoxicity compared to those incorporated with "simple" alkyl side chains (Kumar *et al.*, 2009; Stasiewicz *et al.*, 2008; Stolte *et al.*, 2007b). Those functional groups were thought to impede cellular uptake by membrane diffusion and reduce lipophilicity based interactions with the cell membrane (Stolte *et al.*, 2007b). Taking a closer look at the effects of sub-structural elements of ILs, $[(CF_3SO_2)_2N]^-$ anion and 4-(dimethylamino)pyridinium cation water research 44 (2010) 352–372 361were described to have intrinsic effects of anion and head group on cytotoxicity, respectively (Stolte *et al.*, 2007b). The well known side chain length effect (decrease in EC_{50} values with elongation of the alkyl side chain) could also be confirmed in these studies.

The studies on phytotoxic activity of ILs were conducted mostly on the duckweed, Lemna minor, a common aquatic vascular plant (Jastorff *et al.*, 2005; Larson *et al.*, 2008; Matzke *et al.*, 2007; Stolte *et al.*, 2007a). In general, 1-alkyl-3- methy l imidazolium compounds with longer alkyl chains were more toxic to L. minor than those with short alkyl chain lengths. Imidazolium and pyridinium cations with butyl groups had similar EC_{50}s (the concentrations that produced a 50% reduction in root growth) (39.07 and 32.54 mM, respectively); while the equivalent ammonium cation had a much higher EC_{50} (101.48 mM; i.e., less toxic) (Larson *et al.*, 2008). In consideration of anionic effect, $[(CF_3SO_2)_2N]^-$ was found to cause moderate toxicity to this duckweed (EC_{50} ¼ 6300 mM) (Matzke *et al.*, 2007). On the other hand, this anion had no or even a positive influence on the observed effects on L. minor (Stolte *et al.*, 2007a).

Ecotoxicological literature of ILs to invertebrates mainly focus on the use of *Daphnia magna* as a test organism (Bernot *et al.*, 2005a; Couling *et al.*, 2006; Garcia *et al.*, 2005; Grabinska-Sota and Kalka, 2006; Luo *et al.*, 2008; Nockemann *et al.*, 2007; Pretti *et al.*, 2009; Samori` *et al.*, 2007; Wells and Coombe, 2006; Yu *et al.*, 2009b). *Daphnia* is an important link between microbial and higher trophic levels (McQueen *et al.*, 1986), and has been the subject of hundreds of intensive ecological studies. The results of all studies again observed the well-established link between toxicity and alkyl chain length of the tested ILs containing imidazolium, pyridinium or quaternary ammonium as counter cations. The most toxic compound towards *D. magna* was found to be IM18 Br whereas the least toxic one was IM14 Cl with log10EC50 values of -1.33 and 1.93, respectively. Also, the nature of the anion was suggested to have smaller effects compared to those of the cation. In a recent study, Luo *et al.* (2008) investigated the developmental toxicity of IM18 Br on *D. magna*. It was found that this compound exhibited toxicity on the development of three generation of *D. magna* with the decrease of number of offspring and average brood size correlated to increasing IM18 Br concentrations. This indicated that IM18 Br could cause deleterious effect to the population of *Daphnia* and indirectly disturb freshwater food webs.

2.2 QSAR

The use of bioassays on standard test organisms represents fundamental approach in the definition of ecological risk in the aquatic environment for promising chemicals as ILs (Asiah *et al.*, 2010, Pretti *et al.*, 2009). The environmental hazard assessment of chemicals consists of the identification of the effects that chemicals may have on organisms in the environment and the determination of the concentration of the chemical below which adverse effects in the environmental sphere of concern (e.g., aquatic) are not expected to occur. Development of safer and environmentally friendly processes and products is needed to achieve sustainable production and consumption patterns. Experimental determination of ecotoxicity is required to evaluate the environmental fate of a compound to improve the information related to the factors leading to environmental risks. Several standard procedures with biological systems have been established to evaluate the aquatic toxicity (Luis *et al.*, 2006). However ecotoxicological assays are time and material consuming and can significantly differ. Furthermore, there are theoretically over 1 million ionic liquids, although fewer than 1 thousand have been reported. For these reasons, a method is needed to estimate environmental fate parameters of these agents (Luis *et al.*, 2007).

Fortunately, it may be possible to predict some of the important properties by means of structure-activity correlations (Wilkes, 2004). Structure-activity relationships and quantitative structure-activity correlations, referred to as SARs and QSARs, respectively, are models that can be used to predict the physicochemical and biological properties of molecules (Luis *et al.*, 2007). The basis of any QSAR is that the biological activity of a new or untested chemical can be inferred from the molecular structure or other properties of similar compounds whose activities have already been assessed. In toxicology, changes in structure of a chemical may influence the type and potency of its toxic action; thus models such as structure-activity relationships are used to represent, explain and most importantly, predict phenomena of interest (Schultz *et al.*, 2003). In the field of aquatic toxicity, first-generation quantitative structure-activity relationships, QSARs have developed as scientifically credible tools for predicting the acute toxicity of chemicals when little or no empirical data are available (Auer *et al.*, 1990).

2.2.1 QSAR Steps

According to Thomson (1891), the strategy for QSAR development (i.e in drugs) consists of several iterative steps, based on statistical experimental design and multivariate data analysis, which hopefully lead to the design of compounds with the desired activity profile.

1) Formulation of classes of similar compounds

The first step consists of the selection of the biological activities of interest, the choice of structural domain (structural class) and the choice of structural features to be varied. Since the mechanism of biological action usually differs between different types of compounds, it is not desirable that QSARs are based on compounds too diverse structurally. Thus, the ideal situation corresponds to classes of chemically and biologically similar compounds were within each class, all the compounds are structurally similar and function according to the same mode of action. However, the compounds must be dissimilar enough to cause some systematic change on the biological activity.

The formation of classes of similar compounds consists on dividing the series of compounds of interest into categories on the basis of their chemical structure. This may be achieved according to their general backbone, their substituents, reactivity, and knowledge of the biological mechanism. If the subsequent data analysis reveals that the compounds do not form a homogeneous class, new class should be defined.

2) Quantitative description of structural variation and choice of the QSAR model.

To appropriately describe the structural variation, in general, several descriptor variables are required to contain sufficient relevant information about the biological phenomenon. For that reason the structural description in multivariate. However, is difficult to predict in advance which descriptor variables will be useful.
Thus, it is convenient to have a set of independent design variables, which might have an influence on the biological effect. However, in the optimization of molecules,

where substitution patterns or the whole molecular structure is changed, usually is not possible to discern design variables that can be changed independently of each other.

3) Selection of the training set of compounds (series design).

For any QSAR model, it is of crucial importance that the training set selected to calibrate the model exhibits a well-balanced distribution and contains representative compounds. This calibration can be attained by a systematic selection of the training set, where the major structural features are varied systematically and simultaneously.

4) Synthesis and biological testing.

Provided that the biological testing should be minimized, the basic idea is to subject merely the representative training test to extensive testing, in order to obtain a broad and stable picture of their biological properties. The response matrix contains biological variables that span as many aspects of the biological profiles of the investigated compounds as possible. The more biological tests are performed, the better the stability of the resulting QSAR model is, and this lead to an improved predictive capability. The testing of a few representative compounds saves time and thus money and adheres to the principles of animal welfare. Biological measurements are commonly recorded as dose-response curves, showing the relationships between the administered doses and the responses that they elicit.

5) QSAR development: data analysis.

To calculate the best mathematical expression linking together the physicochemical descriptors and biological responses, information regarding essential features of the chemical and biological data structure is obtained. They may be need to transform some of the descriptors variable, or delete compounds (outliers), exhibiting deviating chemical and/or biological properties. The QSAR analysis also provides information on wether a descriptor variable is relevant for a certain application.

6) Validation and predictions for non-tested compounds.

Finally, the final purpose of a QSAR is to predict the biological activities of non-tested compounds, which belong to the class under investigation. However, the predictive ability of the model first is verified experimentally. This is accomplished by biological testing of some additional compounds in the same way as the training set and then comparing the experimental finding with the values predicted by the QSAR. If the QSAR predicts within acceptable limits, it may be used for a more extensive prognostication. The prediction errors should be compared with the precision and range of the biological measurements obtained.

It is desirable that the compounds in the validation set adequately span the physic-chemical domain and the biological activity range of interest. Conveniently, the validation set may be selected according to a statistical experimental design in order to result in a series of representative compounds.

7) Data analysis and interpretation of results for the proposal of new compounds.

In fact, any QSAR development is an iterative cycle, in which the steps are repeated a number of times, until sufficient knowledge about a class of compounds is obtained in order to either design compounds with the desired activity profile or to include that such a profile cannot be attained.

2.3 DISCOVERIES

2.3.1 Functionalised Side Chain

According to Stefan *et al.* (2007), it is a goal of many researchers to be able to tune the physicochemical properties of ionic liquids via the choice of certain anionic and cationic components when designing a specific ionic liquid ideally suited for a specific process. Such task specific or functionalized ionic liquids are created through incorporation of functional groups into the alkyl chains. For example, thiol and thioether functionality has been used to enhance the extraction of metals. Additionally, ionic liquids with appended nitrile groups have been shown to stabilise catalysts. Hydroxyl groups tend to increase hydrogen bonding strength while ethers can lead to decreases in viscosity. Tertiary amine groups can increase nucleophilicity, while primary amines can be used to capture protons or carbon dioxide.

2.3.2 Length of Alkyl Chain

According to Pretti *et al.* (2009), long chain ammonium salts showed higher toxicity to *Pseudokirchneriella subcapitata* (algae), *Daphnia magna* (cladocerans) and *Danio rerio* (fish), whereas very low toxicities characterized sulfonium- and morpholinium-based ILs. Before that, Cho *et al.* (2007b) have made an experiment about toxicity of imidazolium salt with anion bromide to a phytoplankton *Selenastrum capricornutum.* The results showed that a longer alkyl-chain resulted in stronger inhibition of algal growth which means increase in toxicity.

The results are also agreed by Romero *et al.* (2008). According to them, the shorter chain length of side chain, the lower toxic effect is. They have carried out *linear regression analysis* of the log EC_{50} value vs. the alkyl chain length of R_2 for imidazolium ionic liquids. The following equation has been obtained:

$$logEC_{50} = 5.33 - 0.549nC_{R_2} \qquad [2.1]$$

In Figure 2.3, the log EC_{50} values are plotted vs. the alkyl chain length of R_2 for Romero *et al.* (2008) experiment. The figure shows that a linear relationship is

obtained for the interval $1 \leq nC_{R2} \leq 8$. The validity of this relationship does not depend on anion, and thus can be used to predict the toxicity of the R_2mim ILs.

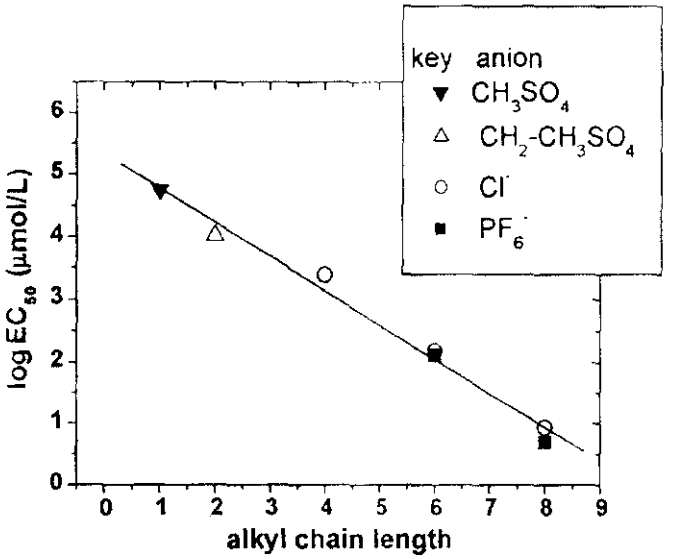


Figure 2.3: Effect of anion and alkyl chain length on the acute toxicity (Microtox®) for 1-alkyl-3-methylimidazolium ionic liquids.

