Development of QSAR Model for Estimating the Toxicity of Ionic Liquids towards *Vibrio Fischeri* Marine Bacteria

By

Nazeerah Binti Mohd Puaat

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

MAY 2012

Universiti Teknologi PETRONAS
Bandar Seri Iskandar
31750 Tronoh
Perak Darul Ridzuan

CERTIFICATION OF APPROVAL

Development of QSAR Model for Estimating the Toxicity of Ionic Liquids towards *Vibrio Fischeri* Marine Bacteria

by

Nazeerah Binti Mohd Puaat

A project dissertation submitted to the
Chemical Engineering Programme
Universiti Teknologi PETRONAS
in partial fulfilment of the requirement for the
BACHELOR OF ENGINEERING (Hons)
(CHEMICAL ENGINEERING)

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.

NAZEERAH BINTI MOHD PUAAT

ABSTRACT

ILs are liquids composed entirely of ions where they are salts with melting point lower than 100°C. Recently, Ionic Liquids (ILs) have received much attention from the scientific community. The use of ILs has been increasing rapidly in broad range of application segment such as chemical reaction, separation process, and many more. Despite their high interest and extraordinary properties, the toxicity of ILs towards aquatic environment needs to be evaluated. This is because, ILs can easily dissolve in water, and this may lead to aquatic pollution and related risks. Toxicity of ILs can be measured experimentally. Indeed, the biological effects of a number of ILs have recently been reported on various species such as bacterium, aquatic organisms and cells. However, due to the large number of ILs, it would be very costly and time consuming to measure the ILs' toxicity using experimental work. Thus, it is necessary to develop mathematical models that can be used to estimate the toxicity of ILs by means of Quantitative Structure Activity Relationship (QSAR). In this work, toxicity of ILs towards *Vibrio Fischeri* marine bacteria is estimated by developing a new QSAR model. A dataset was constructed using effective concentration (EC_{50}) for Vibrio Fischeri marine bacteria collected from different literature and published data. The data was then analysed using summed contributions from the cations, alkyl substitution, and anions. The model employed Multiple Linear Regression (MLR) and polynomial models and the code is written using MATLAB programming language. The result shows that the model is capable of predicting the ILs toxicities accurately, where R^2=96.83% with an average absolute deviation error 4.96%. The model describes and predicts the considered EC_{50} values better than other published models and 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.

ACKNOWLEDGEMENT

First of all, I express my profound gratitude to the Almighty Allah, the most Gracious and the most Benevolent who enables me strength and opportunity to perform this research work within the stipulated time.

I would like to acknowledge and extend my heartfelt gratitude to my respected supervisor Dr. Mohanad El-Harbawi, for his guidance and supervision, during the entire period of this research work and in the preparation of this dissertation. Without his contribution, inspiration, consistent suggestions and constant supervision, it would have been difficult to complete and present this research dissertation. My grateful thanks also go to Mr. M. Ismail Hossain who helped me in doing ILs fragmentation. I am also thankful to Dr. Brahim Belhaouari Samir of the Department of Applied and Fundamental Science who helped me in developing the codes in MATLAB software.

Besides, I would like to thank the management of Universiti Teknologi PETRONAS (UTP) for providing me with a good environment and facilities to complete this project. Also, I would like to take this opportunity to thank to Department of Chemical Engineering of UTP for offering this subject, Final Year Research Project and all Chemical Engineering lecturers for the constant reminder and much needed motivation. It gave me opportunity to explore new thing in Chemical Engineering field.

An honourable mention goes to my families and friends for their understandings and supports in completing this project. Without helps of the particular mentioned above, I would face many difficulties while doing this project.

Last but certainly not the least, I wish everyone the very best and continue to provide excellent guidance and support for innovative research in the future. Thank You.

TABLES OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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

IL – Ionic Liquid

MLR – Multiple Linear Regression

MATLAB – Matrix Laborotary

R^2 - Correlation Coefficient

QSAR – Quantitative Structure Activity Relationship

QSPR – Quantitative Structure Property Relationship

CHAPTER 1

INTRODUCTION

1.1 Background of study

Generally, ILs are liquids composed entirely of ions where they are salts with melting point lower than 100°C. The asymmetry of at least one of the ions, low intermolecular attraction, and the presence of ionic attraction are the reason for their melting points lower than 100°C (Luis et al., 2006). ILs have been the subject of increasing attention due to their unique physicochemical properties such as high thermal stability, large liquid range, high ionic conductivity, high solvating capacity, negligible vapour pressure, and non-flammability that make them ideal solvents for green chemistry (Wasserscheid and Welton, 2009). They are often referred to as designer solvents because of their structure and ability to be modified and produce a set of desired properties (Earl and Seddon, 2000) for a specific purpose, such as solvents, catalysts, extraction process, and electrochemistry.

