A Novel QSPR Model for the Prediction of the Ionic Liquid Toxicities towards Green Algae (*Pseudokirchneriella Subcapitata*)

by

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Dissertation submitted in partial fulfillment of
The requirements for the
Bachelor of Engineering (Hons)
(Chemical Engineering)

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CERTIFICATION OF APPROVAL

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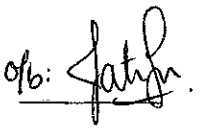
Approved by,



(Dr. Mohanad El-Harbawi)

UNIVERSITI TEKNOLOGI PETRONAS

TRONOH, PERAK

May 2012

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.

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NORA AZIELA BINTI ABDUL RAHMAN

ABSTRACT

Ionic Liquid (IL) are defined as the salt that melting point temperatures are lower than the boiling point of water. ILs can also be called as green solvent due to their undetectable vapor pressure hence can emit no volatile organic compounds. However, many ILs are found to be harmful and toxic to human and environment. Therefore, the toxicity studies of ILs become of great importance. The early study in determining toxicity of ILs was by using experimental works. However, ILs present in a very large combination of cations and anions that synthesized in the market and thousands are added every year which exceed the capacity of experimental works. Besides, experimental works are time consuming, require high cost and can kills aquatic organism. Therefore, Quantitative Structure Activity Relationship (QSAR) models are the best approach to overcome the above matter since QSAR approach is suitable to be used for huge number of chemical in a rational and effective manner. This study aims to develop a QSAR model to predict the toxicities of ILs towards one of green algae which is *Pseudokirchneriella subcapitata* (*P. subcapitata*), previously known as *Selenastrum capricornutum*. The dataset constructed by gathering 61 effective concentrations (EC_{50}) values of various ILs towards *P. subcapitata* from published literature before they being fragmented according to their cations, anions, and alkyl groups. The prediction model will then develop using QSAR approach employing multiple linear regressions (MLR) with polynomial model that will be coded using MATLAB software. To the best of our knowledge, there is no previous model developed based on combination of these two models towards *P. subcapitata*. The proposed model indicates that the model is capable of predicting the IL toxicities accurately, where $R^2 > 100\%$ with an average absolute deviation error 0%. The model has the potential to be used as an alternative to experimental measurement in the determination of EC_{50} values for a wide range of ILs towards *P. subcapitata*. In addition, it can be one of the important references for industrial people who deal with ILs.

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LIST OF ABBREVIATION

AAE – Average Absolute Error

AARD – Average Absolute Relative Deviation

AAD – Average Absolute Deviation

EC_{50} - Effective Concentration

$EC_{50\ cal}$ - Effective Concentration Calculated Values

$EC_{50\ exp}$ – Effective Concentration Experimental Values

EPA – Environmental Protection Agency

IL – Ionic Liquid

LC_{50} - Lethal Concentration

MLR – Multiple Linear Regression

MATLAB – Matrix Laborotary

OECD - Organization for Economic Co-operation and Development

R^2 - Correlation Coefficient

QSAR – Quantitative Structure Activity Relationship

QSPR – Quantitative Structure Property Relationship

CHAPTER 1

INTRODUCTION

1.1 Background of Study

ILs was first discovered back on 1914 by Paul Walden who first successfully prepared ethylammonium nitrate, [EtNH3][NO3], (m.p. 12 °C) by addition of concentrated nitric acid to the ethylamine. After about 100 years, when the first room temperature of ILs with a 1-alkyl-3-methylimidazolium cation was reported, IL began to achieve more attention of scientist (Wilkes et al., 1982). ILs are recently consider as popular solvent due to their liquidous range and non-volatile behavior. One of the essential advantages from IL structure is that, it has certain properties that vary as the structure of the ion are adjusted, making it become designed solvent. Thus, it can be used in a wide range of industry. Usually, the combination fit of cation's and anion's size, geometry and charge distribution determine the physical and chemical properties of ILs. Other ILs unique characteristics are they have high solubility in water, low combustibility, low volatility, non flammability, adjustable viscosity, and high heat capacity and conductivity. With attention to its unique characteristic, which is low volatility and non flammability, ILs is widely known as a green solvent and there are huge interests in their use as solvent for organic synthesis and green chemistry (Kimaru, 2011). Nowadays, since physical and chemical properties of ILs can be adjusted, the ILs usage has extended beyond as green solvent. Therefore, it will definitely be of high demand for its industrial purpose and it can potentially be a waste that needs to be discharge into drainage system (Martyn et al., 2000).

Theoretically there will be 10^{18} possible number of ILs as a result of combination of wide variety of cations and anions. However, the real numbers of ILs will be absolutely lower as compared to the theoretical number of ILs. Recently, there are 300 numbers of ILs commercially available and for about 1000 numbers of ILs are reported in the literature. Showing that huge number of ILs synthesized everyday. Since ILs will possibly become one of the waste that will flow into the

river, the measurement of the ecotoxicological assessment of ILs towards aquatic organism that exist in freshwater system become great important. In general, the ecotoxicological assessment of ILs measured by measuring the toxicity of ILs towards various aquatic organism such as marine bacterium *Vibrio fisheri* (Ranke et al., 2007; Docherty and Kulpa, 2010), algae (Cho et al., 2008a), the freshwater crustacean *Daphnia magna* (Bernot et al., 2005a) and the freshwater snail *Physa acuta* (Bernot et al., 2005b). Figure 1 shows the example of test species for aquatic ecosystem.

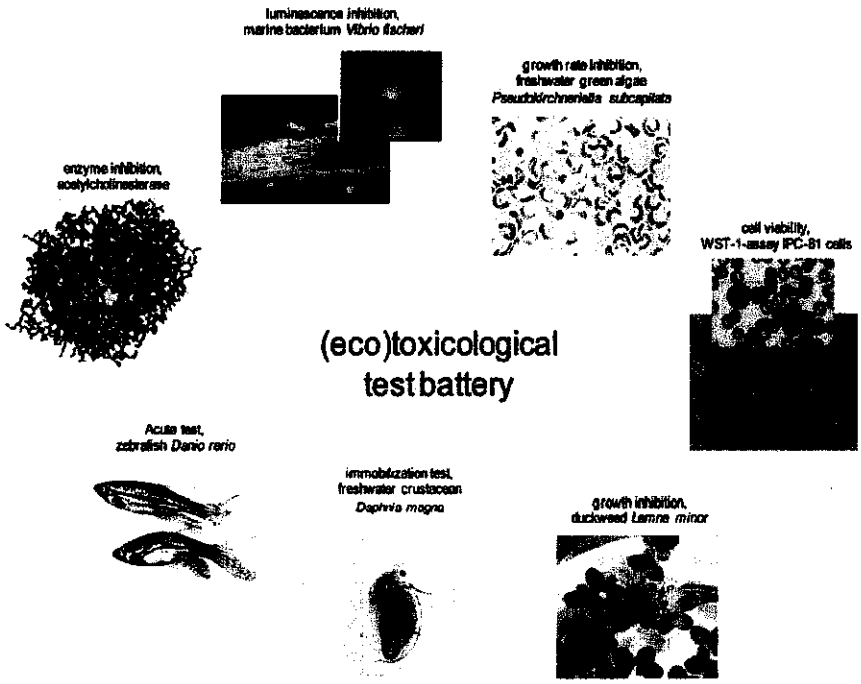


Figure 1. The ecotoxicological test battery considering aquatic and terrestrial compartment as well as different trophic levels including enzymes, luminescent marine bacteria, freshwater green algae, mammalian cells, duckweed, freshwater crustacean and zebrafish.

Aquatic toxicity is basically the measurement of the effect resulting from exposure to a toxic chemical towards aquatic organism. Aquatic organisms are the first organism effected by toxic chemicals that resulting from industrial discharge, agriculture and domestic activities. Since one of the special characteristic of ILs is high solubility in water, they may cause extensive effect on aquatic ecosystem. Therefore, it is important to study the effect of ILs toxicity towards aquatic organism.

Recently, numbers of studies have been conducted towards aquatic organism using experimental works such as Vibrio fisheri (Docherty and Kulpa Jr, 2005), Daphnia magna (Bernot et al., 2005a), algae (Latala et al., 2005), Escherichia coli (Lee et al., 2005) and etc.

As everyone knows, ILs present in a very large combination of cations and anions and due to its infinite numbers of ILs synthesized everyday, it is hard to conduct experimental work to study the effect of toxicity for every single ionic liquid available. Besides, experimental work is time consuming, require high cost and kills aquatic organism since it will be destroyed after the experiment end. Therefore, there is a need to provide faster and more cost-effective approach to determine the aquatic toxicity of ILs (Hossain et al., 2011).

First-principles calculations or structure-property correlation is the best approach as an alternative to experimental works in order to predict the toxicity of ILs (Wilkes, 2004). At the beginning, the first trial of these methods is generally not viable with the current theories. By the same token, the latter method, in the form of quantitative structure-activity [or property] relationships (QSARs or QSPRs), is relatively direct and has been utilize to the modeling and prediction of many physicochemical and biological properties. Above all, QSARs based on group contribution methods have been usually used to determine thermodynamic and other properties from summative contributions of molecular structures. Currently, properties evaluated have comprised boiling points, melting points, aqueous solubilities, toxicities and retention indices, the last with particular focus on medicinal drugs (Schultz et al., 2003; Cao et al., 2010).

In this work, QSAR model is used to estimate the toxicity of ILs towards one of the green algae that exist in freshwater system which is *Pseudokirchneriella subcapitata* (*P. subcapitata*), or previously known as *Selenastrum capricornutum*. QSAR model is the best approach as an alternative to experimental works since QSAR model suitable to be used for huge number of chemical in a rational and effective manner (Xu and Nirmalakhandan, 1998).

1.2 Problem Statement

ILs can be considered as green solvent and it can be used for different type of applications due to their undetectable vapor pressure characteristic. However, some of them are harmful to the environment especially to the aquatic organism. For this reason, it is important to study the toxicity of ILs towards environment especially to the aquatic organism. However, ILs toxicity data are either scarce and sometimes absent due to the large number of ILs synthesize every year. Therefore, it is desirable to have predictive models for calculating the ILs properties as alternative to the experimental measurement since experimental works is time consuming and require high cost. In this study, QSAR model is used to predict the toxicity of various ILs towards one of the green algae that exist in fresh water system which is *P. subcapitata*. By having this predictive model, the harmful effect of ILs towards environment can be minimized and it can be used as reference for industrial people who have to deal with discharging ILs into drainage system.

1.3 Objective of Study

The objectives of this study are:

1. To develop a novel QSAR model for estimating the toxicity of ILs towards *P. subcapitata* by using MATLAB software.

1.4 Scope of Study

Generally, toxicity study of ILs can be done using experimental works and mathematical modeling. Experimental works is time consuming and require high cost. Thus, in this study the main target is to develop a model that is capable to predict the toxicity of ILs on *P. subcapitata* using QSAR approach. The QSAR model employs a combination of MLR and polynomial method. In addition to that, it is also aims to study the effect of functional groups of ILs on toxic effect. *P. subcapitata*, one of the green algae is choose to be the study species since other

species such as *Vibrio fisheri* (marine bacterium), IPC-81 (leukemia rat cell line), *Daphnia magna* (freshwater crustacean), and *Acetylcholinesterase* (enzyme) have been already studied and different QSAR model have been developed by other author. From this model, the effect of toxicity of various ILs can be predicted and it can use as another alternative to experimental assessment.

1.5 Feasibility and Relevancy of Study

This study is relevant since IL is one of the focus fields of study in Chemical Engineering Department. Plus, this study is related to health, safety and environment field. The study does not involve any experimental work that time consuming and the MATLAB software is already available in Statistic Lab. Therefore, it is feasible to be finished within the time range.

CHAPTER 2

LITERATURE REVIEW

1.1 Introduction

In recent years, ILs that being composed by entirely ions, are new types of solvents that have generated a huge interest in many type of applications (Welton, 1999). The major properties of ILs are usually depending on the choice of base cation and anion. Thus, making ILs become design solvent that properties can be tuned by the variation of the length and branching of the alkyl groups incorporated into the cation (Pham et al., 2008b). ILs has numerous unique properties which provide a medium for various types of chemical processes.