2.3.3 Structure of Cation

According to Pretti *et al.* (2009), ILs not having a surfactant-like structure were generally not toxic for *Danio rerio*. However, many show a toxicity to *Daphnia magna* and *Pseudokirchneriella subcapitata, which* is strongly affected by the cationic head group. It decreases on going from aromatic heterocyclic nitrogen-containing compounds (pyridinium and imidazolium) to non-aromatic cyclic and acyclic compounds (pyrrolidinium, ammonium and morpholinium).

Stock *et al.* (2004), have made experiment on the effect of ionic liquids on acetylcholinesterase (an enzyme that breaks down acetylcholine, stopping excitation of a nerve after transmission of an impulse). The results clearly show a dependency of inhibitory potency on the core structural elements of the ionic liquids. The strongest inhibition was obtained with ionic liquids containing a positively charged nitrogen (pyridinium and imidazolium). The ionic liquid with pyridinium as the cationic core structure inhibited the enzyme slightly stronger than imidazolium analogue. The ionic liquid based on phosphonium was less inhibitory. The results are shown in Figure 2.4 below;

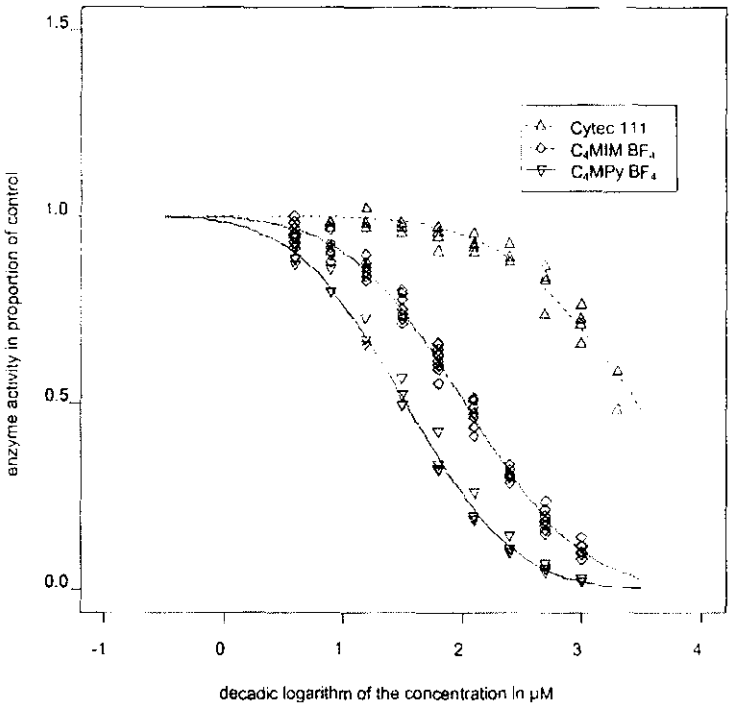


Figure 2.4: Concentration-response curves for ionic liquids with different core structures of the cation. The anion is BF_4.

According to Stefan *et al.* (2007), for *Vibrio fischeri* the non-aromatic compounds of head group of ionic liquids exhibited a low toxicity, whereas the aromatic substances showed an increased toxicity.

2.3.4 Anion

Romero *et al.* (2008) and Stefan *et al.* (2007) have done experiment of ionic liquids with different anions (Cl, Br, PF_6 and BF_4). They have concluded that anion has a low effect on toxicity. On the other hand, Stefan *et al.* (2006) used a formula to calculate the anion effect ratio (AR) for imidazolium ionic liquids.

The cytotoxicity of the ionic liquid is normalized on the cytotoxicity of the ionic liquids with chloride as counterion. Since the chloride anion does not exhibit intrinsic cytotoxic effects, these reference ionic liquids were taken to build up a linear regression line. This base line represents the intrinsic cytotoxicity of the cation moiety and is therefore used as benchmark for anion effects in the cytotoxicity assay. The formula is as below,

$$AR = \frac{EC_{50}(R_xMIMCl)}{EC_{50}(R_xMIMY)} \qquad [2.2]$$

From the formula, it is shown that anions in ionic liquids with AR values < 5 are non-cytotoxic or only marginally altering the cytotoxicity of the ionic liquids. In contrast, anions exhibiting AR values > 5 are viewed as significantly influencing the cytotoxicity of the corresponding ionic liquids.

2.3.5 Lipophilicity

Stefan *et al.* (2007) have done experiment on marine bacteria *Vibrio fischeri*, the limnic green algae *Scenedesmus vacuolatus* and the fresh water plant *Lemnar minor*. In this study, the analysed set of about 40 ionic liquids confirmed the interdependency between lipophilicity – as derived from gradient HPLC and ecotoxicity. The toxicity was clearly reduced for the test organisms (partially by seven orders of magnitude) when short functionalized side chains were used instead of non-polar alkyl chains. Furthermore, they can demonstrate strong interactions of hydrophobic ionic liquid cations with two different types of common biological lipid bilayers, indicating that the membrane system of organisms is probably a primary target site of toxic action.

Further research was made by Studzinska and Buszewski (2009) about lipophilicity/hydrophobicity. The experiment was done on watercress (*Lepidium sativum* L.). It was found that the seedling growth is increasing with the decrease in ionic liquid hydrophobicity.

2.3.6 Modification to Imidazolium Ionic Liquid

According to Pretti *et al.* (2009), in the case of imidazolium-based ILs, it is suggested that (i) maintaining practically constant molecular weight, substitution of one or two carbon atoms of the longer alkyl chain with a more electronegative atom (chlorine or oxygen) reduces the acute toxicity to *D. magna* and *P. subcapitata*; (ii) the substitution of an alkyl chain with a hydrogen atom reduces toxicity, too; and (iii) the presence of $[Tf_2N]^-$ anion increases the toxicity, compared to halides, for *P. subcapitata*.

2.3.7 QSAR

Group contribution methods have been applied to estimate physicochemical properties of interest in the environmental behavior of chemicals. These include melting point, boiling point, vapor pressure, Henry's Law constant, octanol-water partition coefficient and water solubility (Allen and Shonnard, 2002 and Trohalaki *et al.*, 2004). Some studies have been performed on the relationship between toxicity and chemical structure for several compounds (Beteringhe and Balaban, 2004), several QSARs which predict toxicity values for *Pimephales promelas*, green algae and fish (Russom *et al.*, 1997, Lie *et al.*, 2003, Verhaar *et al.*, 1995) have been developed and some QSARs estimate *Vibrio fischeri* toxicity for specific groups of compounds using molecular and physicochemical descriptors (Agrawal and Khadikar, 2002 and Luis *et al.*, 2007).

A QSAR model to estimate the ecotoxicity (EC_{50} *Vibrio fischeri*) of ionic liquids has been carried out by Luis *et al.* (2010) based on previous works and performing the application to 96 data. The model shows a group contribution methods that considers three main groups of descriptors in the ionic liquids structure: anion, cation and substitutions (carbon linked to cation). Based on these three descriptors, their contribution to the ecotoxicity of the ionic liquids has been evaluated by mean of multilinear regression model. The data range for log EC_{50} values between 5 and -0.23 where it covers 9 kinds of cations and 17 anions. The results were well correlated (r^2 = 0.924) but some influences cannot be statistically established (e.g for pyrrolidinium, morpholinium and piperidinium based ionic liquids). The main contribution to the ecotoxicity is associated to the alkyl chain length which is in good agreement with recent literature. Anions group tend to decrease the ecotoxicity due to their negative sign. However, when caprylate anion is present in the molecule, higher toxicity is expected because its contribution takes a value of 0.28.

Yan *et al.* (2010) applied QSAR approach to melting point of ionic liquids of imidazolium bromides and imidazolium chlorides. The cationic structures of these ILs were optimized by means of Hyperchem software and MOPAC program. QSAR module of Materials Studio software and Genetic Algorithm (GA) programs were employed to calculate and select the structure descriptors of ILs, then prediction

models correlating the selected structure descriptors and melting points of ionic liquids were set up by using the multiple linear regressions (MLR) method and the back-propagation artificial neural network (BP ANN) method, separately. Finally, the obtained QSAR models, including MLR model and BP ANN model, were validated by external test sets. In this work, three data sets, which were 30 imidazolium bromides, 20 imidazolium chlorides and the emerging of above two data sets respectively, were used to investigate the QSAR correlation of the melting points of ILs. The results demonstrated that the prediction mean absolute errors (MAEs) of MLR models for test sets of those three data sets were in the order of 20.52 K, 13.59 K, and 21.95 K, and the prediction MAEs of BP ANN models were 8.77 K, 4.98 K and 9.31 K, respectively. It is indicated that the predictions of two models for all melting points of ILs were reliable, and the prediction precision of BP ANN model was higher than that of MLR model. That might denote that relationship between melting points and the cationic structure descriptors were not just linear, and nonlinear modeling might be more rational.

Using previously published toxicity data as well as a small set of heretofore-unpublished results, quantitative structure–property relationship models are developed by Couling *et al.* (2006) to assess the factors that govern the toxicity of a range of different ionic liquids to two aquatic organisms (*Vibrio fischeri* and *Daphnia magna*). With at most four molecular descriptors, $\log_{10}$ EC_{50} and $\log_{10}$ LC_{50} data are reproduced with an R^2 of 0.78–0.88. Besides the well-established link between toxicity and alkyl chain length on imidazolium, pyridinium and quaternary ammonium-based ionic liquids, the models predict that toxicity increases slightly with the number of nitrogen atoms in an aromatic cation ring. All other things being equal, toxicity is expected to show the trend with cation type of ammonium < pyridinium < imidazolium < triazolium < tetrazolium. In addition, toxicity is expected to decrease with ring methylation as well as with an increase in the number of negatively charged atoms on the cation. The anion plays a secondary role in toxicity for the compounds studied here, although the presence of positively charged atoms on the anion is predicted to slightly increase toxicity.

Trohalaki *et al.* (2004) have presented QSARs for the melting points and liquid densities of a new class of energetic ionic liquids that can be used in the design of

additional 1-substituted-4-amino-1,2,4-triazolium bromide and nitrate salts. They optimized the molecular geometries of the cations of the ionic liquids using ab initio quantum chemical methods. Melting-point QSPRs were then derived from molecular orbital, thermodynamic, and electrostatic descriptors. Good correlations with the experimental data were found. The correlation coefficients for three-parameter melting-point QSARs and for one-parameter density QSARs exceed 0.9 which are r^2 = 0.914 for bromide salt and r^2 = 0.933 for nitrate salt. Although some of the descriptors that appear in our QSARs were designed to describe chemical reactions, Trohalaki *et al.* (2004) infer that they serve in this study to quantify interactions between the cation and anion.

2.4 MULTILINEAR REGRESSION ANALYSIS

Multilinear regression analysis will be used to calculate the group (anions, cations and substitutions) contribution on toxicity. According to Asiah *et al.* (2010), regression analysis is a conceptually simple method for investigating functional relationships among variables. The relationship is expressed in the form of an equation or a model connecting the response or dependent variable and one or more explanatory or predictor variables (Chatterjee and Hadi, 1938). In this research, the response or dependent would be dimensionless toxicity Y^* which is expressed in Equation (3.1) while the explanatories or predictors are anions, cations and substitutions.

We denote the response variable by Y and the set of predictor variables by X_1, X_2, ..., X_P, where p denoted the number of predictor variables. The true relationship between Y and X_1, X_2, ..., X_P can be approximated by the regression model

$$Y = f(X_1, X_2 \ldots, X_p) + \varepsilon \tag{2.3}$$

where, ε is assumed to be a random error representing the discrepancy in the approximation. It accounts for the failure of the model to fit the data exactly. The function f(X_1, X_2, ..., X_P) describes the relationship between Y and X_1, X_2, ..., X_P . An example is the linear regression model

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_p X_p + \varepsilon \tag{2.4}$$

where, $\beta_0, \beta_1, \ldots, \beta_p$, is called the regression parameters or coefficients, are unknown constant to be determined (estimated) from the data. We follow the commonly used notational convention of denoting unknown parameter by Greek letters.