Theoretically, there are well over one million ILs which can be synthesized, though only about one thousand have been reported to date (Luis et al. 2007). Despite the attractive properties of ILs, due to their often high solubility in water, the future possible industrial discharge of spent or waste ILs into natural water bodies such as sea may have significant toxicological impact to the aquatic organisms (Hossain et al., 2011). Thus there is the necessity to evaluate the ecological risks posted by ILs towards the aquatic environment.

Aquatic organisms are the first recipient of any toxic substances generated by industries due to its wastewater discharge into the sea. As ILs are soluble in water, they may cause a large impact on the aquatic bodies. *Vibrio fischeri* marine bacteria, one of the aquatic organisms, are bioluminescent bacteria. It is found predominantly in symbiosis with marine animal, and survive in decaying organic matter, thus making it suitable to be one of the testing species for ecotoxicology. It is necessary to measure the toxicity of ILs towards aquatic organisms because it may give impact to

the survival, growth, reproduction and behaviour of the organisms. It is also important to provide an easy way to predict the toxicity of ILs towards *Vibrio fischeri* marine bacteria due to rapid increase of the used of ILs in industries. The result from this study can be beneficial not only to industrial people who used ILs in their industrial processes, but also beneficial in keeping the aquatic environment clean and safe.

Generally, toxicity is the exposure concentration of the chemicals to the environment which can kill the population of the certain organisms. Toxicity is usually expressed by Effective Concentration (EC_{50}), Lethal Concentration (LC_{50}) or Inhibitory Concentration (IC_{50}). A general concept of toxicology is that effects are dose-dependant. The dose of the chemicals is defined as mgL^{-1}, mM, μM or ppm. Toxicity of ILs can be measured using experiment. Indeed, the biological effects of a number of ILs have recently been reported on various species such as bacterium, aquatic organisms and cells. However, such experimental investigations are time consuming and require significant physical and chemical resources. Since there are very large numbers of ILs, experimental investigations may not be the convenience approach to evaluate the toxicities of ILs towards *Vibrio fischeri* marine bacteria. There is, therefore, a need to provide a faster and more cost-effective approach to assess the aquatic toxicity of ILs.

Quantitative Structure-Activity Relationship (QSAR) method was first developed in nineteenth century by E. J. Mills, (1884) who proposed a quantitative structure-property relationship (QSPR) models to predict melting points and boiling points of homologous series. Nowadays, QSAR method has been widely used in predicting various properties or activities. In this work, new mathematical models have been developed that expresses the toxicities (EC_{50} values) of a wide variety of ILs towards bioluminescence bacteria *Vibrio fischeri*, by means of QSAR method. This model afford a practical, cost-effective and convenient alternative to experimental toxicological assessments of many ILs.

1.2 Problem statement

Over the past decade, ILs which can be considered as green solvent have become very popular and are being used vastly in industries mostly to replace the conventional solvents. Due to their high solubility in water, it is important to evaluate the toxicity of ILs so that future possible industrial discharge of spent ILs into the sea will not pollute the aquatic environment and bring no harm to the population of aquatic organisms. Experimental work is the standard procedure to evaluate the toxicological risks of ILs but it is time consuming, requires specific chemical and physical resources, and using costly apparatus. Currently, there are several (QSAR) models that have been developed to estimate the toxicity of ILs towards *Vibrio fischeri* marine bacteria and other aquatic organisms. However, these models were developed based on dataset that have limited number of ILs, thus accuracy of the developed model are not very high. For this reason, the proposed model can enhance the accuracy.

1.3 Research objectives

This project focuses to develop a novel mathematical model as an alternative to experimental assessments which are more practical, faster, and cost-effective approach to predict the toxicity of ILs.