Apart from that, one of ILs unique properties is the undetectable vapor pressure which is this decrease the risk of exposure and loss of solvent by evaporation. As a result, the risk of air pollution decreased as well. Therefore, ILs listed as environmentally friendly solvent that achieve great attention in a number of chemical industry as potential "green" substitutes (Cho et al., 2008b). Due to their strong acceptance in an industrial chemistry, ILs have high probability to be release into watercourse through effluent discharges that may affect aquatic ecosystem for a long period of time. However, the effect of ILs towards aquatic organism is mainly unknown (Couling et al., 2005). For this reason, several study conducted to examine the toxicity of ILs towards aquatic organism by using standard experimental ecotoxicological assessment that apply bioassays on specified organism to measure the ecological risk posed by test substance (Pretti et al., 2009).

To the date, some biological effects for several number of ILs have been reported on various aquatic organisms. The early study conducted to examine the toxic hazard nature of ILs is by Stepnowski and co-workers towards Baltic algae which is *Oocystis submarina* and *Cyclotella meneghiniana* (Zhao et al., 2006).

1.2 Green Chemistry: Ionic Liquids as Green Solvent

One of the branches in chemistry is green chemistry. It is also can be called sustainable chemistry where the main philosophy is to encourage the design or process in any industry that can decrease or remove the use and generation of hazardous materials (Anastas and Warner, 1998). This philosophy concentrate on industrial application where the industry can minimize the hazard release and at the same time can maximize the efficiency of the chemical.

The common organic solvent are usually toxic, flammable and have high volatility in which if the solvent release to environment, it will cause pollution and can have potentially devastating effects. Thus, it is important to design a safe and environmental friendly solvent in the developing a clean manufacturing process. ILs is the best solvent that potentially can replace the common volatile organic solvent including those prohibited by the Montreal protocol of 1989 (Anastas and Warner, 1998) due to ILs has undetectable vapor pressure. Thus, it is cited as a significant element of green chemistry.

From the study conducted, most of imidazolium based ILs were shown to be badly biodegradable and based on the investigation condition, it will not be used as a source of carbon for bacteria (Remoro et al., 2008). This fact proved by a number of studies which shows that the imidazoilum based ILs have a toxicities that depends on the length of the n-alkyl chain in its cation while there is no distinct effect on EC_{50} values due to the effect of anion (Pretti et al., 2009; Bernot et al., 2005b; Wells and Coombe 2006; Ranke et al., 2004; Cho et al., 2008b; Matzke et al., 2010; Samori et al., 2007; Samori, C., 2011; Pham et al., 2010).

In order to get better understanding for the process developments, it is important to know the properties of ILs itself such as density, viscosity, surface tension, flammability, thermal stability, vapor pressure and etc. ILs are generally consist

of ions and it exist as liquid below 100°C. They are thermally stable and it has a very low vapor pressure. Thus, they are of a huge interest in green chemistry due to their certain properties.

1.3 Ionic Liquids

The first documented observation of ILs was back in early 19th century during Friedel-Craft reactions by formation of 'red oil' liquid characterized. The starting of ILs modern era began with discovery 1-butylpyridinium chloride-aluminum chloride mixture. In 1970s an alternative methods which is computer methods developed to predict the electrochemical properties of ammonium salts. On late 1990s to present, limitless possibilities of a new ionic salt have been developed to fill specific branch of industrial chemistry which shows that ILs synthesis rapidly growth so far (Wilkes, 2002).

The capability of changing the chemical, physical and biological properties of ILs, by changing the properties of its anion and cation, has been the important driving force behind the huge interest in fast growing industrial chemistry. A few examples of cation and anion are shown in the Figure 2 below.

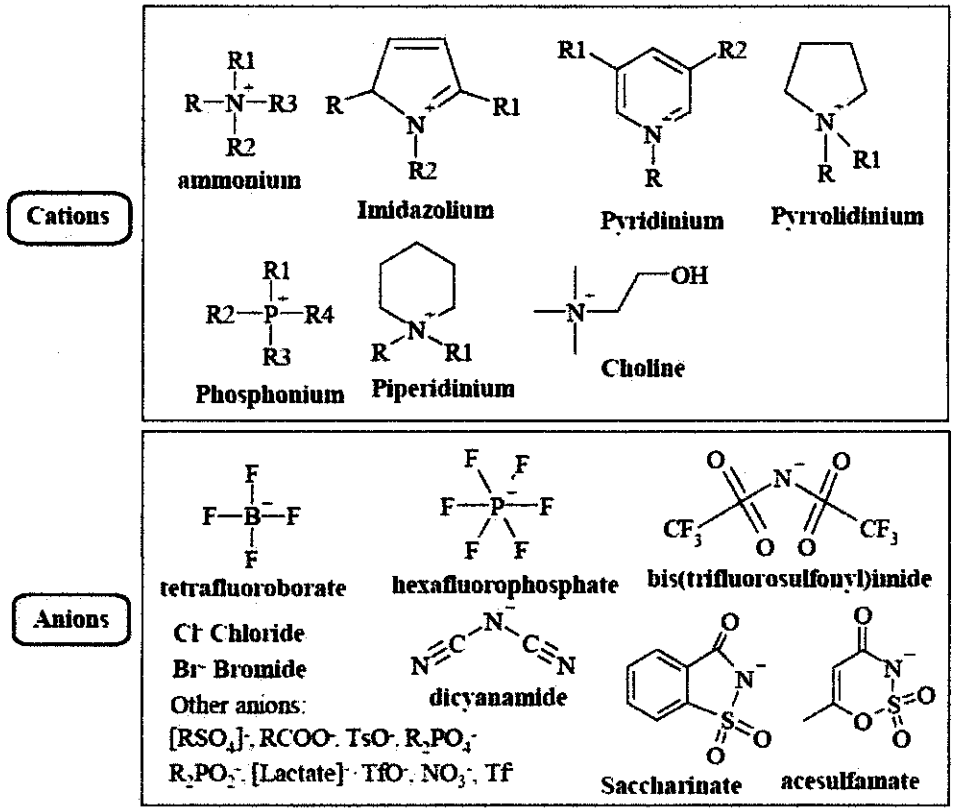


Figure 2: Examples of cation and anion of ILs (Hosssain, 2012)

The most common cation based usually consists of ammonium, imidazolium, pyridinium, pyrrolidinium, phosphonium, piperidinium and choline. While, the most common anion based commonly consist of tetrafluoroborate, hexafluorophosphate, bis(trifluorosulfonyl)imide, dicyanamide, saccharinate, acesulfamate, and some halogen ions group (Hosssain, 2012).

ILs basically consists of positive and negatively charge ion, unlike organic compound that made up of molecules. This combination of cation and anion will determine their respective properties. This tuned characteristic can't be found in any conventional organic solvent (Fumino et al., 2008). Their popularity in industrial chemistry can be shown by the count of publication with the topic "ionic liquids" obtained by a topical search (keyword "ionic liquids") in the chemical abstract of December 29, 2010 (Hossain, 2012). Figure 3 shows publication per year dealing with ILs.

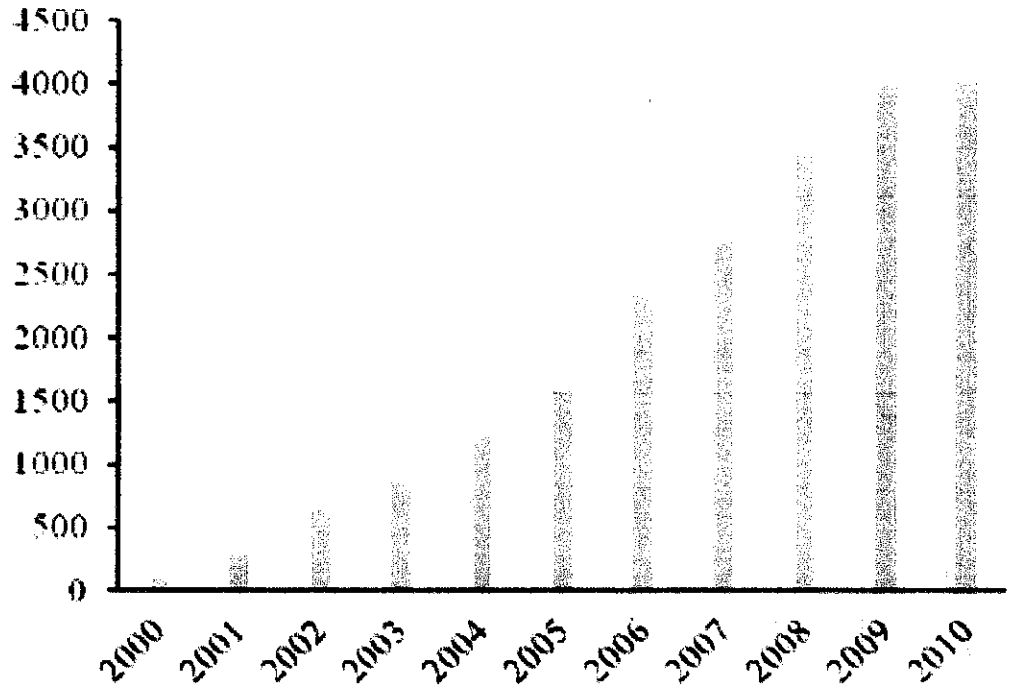


Figure 3: Publication per year dealing with ILs (Hossain, 2012)

ILs can be apply to many type of industries due to it tunable property that vary with respect to the length and alkyl chain and the ionic precursor (Fuler et al., 1997; Huddleston et al., 2001). In addition to that, it becomes high demand in chemical industry because of its one unique characteristic which is immeasurably vapor pressure and they are not flammable. Recently, ILs application in organic synthesis, catalysis, or biocatalysis as reaction medium has been well reported (Earle and Seddon, 2000; Wasserscheid and Keim, 2000; Welton, 1999). ILs also can be used in extraction, separation process (Domanska et al., 2007 and Cassol et al., 2007), metal refining, fuel-cell electrolyte and other type of industry. Figure 4 shows the application of ILs in several branch of industry.

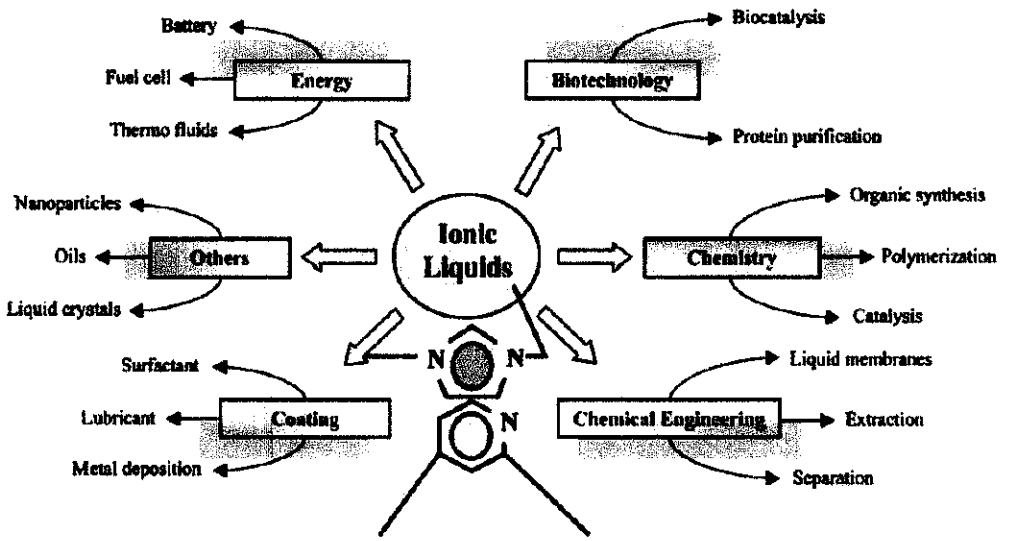


Figure 4: Application of ILs.

According to Gordon, (2001), there are a lot of benefits in performing many reactions in ILs due to the development in process economics, reaction activity, selectivity and also yield. Above all, ILs can be say as future important solvent since it can be apply to many types of industry due to its unique properties that doesn't exist in any other solvent.