The predictor or explanatory variables are also called by other names such as independent variables, covariates, regressors, factors, and carriers. The name independent variable, though commonly used, is the least preferred because in practice the predictor variables are rarely independent of each other.

CHAPTER 3
METHODOLOGY

For this research, multilinear regression analysis is the method that will be used to predict toxicity of ionic liquid. Due to time consumption, this research will only focus on *Daphnia magna* as tested organism. Below are the steps to get through the result.

Step 1: Collect log EC_{50} data for ionic liquid toxicity on *Daphnia magna*.

Step 2: To avoid the influence of the absolute values of the data to group contribution method calculations, the dimensionless toxicity, Y^*, between 0 and 1 is defined as below for all ionic liquids.

$$Y^* = \frac{\log EC_{50\_max} - \log EC_{50}}{\log EC_{50\_max} - \log EC_{50\_min}} \quad (3.1)$$

where log EC_{50_max} and log EC_{50_min} are, respectively, the maximum and minimum values of log EC_{50} in the database showing the application range of the model.

Step 3: Analyze structure of ionic liquid in a table form such as Table 3.1,

Table 3.1: Ionic liquids toxicities, dimensionless toxicity, and group contribution descriptors.

Compound	$LogEC_{50}$ (μmol/L)	Y	Br^-	Cl^-	BF_4^-	PF_6^-	TF_2N^-	*Imida*	*Pyrid*	*Ammon*	*Pyrrol*	C_5	C_6	R	R_1
bmimCl	1.85	0.05	0	1	0	0	0	1	0	0	0	0	0	4	1
bmimBr	1.56	0.11	1	0	0	0	0	1	0	0	0	0	0	4	1
bmimBF4	1.79	0.06	0	0	1	0	0	1	0	0	0	0	0	4	1

where Br^-, Cl^-, BF_4^-, PF_6^- and TF_2N^- are set of anions; *Imida, Pyrid, Ammon, Pyrro,* C_5 and C_6 are *Imidazolium, Pyridinium, Ammonium, Pyrrolidinium, dimethylamino Pyridinium* and *piperidino Pyridinium* respectively; R is long-alkyl-chain substitution and R_1 is an additional short chain (methyl). These variables have a nonzero value when the group is present in the molecule.

Step 4: Estimate QSAR by summation of the contribution of each group as shown in Equation 3.2.

$$Y = \sum_i a_i A_i + \sum_j c_j C_j + \sum_k s_k S_k \quad (3.2)$$

where A_i, C_j and S_k are the molecular descriptors for ionic liquids, a_i, c_j, and s_k are the contribution of each group of the toxicity, and the summation is taken over all group, subscripts indicate anions (i), cations (j), and substitutions (k).

To estimate QSAR, we need to apply multilinear regression analysis on the data. According to Chatterjee and Hadi, (1938), we present the standard results of multiple regression analysis in matrix notation. Let us define the following matrices:

$$\boldsymbol{Y} = \begin{bmatrix} y_1 \\ y_2 \\ \vdots \\ y_n \end{bmatrix}, \boldsymbol{X} = \begin{bmatrix} x_{10} & x_{11} & \cdots & x_{1p} \\ x_{10} & x_{21} & \cdots & x_{2p} \\ \vdots & \vdots & & \vdots \\ x_{n0} & x_{n1} & \cdots & x_{np} \end{bmatrix}, \boldsymbol{\beta} = \begin{bmatrix} \beta_0 \\ \beta_1 \\ \vdots \\ \beta_p \end{bmatrix} \boldsymbol{\varepsilon} = \begin{bmatrix} \varepsilon_1 \\ \varepsilon_2 \\ \vdots \\ \varepsilon_n \end{bmatrix},$$

For this research, the matrix represent Y* and all descriptor as below,

$$\boldsymbol{Y} = \begin{bmatrix} y_1 \\ y_2 \\ \vdots \\ y_n \end{bmatrix}, \boldsymbol{X} = \begin{bmatrix} x_{10} & A_{11} & \cdots & R_{12} \\ x_{10} & A_{21} & \cdots & R_{22} \\ \vdots & \vdots & & \vdots \\ x_{n0} & A_{n1} & \cdots & R_{n2} \end{bmatrix}, \boldsymbol{\beta} = \begin{bmatrix} \beta_0 \\ \alpha_2 \\ \vdots \\ s_2 \end{bmatrix} \boldsymbol{\varepsilon} = \begin{bmatrix} \varepsilon_1 \\ \varepsilon_2 \\ \vdots \\ \varepsilon_n \end{bmatrix},$$

The above matrix can be expressed as linear equation as below,

$$\boldsymbol{Y} = \boldsymbol{X\beta} + \boldsymbol{\varepsilon} \quad (3.3)$$

where $x_{i0} = 0$ for all I since the graph will go through origin. The assumptions made about ε for least squares estimation are

$$E(\varepsilon) = 0, and\ Var(\varepsilon) = E(\varepsilon\varepsilon^T) = \sigma^2 \boldsymbol{I}_n$$

where $E(\varepsilon)$ is the expected value (mean) of ε, $\boldsymbol{I}_n$ is the identity matrix order n, and ε^T is the transpose of ε. Accordingly, ε_i's are independent and have a zero mean and constant variance. This implies that

$$E(\boldsymbol{Y}) = \boldsymbol{X\beta}$$

The least squares estimator $\hat{\beta}$ of β is obtained by minimizing the sum of squared deviations of the observations from their expected values. Hence the least squares estimators are obtained by minimizing $S(\beta)$, where

$$S(\beta) = \varepsilon^T\varepsilon = (\boldsymbol{Y} - \boldsymbol{X\beta})^T(\boldsymbol{Y} - \boldsymbol{X\beta})$$

Minimization of $S(\beta)$ leads to the system of equations

$$(\boldsymbol{X}^T\boldsymbol{X})\hat{\beta} = \boldsymbol{X}^T\boldsymbol{Y} \quad (3.4)$$

This is the system of *normal equations*. Assuming that $(\boldsymbol{X}^T\boldsymbol{X})$ has an inverse, the least squares estimates $\hat{\beta}$ can be written explicitly as

$$\hat{\beta} = (\boldsymbol{X}^T\boldsymbol{X})^{-1}\boldsymbol{X}^T\boldsymbol{Y} \tag{3.5}$$

from which it can be seen that $\hat{\beta}$ is a linear function of **Y**. The vector of fitted values $\hat{\boldsymbol{Y}}$ corresponding to the observed Y is

$$\hat{\boldsymbol{Y}} = \boldsymbol{X}\hat{\beta} = \boldsymbol{P}\boldsymbol{Y} \tag{3.6}$$

where

$$\boldsymbol{P} = \boldsymbol{X}(\boldsymbol{X}^T\boldsymbol{X})^{-1}\boldsymbol{X}^T \tag{3.7}$$

is known as the *hat* or *projection* matrix. The vector of residuals is given by

$$\boldsymbol{e} = \boldsymbol{Y} - \hat{\boldsymbol{Y}} = \boldsymbol{Y} - \boldsymbol{P}\boldsymbol{Y} = (\boldsymbol{I}_n - \boldsymbol{P})\boldsymbol{Y} \tag{3.8}$$

The properties of the least squares estimators are:

1. $\hat{\beta}$ is an unbiased estimator of β (that is, $E(\hat{\beta}) = \beta$) with variance-covariance matrix $Var(\hat{\beta})$, which is

   $$Var(\hat{\beta}) = E(\hat{\beta} - \beta)(\hat{\beta} - \beta)^T = \sigma^2(\boldsymbol{X}^T\boldsymbol{X})^{-1} = \sigma^2\boldsymbol{C}$$

   where

   $$\boldsymbol{C} = (\boldsymbol{X}^T\boldsymbol{X})^{-1} \tag{3.9}$$

   Of all unbiased estimators of β that are linear in the observations, the least squares estimator has minimum variance. For this reason, $\hat{\beta}$ is said to be the *best linear unbiased estimator* (BLUE) of β.

2. The residual sum of squares can be expressed as

   $$e^Te = \boldsymbol{Y}^T(\boldsymbol{I}_n - \boldsymbol{P})^T(\boldsymbol{I}_n - \boldsymbol{P})\boldsymbol{Y} = \boldsymbol{Y}^T(\boldsymbol{I}_n - \boldsymbol{P})\boldsymbol{Y} \tag{3.10}$$

   The last equality follow because $(\boldsymbol{I}_n - \boldsymbol{P})$ is a symmetric idempotent matrix.

3. An unbiased estimator of σ^2 is

   $$\hat{\sigma}^2 = \frac{\boldsymbol{e}^T\boldsymbol{e}}{n-p-1} = \frac{\boldsymbol{Y}^T(\boldsymbol{I}_n-\boldsymbol{P})\boldsymbol{Y}}{n-p-1} \tag{3.11}$$

   With the added assumptions that the ε_i's are normally distributed we have the following results:

4. The vector $\hat{\beta}$ has a (p+1)-variate normal distribution with mean vector $\beta$and variance-covariance matrix $\sigma^2\boldsymbol{C}$. The marginal distribution of $\hat{\beta}_j$ is normal with mean β_j and variance $\sigma^2 c_{jj}$, where c_{jj} is the jth diagonal element of **C** in Equation (3.9). Accordingly, the standard error of β_j is

$$s.e.(\hat{\beta}_j) = \hat{\sigma}\sqrt{c_{jj}} \tag{3.12}$$

and the covariance of $\hat{\beta}_i$ and $\hat{\beta}_j$ is $\boldsymbol{Cov}(\hat{\beta}_i, \hat{\beta}_j) = \sigma^2 c_{jj}$.

5. The quantity $W = e^T e/\sigma^2$ has an x^2 distribution with (n-p-1) degrees of freedom.
6. $\hat{\beta}$ and $\hat{\sigma}^2$ are distributed independently of one another.
7. The vector of fitted values $\hat{Y}$ has a singular InI-variate normal distribution with mean $E(\hat{\boldsymbol{Y}})=\mathbf{X}\beta$ and variance-covariance matrix $Var(\hat{\boldsymbol{Y}}) = \sigma^2 \boldsymbol{P}$.
8. The residual vector **e** has a singular n-variate normal distribution with mean $E(\mathbf{e})=\mathbf{0}$ and variance-covariance matrix $Var(\boldsymbol{e}) = \sigma^2(\boldsymbol{I}_n - \boldsymbol{P})$.
9. The predicted value $\hat{y}_0$ corresponding to an observation vector $x_0 = (x_{00}, x_{01}, x_{02}, \ldots, x_{0p})^T$, with $x_{00} = 0$ is

$$\hat{y}_0 = x_0^T \hat{\beta} \tag{3.13}$$

and its standard error is

$$s.e.(\hat{y}_0) = \hat{\sigma}\sqrt{1 + x_0^T (\boldsymbol{X}^T \boldsymbol{X})^{-1} x_0} \tag{3.14}$$

The mean response μ_0^T corresponding to x_0^T is

$$\hat{\mu}_0 = x_0^T \hat{\beta} \tag{3.15}$$

with a standard error

$$s.e.(\hat{\mu}_0) = \hat{\sigma}\sqrt{x_0^T (\boldsymbol{X}^T X)^{-1} x_0} \tag{3.16}$$