The objectives of this project are;

I. To develop a new mathematical model using QSAR approach for prediction of toxicity of ILs based on the up to date existing data published towards *Vibrio fischeri* marine bacteria.

II. To validate the results from the developed model with other results published by established models.

1.4 Scope of study

There is a necessity to study the toxicological effect posted by various ILs used in industries. Toxicity of ILs normally measured using experimental works. However, such experimental measurements are time-consuming, costly, and require various

physical and chemical resources. Therefore, this study intends to develop a mathematical model that can be used to estimate the toxicity of various ILs towards *Vibrio fischeri* marine bacteria. This is the more practical, cost-effective, and faster approach to assess the aquatic toxicity of ILs. This is theoretical work and no experimental work is involved in this project. In order to develop the model, the ILs data that had been gathered from various literatures need to be analysed by fragmenting all the ILs components based on the cations, anions, and the alkyl substitutions. Based on the constructed dataset, the model can be developed using MATLAB software. Familiarization of MATLAB software is needed in order to write the code correctly thus developing a good model that can produce the best results.

Tools required in this project are Microsoft Excel 2007 to develop the dataset and MATLAB 7.8.0.347 software to write the code.

1.5 Relevancy and feasibility of the project

This project is relevant since it is related to student background of study which is Chemical Engineering. The knowledge of Organic Chemistry is very much applied in order to do data fragmentation of the ILs structure. The knowledge of Engineering Mathematics and Probability and Statistics is also applied in developing the code for MATLAB software. Study of ILs is also one of the fields that are emphasized by Chemical Engineering department. This project will contribute a lot in the field of ILs and Health, Safety, and Environement (HSE) field since it will provide alternatives to the experimental work in order to determine the toxicity of ILs.

This project is a theoretical based project and did not require any laboratory work. The model is developed based on toxicity data collected from published literature by using MATLAB software. Thus, this project is feasible within the scope and time frame.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This chapter provides a review on the related research work in the field of toxicity of ILs done by other researcher. The review includes experimental work as well as theoretical work.

ILs are composed of ions only and they are fluid below 100°C due to asymmetry of one of the ions. They are thermally stable with liquid range up to 300°C compared to 100°C for water and show very low vapour pressure, being of great interest due to these properties. ILs typically consist of bulky organic cations, such as imidazolium, pyridinium, ammonium, phosphonium, etc. paired with various anions, such as hexaflurophosphate (PF_4^-), bromide (Br^-), etc (Pernak et al., 2005). Currently, there were millions of ILs have been synthesized and commercially available. Although intensive information and data regarding IL's physical and thermodynamic properties have been reported and continuously published, only limited data with regard to the toxicity and ecotoxicity of ILs'were reported. Several papers that have been reported on toxicity of ILs were reported by Bernot et al., (2005), Latala et al., (2005), Pretti et al., (2006), etc.

In general, the toxicity of chemicals can be measured using standard experimental procedure. Indeed, the biological effects of a number of ILs have been recently been reported on various aquatic organisms such as marine bacterium *Vibrio fischeri* (Ranke et al., 2004), algae (Cho et al., 2008), the freshwater crustacean Daphnia magna (Bernot et al., 2005a), the freshwater snail Physa acuta (Bernot et al., 2005b) and many more. Although experimental study can give the toxicity or value of EC_{50} (effective concentration where 50% of maximum effect detected) for ILs tested, however, such experimental investigations are time consuming and require some chemical and physical resource. In principle, there are well over one million ILs which can be synthesized, though only about one thousand have been reported to

date (Luis et al., 2007). Therefore, there is a need to provide a faster and more cost effective approach to evaluate the toxicity of ILs. The used of mathematical model that can expresses the toxicities (EC50 values) of a wide variety of ionic liquids (ILs) towards aquatic organisms by means of a quantitative structure activity relationship (QSAR) is one of the alternative to experimental assessment.

2.2 Ionic Liquids (ILs)

The field of ILs was started by Paul Walden with his report on the physical properties of ethylammonium nitrate ([EtNH3][NO3]; mp 13–14 uC) which was formed by the neutralisation of ethylamine with concentrated nitric acid in 1914 (Plechkova & Seddon, 2008). The ever increasing interest in ILs grows from their interesting physical properties and the simplicity by which they can be adapted for a specific system. The most common cations consist of imidazolium, pyrrolidinium, pyridinium, tetraalkylammonium, and tetraalkylphosphonium. Billions of different structures of ILs can be synthesized based on the combination of cations and anions. Some cation and anion combinations comprising the main roots of the ILs are shown in Figure 1.