With attention to its tunable properties, it is hard to generalize their properties that can represent all ILs that already exist. Usually, the writer emphasize on their difference in properties but not their similarities. Some of the properties that examine few years back are now subject to change. For example, thermal stability was certainly overestimated in the past. These changing due to several reasons which is because of an evolution toward a better knowledge of ILs and better characterization with improved knowledge and quantification of its impurities have been accomplished in recent years. Some of properties that usually reported for ILs are melting point, volatility, flammability, thermal and chemical stability, conductivity, density, viscosity, polarity, toxicity and surface tension (Oliver-Bourbigou et al., 2010). It is important to study the toxicity of ILs since ILs toxicity data are either scarce and sometimes absent due to the large number of ILs synthesize every year

1.4 Toxicological Studies of ILs

Toxicology study is basically a study of bad effect of any chemical on living organism whether it is human, animal, plant or microbe. The bad effect refer to the effect that can cause life threatening injury to at least something that can be consider as a minor injury. Toxicology includes many branch of disciplinary science such as chemistry, biology, pharmacology, molecular biology and medicine.

ILs have insignificant vapor pressure which mean it has significant solubility in water. Therefore, this can be one of the reasons that lead to releasing ILs into environment such as industrial discharge into natural water system. The release of ILs can cause water pollution, and for this reasons it can kills aquatic organism and disturb aquatic ecosystem. Furthermore, ILs are also difficult to be decomposed by microorganism (Garcia et al., 2005). Thus, it can be conclude that ILs can give environment risks to aquatic ecosystem and toxicities study of ILs towards aquatic organism become foremost importance.

Generally, ecotoxicity test is performed for terrestrial organisms including avian, mammalian, and earthworm. The Organization for Economic Cooperation and Development (OECD) test guideline has developed specific test to test toxicity level in organism. LC_{50} is the acute toxicity test which tests for the concentrate of tissue at which it is lethal to 50% within 96 hours. The test may start with eggs, embryos, or juveniles and last from 7 to200 days. EC_{50} is the effective concentration at 50% which is the concentration which causes adverse effects in 50% of the test organisms. These two tests are usually conducted to test the toxicity of chemicals.

Recently, a number of toxicity of ILs study towards certain organism have already been documented such as *Vibrio fisheri* (Docherty and Kulpa Jr, 2005), *Daphnia magna* (Bernot et al., 2005a), animal cells (Stepnowski et al., 2004),

algae (Latala et al., 2005), *Escherichia coli* (Lee et al., 2005), lactic acid producing bacteria (Matsumoto et al., 2004a, b) and *acetylcholinesterase* (Stock et al., 2004).

Above study have been conducts experimentally. However, experimental works require more time and not feasible to do it for every single IL exists. For this reason, alternative methods need to be develop in order to predict toxicity of ILs in fast and effective manner. QSAR is the best approach as an alternative to experimental works due to it is suitable to be used for large number of data.

1.5 Experimental Study of Toxicity of ILs

In early study of toxicity of ILs, the experimental study has been used to determine the toxicity of ILs towards several organisms such as fish, marine bacteria, human cell and algae. Experimental study conducted has been very useful in creating a guideline for the selection of ILs that gives low effect of toxicity. And those studies provide general references to determine the toxicity of ILs based on its cation and anion.

One of the experimental studies conducted to study the effect of toxicity towards fish is towards *Danio rerio* (D. rerio) which is formerly known as zebrafish (Pretti et al., 2006). In this study, they used 15 widely used of ILs. However, on 2009, another experimental study conducted to verify the result in 2006. In the latest study, they used three widely used ILs (1-butyl-3methylimidazolium bis(triflimide),[bmim][Tf_2N], butylpyridinium bis(triflimide), [bpy][Tf_2N], and N,N-methylbutylpyrrolidinium bis(triflimide),[bmpyrr][Tf_2N] and 15 less common salt.

This study measured the acute toxicity of ILs towards *D. rerio* by measuring their lethal effect after 96 hours of exposure towards the respective ILs in a static test. The testing was started by a limit test which at the concentration of 100

mg/L of respective ILs. So that, it can demonstrate that the LC_{50} was bigger than this concentration. If the case of mortality appears in the limit test, then the full LC_{50} study was conducted. Generally, all the experimental tests conducted follow the OECD Guideline no. 203 (OECD, 1992). Since the experiments used fish as the study species, the procedure and animals management were performed by following the provision of the EC Council Directive 86/609 EEC, recognized, and adopted by the Italian Government (DL 27.01.1992, no. 116). During the test conducted, the fish were kept in laboratory with daily photoperiod of 12 hours and the water temperature was kept at 23°C. During the test, there is no food provided to the fish. On the day of the experiment, 10 fish were put in 5-L-glass aquarium that contained control solution (rearing water) and aerated to restore the concentration of dissolved oxygen to at least 90% of its air saturation value.

The preliminary limit test was conducted in order to illustrate that the EC_{50} was greater than this concentration. If the EC_{50} is lower, then a full test was conducted on chosen ILs. Apart from doing the toxicity test with sample, test with potassium dichromate also been carried out to check the reliability of the test procedure (Pretti et al., 2009). Both study in 2006 and 2009 used the same experimental procedure. The data for all tested ILs are usually present in table form as shown in Table 1.

Table 1: EC_{50}s of some ILs

ILs	Limit test/full test EC_{50} 48 hours mg/L	mM
[C2Clmim][Cl]	>100	>552
[C2Clmim][Tf_2N]	>100	>234
[C2OHmim][Tf_2N]	>100	>246
[C3OHmim][Cl]	>100	>566
[TmSiMmim][Br]	>100	>402
[Hmim][Cl]	>100	>844
[HC2Clim][Cl]	>100	>599

$[C_2(Him)_2]_2[Cl]$	>100	>425
$[Chol][PF_6]$	>100	>401
[emmor][Br]	>100	>476
[ebmor][Br]	>100	>397
[ETHT][Br]	>100	>508
$[C_2C_2C_2S][Br]$	>100	>505

Above information become significant in determining toxicity of ILs towards fish. From the experimental result, it is shows that these ILs found to be toxic to fish (Pretti et al., 2006). These data justify the experimental study on 2006 and as a conclusion, the use of these ILs as solvents should be avoided, if possible in future.

Another experimental study conducted was to study the toxicity of ILs towards algae. Algae are usually acts as producer in food chains which makes their ecology is important for sustaining other animal. Algae were used as the study species since it has a short life cycle which can react rapidly to any environmental change (Blaise, 1993; Lewis, 1995). Besides, another characteristic that contribute in deciding algae as the study species is their capability to split water which it can create molecular oxygen photosynthesis and at the same time can assimilating carbon dioxide. In addition to that, algae assays is much more simple, quick, and less expensive as compared to bioassays that using other organism (Latala et al., 2005). For this reason, test procedure using algae are important in estimating the pollution effect that exists in aquatic ecosystem. Generally, procedure for algae assays based on algae growth conducted according to the standard U.S Environmental Protection Agency and Organization for Economic Cooperation and Development.

Up to now, several studies have been conducted using algae to study the effect of ILs towards aquatic ecosystem. (Cho et al., 2007a; Cho et al., 2008 a, b, c; Grabinska-Sota and Kalka, 2006; Kulacki and Lamberti, 2008; Latala et al., 2005; Matzke et al., 2007; Matzke et al., 2008; Pham et al., 2008a, b; Pretti et al., 2009; Stolte et al., 2007; Wells and Coombe, 2006).

One of the previous study conducted by Cho and co-workers used *P. subcapitata* in order to determine the effect of anions, different head groups and side chain of ILs towards algae growth rate and photosynthesis activity (Pham et al., 2008b). In this study, it was found that ILs toxicity gives significant impact on growth rate as compared to photosynthesis activity. For side chain effect, it was found that as the alkyl chain length increase, the toxicity increase (Cho et al., 2007a; Pham et al., 2008b). With respect to anion effect, the study showed *P. subcapitata* was sensitive according to the order $[SbF6]^- > [PF6]^- > [BF4]^- > [CF3SO3]^- > [C8H17OSO3]^- > [Br]^- \approx [Cl]^-$. In short, by releasing ILs into the aqueous system, it can become more dangerous than this experimental data with fresh ILs.

Moreover, another study conducted by Latala et al., 2005, that used marine algae which is *Oocystis submarina* (green algae) and *Cyclotella meneghiniana* as studied species. From the study, it was found that these two species different in their ability to recover from IL exposure. In this case, due to the reduced permeability of IL cations through the algae cell walls, it causes lower IL toxicity. From this study, it will be very useful in providing information in assessment of ILs towards marine ecosystem. Usually, this kind of information and analysis can be extract from experimental works making these studies as important references for future works.

Many experimental standard tests have been conducted towards many type of species to determine the toxicity of ILs. However, this experimental works are time consuming and require high cost. Plus, there is thousands of ILs added in market each year which exceed the experimental methods capacity. For this reason, another alternative were introduce in order to measure the toxicity of ionic liquid via first–principles calculations or structure-property correlations. However, this approach is not practicable with current theories. Thus, lead to introducing another method which is called quantitative structure-activity [or property] relationship (QSAR or QSPR) model (Hossain et al., 2011).

1.6 Quantitative Structure Activity Relationship (QSAR) Approach

In nineteenth century, the QSAR model was first introduced by E. J. Mills by proposed a QSPR model in order to determined melting points and boiling points in homologous series (Mills, 1884). Start from that date, many QSAR models have been developed in order to predict numerous properties of ILs such as melting point (Katritzky et al., 2002; Karthikeyan and Bender 2005; Eike et al., 2003; Varnek et al., 2007), viscosity (Palomar et al., 2007; Tochigi and Yamamoto, 2007; Eiden et al., 2010; Li et al., 2008), and heat capacity (Gardas, and Coutinho, 2008; Preiss et al., 2009). Later on, toxicity also being predicted by using the same approach by number of researcher (Luis et al., 2007; Luis et al., 2010; Arning et al., 2008; Garcia et al., 2008; Bruzzone et al., 2011; Cronin, M. T. D. 2002; Torrecilla et al., 2010; Irabien et al., 2009)

Based on Environmental Protection Agency (EPA), QSAR is mathematical model that is used to predict the toxicity based on the physical properties of the chemicals structure. QSAR has several methodologies that have been develop such as single model method, group contribution method, random forest method and etc. QSAR based on group contribution method is prediction made by using multiple linear regression models.

In the study of toxicology, a lot of studies have been conducted to various type of organism since 2006. According to Couling et al., (2006), the first QSAR model was develop towards *Vibrio fischeri* and *Daphnia Magna* that gives the correlation coefficient from 0.78-0.88. On 2007, another QSAR model again develop by Luis et al., (2007) towards *Vibrio fischeri* that manage to improve the previous model which gives the correlation coefficient, R^2 up to 0.925 and also manage to add more number of IL. The model developed using different software which is polymath 5.0 software. In 2010, the same researcher again develop QSAR model towards *Vibrio fisheri* using Polymath software but with more numbers of ILs that gives R^2 value 0.924. It is shows that, many studies conducted in order to improve the R^2 value so that better prediction of toxicity of ILs can be achieved.

The summary of the developed QSAR model for several types of species are as Table 2.

Table 2: Developed QSAR model for several species

Method	Organism	Number of IL	Correlation coefficient (R^2)	Software used	Error	Reference
Genetic function approximation (GFA)	Vibrio fisheri; Daphnia magna	25 17	0.78-0.88	MOPAC	na	Couling et al. (2006)
MLR	Vibrio fisheri	43	0.925	Polymath 5.0	0.0051	Luis et al. (2007)
Linear regression model	Leukemia rat cell line; IPC-81	74	0.78	R 2.12.1	0.35	Ranke et al. (2007)
Spectral-SAR vectorial model	Vibrio fisheri	22	Na	HyperChem Program	Na	Lacrama et al. (2007)
Topological sub-structural molecular design (TOPS-MODE)	Caco-2 cells	15	0.98	MODESLAB 1.5	Na	Garcia-Lorenzo et al. (2008)

MLR, Radial Basisi(RB) multilayer perception (MLP, neural network (NN).	Leukemia rat cell line; IPC-81; Acetylcholinesterase (AChE)	153	MLR, R^2= 0.867 (IPC) MLR, R^2= 0.814 (AChE) NN, R^2= 0.982 (IPC) NN, R^2= 0.973 (AChE)	MATLAB and Statgraphics Plus	0.22 and 0.38	Torrecilla et al. (2009)
MLR	Vibrio fisheri	96	0.924	Polymath 5.0	Na	Luis et al. (2010)
MLR and polynomial methods	Daphnia Magna	64	0.974	MATLAB 7.8.0.347	0.0283	Hossain et al. (2011)
Partial Least Squares-Discriminant Analysis	Vibrio Fisheri	148	na	MATLAB	na	Guerra, Irabien (2011)

Based on summary above and to the best of my knowledge, there is no study had been develop to predict the toxicity of ILs towards green algae *P. subcapitata* by using QSAR model. In this study, since this is the first model that used *P. subcapitata* as study species, the aim is to develop a model with the highest R^2 value with the highest number of IL possible. Therefore it can be used to predict the toxicity of ionic towards *P. subcapitata* by using MLR and polynomial method that in future can be used as a reference to industrial people. This model cannot be compared to any model since there is no previous QSAR model been developed towards *P. subcapitata*. This model can be one of the alternative approaches that can replace previous experimental works.