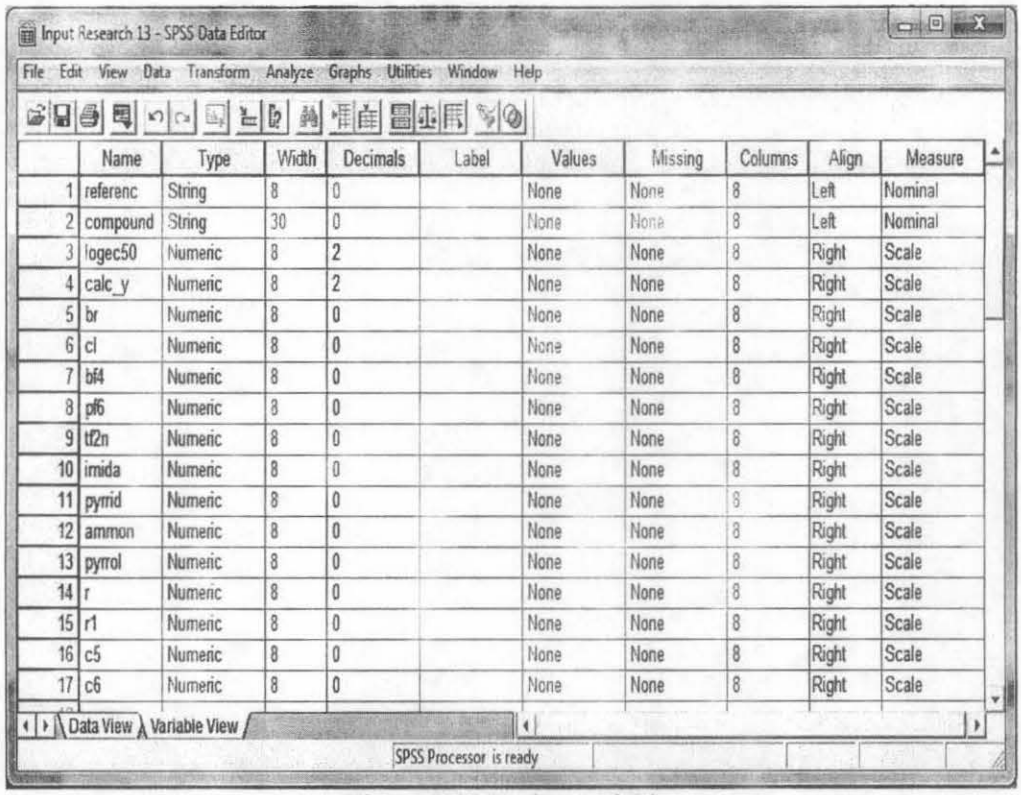
10. The 100(1-α)% joint confidence region for the regression parameters β is given by

$$\left\{\beta: \frac{(\beta-\hat{\beta})^T (X^T X)(\beta-\hat{\beta})}{\hat{\sigma}^2(p+1)} \leq F_{(p+1,n-p-1,\alpha)}\right\} \tag{3.17}$$

Which is an ellipsoid centered at $\hat{\beta}$.

As it is complex and time consuming to calculate it by hand, software such as Polymath and SPSS 11.5 can be used to analyze the data. For this research, SPSS 11.5 will be used since it is already in the lab. The steps to estimate QSAR by using SPSS is shown below.

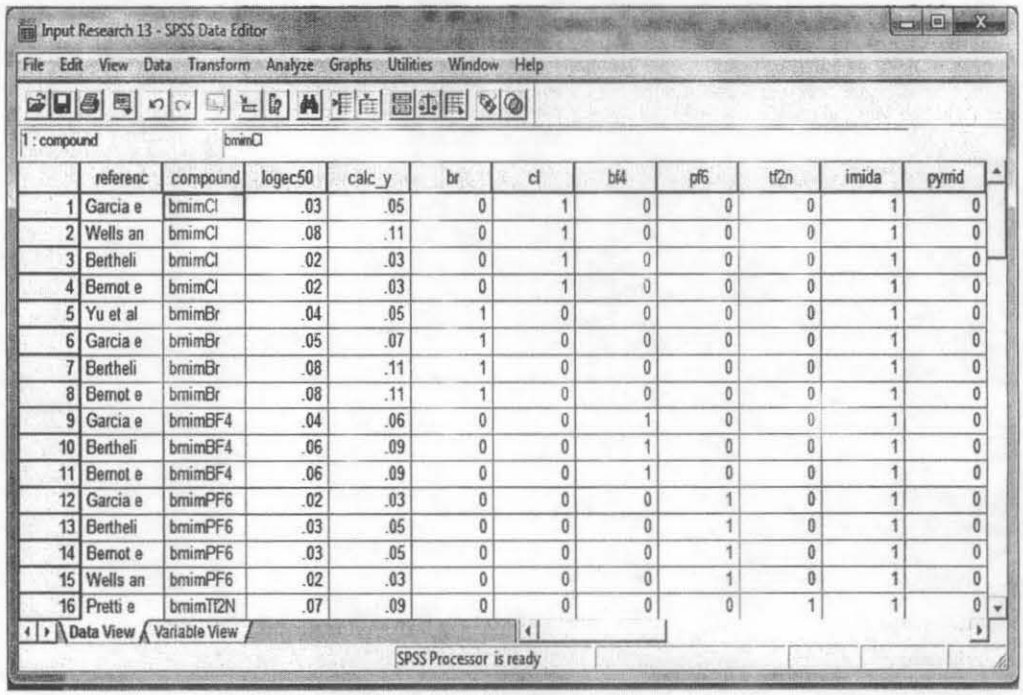
Step 4.1: Name the variable.



	Name	Type	Width	Decimals	Label	Values	Missing	Columns	Align	Measure
1	referenc	String	8	0		None	None	8	Left	Nominal
2	compound	String	30	0		None	None	8	Left	Nominal
3	logec50	Numeric	8	2		None	None	8	Right	Scale
4	calc_y	Numeric	8	2		None	None	8	Right	Scale
5	br	Numeric	8	0		None	None	8	Right	Scale
6	cl	Numeric	8	0		None	None	8	Right	Scale
7	bf4	Numeric	8	0		None	None	8	Right	Scale
8	pf6	Numeric	8	0		None	None	8	Right	Scale
9	tf2n	Numeric	8	0		None	None	8	Right	Scale
10	imida	Numeric	8	0		None	None	8	Right	Scale
11	pyrid	Numeric	8	0		None	None	8	Right	Scale
12	ammon	Numeric	8	0		None	None	8	Right	Scale
13	pyrrol	Numeric	8	0		None	None	8	Right	Scale
14	r	Numeric	8	0		None	None	8	Right	Scale
15	r1	Numeric	8	0		None	None	8	Right	Scale
16	c5	Numeric	8	0		None	None	8	Right	Scale
17	c6	Numeric	8	0		None	None	8	Right	Scale

Figure 3.1 Naming variable

Step 4.2: Key in the data.



	referenc	compound	logec50	calc_y	br	cl	bf4	pf6	tf2n	imida	pyrid
1	Garcia e	bmimCl	.03	.05	0	1	0	0	0	1	0
2	Wells an	bmimCl	.08	.11	0	1	0	0	0	1	0
3	Bertheli	bmimCl	.02	.03	0	1	0	0	0	1	0
4	Bernot e	bmimCl	.02	.03	0	1	0	0	0	1	0
5	Yu et al	bmimBr	.04	.05	1	0	0	0	0	1	0
6	Garcia e	bmimBr	.05	.07	1	0	0	0	0	1	0
7	Bertheli	bmimBr	.08	.11	1	0	0	0	0	1	0
8	Bernot e	bmimBr	.08	.11	1	0	0	0	0	1	0
9	Garcia e	bmimBF4	.04	.06	0	0	1	0	0	1	0
10	Bertheli	bmimBF4	.06	.09	0	0	1	0	0	1	0
11	Bernot e	bmimBF4	.06	.09	0	0	1	0	0	1	0
12	Garcia e	bmimPF6	.02	.03	0	0	0	1	0	1	0
13	Bertheli	bmimPF6	.03	.05	0	0	0	1	0	1	0
14	Bernot e	bmimPF6	.03	.05	0	0	0	1	0	1	0
15	Wells an	bmimPF6	.02	.03	0	0	0	1	0	1	0
16	Pretti e	bmimTf2N	.07	.09	0	0	0	0	1	1	0

Figure 3.2 Keying in data.

Step 4.3: Select Analyze – Regression – Linear.

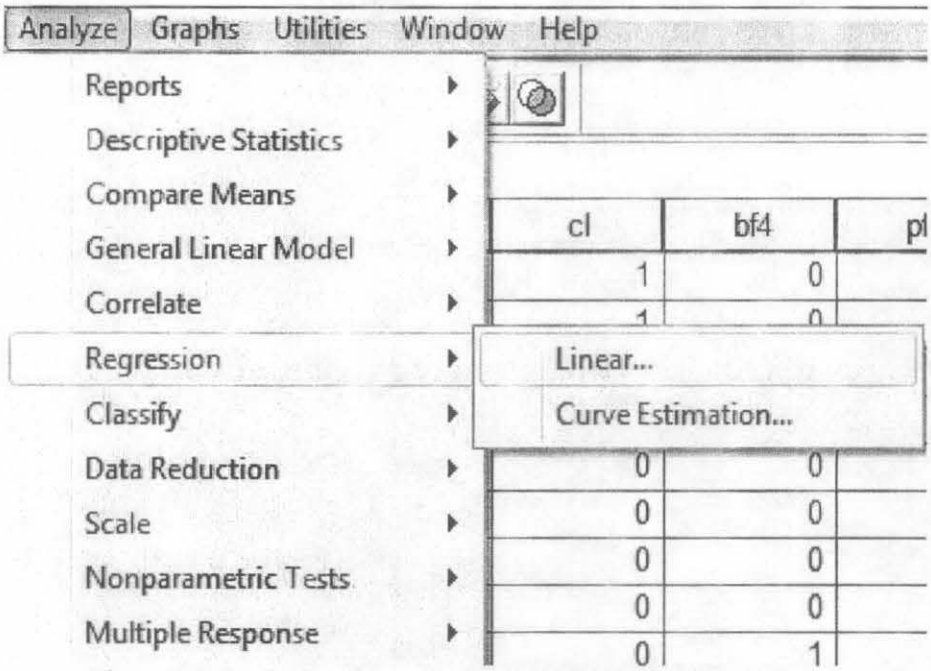


Figure 3.3 Analyzing data using multilinear regression analysis.

Step 4.4: Choose dependent and independent variables. The software will form matrix equation (refer to Equation 3.3)

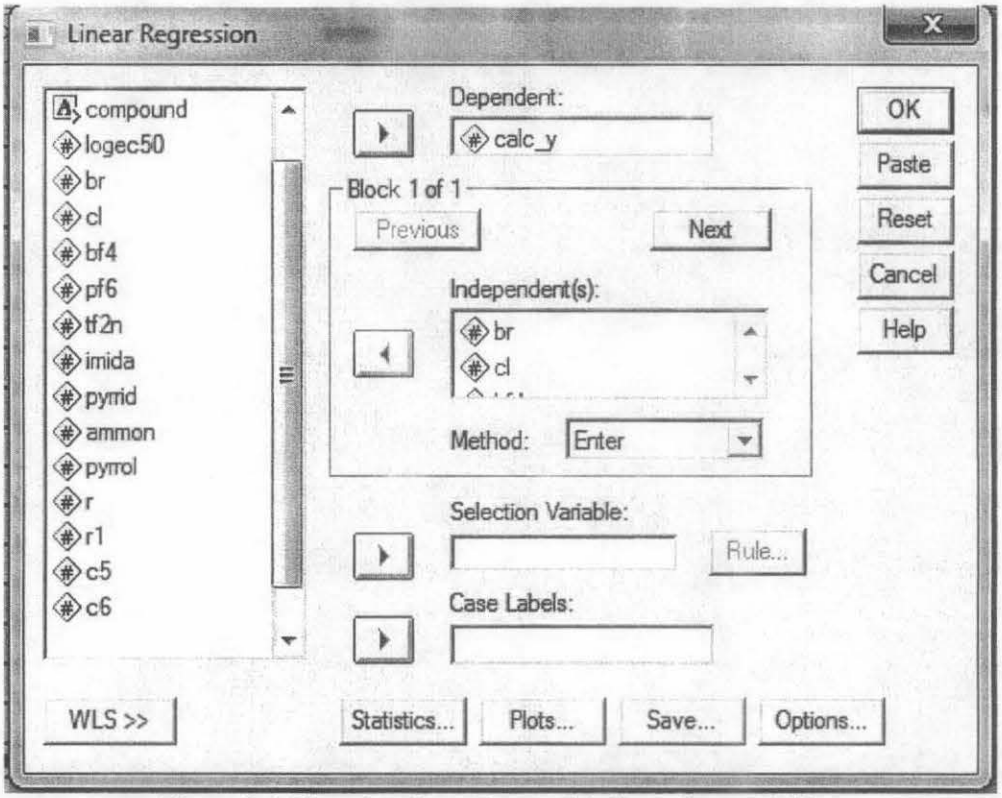


Figure 3.4 Choosing dependent and independent variable.