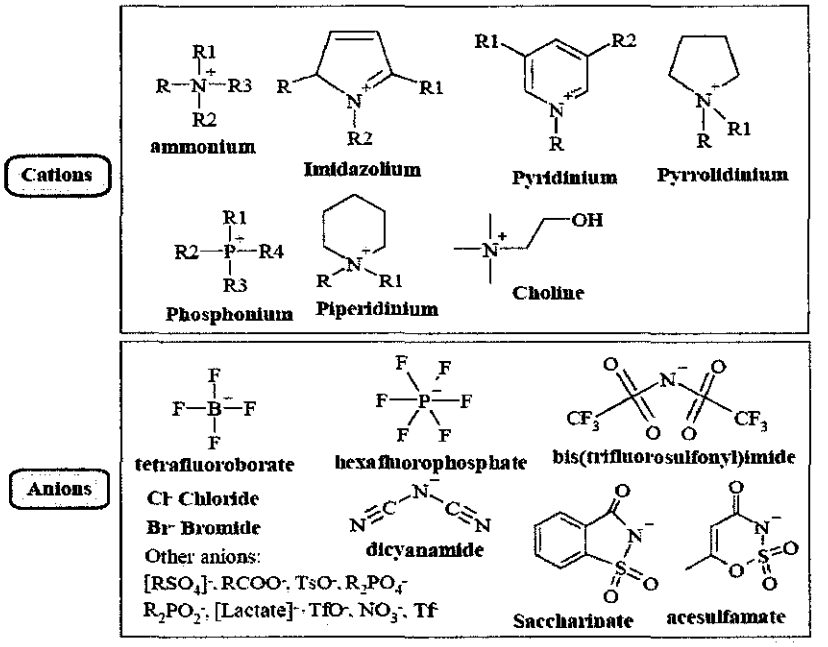


Figure 1: Examples of cation and anion pairs used to form the ILs.

According to the variety of ILs, they can be classified as seen in Figure 2, although it can't give an actual idea of all the cations and anions of the ILs' world.

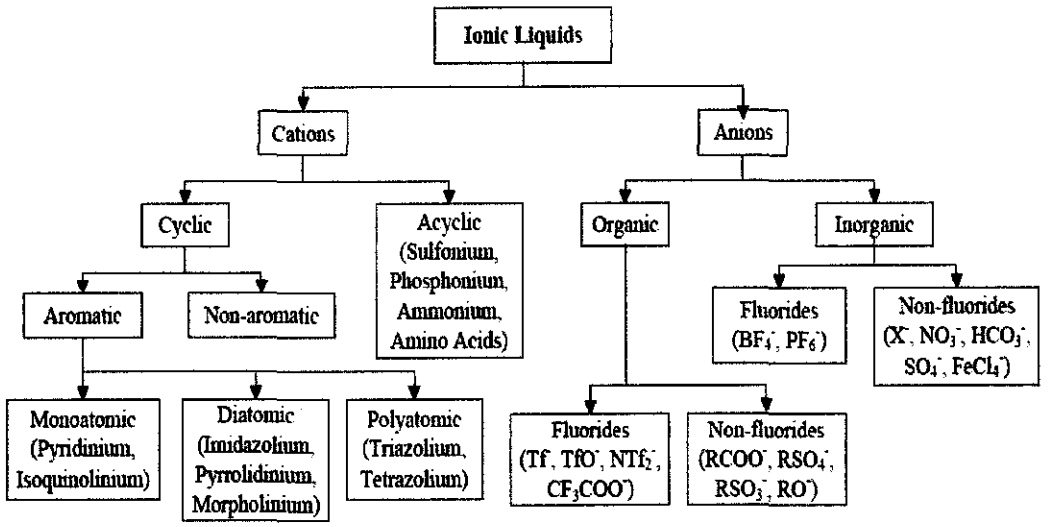


Figure 2: Classification of ILs (Hossain, 2012).