CHAPTER 3
METHODOLOGY

3.1 Introduction

Toxicity study of ILs already been measured by using experimental procedure. However, experimental works is time consuming, require high cost and killed the studied species. In addition, thousand of ILs added in the market each year. It is impossible to predict toxicity of each available ILs using experimental works. Therefore, the prediction can be done theoretically by using mathematical modeling. In this chapter, the content will focus more on the development of the model to be used in estimating the toxicity of ILs. The model will be developed using QSAR model that employ a combination of MLR and polynomial methods. MATLAB programming language will be used to write the code and develop the model.

3.2 Quantitative Structure-Activity Relationship (QSAR)

QSAR model was first discovered by Hansch and Fugita (Martin, Y. C. 2012). In their work, QSAR model being used in order to come out with a linear equation to predict the next molecule to be synthesized using a few number of molecules of a given chemotype. There is advantages by using QSAR approach which is the equation produced were basically simple and easily interpretable which gives the users more confident in the model prediction. Recently, QSAR has become an important method in determining toxicity of ILs since it is cost effective, faster and environmental friendly. Generally, QSAR is basically a mathematical equation that is that determined from a set of molecules with known activities via computational methods. Many statistical methods can be used to determine the correct form of the relationship between the structure of the ionic liquid and their toxicity.

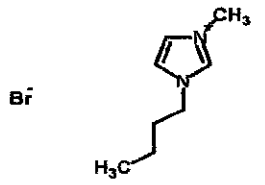
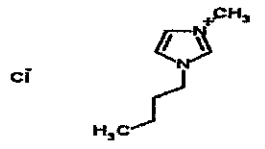
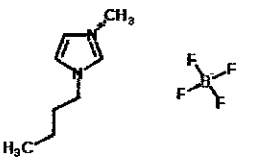
3.3 Data Collection

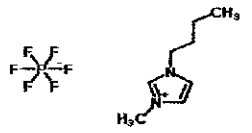
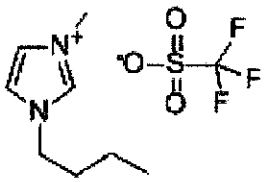
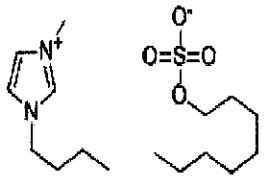
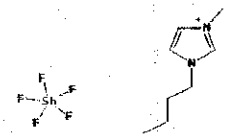
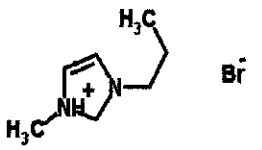
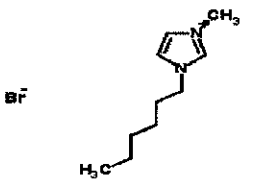
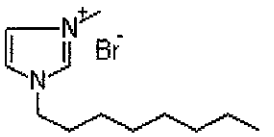
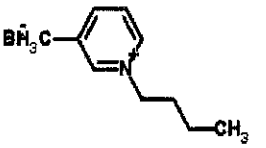
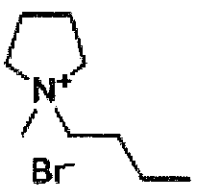
EC_{50} values for the list of ILs used in this work have been collected from published literature. These data can be found in the work of Cho et al., (2007a), Cho et al., (2007b), Cho et al., (2008a), Pham, et al. (2008a), Wells, et al., (2006), Pretti, et al., (2009).

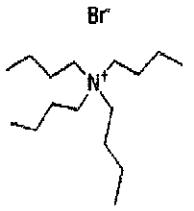
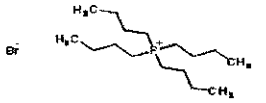
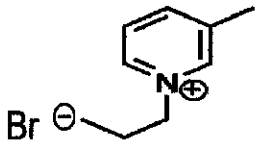
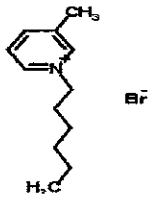
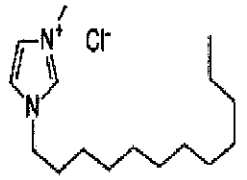
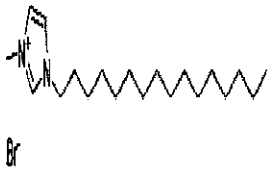
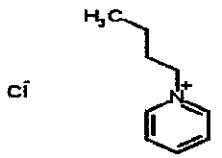
3.4 Dataset Preparation

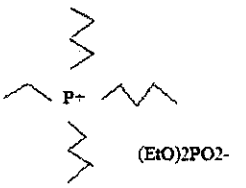
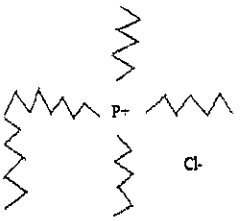
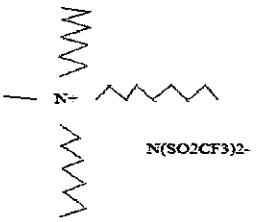
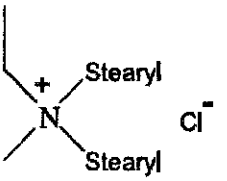
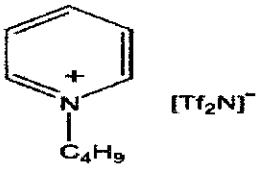
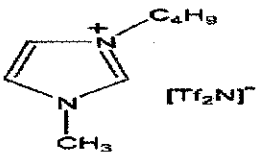
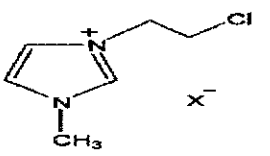
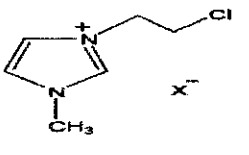
The first step in preparing the dataset is to design the 'molecular structure table' based on the molecular fragments (groups) (description of molecular structure of pure compounds). The molecular structure table comprise number of functional groups such as halogenated compounds, hydrocarbon, and several cations and anions. The earliest step in constructing the molecular structure table is to gather all the molecular structure for every single IL. Based on the molecular structure, it will be fragmented into their respective cation, anion and alkyl groups. Table 3 below shows the example of molecular structure for several types of ILs.

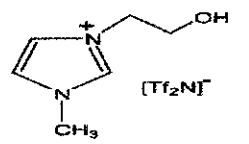
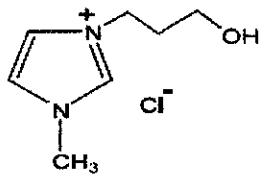
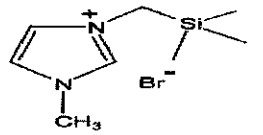
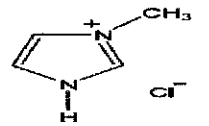
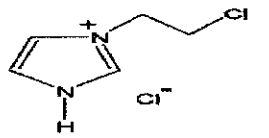
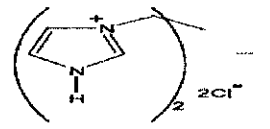
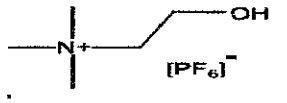
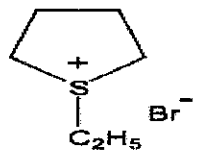
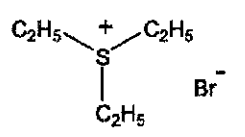
Table 3: Example of ionic liquid's molecular structure

No.	Ionic Liquid	Abbreviation	Structure
1	**1-butyl-3-methylimidazolium Bromide**	**[BMIM][Br]**	
2	**1-butyl-3-methylimidazolium Chloride**	**[BMIM][Cl]**	
3	**1-butyl-3-methylimidazolium Tetrafluoroborate**	**[BMIM][BF4]**	

4	1-butyl-3-methylimidazolium Hexafluorophosphate	[BMIM][PF6]	
5	1-butyl-3-methylimidazolium Trifluoromethylsulfonate	[BMIM] [CF3SO3]	
6	1-butyl-3-methylimidazolium Octylsulfate	[BMIM] [C8H17SO4]	
7	1-butyl-3-methylimidazolium Hexafluoroantimonate	[BMIM] [SbF6]	
8	1-propyl-3-methylimidazolium Bromide	[PMIM][Br]	
9	1-hexyl-3-methylimidazolium Bromide	[HMIM][Br]	
10	1-octyl-3-methylimidazolium bromide	[OMIM][Br]	
11	1-butyl-3-methylpyridinium Bromide	[BMPy][Br]	
12	1-butyl-1-methylpyrrolidinium Bromide	[BMPyrr][Br]	

13	**Tetrabutylammonium Bromide**	**[TBA][Br]**	
14	**Tetrabutylphosphonium Bromide**	**[TBP][Br]**	
15	**1-propyl-3-methylpyridinium Bromide**	**[PMPy][Br]**	
16	**1-hexyl-3-methylpyridinium Bromide**	**[HMPy][Br]**	
17	**1-octyl-3-methylpyridinium Bromide**	**[OMPy][Br]**	
18	**1-dodecyl-3-methylimidazolium Chloride**	**[DMIM][Cl]**	
19	**1-cetyl-3-methylimidazolium Chloride**	**[CMIM][Cl]**	
20	**1-butylpyridinium Chloride**	**[BPy][Cl]**	

21	**Butyl Ethyl Phosphonium Diethylphosphate**	**[BEP][$(EtO)_2PO_2$]**	
22	**Hexyl Tetradecyl Phosphonium Chloride**	**[HTP][Cl]**	
23	**Octyl Methyl Ammonium N-fluorobis(trifluoromethanesulfonyl)imide**	**[OMA][N(SO2 CF3)2]**	
24	**AMMOENG 130**	**AMMOENG 130**	
25	**Butyl Pyridinium Bis(triflimide)**	**[BPy][Tf_2N]**	 [bpy][Tf_2N], **IL3**
26	**1-butyl-3-methylimidazolium Bis(triflimide)**	**[BMIM][Tf2N]**	 [bmim][Tf_2N], **IL5**
27	**3-(2-cloroethyl)-1-methylimidazolium Chloride**	**[C2ClMIM][Cl]**	 [C_2Clmim]Cl, **IL6** [C_2Clmim][Tf_2N], **IL7**
28	**3-(2-cloroethyl)-1-methylimidazolium Bis(triflimide)**	**[C2ClMIM] [Tf2N]**	 [C_2Clmim]Cl, **IL6** [C_2Clmim][Tf_2N], **IL7**

29	3-(2-hydroethyl)-1-methylimidazolium Bis(triflimide)	[C2OHMIM][Tf2N]	 [C2OHmim][Tf2N], IL8
30	3-(2-cloroethyl)-1-methylimidazolium Chloride	[C3OHMIM][Cl]	
31	1-methyl-3-[(trimethylsilyl)methyl]-imidazolium Bromide	[TMiMMIM][Br]	 [TMSiMmim]Br, IL10
32	1-hexyl-3-methylimidazolium Chloride	[HMIM][Cl]	 [Hmim]Cl, IL11
33	1-(2-Chloroethyl)imidazolium Chloride	[HC2ClIM][Cl]	 [HC2Clim]Cl, IL12
34	1,1'-(1,2-ethanediyl)bis-imidazolium Chloride	[C2(HIM)2]2[Cl]	 [C2(Him)2]2Cl, IL13
35	Choline hexafluorophosphate	[Chol][PF_6]	 [chol][PF_6], IL14
36	Ethyltetrahydrothiophenium Bromide	[ETHT][Br]	 [ETHT]Br, IL17
37	Triethylsulfonium Bromide	[TES][Br]	 [$C_2C_2C_2S$]Br, IL18

After completing the molecular structure table, the next step is to construct dataset by fragment the IL into their descriptors. The descriptor, such as substituent, cations and anions, used in this study was summarized in Table 4.