Step 4.5: Select 'Statistic' and choose coefficient that wanted to be shown.

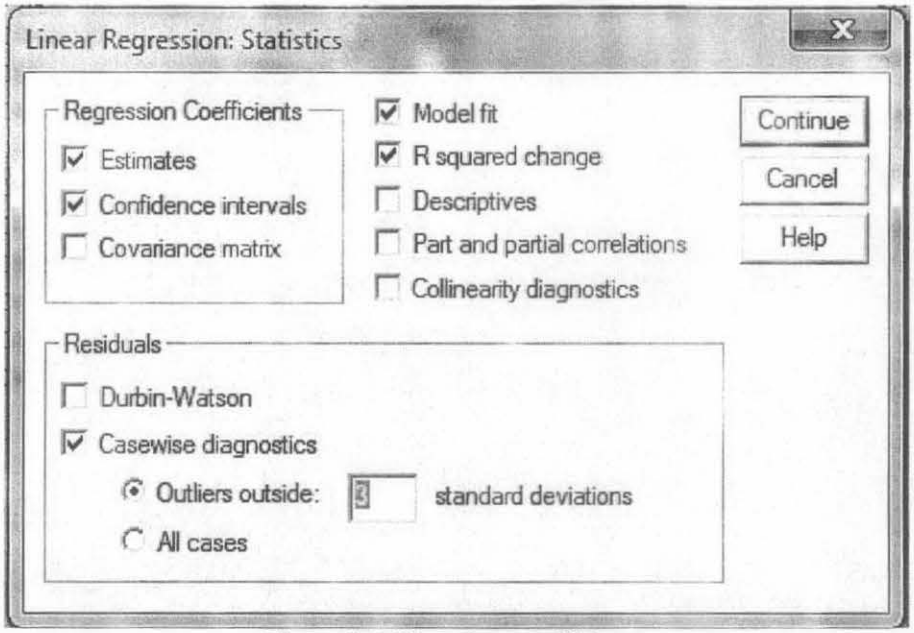


Figure 3.5 Choosing coefficient.

Step 4.6: Select 'Plot' and choose the axis of graph to be plotted. For this research, the graph of Y^* vs predicted value (adjusted) is chose.

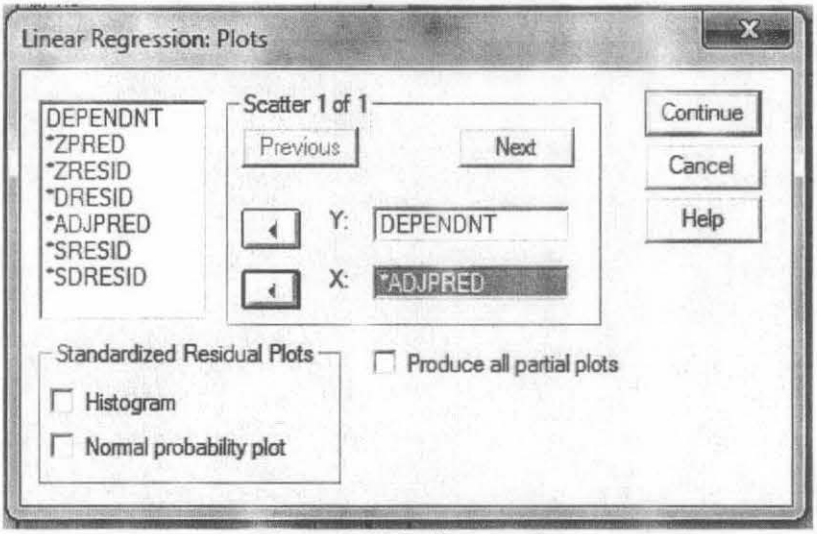


Figure 3.6 Plotting graph.

Step 4.7: Select **'Option'** and unselect constant because the graph of toxicity should be go through origin.

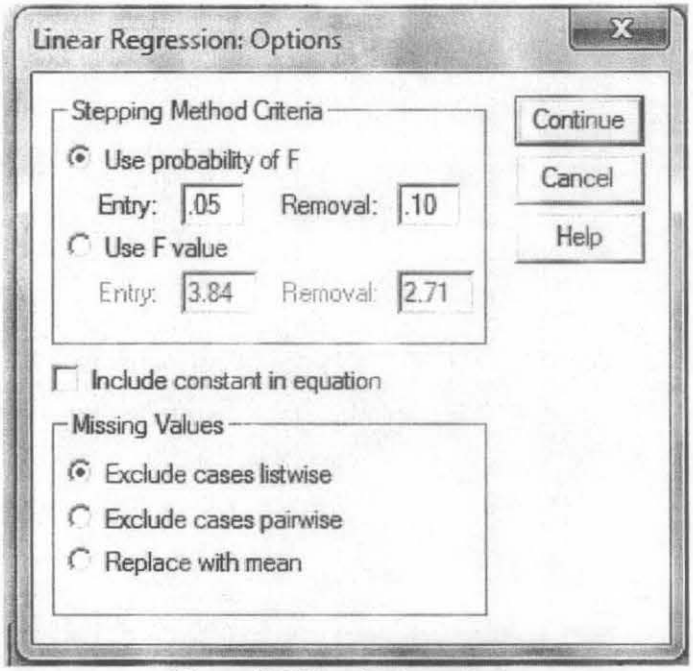


Figure 3.7 Unselect constant.

Step 4.8: Click 'OK'. The result will appear.

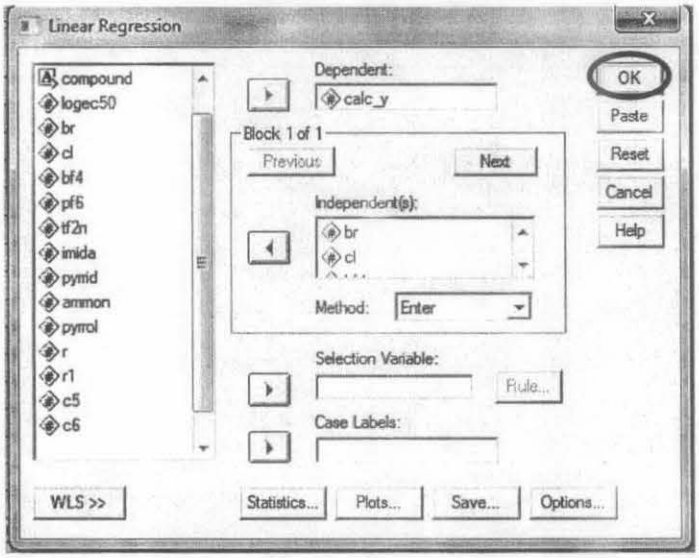


Figure 3.8 Click OK.

The summary of the methodology is as Figure 3.9.

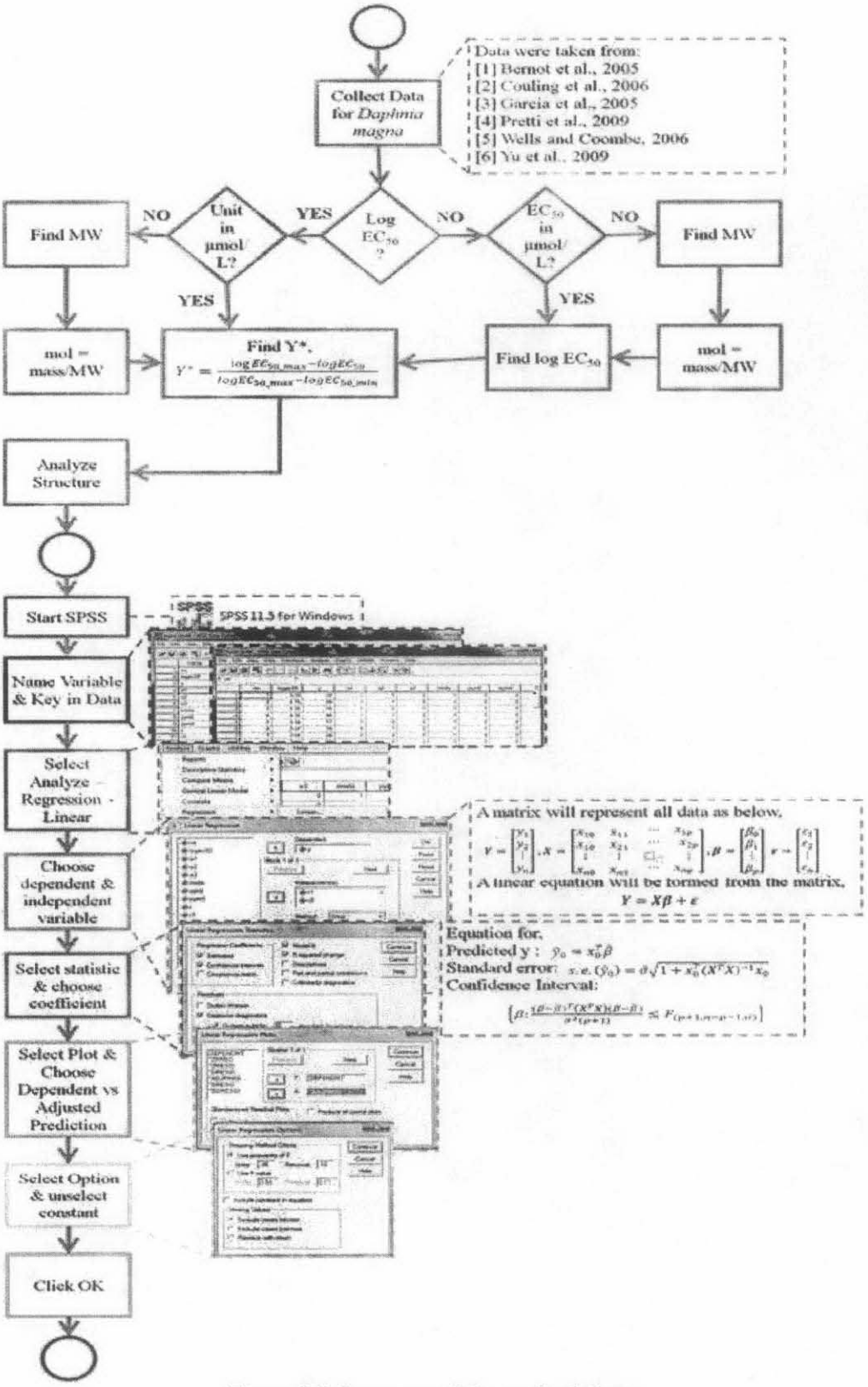


Figure 3.9 Summary of the methodology.

CHAPTER 4

RESULTS AND DISCUSSION

Log EC_{50} values for the ionic liquids included in this study are shown in Table 4.1. According to group contribution methods, properties of a molecule can be assumed to be the summation of the contributions of its atom and/or fragments. In this work, the structure of ionic liquids has been based on three main contributions: anions (A), cations (C) and substitutions (S). Anions were Br^-, Cl^-, BF_4^-, PF_6^- and Tf_2N^-. Since BF_4^- and PF_6^- have similar contribution values, they are grouped. Cations were imidazolium, pyridinium, ammonium, pyrrolidinium, dimetylamino pyridinium and piperinido pryridinium (Imida, Pyrid, Ammon, Pyrrol, C_5 and C_6 respectively). Substitutions were R which is long n-alkane chain and R_1 which is an additional short chain (methyl).