Table 4: List of descriptor used in constructing the dataset

Fragment Name	Structure	Fragment Name	Structure
	Substituent		
$-CH_3$	Methyl	-OH	hyddroxyl
$>CH_2$	Methylene	-NH	Hydrogen atom attached with nitrogen atom
$-NCH_2$	Methylene group attached with nitrogen atom	-PH	Hydrogen atom attached with phosphorus atom
$-NCH_3$	Methyl group attached with nitrogen atom	-CCl	Chlorine atom attached with carbon atom
$-SCH_2$	Methylene group attached with sulfur atom	-CSi	Silica atom attached with carbon atom
$-PCH_2$	Methylene group attached with phosphorus atom	-CO	Oxygen atom attached with carbon atom
	IL families		
Imida	Imidazolium	Ammo	Ammonium
Pyrid	Pyridinium	Phos	Phosphonium
Pyrrol	Pyrrolidinium	Sulfo	Sulfonium
THT	Thiophenium		
	Anions		
Cl^-	Chloride	R in anion	Alkyl group in anion (R=0 indicates only H)
Br^-	Bromide	Tf_2N^-	Bis(triflimide)
BF_4^-	Tetrafluoroborate	SbF_6^-	Hexafluoroantimonate

PF_6^-	Hexafluorophosphate	$(EtO)_2PO_2^-$	Diethylphosphate
$CF_3SO_3^-$	Trifluoromethylsulfonate	$N(SO_2CH_3)_2^-$	N-fluorobis(trifluoromethanesulfonyl)imide
SO_4^-	Sulfate		

List of descriptor table (Table 4) will be used to construct a dataset based on descriptor discuss above for MATLAB code to process the data comprised in this dataset and predict the best fit model (the model which can give the most accurate results between the experimental and predicted values). The accuracy of the obtained results will be depending on how accurate the data set table build. Table 5 shows the dataset that need to be completed by manual fragmentation.

Table 5: Dataset for prediction of ILs toxicity

IL number	Ionic Liquid	Log EC50		Ref
1	[BMIM][Br]	3.329804522		Cho et al. 2008
2	[BMIM][Br]	3.459995256		Cho et al. 2007.
3	[BMIM][Br]	3.359835482		Cho et al. 2007.
4	[BMIM][Br]	3.019946682		Cho et al. 2007.
5	[BMIM][Br]	3.330007701		Pham et al. 2008
6	[BMIM][Cl]	3.459995256		Cho et al. 2008
7	[BMIM][Cl]	2.343789756		Wells et al. 2006
8	[BMIM][BF4]	3.400019635		Cho et al. 2008
9	[BMIM][BF4]	3.539953842		Pham et al. 2008
10	[BMIM][BF4]	2.170261715		Pham et al. 2008
11	[PMIM][Br]	3.289811839		Cho et al. 2007.
12	[PMIM][Br]	3.459995256		Cho et al. 2007.
13	[PMIM][Br]	3.139879086		Cho et al. 2007.
14	[HMIM][Br]	2.56937391		Cho et al. 2007.
15	[HMIM][Br]	2.539953842		Cho et al. 2007.
16	[HMIM][Br]	2.459392488		Cho et al. 2007.
17	[OMIM][Br]	1.650307523		Cho et al. 2007.
18	[OMIM][Br]	1.679972694		Cho et al. 2007.
19	[OMIM][Br]	1.582063363		Cho et al. 2007.
20	[BMPy][Br]	3.46		Cho et al. 2008.
21	[BMPy][Br]	3.7		Cho et al. 2008.
22	[BMPyrr][Br]	3.67		Cho et al. 2008.
23	[BMPyrr][Br]	3.97		Cho et al. 2008.
24	[BMPyrr][Br]	4.09		Cho et al. 2008.
25	[TBA][Br]	2.97		Cho et al. 2008.
26	[TBA][Br]	3.68		Cho et al. 2008.
27	[TBA][Br]	3.35		Cho et al. 2008.
28	[TBP][Br]	1.9		Cho et al. 2008.
29	[TBP][Br]	2.19		Cho et al. 2008.
30	[TBP][Br]	2.35		Cho et al. 2008.
31	[BMPy][Br]	3.69		Cho et al. 2008.
32	[BMIM][PF6]	2.199894174		Wells et al. 2006

3.5 Model Development

The models that being develop in this work are based on statistical methods such as MLR, polynomial regression, partial least square, or non-linear methods such as support vector machines, artificial neural network, etc. All these methods were used to develop mathematical model that connects experimental measurement with a set of chemical descriptor. In this work, a new model developed based on a combination between Multiple Linear Regression (MLR) method and polynomial method. The dataset consist response variable which can be denoted as Y, while the predictor variables is denoted as X_1, X_2, ..., X_p, where p represents the total number of predictor variables. The true relationship between Y and X_1, X_2, ...,X_p is approximated by a regression model (Chatterjee and Hadi, 2006) expressed as:

$$Y = f(X_1, X_2, \ldots X_p) + \varepsilon \quad (1)$$

Where, ε is defined as a normal random error expressing the discrepancy in the approximation.

The linear form of Eq. (2) can be expressed as:

$$Y = \beta_o + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_p X_p + \varepsilon \quad (2)$$

Where $\beta_o, \beta_1, \ldots \beta_p$, are defined as regression coefficients.

Eq. (2) was modified by integration with the polynomial model Eq. (3) (Angelov, 2002) and the interaction between the two models (ωX_i) can be described by Eq. (4):

$$Y = \gamma_0 + \gamma_1\omega^1 + \gamma_2\omega^2 + ... + \gamma_m\omega^m + \varepsilon \quad (3)$$

$$Y = \alpha_0 + \sum_{i=1}^{n}\alpha_i X_i + \sum_{i=1}^{m}\gamma_i\omega^i + \sum_{i=n+1}^{k}\delta_i\omega X_i \quad (4)$$

where,

$\alpha_i = \beta_i$ for $i \le n$; $\gamma_i = \beta_{i+n}$ for $i \le m$; $\delta_i = \beta_{i+n+m}$ for $i \le k$; $n+m+k = p$,

$\alpha_0, \alpha_1, ...\alpha_n$ are the parameters for MLR model,

$\gamma_1 .....\gamma_m$ are the parameters for polynomial model, and

$\delta_1 .....\delta_k$ are the parameters for the interaction between the descriptors.

MATLAB software version 7.8.0.347 was used to develop the code and estimate the ILs toxicities based on the algorithm illustrated by Figure. 7. The accuracy of the develop model is confirm using the average relative deviation (ARD) (Eq. 5), average absolute relative deviation (AARD) (Eq. 6), average absolute error (AAE) (Eq. 7) and standard deviation (square root of the variance) ($\hat{\sigma}^2$) (Eq. 8) between the predicted values of EC_{50} and the experimental data.

$$ARD = \frac{100}{N}\sum_{i=1}^{N}\left(\frac{EC50_{Cal} - EC50_{Exp}}{EC50_{Exp}}\right)_i \quad (5)$$

$$AARD = \frac{100}{N}\sum_{i=1}^{N}\left|\frac{EC50_{Cal} - EC50_{Exp}}{EC50_{Exp}}\right|_i \quad (6)$$

$$AAE = \frac{100}{N}\sum_{i=1}^{N}\left|EC50_{Cal} - EC50_{Exp}\right|_i \quad (7)$$

$$\hat{\sigma}^2 = \frac{\sum_{i=1}^{N}\left(EC50_{Cal} - \bar{EC50}_{Cal}\right)_i^2}{N-1} \quad (8)$$

where,

N is the number of the substances,

EC_{50cal} is the EC_{50} calculated values,

EC_{50exp} is the EC_{50} experimental values, and

$\overline{EC}_{50cal}$ is the mean EC_{50} values .

The selection of significant coefficients based on the descriptors, which construct a relationship between the EC_{50} of each ILs and their molecular structures, is an important step in QSPR modeling. For this purpose, MLR model combined with polynomial models were used to determine the important coefficients based on functional group contributions as given by Eq. (4) and the calculations were performed using MATLAB programming language. The code was written using a 80%-20% training-testing split. In the training process, the code trained the data to obtain the optimal coefficients based on the least square error between the EC_{50} predicted and experimental values. In order to compress the number of coefficients without decreasing the accuracy of the models, R^2, hypothesis testing was applied for each coefficient to test its significance in the developed model. The most significant coefficients obtained from the hypothesis testing were only selected and used in Eq. (4) to predict the EC_{50} values and coefficients with small values were eliminated. Figure 5 shows the algorithm used for the estimation of toxicity using MATLAB 7.8.0.347

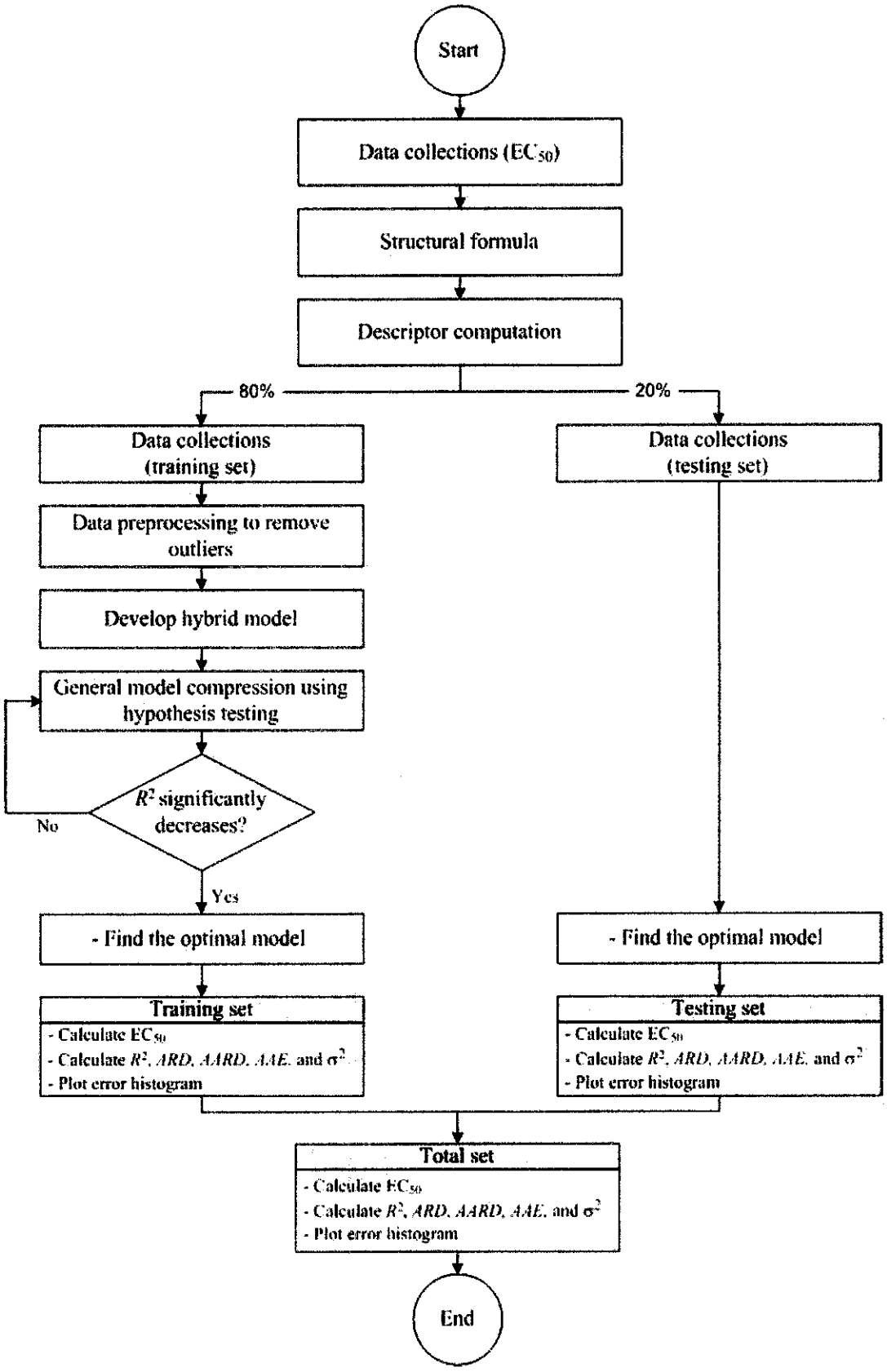


Figure 5: Algorithm for the estimation of toxicity using MATLAB 7.8.0.347

CHAPTER 4
RESULT AND DISCUSSION

4.1 Introduction

This chapter discussed the method that used to determine the toxicity of ILs towards *P. subcapitata* using QSAR approache. As mention in chapter two, measurement of EC_{50} for different ILs towards *P. subcapitata* have been done by different researcher using experimental study. However, this is not easy to perform as other may think since huge number of ILs is added each year in the market. Therefore, a new QSAR model will be introduced and discussed in this study. To the best of our knowledge, there is no any reported model to predict the toxicity of ILs towards P. subcapitata.

4.2 QSAR model for estimating the toxicity of ILs

4.2.1 Dataset preparation

To obtain an accurate QSAR model, a reliable experimental dataset have to be selected. In this work, the dataset was construct using 61 number of ILs from six different literatures (Cho et al., 2007a; Cho et al., 2007b; Cho et al., 2008a; Pham., et al. 2008a; Wells., et al. 2006; Pretti et al., 2009). A complete list of ILs involves and their toxicity value presented in previous chapter. The dataset will be used by the code to develop the model to predict the toxicity of ILs towards *P. subcapitata*.

4.2.2 Group Contribution

In this study, the first step is to construct the group contribution. The group contribution is constructed by gathered the molecular structure of each ionic liquid involved. All the ILs is manually fragmented into their cation, anion and alkyl substituent based on the molecular structure as discussed in previous chapter.

4.2.3 Result and Model Acccuracy.

The model was developed using MATLAB 7.8.0.347 programming language in order to find the optimum quantitative relationship between the predictor variables (ILs fragmentation) and response variables (toxicity values). The result presented by comparison between predicted and experimental values graph and results error histogram graph. The results were produce by the best optimum model generated by MATLAB programming language.

The toxicity model was written using 80%-20% training and predicting set. The data from 1 to 49 were used for training purpose while the data from 50 to 61 were used for prediction purpose. In the training set, the data is used to calculate the coefficient of the prediction model (α_i, γ_i, δ_i,) in Eq. (5) using square error method. While in the prediction set, the data is used to test how good is the model has been trained and the accuracy of the prediction model together with the coefficient obtained from the training set.

For the toxicity prediction model, a good fit was obtained between the predicted and experimental toxicity values which gave R^2 of 100%. Based on the R^2 value and Figure 7, it indicates that the error values are very close between the training and testing sets. This clearly shows that the proposed model is reliable for training and testing abilities. The fragments and their contribution in the molecular structure of the individual IL were listed in Table 6 and the statistical parameters of the developed models show in Table 7 below.

Table 6 :Fragmentations and predicted values of ILs for the toxicities on *P. subcapitata*.

IL number	Ionic Liquid	CH_3-	CH_2	N-CH_3	N-CH_2	C-CH_3	S-CH_2	P-CH_2	OH	H-N	P-H	CCl	CSi	CO	Imida	Pyrid	Pyrrol	Ammo	Phos	Sulfo	THT	Cl-	Br-	BF_4^-	PF_6^-	$CF_3SO_3^-$	SO_4^-	R	SbF_6^-	Tf_2N^-	$(EtO)_2PO_2^-$	$N(SO_2CF_3)_2^-$	Log Ec_{50} (Experimental value)	Log Ec_{50} (Proposed methods)	Reference
1	[BMIM][Br]	1	2	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	3.329804522	3.329804522	Cho et al. 2008
2	[BMIM][Br]	1	2	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	3.459995256	3.459995256	Cho et al. 2007.
3	[BMIM][Br]	1	2	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	3.359835482	3.359835482	Cho et al. 2007.
4	[BMIM][Br]	1	2	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	3.019946682	3.019946682	Cho et al. 2007.
5	[BMIM][Br]	1	2	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	3.330007701	3.330007701	Pham et al. 2008
6	[BMIM][Cl]	1	2	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	3.459995256	3.459995256	Cho et al. 2008
7	[BMIM][Cl]	1	2	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2.343789756	2.343789756	Wells et al. 2006
8	[BMIM][BF4]	1	2	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	3.400019635	3.400019635	Cho et al. 2008
9	[BMIM][BF4]	1	2	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	3.539953842	3.539953842	Pham et al. 2008
10	[BMIM][BF4]	1	2	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2.170261715	2.170261715	Pham et al. 2008
11	[PMIM][Br]	1	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	3.289811839	3.289811839	Cho et al. 2007.
12	[PMIM][Br]	1	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	3.459995256	3.459995256	Cho et al. 2007.
13	[PMIM][Br]	1	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	3.139879086	3.139879086	Cho et al. 2007.
14	[HMIM][Br]	1	4	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	2.56937391	2.56937391	Cho et al. 2007.
15	[HMIM][Br]	1	4	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	2.539953842	2.539953842	Cho et al. 2007.
16	[HMIM][Br]	1	4	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	2.459392488	2.459392488	Cho et al. 2007.
17	[OMIM][Br]	1	6	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1.650307523	1.650307523	Cho et al. 2007.
18	[OMIM][Br]	1	6	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1.679972694	1.679972694	Cho et al. 2007.
19	[OMIM][Br]	1	6	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1.582063363	1.582063363	Cho et al. 2007.
20	[BMPy][Br]	1	2	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	3.46	3.46	Cho et al. 2008.
21	[BMPy][Br]	1	2	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	3.7	3.7	Cho et al. 2008.
22	[BMPyrr][Br]	1	2	0	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	3.67	3.67	Cho et al. 2008.
23	[BMPyrr][Br]	1	2	0	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	3.97	3.97	Cho et al. 2008.
24	[BMPyrr][Br]	1	2	0	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	4.09	4.09	Cho et al. 2008.
25	[TBA][Br]	4	8	0	4	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	2.97	2.97	Cho et al. 2008.
26	[TBA][Br]	4	8	0	4	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	3.68	3.68	Cho et al. 2008.
27	[TBA][Br]	4	8	0	4	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	3.35	3.35	Cho et al. 2008.
28	[TBP][Br]	4	8	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1.9	1.9	Cho et al. 2008.
29	[TBP][Br]	4	8	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	2.19	2.19	Cho et al. 2008.
30	[TBP][Br]	4	8	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	2.35	2.35	Cho et al. 2008.

31	[BMPy][Br]	1	2	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	3.69	3.69	Cho et al. 2008.
32	[BMIM][PF6]	1	2	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2.199894174	2.199894174	Wells et al. 2006
33	[BMIM][PF6]	1	2	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	3.11991541	3.11991541	Cho et al. 2008
34	[PMPy][Br]	1	1	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	-1.270025714	-1.270025714	Pham et al. 2008.
35	[HMPy][Br]	1	4	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	-2.829738285	-2.829738285	Pham et al. 2008.
36	C8MPy Br	1	6	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	-4.259637311	-4.259637311	Pham et al. 2008.
37	[DMIM][Cl]	1	10	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	-2.415656141	-2.415656141	Wells et al. 2006
38	[CMIM][Cl]	1	14	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	-1.921813314	-1.921813314	Wells et al. 2006
39	[SMIM][Cl]	1	6	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	-1.458139889	-1.458139889	Wells et al. 2006
40	[Bpy][Cl]Cl	1	2	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2.56520306	2.56520306	Wells et al. 2006
41	[BEP][$(EtO)_2PO_2$]	4	6	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1.207958689	1.207958689	Wells et al. 2006
42	[HTP][Cl]	4	42	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	-1.091457588	-1.091457588	Wells et al. 2006
43	[OMA][N(SO2CF3)2]	3	81	1	3	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	-1.048260933	-1.048260933	Wells et al. 2006
44	[[C2H4O(C2H4O)4Me]CH3N][MeSO4]	3	03	1	2	0	0	0	0	0	0	0	0	5	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	-0.962428532	-0.962428532	Wells et al. 2006
45	AMMOENG 130	2	6	1	3	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0.146128036	0.146128036	Pretti et al. 2009.
46	[BPy][Tf_2N]	1	2	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1.227886705	1.227886705	Pretti et al. 2009.
47	[BMIM][Tf_2N]	1	2	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1.800717078	1.800717078	Pretti et al. 2009.
48	[C2ClMIM][Cl]	0	1	1	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2.624385241	2.624385241	Pretti et al. 2009.
49	[C2ClMIM] [Tf2N]	0	1	1	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2.223236273	2.223236273	Pretti et al. 2009.
50	[C2OHMIM] [Tf2N]	0	1	1	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2.143639235	2.143639235	Pretti et al. 2009.
51	[C3OHMIM][Cl]	0	1	1	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2.228913406	2.228913406	Pretti et al. 2009.
52	[TMiMMIM][Br]	3	1	1	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1.633468456	1.633468456	Pretti et al. 2009.
53	[HMIM][Cl]	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2.8409212	2.8409212	Pretti et al. 2009.
54	[HC2ClIM][Cl]	0	1	0	1	0	0	0	0	1	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2.675778342	2.675778342	Pretti et al. 2009.
55	[C2(HIM)2]2[Cl]	2	0	0	2	0	0	0	0	2	0	0	0	0	2	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	2.460897843	2.460897843	Pretti et al. 2009.
56	[Chol][PF6]	0	1	3	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2.180699201	2.180699201	Pretti et al. 2009.
57	[ETHT][Br]	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	2.481012421	2.481012421	Pretti et al. 2009.
58	[BMIM][CF3SO3]	1	2	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	3.340047318	3.340047318	Cho et al. 2008
59	[BMIM][C8H17SO4]	1	2	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	8	0	0	0	0	3.350054094	3.350054094	Cho et al. 2008
60	[BMIM][SbF6]	1	2	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2.130333768	2.130333768	Cho et al. 2008
61	[TES][Br]	3	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	2.342027688	2.342027688	Pretti et al. 2009.

Table 7 : The statistical parameters of the developed models

Statistical parameters	Training set	Testing set	The whole dataset
No. of compounds	49	12	61
R^2 (%)	1	1	1
ARD (%)	$6 * 10^{-14}$	$2 * 10^{-14}$	$2 * 10^{-14}$
AARD (%)	$9 * 10^{-14}$	$5 * 10^{-14}$	$4 * 10^{-14}$
AAE (%)	$1 * 10^{-13}$	$4 * 10^{-14}$	$5 * 10^{-14}$
σ^2	$5 * 10^{-28}$	$2 * 10^{-28}$	$2 * 10^{-28}$

The proposed model exhibit a good fit with highest R^2 which is 100%. This clearly shows that the proposed method which is the combination of MLR and polynomial method can increase the accuracy of the results. It is because of the combination of this two methods will consider more predictor variables (X_1, X_2,X_3, …, X_n) into account when MATLAB code process the data and during the optimization in order to get the best coefficient (α_i, γ_i, δ_i, λ_i) to be used in the proposed model. The best significant coefficient will be used in Eq (4) to get the best predictive model to estimate toxicity of ILs. Generally, in order to consider any QSPR model is reliable, the model has to be further validated. In this study, the proposed model have been validated in prediction set in which 20% random selection of ILs in the dataset used to validated the proposed model. The predicted result was then validated against the experimental values. From the validation, it is shows that the predicted values and experimental values is consistent without arise any significant deviation. A good fit is obtained with square correlation coefficient, R2=100% as illustrated in Figure 6. The ARD of $2 * 10^{-14}$, an AARD of $4 * 10^{-14}$, an AAE $5 * 10^{-14}$and σ^2 of $2 * 10^{-28}$. It can be shows in Figure 7 – Figure 9. Error histogram graphs show that there are 59 values having errors with less than $2x10^{-13}$% between the predicted results and experimental data.