The data set has been fitted to the QSAR model by multilinear regression analysis using SPSS. The contributions (a_i, c_j, and s_k) of each group are shown in Table 4.2 according to equation [2] and a good fitting was achieved, with $n = 44$, $r^2 = 0.934$, $r_{adj}^2 = 0.910$ and standard error of estimate = 0.149.

A comparison between the calculated data (from log EC_{50}) for the dimensionless toxicity Y^* of ionic liquids and the prediction based on the novel estimation method was performed. Figure 4.1 shows the parity plot.

From the results, it can be assumed that imidazolium, pyridinium, ammonium, pyrrolidinium, dimethilamino pryridinium and piperidino pyridinium groups contribute about 20% decrease, 5% increase, 86% decrease, 31% decrease, 24% increase and 40% increase to the toxicity, respectively. In addition, contributions of anions and cations to the toxicity are negative, which means that the presence of anions leads to a decrease in the toxic effect of cation. The anion group Cl^- and PF_6^- show a negligible difference between their contributions. For each carbon atom added to R chains, an increase in toxicity of about 7% is produced. An additional methyl group in the molecule decreases the toxicity by about 14%. Figure 4.2 shows a schematic way to infer the least and most toxic ionic liquids.

Table 4.1: Data for Ionic liquid toxicities in μmol/L (log EC_{50}), dimensionless toxicity (Y^*), and group contribution descriptor.

No	Compound	Log EC50 (μmol/L)	Y	Br^-	Cl^-	BF_4^-	PF_6^-	TF_2N^-	*Imida*	*Pyrid*	*Ammon*	*Pyrrol*	C_5	C_6	R	R_1
1	bmimCl	1.85	0.05	0	1	0	0	0	1	0	0	0	0	0	4	1
2	bmimCl	1.57	0.11	0	1	0	0	0	1	0	0	0	0	0	4	1
3	bmimCl	1.93	0.03	0	1	0	0	0	1	0	0	0	0	0	4	1
4	bmimCl	1.93	0.03	0	1	0	0	0	1	0	0	0	0	0	4	1
5	bmimBr	1.84	0.05	1	0	0	0	0	1	0	0	0	0	0	4	1
6	bmimBr	1.78	0.07	1	0	0	0	0	1	0	0	0	0	0	4	1
7	bmimBr	1.56	0.11	1	0	0	0	0	1	0	0	0	0	0	4	1
8	bmimBr	1.56	0.11	1	0	0	0	0	1	0	0	0	0	0	4	1
9	bmimBF4	1.79	0.06	0	0	1	0	0	1	0	0	0	0	0	4	1
10	bmimBF4	1.67	0.09	0	0	1	0	0	1	0	0	0	0	0	4	1
11	bmimBF4	1.67	0.09	0	0	1	0	0	1	0	0	0	0	0	4	1
12	bmimPF6	1.95	0.03	0	0	0	1	0	1	0	0	0	0	0	4	1
13	bmimPF6	1.85	0.05	0	0	0	1	0	1	0	0	0	0	0	4	1
14	bmimPF6	1.85	0.05	0	0	0	1	0	1	0	0	0	0	0	4	1
15	bmimPF6	1.93	0.03	0	0	0	1	0	1	0	0	0	0	0	4	1
16	bmimTf2N	1.65	0.09	0	0	0	0	1	1	0	0	0	0	0	4	1
17	hmimCl	1.09	0.22	0	1	0	0	0	1	0	0	0	0	0	6	1
18	hmimBr	1.06	0.23	1	0	0	0	0	1	0	0	0	0	0	6	1
19	hmimBF4	1.13	0.21	0	0	1	0	0	1	0	0	0	0	0	6	1
20	omimCl	0.54	0.35	0	1	0	0	0	1	0	0	0	0	0	8	1
21	omimBr	0.54	0.35	1	0	0	0	0	1	0	0	0	0	0	8	1
22	omimBF4	0.66	0.32	0	0	1	0	0	1	0	0	0	0	0	8	1

23	C_{10}mimBr	-0.31	0.54	1	0	0	0	0	1	0	0	0	0	0	10	1
24	C_{12}mimCl	-1.82	0.88	0	1	0	0	0	1	0	0	0	0	0	12	1
25	C_{12}mimbR	-0.82	0.66	1	0	0	0	0	1	0	0	0	0	0	12	1
26	C_{18}mimCl	-2.00	0.92	0	1	0	0	0	1	0	0	0	0	0	16	1
27	C_{18}mimCl	-2.34	1.00	0	1	0	0	0	1	0	0	0	0	0	18	1
28	bpyCl	2.07	0.00	0	1	0	0	0	0	1	0	0	0	0	4	0
29	bpyTf_2N	0.62	0.33	0	0	0	0	1	0	1	0	0	0	0	4	0
30	bmpyr Tf_2N	1.94	0.03	0	0	0	0	1	0	0	0	1	0	0	4	1
31	$C_{12}C_3$NBr	0.09	0.45	1	0	0	0	0	0	0	1	0	0	0	12	3
32	$C_{14}C_3$NBr	-0.38	0.56	1	0	0	0	0	0	0	1	0	0	0	14	3
33	$C_{16}C_3$NBr	-0.45	0.57	1	0	0	0	0	0	0	1	0	0	0	16	3
34	ompyBr	-2.6	0.73	1	0	0	0	0	0	1	0	0	0	0	8	1
35	hmpyBr	-2.41	0.70	1	0	0	0	0	0	1	0	0	0	0	6	1
36	bmpyBr	-1.24	0.52	1	0	0	0	0	0	1	0	0	0	0	4	1
37	omimBr	-4.33	1.00	1	0	0	0	0	1	0	0	0	0	0	8	1
38	hmimBr	-2.22	0.67	1	0	0	0	0	1	0	0	0	0	0	6	1
39	bmmpyBr	-1.01	0.48	1	0	0	0	0	0	1	0	0	0	0	4	2
40	hpyBr	-1.93	0.63	1	0	0	0	0	0	1	0	0	0	0	6	0
41	hmmimBr	-2.19	0.67	1	0	0	0	0	1	0	0	0	0	0	6	2
42	hPiPyBr	-3.66	0.90	1	0	0	0	0	0	1	0	0	0	1	6	0
43	HDMAPyBr	-3.28	0.84	1	0	0	0	0	0	1	0	0	1	0	6	0
44	HMDMAPyBr	-2.79	0.76	1	0	0	0	0	0	1	0	0	1	0	6	1

Reference: 1, 6, 9, 12, 17, 19, 20, 22, 31, 32 and 33 from Garcia *et al.* (2005); 2, 15, 24, 26, 27 and 28 from Wells & Coombe (2006); 3, 7, 10 and 13 from Berthelin (1999); 4, 8, 11 and 14 from Bernot *et al.* (2005); 5, 18, 21, 23 and 25 from Yu *et al.* (2009); 16, 29 and 30 from Pretti *et al.* (2009); 34 to 44 from Couling *et al.* (2006).

Table 4.2: Group contribution to the dimensionless toxicity.

Group	Molecular descriptor	Comments	Contribution	95% Confidence Interval	
				Lower	Upper
Anion (A)	Br^-	Influence of Br^- anion. Value = 1 if it exists and 0 if not.		[a]	
	Cl^-	Influence of Cl^- anion. Value = 1 if it exists and 0 if not.	-0.171	-0.303	-0.039
	BF_4^-	Influence of BF_4^- anion. Value = 1 if it exists and 0 if not.	-0.152	-0.317	0.013
	PF_6^-	Influence of PF_6^- anion. Value = 1 if it exists and 0 if not.	-0.176	-0.358	0.006
	TF_2N^-	Influence of TF_2N^- anion. Value = 1 if it exists and 0 if not.	-0.075	-0.317	0.168
Cation (C)	*Imida*	Influence of Imidazolium cation. Value = 1 if it exists and 0 if not.	-0.197	-0.420	0.026
	Pyrrid	Influence of Pyrridinium cation. Value = 1 if it exists and 0 if not.	0.053	-0.138	0.244
	Ammon	Influence of Ammoinium cation. Value = 1 if it exists and 0 if not.	-0.862	-1.391	-0.334
	Pyrrol	Influence of Pyrrolidinium cation. Value = 1 if it exists and 0 if not.	-0.308	-0.758	0.141
	C_5	Influence of dimethylamino pyridinium. Value = 1 if it exists and 0 if not.	0.240	-0.010	0.491
	C_6	Influence of piperidino pyridinium. Value = 1 if it exists and 0 if not.	0.397	0.049	0.745
Substitution (S)	R	Influence of number of carbons in long chain (R:1 to 18)	0.075	0.059	0.091

	R_1	Influence of additional short chain in the molecule (R_1:1 to 3)	0.113	-0.034	0.260

a By using SPPS, Bromide is excluded variable which means more data that have different anions (rather than Br) should be add.

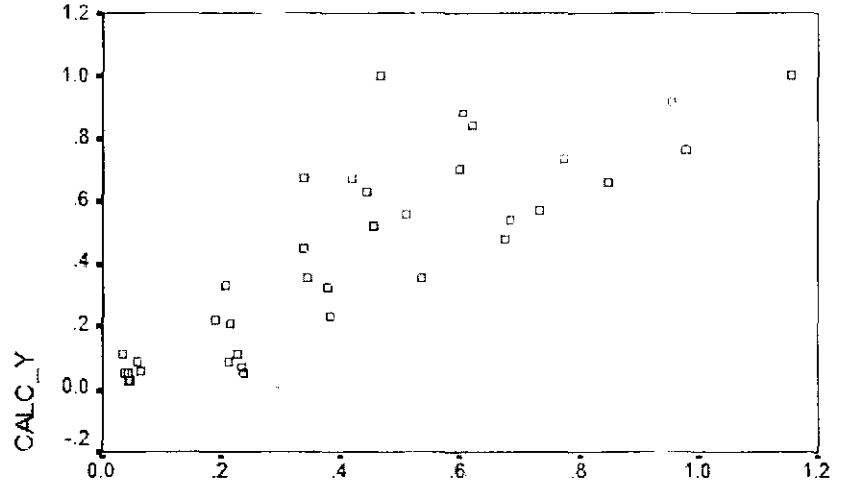


Figure 4.1: Calculated data (from log EC_{50}) for Y^* using Equation [1] and predicted Y^* using SPSS.

	- ⟶ +
Anions	PF_6 < Cl < BF_4 < TF_2N
Cations	Ammonium<Pyrrolidium<Imidazolium< Pyridinium<Dimethylamino Pyridinium<Piperidino Pyridinium
Substitutions	R < R_1

Figure 4.2: Ecotoxicity order of anions, cations and substitutions.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

A novel group contribution method, QSAR, has been developed for ionic liquids to estimate the EC_{50} for *Daphnia magna*. The method is based on the prediction of dimensionless toxicity, *Y**, by the summation of the group contributions: anion, cation and substitution. 44 data for log EC_{50} of ionic liquids was assembled. The data range for log EC_{50} values between 2.07 and -4.33, which is the experimental range of ecotoxicity covered by literature results. The result were well correlated ($r^2 = 0.934$, $r^2 = 0.910$ and variance = 0.022). From the results, it can be concluded that the toxicity contribution in increasing order for anions is hexafluorophosphate (PF_6^-) < chloride (Cl^-) < tetrafluoroborate (BF_4^-) < bis((trifluoromethyl)sulfonyl)imide (TF_2N^-), for cations is ammonium (N) < pyrrolidinium (pyr) < imidazolium (im) < pyridinium (py) < dimethylamino pyridinium (DMApy) < piperidino pyridinium (pipy), and while for alkyl is R < R_1 with R is long *n*-alkane chain and R_1 is an additional short chain (methyl). In general, anion will decrease the toxicity of ionic liquids, while cation and substitution will increase the toxicity of ionic liquids. The contributions to the QSAR allow one to estimate the influence of each group has on the EC_{50}.