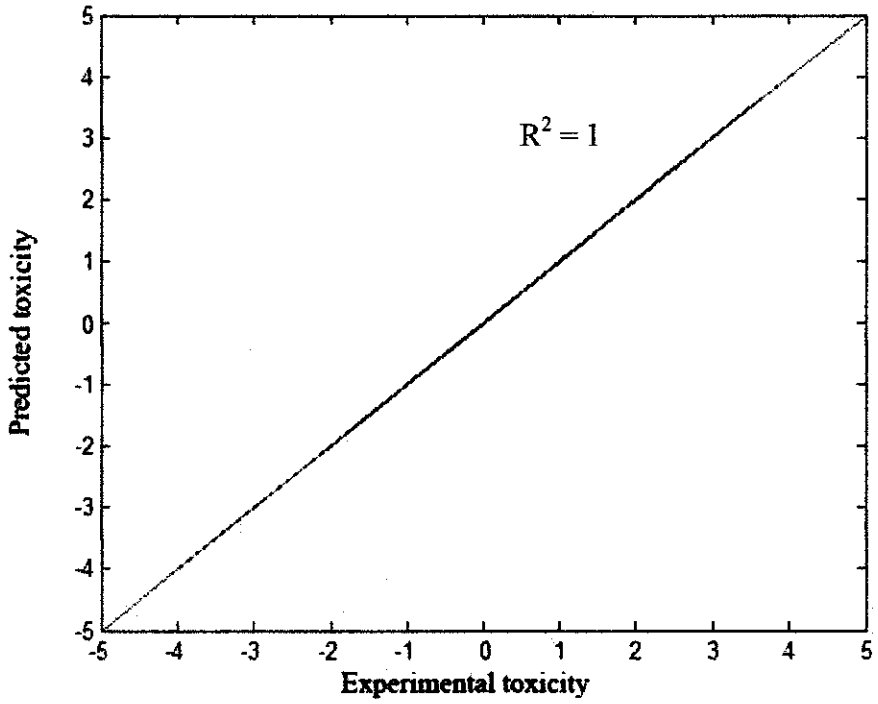


Figure 6 : Comparison between predicted and experimental toxicity value

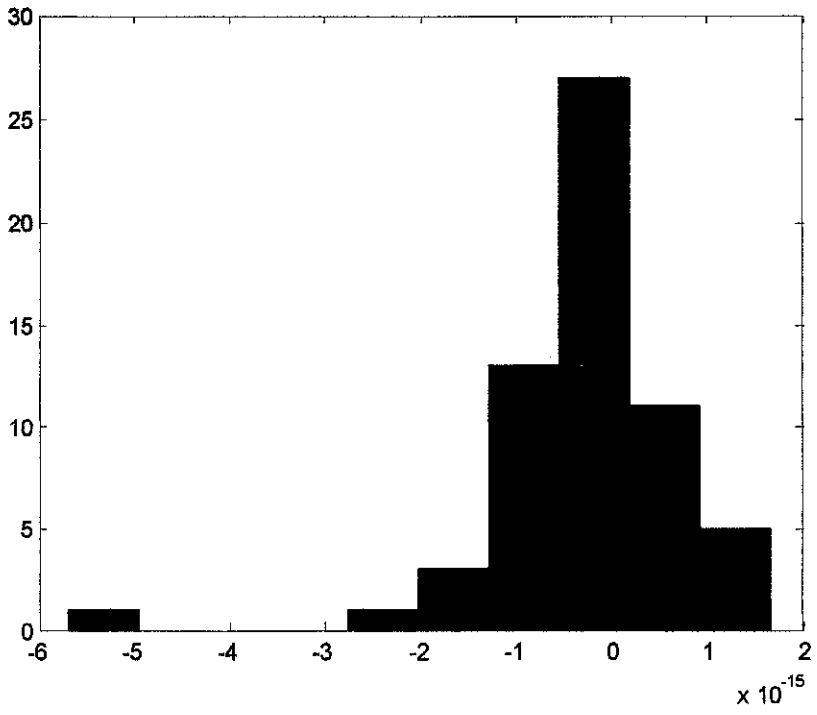


Figure 7: ARD histogram for the toxicity model

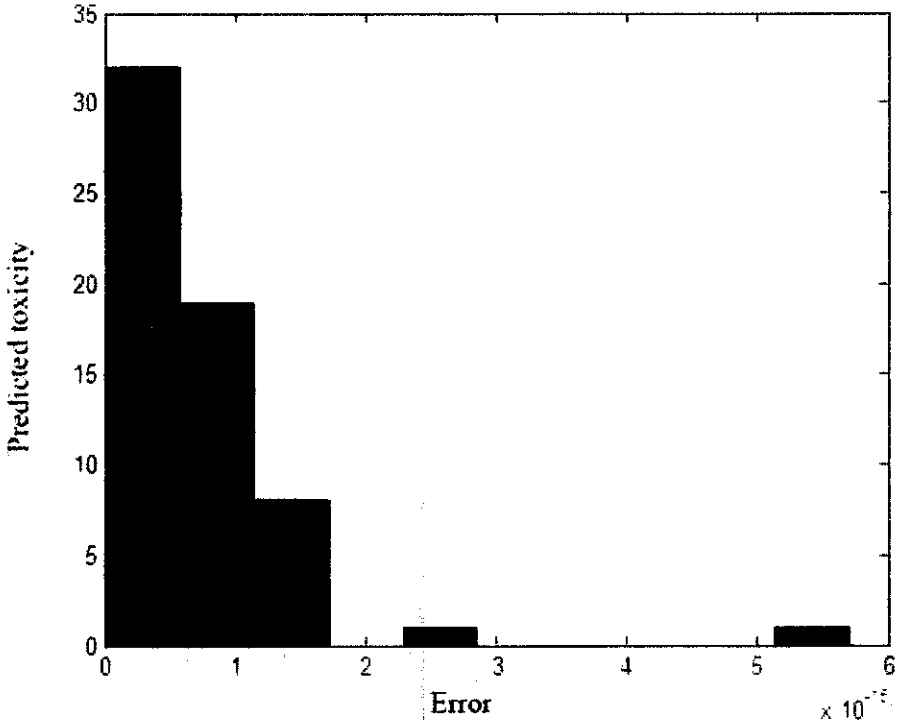


Figure 8 : AARD histogram for the toxicity model

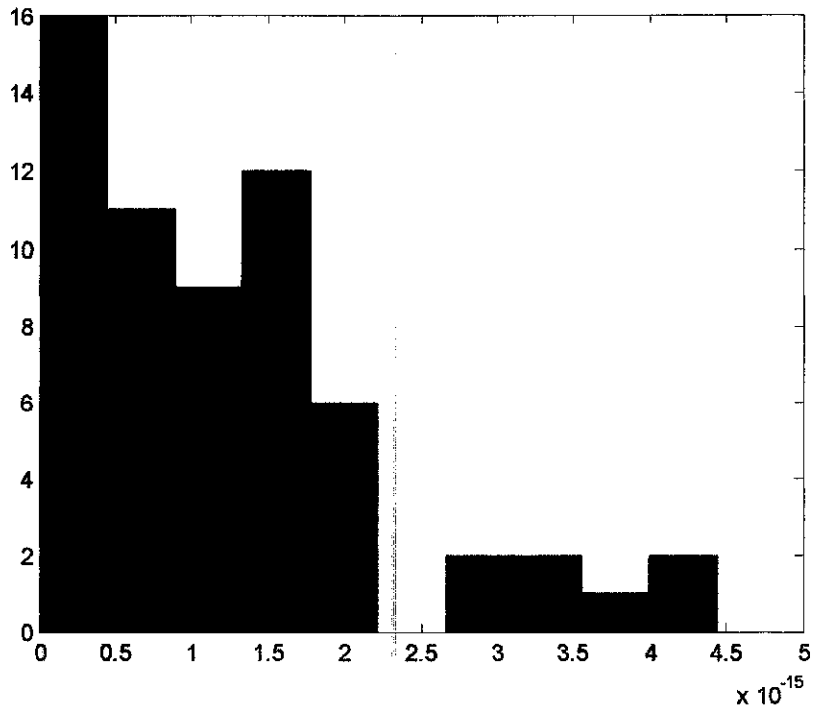


Figure 9 : AAE histogram for the toxicity model

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In this work, we proposed a new model for the prediction of the toxicity of ILs towards *P. subcapitata* based on QSAR approach. The model is developed using a data set of 61 pure ILs and the code was written using MATLAB programming language that employ the combination of MLR, and polynomial methods. The proposed model shows a very accurate result with high accuracy which is the R^2 value is equal to 100%. Moreover, to the best of our knowledge, this is the first QSAR study conducted towards *P. subcapitata* to predict the toxicity of ILs. Thus, this study cannot be compared to any previous QSAR study. Therefore, this model can be one of the reliable models and also can be considered as an alternative to experimental measurements of any ILs toxicity determination towards *P. subcapitata.*

5.2 Recommendation

In future, this study can be further improved by adding more numbers of ILs in the dataset in order to cover wide range of ILs, Thus, can achieve more accurate and reliable prediction model.

REFERENCES

Anastas, P. T. and Warner, J. C., Green Chemistry: Theory and Practice: Oxford University Press, New York, 1998.

Angelov, A. A. 2002. Evolving rule-based models: a tool for design of flexible adaptive systems, Physica-Verlag: A Springer-Verlag Company.

Arning, J., Stolte, S., Boschen, A., Stock, F., Pitner, W.-R., Welz-Biermann, U., Jastorff, B. and Ranke, J. (2008). Qualitative and quantitative structure activity relationships for the inhibitory effects of cationic head groups, functionalised side chains and anions of ionic liquids on acetylcholinesterase, Green Chemistry, 10(1), 47-58.

Bernot, R.J., Brueseke, M.A., Evans-White, M.A., Lamberti, G.A., 2005a. Acute and chronic toxicity of imidazolium based ionic liquids on Daphnia magna. Environ. Toxicol. Chem. 24, 87-92.

Bernot, R. J., Kennedy, E. E., Lamberti, G. A., 2005b. Effects of ILs on the survival, movement, and feeding behavior of the fresh water snail Physa acuta. Environ. Toxicol. Chem. 24, 1759-1765.

Blaise, C.R., 1993. In: Richardson, M.L. (Ed.), Ecotoxicology Monitoring. VCH,Weinheim, pp. 83–108.

Bruzzone, S., Chiappe, C., Focardi, S. E., Pretti, C. and Renzi, M. (2011). Theoretical descriptor for the correlation of aquatic toxicity of ionic liquids by quantitative structure–toxicity relationships, Chemical Engineering Journal, 175(0), 17-23.

Cao, D.S., Liang, Y.Z., Xu, Q.S., Chen, X., 2010. A new strategy of outlier detection for QSAR/QSPR, J. Comput. Chem. 31, 592-602

Cassol, C., Umpierre, A., Ebeling, G., Ferrera, B., Chiaro, S. and Dupont, J. (2007). On the Extraction of Aromatic Compounds from Hydrocarbons by Imidazolium Ionic Liquids, International Journal of Molecular Sciences, 8(7), 593-605

Chatterjee, S. and Hadi, A. S. 2006. Regression Analysis by Example, fourth ed. John Wiley, New York.

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

Cho, C., Pham P. T., T., - Jeon, Y., & - Yun, Y. 2007b- Influence of anions on the toxic effects of ionic liquids to a phytoplankton selenastrum capricornutum - The Royal Society of Chemistry

Cho, C., Jeon, Y., Pham, T. P. T., Vijayaraghavan, K., & Yun, Y. 2008a. The ecotoxicity of ionic liquids and traditional organic solvents on microalga selenastrum capricornutum. Ecotoxicology and Environmental Safety, 71(1), 166-171. doi:10.1016/j.ecoenv.2007.07.001

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

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.

Couling, D.J., Bernot, R.J., Docherty, K.M., Dixon, J.K., Maginn, E.J., 2005. Acessing the factors responsible for ionic liquid toxicity to aquatic organism

via quantitative structure-property relationship modeling. Green Chem, 8, 82-90

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 Chem. 8, 82–90.

Cronin, M. T. D. (2002). The current status and future applicability of quantitative structure-activity relationships (QSARs) in predicting toxicity, Alternative to laboratory animals (ATLA), 30(2), 81-84.

Docherty, K.M., Kulpa, C.F., 2005. Toxicity and antimicrobial activity of imidazolium and pyridinium ionic liquids. Green Chem. 7, 185–189.

Docherty, K. M., Joyce, M. V., Kulacki, K. J., Kulpa, C. F., 2010. Microbial biodegradation and metabolite toxicity of three pyridinium-based cation ILs. Green Chem. 12, 701-712.

Domańska, U., Pobudkowska, A. and Królikowski, M. (2007). Separation of aromatic hydrocarbons from alkanes using ammonium ionic liquid C2NTf2 at T =298.15 K, Fluid Phase Equilibria, 259(2), 173-179.