5.2 RECOMMENDATION

Based on steps for QSAR, experiment of other untested ionic liquids should be done on *Daphnia magna* to ensure validity of model. Besides that, more data should be gathered to get a more accurate model. Gantt chart for the experiment and modeling should also be renewed to ensure that time is optimized.

REFERENCES

Asiah N.M., Ismail M.H. and Mohanad E.H., (2010), "Modelling and Simulation of Ecotoxic Properties for Ionic Liquids Using QSAR," *UTP Research Paper Conference 2010, 22 October 2010.*

Anthony, J.L., Maginn, E.J., Brennecke, J.F., (2001), "Solution thermodynamics of imidazolium-based ionic liquids and water." *J. Phys. Chem. B 105, 10942–10949.*

Atkins, M.P., Davey, P., Fitzwater, G., Rouher, O., Seddon, K.R. and Swindall, J., (2004), *Ionic Liquids: A Map for Industrial Innovation,* Report Q001, QUILL, Belfast.

Babalola, G.O., (1998), "Anti-bacterial activity of synthetic N-heterocyclic oxidizing compounds," *Lett. Appl. Microbiol. 26, 43–46.*

Bernot, R.J., Brueseke, M.A., Evans-White, M.A., and Lamberti, G.A., (2005), "Acute and Chronic toxicity of Imidazolium Based Ionic Liquids on *Daphnia magna*," *Environmental Toxicology and Chemistry, vol. 24(1), pp. 87–92.*

Bernot, R.J., Kennedy, E.E., Lamberti, G.A., (2005), "Effects of ionic liquids on the survival, movement, and feeding behavior of the fresh water snail *Physa acuta*," *Environmental Toxicology and Chemistry, vol. 24, pp. 1759–1765.*

Canton, J.H., Adema, D.M.M., (1978), "Reproducibility of short-term and reproduction toxicity experiments with *Daphnia magna* and comparison of the sensitivity of *Daphnia magna* with *Daphnia pulex* and *Daphnia cucullata* in short-term experiments," *Hydrobiologia, vol. 59, pp. 135–140.*

Chatterjee, S., Hadi, A.S., (1938), *Regression Analysis by Example*, United States of America, John Wiley & Sons

Chemistry Innovation KTN (2006), "Ionic Liquids".

Cho, C.W., You, C.J., Thi, P.T.P., Vijayaraghavan, K. and Yeoung, S.Y., (2007a), "The ecotoxicity of ionic liquids and traditional organic solvents on microalga *Selenastrum capricornutum,*" *Ecotoxicology and Environmental Safety 71 (2008) 166-171.*

Cho, C.W., Thi, P.T.P., You, C.J., Vijayaraghavan, K., Woo, S.C. and Yeoung, S.Y., (2007b), "Toxicity of imidazolium salt with anion bromide to a phytoplankton *Selenastrum capricornutum*: Effect of alkyl chain length." *Chemosphere 69 (2007) 1003-1007.*

Cho, C.W., Pham, T.P.T., Jeon, Y.C., Vijayaraghavan, K., Choe, W.S., Yun, Y.S., (2007). "Toxicity of imidazoliumsalt with anionbromide to a phytoplankton Selenastrum capricornutum: effect of alkylchain length," *Chemosphere 69, 1003–1007.*

Cho, C.W., Pham, T.P.T., Jeon, Y.C., Yun, Y.S., (2008a), "Influence of anions on the toxic effects of ionic liquids to a phytoplankton Selenastrum capricornutum," *Green Chem. 10, 67–72.*

Cho, C.-W., Jeon, Y.-C., Pham, T.P.T., Vijayaraghavan, K., Yun, Y.S., (2008b), "The ecotoxicity of ionic liquids and traditional organic solvents on microalga Selenastrum capricornutum," *Ecotoxicol. Environ. Saf. 71, 166–171.*

Cho, C.W., Pham, T.P.T., Jeon, Y.C., Min, J., Jung, H.Y., Lee, D.S., Yun, Y.S., (2008c), "Microalgal photosynthetic activity measurement system for rapid toxicity assessment," *Ecotoxicology 17, 455–463.*

Cieniecka-Roslonkiewicz, A., Pernak, J., Kubis-Feder, J., Ramani, A., Robertson, A.J., Seddon, K.R., (2005), "Synthesis, anti-microbial activities and anti-electrostatic properties of phosphonium based ionic liquids," *Green Chem. 7, 855–862.*

Couling, D.J., Bernot, R.J., Docherty, K.M., Dixon, J.K., Maginn, E.J., (2006), "Assessing the factors responsible for ionic liquid toxicity to aquatic organisms

via quantitative structure–property relationship modeling," *Green Chemistry, vol. 8, pp. 82–90.*

Cowgill, U.M., Milazzo, D.P., (1991), "The sensitivity of *Ceriodaphnia dubia* and *Daphnia magna* to seven chemicals utilizing the three-brood test," *Archives of Environmental Contamination and Toxicology, vol. 20, pp. 211–217.*

Docherty, K.M., Kulpa, C.F. (2005), "Toxicity and antimicrobial activity of imidazolium and pyridinium ionic liquids," *Green Chemistry, vol. 7, pp. 185–189, 2005.*

Docherty, K.M., Dixon, J.K., Kulpa, C.F., (2007), "Biodegradability of imidazolium and pyridinium ionic liquids by an activated sludge microbial community," *Biodegradation 18, 481–493.*

Fitzwater, G., Geissler, W., Moulton, R., Plechkova, N.V., Robertson, A., Seddon, K.R., Swindall, J. and Wan Joo, K., (2005). *Ionic Liquids: Source of Innovation,* Report Q002, QUILL, Belfast.

Fuller, J., Carlin, R.T. and Osteryoung, R.A., (1997), "The room temperature ionic liquids 1-ethyl-3-methylimidazolium tetrafluoroborate: electrochemical couples and physical properties," *J. Electrochem. Soc. 144 (11), 3881-3886.*

Garcia, M.T., Gathergood, N., and Scammells, P.J., (2005),"Biodegradable ionic liquids Part II. Effect of the anion and toxicology," *Green Chemistry, vol. 7, pp. 9–14.*

Grabinska-Sota, E., Kalka, J., (2006), "Toxicity ofimidazoliumchlorides to aquatic organisms," *Polish J. Environ. Stud. 15, 405–409.*

Guilhermino, L., Diamantino, T., Silva, M.C., Soares, A.M.V.M., (2000), "Acute toxicity test with *Daphnia magna*: An alternative to mammals in the prescreening of chemical toxicity?," *Ecotoxicology and Environmental Safety, vol. 46, pp. 357–362.*

Ioana S., (2009), "Bioavailability and biological properties of several selected ionic liquids in Testsystem AquaHab®."

Jastorff, B., Mo¨lter, K., Behrend, P., Bottin-Weber, U., Filser, J., Heimers, A., Ondruschka, B., Ranke, J., Schaefer, M., Schro¨der, H., Stark, A., Stepnowski, P., Stock, F., Sto¨rmann, R., Stolte, S., Welz-Biermann, U., Ziegert, S., Tho¨ming, J., (2005), "Progress in evaluation of risk potential of ionic liquids-basis for an eco-design of sustainable products," *Green Chem. 7, 362–372.*

Kaniewska-Prus, M., (1982), "The effect of ammonia, chlorine, and chloramines toxicity on the mortality of *Daphnia magna* Straus," *Polish Archives of Hydrobiology, vol. 29, pp. 607-624.*

Keith, E.J., (2007), "What's an Ionic Liquids" in *The electrochemical Society Interface,* Canada.

Kelman, D., Kashman, Y., Rosenberg, E., Ilan, M., Ifrach, I., Loya, Y., (2001), "Antimicrobial activity of the reef sponge Amphimedon viridis from the Red Sea: evidence for selective toxicity," *Aquat. Microb. Ecol 24, 9–16.*

Kulacki, K.J., Lamberti, G.A., (2008), "Toxicity of imidazolium ionic liquids to freshwater algae," *Green Chem. 10, 104–110.*

Kumar, R.A., Papai¨conomou, N., Lee, J.-M., Salminen, J., Clark, D.S., Prausnitz, J.M., (2009), "In vitro cytotoxicities of ionic liquids: effect of cation rings, functional groups, and anions," *Environ. Toxicol. 24, 388–395.*

Larson, J.H., Frost, P.C., Lamberti, G.A., (2008), "Variable toxicity of ionic liquid-forming chemicals to Lemna minor and the influence of dissolved organic matter," *Environ. Toxicol. Chem. 27, 676–681.*

Latala, A., Stepnowski, P., Nedzi, M., Mrozik, W., (2005), "Marine toxicity assessment of imidazolium ionic liquids: acute effects on the Baltic algae *Oocystis*

submarina and *Cyclotella meneghiniana*," *Aquatic Toxicology, vol. 73, pp. 91–98, 2005.*

Li, G., Shen, J., Zhu, Y., (1998), "Study of pyridinium-type functional polymers. II. Antibacterial activity of soluble pyridinium-type polymers," *J. Appl. Polym. Sci. 67, 1761–1768.*

LeBlanc, G.A., (1980), "Acute toxicity of priority pollutants to water flea (*Daphnia magna*)," *Bulletin of Environmental Contamination and Toxicology, vol. 24, pp. 684-691.*

Lozano, P., Garcia-Verdugo, E., Piamtongkam, R., Montague, K., Karbass, N., De Diego, T., Burguete, M.I., Luis, S.V. and Iborra, J.L., (2007), "Biocatalysis in supported ionic liquids," *2nd International Congress on Ionic Liquids*, Yokohama, Japan.

Luis, P., Ortiz, I., Aldaco, R., Irabien, A., (2006), "A novel group contribution method in the development of a QSAR for predicting the toxicity (*Vibrio fischeri* EC_{50}) of ionic liquids," *Ecotoxicology and Environmental Safety, vol. 67, pp. 423–429.*

Luis, P., Garea, A., and Irabien, A., (2010), "Quantitative structure –activity relationships (QSARs) to estimate ionic liquids ecotoxicity EC_{50} (*Vibrio fischeri*)," *Journal of Molecular Liquids 152 (2010) 28-33.*

Luo, Y.R., Li, X.Y., Chen, X.X., Zhang, B.J., Sun, Z.J., Wang, J.J., (2008), "The developmental toxicity of 1-butyl-3-octylimidazolium bromide on Daphnia magna," *Environ. Toxicol 23, 736–744.*

Macfarlane, D.R., Dean, P.M., Turanjanin, J., Scott, J.L., Yoshizawa-Fujita, M., Hough, W.L., Smiglak, M., Rodriguez, H., Swatloski, R.P., Spear, S.K., Daly, D.T., Pernak, J., Grisel, J.E., Carliss, R.D., Soutullo, M.D., Davis, J.H. and Rogers, R.D. (2007), "Drug ionic liquids – A new phase for the pharmaceutical world," *2nd International Congress on Ionic Liquids*, Yokohama, Japan.