Earle, M.J., Seddon, K.R., 2000. Ionic liquids. Green solvents for the future. Pure Appl. Chem. 72, 1391–1398

Eiden, P., Bulut, S., Ko chner, T., Friedrich, C., Schubert, T. and Krossing, I. (2010). In Silico Predictions of the Temperature-Dependent Viscosities and Electrical Conductivities of Functionalized and Nonfunctionalized Ionic Liquids, The Journal of Physical Chemistry B, 115(2), 300-309.

Eike, D. M., Brennecke, J. F. and Maginn, E. J. (2003). Predicting melting points of quaternary ammonium ionic liquids, Green Chemistry, 5(3), 323-328.

Fuler, J., Carlin, R.T., Osteryoung, R.A., 1997. The room temperature ionic liquids 1-ethyl-3-methylimidazolium tetrafluoroborate: electrochemical couples and physical properties. J. Electrochem. Soc. 144, 3881–3886.

Fumino, K., Wulf, A. and Ludwig, R. (2008). Strong, Localized, and Directional Hydrogen Bonds Fluidize Ionic Liquids, Angewandte Chemie International Edition, 47(45), 8731-8734.

Garcia, M. T., Gathergood, N. and Scammells, P. J. (2005). Biodegradable ionic liquids Part II. Effect of the anion and toxicology, Green Chemistry, 7(1), 9-14.

Garcia-Lorenzo, A., Tojo, E., Tojo, J., Teijeira, M., Rodriguez-Berrocal, F. J., Gonzalez, M. P. and Martinez-Zorzano, V. S. (2008). Cytotoxicity of selected imidazolium-derived ionic liquids in the human Caco-2 cell line. Sub-structural toxicological interpretation through a QSAR study, Green Chemistry, 10(5), 508-516.

Gardas, R. L. and Coutinho, J. o. A. P. (2008). A Group Contribution Method for Heat Capacity Estimation of Ionic Liquids, Industrial & Engineering Chemistry Research, 47(15), 5751-5757.

Gordon, C.M., 2001. New developments in catalysis using ionic liquids. Appl. Catal. A 222, 101–117.

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

Hossain, M. I., Samir, B. B., El-Harbawi, M., Masri, A. N., Abdul Mutalib, M. I., Hefter, G., & Yin, C. (2011). Development of a novel mathematical model using a group contribution method for prediction of ionic liquid toxicities. Chemosphere, 85(6), 990-994. doi:10.1016/j.chemosphere.2011.06.088

Hossain, M. I. 2012, synthesis, physical properties and toxicity studies of ionic liquids to predict the hazard potential on human and the environment, Ph. D. Thesis, Universiti Teknologi PETRONAS, Malaysia

Huddleston, J.G., Visser, A.E., Reichert, W.M., Willauer, H.D., Broker, G.A., Rogers, R.D., 2001. Characterization and comparison of hydrophilic and hydrophobic room temperature ionic liquids incorporating the imidazolium cation. Green Chem. 3, 156–164.

Irabien, Á., Garea, A., Luis, P., Jacek, J. and Jan, T., Hybrid Molecular QSAR Model for Toxicity Estimation: Application to Ionic Liquids, in 19th European Symposium on Computer Aided Process Engineering - ESCAPE19 2009, pp. 63-67.

Katritzky, A. R., Jain, R., Lomaka, A., Petrukhin, R., Karelson, M., Visser, A. E. and Rogers, R. D. (2002). Correlation of the melting points of potential ionic liquids (imidazolium bromides and benzimidazolium bromides) using the CODESSA program, Journal of Chemical Information and Computer Sciences, 42(2), 225-231.

Karthikeyan, M., Glen, R. C. and Bender, A. (2005). General melting point prediction based on a diverse compound data set and artificial neural networks, Journal of Chemical Information and Modeling, 45(3), 581-590.

Kimaru, I. 6 Dec 2011 < http://www.laboratory-journal.com/science/chemistry-physics/ionic-liquids-odyssey>

Kulacki, K. J. and Lamberti, G. A. (2008). Toxicity of imidazolium ionic liquids to freshwater algae, Green Chemistry, 10(1), 104-110.

Latala, A., Stepnowski, P., N,edzi, M., Mrozik, W., 2005. Marine toxicity assessment of imidazolium ionic liquids: Acute effects on the Baltic algae Oocystis submarina and Cyclotella meneghiniana. Aquat. Toxicol. 73, 91–98.

Lee, S.-M., Chang, W.-J., Choi, A.-R., Koo, Y.-M., 2005. Influence of ionic liquids on the growth of Escherichia coli. Korean J. Chem. Eng. 22 (5), 687–690.

Lewis, M.A., 1995. In: Rand, G.M. (Ed.), Fundamentals of Aquatic Toxicology: Effects, Environment Fate, and Risk Assessment, second ed. Taylor and Francis, Washington, DC, USA, pp. 135–170.

Li, H., Ibrahim, M., Agberemi, I. and Kobrak, M. N. (2008). The relationship between ionic structure and viscosity in room-temperature ionic liquids, The Journal of chemical physics, 129 124507.

Luis, P., Ortiz, I., Aldaco, R. and Irabien, A. (2007). A novel group contribution method in the development of a QSAR for predicting the toxicity (Vibrio fischeri EC50) of ionic liquids, Ecotoxicology and Environmental Safety, 67(3), 423- 429.

Luis, P., Garea, A. and Irabien, A. (2010). Quantitative structure-activity relationships (QSARs) to estimate ionic liquids ecotoxicity EC50 (Vibrio fischeri), Journal of Molecular Liquids, 152(1-3), 28-33.

Martin, Y. C. (2012). Hansch analysis 50 years on. Wiley Interdisciplinary Reviews: Computational Molecular Science, 2(3), 435-442. doi: 10.1002/wcms.1096

Martyn J. Earle and Kenneth R. Seddon, 2000. ILs. Green solvents for the future. The QUILL Centre, Stranmillis Road, The Queen's University of Belfast, Northern Ireland, BT9 5AG, UK

Matsumoto, M., Mochiduki, K., Kondo, K., 2004a. Toxicity of ionic liquids and organic solvents to lactic acid-producing bacteria. J. Biosci. Bioeng. 98, 344–347.

Matsumoto, M., Mochiduki, K., Fukunishi, K., Kondo, K., 200b4. Extraction of organic acids using imidazolium-based ionic liquids and their toxicity to Lactobacillus rhamnosus. Sep. Purif. Technol. 40, 97–101.

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.

Matzke, M., Arning, J., Ranke, J., Jastorff, B. and Stolte, S., Design of Inherently Safer Ionic Liquids: Toxicology and Biodegradation vol. 6: Wiley-VCH Verlag GmbH & Co. KGaA, 2010.

Mills, E. J. (1884). On melting-point and boiling-point as related to chemical composition, Philosophical Magazine, 17(105), 173-187.

OECD, 1992. Fish acute toxicity test. OECD Guideline for testing chemicals, no. 201. Organization for Economic Co-operation and Development.

Olivier-Bourbigou, H., Magna, L., & Morvan, D. (2010). Ionic liquids and catalysis: Recent progress from knowledge to applications. Applied Catalysis A: General, 373(1–2), 1-56. doi:10.1016/j.apcata.2009.10.008

Palomar, J., Torrecilla, J. S., Ferro, . R. and Rodr guez, F. (2008). Development of an a Priori Ionic Liquid Design Tool. 1. Integration of a Novel COSMO-RS Molecular Descriptor on Neural Networks, Industrial & Engineering Chemistry Research, 47(13), 4523-4532.

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(2), 352-372.

Preiss, U. P. R. M., Slattery, J. M. and Krossing, I. (2009). In Silico Prediction of Molecular Volumes, Heat Capacities, and Temperature-Dependent Densities of Ionic Liquids, Industrial & Engineering Chemistry Research, 48(4), 2290-2296.

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 Chem. 8, 238–240

Pretti, C., Chiappe, C., Baldetti, I., Brunini, S., Monni, G., & Intorre, L. (2009). Acute toxicity of ionic liquids for three freshwater organisms: Pseudokirchneriella subcapitata, daphnia magna and danio rerio. Ecotoxicology and Environmental Safety, 72(4), 1170-1176. doi:10.1016/j.ecoenv.2008.09.010

Ranke, J., Muller, A., B-Weber, U., Stock, F., Stolte, S., Arning, j., Stormann, R., Jastorff, B., 2007. Lipophilicity parameters for ionic liquid cations and their correlation to in vitro cytotoxicity. Ecotoxicol. Environ. Safety 67, 430-438.

Ranke, J., Mölter, K., Stock, F., Bottin-Weber, U., Poczobutt, J., Hoffmann, J., Ondruschka, B., Filser, J. and Jastorff, B. (2004). Biological effects of imidazolium ionic liquids with varying chain lengths in acute Vibrio fischeri and WST-1 cell viability assays, Ecotoxicology and Environmental Safety, 58(3), 396-404.

Romero, A., Santos, A., Tojo, J. and Rodríguez, A. (2008). Toxicity and biodegradability of imidazolium ionic liquids, Journal of hazardous materials, 151(1), 268-273.

Samorì, C., Pasteris, A., Galletti, P. and Tagliavini, E. (2007). Acute toxicity of oxygenated and nonoxygenated imidazolium based ionic liquids to Daphnia magna and Vibrio fischeri, Environmental Toxicology and Chemistry, 26(11), 2379-2382.

Samorì, C., Ionic Liquids and their biological effects towards microorganisms, in Ionic liquids: analytical and environmental issues. vol. 15, P. Stepnowski, Ed., ed: Bentham Science Publishers Ltd., 2011, pp. 1888-1904

Stepnowski P, Skladanowski A C, Ludwiczak A, Laczy´nska E, 2004. Evaluating the cytotoxicity of ionic liquids using human cell line Hela. Human & Experimental Toxicology, 23(11): 513–517.

Stock, F., J. Hoffmann, J. Ranke, R. Sto¨rmann, B. Ondruschka and B. Jastorff, Green Chem., 2004, 6, 286–290.

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.

Tochigi, K. and Yamamoto, H. (2007). Estimation of Ionic Conductivity and Viscosity of Ionic Liquids Using a QSPR Model, The Journal of Physical Chemistry C, 111(43), 15989-15994.

Torrecilla, J. S., Palomar, J., Lemus, J. and Rodriguez, F. (2010). A quantumchemical- based guide to analyze/quantify the cytotoxicity of ionic liquids, Green Chemistry, 12(1), 123-134.

Varnek, A., Kireeva, N., Tetko, I. V., Baskin, II and Solov'ev, V. P. (2007). Exhaustive QSPR Studies of a Large Diverse Set of Ionic Liquids: How Accurately Can We Predict Melting Points?, Journal of Chemical Information and Modeling, 47(3), 1111-1122.

Walden, P. (1914). Molecular weights and electrical conductivity of several fused salts., Bulletin of the Russian Academy of Sciences, 8(405-422.

Wasserscheid, P., Keim, W., 2000. Ionic liquids–New ''solutions'' for transition metal catalysis. Angew. Chem. Int. Ed. 39, 3772–3789.

Wells, A. S. and Coombe, V. T. (2006). On the freshwater ecotoxicity and biodegradation properties of some common ionic liquids, Organic Process Research & Development, 10(4), 794-798.

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

Wilkes, J. S.; Levisky, J.A.; Wilson, R.A. & Charles L.H. (1982). Diaklyimidazolium chloroaluminate melts: A new class of room-temperature ionic liquids for electrochemistry, spectroscopy, and synthesis. Inorg. Chem. 21, 1263–1264.

Wilkes, J. S. Green Chem. 2002, 4, 73-80

Wilkes, J. S., 2004. Properties of ionic liquid solvents for catalysis. J. Mol. Catal. A Chem. 214, 11-17.

Xu S.,Nirmalakhandan N., 1998. Use of QSAR models in predicting joint effects in multi-component mixtures of organic chemicals. Civil Agricultural and Geological Engineering Department, New Mexico State University, Las Cruces, NM 88003, USA

Zhao, D., R. Feng, and Y. Guo, "Revisiting Characteristics of Ionic Liquids: A Review for Further Application Development," Journal of Environmental Protection, Vol. 1 No. 2, 2010, pp. 95-104. doi: 10.4236/jep.2010.12012.