Matzke, M., Stolte, S., Thiele, K., Juffernholz, T., Arning, J., Ranke, J., Welz-Biermann, U., Jastorff, B., (2007), "The influence of anion species on the toxicity of 1 alkyl-3-methylimidazolium ionic liquids observed in an (eco)toxicological test battery," *Green Chem. 9, 1198–1207.*

Matzke, M., Stolte, S., Bo¨ schen, A., Filser, J., (2008), "Mixture effects and predictability of combination effects of imidazolium based ionic liquids as well as imidazolium based ionic liquids and cadmium on terrestrial plants (*Triticum aestivum*) and limnic green algae (*Scenedesmus vacuolatus*)," *Green Chem. 10, 784–792.*

McFarlane, J., Ridenour, W.B., Luo, H., Hunt, R.D., Depaoli, D.W., Ren, R.X., (2005), "Room temperature ionic liquids for separating organics from produced water," *Sep. Sci. Technol 40, 1245–1265.*

McQueen, D.J., Post, J.R., Mills, E.L., (1986), "Trophic relationships in freshwater pelagic ecosystems," *Can. J. Fish. Aqua. Sci. 43, 1571–1581.*

Mount, D.I., Norberg, T.J., (1984), "A seven-day life cycle cladoceran toxicity test," *Environmental Toxicology and Chemistry, vol. 3, pp. 425-434, 1984*

Nockemann, P., Thijs, B., Driesen, K., Janssen, C.R., Hecke, K.V., Meervelt, L.V., Kossmann, S., Kirchner, B., and Binnemans, K., (2007), "Choline Saccharinate and Choline Acesulfamate: Ionic Liquids with Low Toxicities," *Journal of Physical Chemistry B, vol. 111, pp. 5254-5263.*

Pernak, J., Rogo_ za, J., Mirska, I., (2001a), "Synthesis and antimicrobial activities of new pyridinium and benzimidazolium chlorides," *Eur. J. Med. Chem. 36, 313–320.*

Pernak, J., Kalewska, J., Ksycin´ ska, H., Cybulski, J., (2001b), "Synthesis and anti-microbial activities of some pyridinium salts with alkoxymethyl hydrophobic group." *Eur. J. Med. Chem. 36, 899–907.*

Pernak, J.,Chwala, P., (2003a), "Synthesis and anti-microbial activities of choline-likequaternaryammoniumchlorides," *Eur. J.Med.Chem. 38, 1035–1042.*

Pernak, J., Sobaszkiewicz, K., Mirska, I., (2003b), "Anti-microbial activities of ionic liquids," *Green Chem. 5, 52–56.*

Pernak, J., Goc. I., Mirska, I., (2004a), "Anti-microbial activities of protic ionic liquids with lactate anion," *Green Chem. 6, 323–329.*

Pham, T.P.T., Cho, C.W., Vijayaraghavan, K., Min, J., Yun, Y.S., (2008a), "Effect of imidazolium-based ionic liquids on the photosynthetic activity and growth rate of *Selenastrum capricornutum*," *Environ. Toxicol. Chem. 27, 1583–1589.*

Pham, T.P.T., Cho, C.W., Min, J., Yun, Y.S., (2008b), "Alkyl-chain length effects of imidazolium and pyridinium ionic liquids on photosynthetic response of *Pseudokirchneriella subcapitata*," *J. Biosci. Bioeng 105, 425–428.*

Pham, T.P.T, Cho, C.W., and Yun, Y.S, (2010), "Environmental fate and toxicity of ionic liquids: A review," *Water Research 44 (2010) 352-372*

Pitner, W.R., Colnot, T. and Welz-Biermann, U., (2007), "Toxicological Screening and Assessment of Ionic Liquids," *2nd International Congress on Ionic Liquids*, Yokohama, Japan.

Pretti, C., Chiappe, C., Pieraccini, D., Gregori, M., Abramo, F., Monni, G., Intorre, L., (2006), "Acute toxicity of ionic liquids to the zebra fish (*Danio rerio*)," *Green Chemistry, vol. 8, pp. 238–240.*

Pretti, C., Chiappe, C., Baldetti, I., Brunini, S., Monni, G., Intorre, L., (2009), "Acute toxicity of ionic liquids for three fresh water organisms: *Pseudokirchneriella subcapitata*, *Daphnia magna* and *Danio rerio*," *Ecotoxicology and Environmental Safety, 72, 1170–1176.*

Pretti, C., Cinzia, C., Ilaria, B., Sara, B., Gianfranca, M. and Luigi, I., (2009), "Acute Toxicity of ionic liquids for three freshwater organism: *Pseudokirchneriella subcapitata, Daphnia magna and Danio rerio,*" *Ecotoxicology and Environmental Safety 72 (2009) 1170-1176.*

Ranke, J., "Joint bioaccumulation and joint biological activity as ecotoxicological risk indicators," *UFT Centre for Environmental Research and Environmental Technology,* University of Bremen, Germany.

Ranke, J., Moelter, K., Stock, F., Bottin-Weber, U., Poczobutt, J., Hoffmann, J., Ondruschka, B., Filser, J., Jastorff, B., (2004), "Biological effects of imidazolium ionic liquids with varying chain lengths in acute Vibrio fischeri and WST-1cell viability assays," *Ecotoxicology and Environmental Safety, vol. 58, pp. 396–404.*

Ranke, J.,Mu¨ ller, A., Bottin-Weber, U., Stock, F., Stolte, S., Arning, J., Sto¨rmann, R., Jastorff, B., (2007a), "Lipophilicity parameters for ionic liquid cations and their correlation to in vitro cytotoxicity," *Ecotox. Environ. Saf 67, 430–438.*

Romero, A., Santos, A., Tojo, J. and Rodriguez, A., (2008), "Toxicity and biodegradability of imidazolium ionic liquids," *Journal of Hazardous Materials 151 (2008) 268-273.*

Samorì, C., Pasteris, A., Galletti, P., Tagliavini, E., (2007), "Acute toxicity of oxygenated and nonoxygenated imidazoliumbased ionic liquids to Daphnia magna and Vibrio fischeri," *Environ. Toxicol. Chem. 26, 2379–2382.*

Sewell, (2008), "Reduction in the numbers of fish used in aquatic acute toxicity testing." *Poster presentation at SETAC Europe 18th Annual Meeting,* abstract no MO 253.

Stasiewicz, M., Mulkiewicz, E., Tomczak-Wandzel, R., Kurmirska, J., Siedlecka, E.M., Golebiowski, M., Gajdus, J., Czerwicka, M., Stepnowski, P., (2008) "Assessing toxicity and biodegradation of novel, environmentally benign ionic liquids (1-alkoxymethyl-3-hydroxypyridinium chloride, saccharine and

acesulfamates) on cellular and molecular level," *Ecotox. Environ. Saf 71, 157–165.*

Stefan, S., Jurgen, A., Ulrike, B.W., Marianne, M., Franke, S., Karen, T., Marc, U., Urs, W.B., Bernd, J. and Johannes, R., (2006). "*Anion effect on the cytotoxicity of ionic liquids.*" <www.rsc.org/greenchem>.

Stefan, S., Marianne, M., Jurgen, A., Andrea, B., William, R.P., Urs, W.B., Bernd, J. and Johannes, R., (2007). "*Effects of different head groups and functionalized side chains on the aquatic toxicity of ionic liquids.*" <www.rsc.org/greenchem>.

Stock, F., Hoffmann, J., Ranke, J., Stormann, R., Ondruschka, B. and Jastorff, B., (2004), "Effects of ionic liquids on the acetylcholinesterase – a structure-activity relationship consideration," *The Royal Society of Chemistry 2004, Green Chem.*, 2004, 6, 286-290.

Stolte, S., Arning, J., Bottin-Weber, U., Matzke, M., Stock, F., Thiele,K., Uerdingen, M., Welz-Biermann, U., Jastorff, B., Ranke, J., (2006), "Anion effects on the cytotoxicity of ionic liquids," *Green Chem. 8, 621–629.*

Stolte, S., Matzke, M., Arning, J., Bo¨ schen, A., Pitner, W.-R., Welz-Biermann, U., Jastorff, B., Ranke, J., (2007a), "Effects of different head groups and functionalized side chains on the aquatic toxicity of ionic liquids," *Green Chem. 9, 1170–1179.*

Stolte, S., Arning, J., Bottin-Weber, U., Mu¨ ller, A., Pitner, W.-R., Welz-Biermann, U., Jastorff, B., Ranke, J., (2007b), "Effects of different head groups and functionalized side chains on the cytotoxicity of ionic liquids," *Green* Chem. 9, 760–767.

Studzinska, S. and Buszewski, B., (2009), "Study of toxicity of imidazolium ionic liquids to watercress (*Lepidium sativum L.*)," *Anal Bioanal Chem. 393:983-990.*

Thomas, S., (2005), "A brief history of IOLITEC," *Ionic Liquids Technologies GmbH & Co. KG,* Germany.

Tong, Z., Huailin, Z., Hongjun, J., (1996), "Chronic toxicity of acrylonitrile and acetonitrile to *Daphnia magna* in 14-d and 21-d toxicity tests," *Bulletin of Environmental Contamination and Toxicology, vol. 57, pp. 655–659.*

Torrecilla, J.S., Garcı´a, J., Rojo, E., Rodrı´guez, R., (2009), "Estimation of toxicity of ionic liquids in leukemia rat cell line and acetylcholinesterase enzyme by principal component analysis, neural networks and multiple linear regressions," *J. Hazard. Mater 164, 182–194.*

Trohalaki, S., Pachter, R., Drake, G.W. and Hawkins, T, (2004), "Quantitative Structure-Property relationship for melting points and densities of ionic liquids," OMB No. 0704-0188.

Ue, M., (2007), "Application of ionic liquids to double-layer capacitors," *2nd International Congress on Ionic Liquids*, Yokohama, Japan.

Vega, M.P., Pizarro, R.A., (2000), "Oxidative stress and defence mechanisms of the fresh water cladoceran Daphnia longispina exposed to UV radiation," *Journal of Photochemistry and Photobiology B: Biology, vol. 54, pp. 121–125.*

Wells, A.S., and Coombe, V.T., (2006), "On the Freshwater Ecotoxicity and Biodegradation Properties of Some Common Ionic Liquids," *Organic Process Research & Development, vol. 10, pp. 794–798.*

Welton, T., (1999), "Room-temperature ionic liquids, solvents for synthesis and catalysis," *Chem. Rev. 99, 2071-2083.*

Wong, D.S.H., Chen, J.P., Chang, J.M., Chou, C.H., (2002), "Phase equilibria ofwater and ionic liquids [emim][PF6] and [bmim][PF6]," *Fluid Phase Equilibr 194–197, 1089–1095.*

Yanagida, S., Masaki, N. and Suzuki, K., (2007), "Unique role of ionic liquids in solid-state dye-sensitized solar cells," *2nd International Congress on Ionic Liquids*, Yokohama, Japan.

Yan, C., Han, M., Wan, H., and Guan, G, (2010), "QSAR correlation of the malting points for imidazolium bromides and imidazolium chlorides ionic liquids," *Fluid Phase Equilibria 292 (2010) 104-109*

Yu, M., Wang, S.H., Luo, Y.R., Han, Y.W., Li, X.Y., Zhang, B.J., Wang, J.J., (2009) "Effects of the 1-alkyl-3-methylimidazolium bromide ionic liquids on the antioxidant defense system of *Daphnia magna*," *Ecotoxicology and Environmental Safety, vol. 72, pp. 1798–1804.*

Zhang, S., Lu, X., Zhou, Q., Li, X., Zhang, X., Li, S., (2009), *Ionic Liquids Phyisicochemical Properties*, UK, Elsevier.

APPENDICES

Appendix I: Gantt Chart for Second Semester

No.	Detail/ Week	1	2	3	4	5	6	7		8	9	10	11	12	13	14
1	Project Work Continue	■	■	■												
	Studying Multilinear Regression		■	■	■											
	Studying SPSS					■										
	Run Example on SPSS	■	■	■	■	■										
2	Submission of Progress Report 1					/										
3	Project Work Continue					■	■	■								
	Gather Data from Literature Review						■									
	Analyze Data							■								
	Run Data on SPSS						■	■								
4	Submission of Progress Report 2								MID SEM BREAK	/						
5	Seminar (compulsory)										■	■	■			
5	Project work continue									■	■	■	■			
	Analyze Result Obtained									■	■	■	■			
6	Poster Exhibition											/				
7	Submission of Dissertation (soft bound)													/		
8	Oral Presentation														/	
9	Submission of Project Dissertation (Hard Bound)															/