ILs are gaining strong interests from both academic and industrial fields due to its unique properties. ILs also show high capability to dissolve a wide variety of materials; including salts, fats, proteins, amino acids, surfactants, sugars, and polysaccharides as well as organic molecules including crude oil, inks, plastic, and DNA in a large liquidus range of about 300 °C (Hossain, 2012). The chemical and physical properties of ionic liquids can be tuned by selecting a certain anion and cation combination (Ranke and Jastorff, 2000). ILs have low melting point and sometimes the melting point of ILs can be far below 0 °C (Cho et al., 2008). Furthermore, unlike volatile organic compounds, ILs does not evaporate, but they decompose at high temperatures. The decomposition temperature depends on the IL, and particularly on the anion (Anastas and Warner, 1998). The properties of ILs have been extensively studied by so many researchers and they conclude that their unique properties have drawn attention into using ILs as replacement for other volatile organic compounds. The most important properties of ionic liquids are: thermal stability, low vapour pressure, electric conductivity, tunable solubility, liquid crystal structures, high electroelasticity, high heat capacity and inflammability. These properties enable the use of ionic liquids in a wide range of applications, as shown in Fig. 3 (Romero et al. 2008)

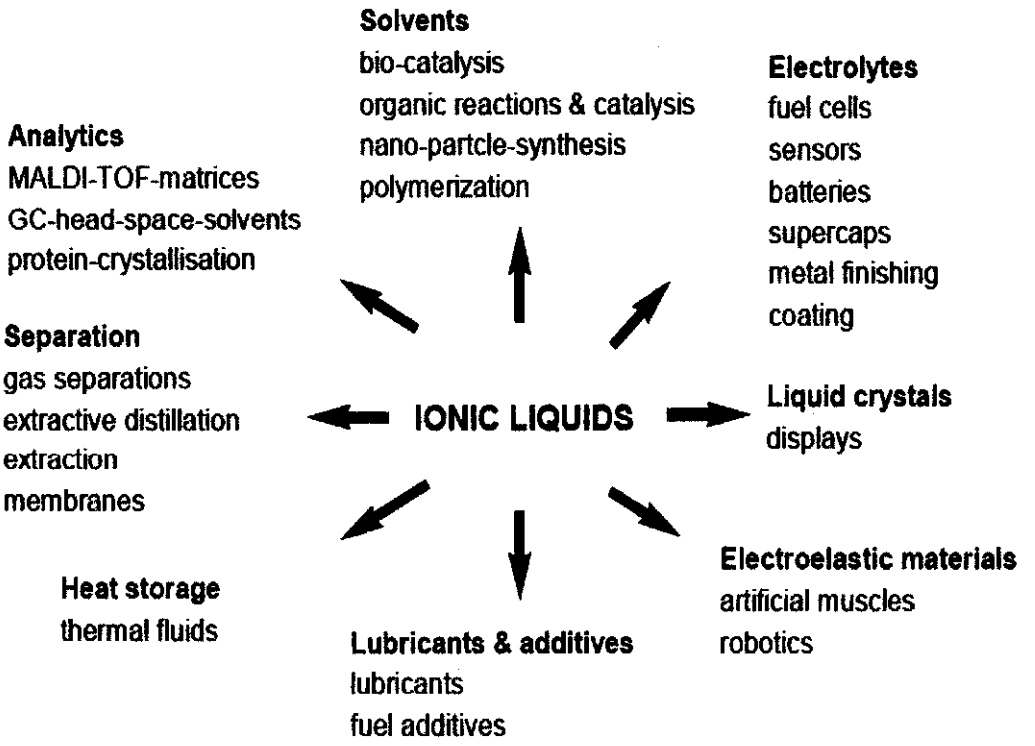


Figure 3: Application of ILs

2.2.1 Toxicity of ILs towards aquatic organisms

According to Society of Toxicology, toxicology is the study of the adverse effects of chemical, physical or biological agents on living organisms and the ecosystem, including the prevention and amelioration of such adverse effects. Generally, the survival of human beings depends on the survival of other species (plants and animals alike); on the availability of clean water, air, and soil; and on the availability of energy (Zakrzewski, 2002). The release of chemicals into the environment can resulted in global impacts. Large exposures of chemicals can disrupt the ecological systems that exist in rivers, lakes, oceans, streams, wetlands, forests and fields thus affecting human health. Figure 4 below described some potential routes in assessing the chemical exposure to the public.

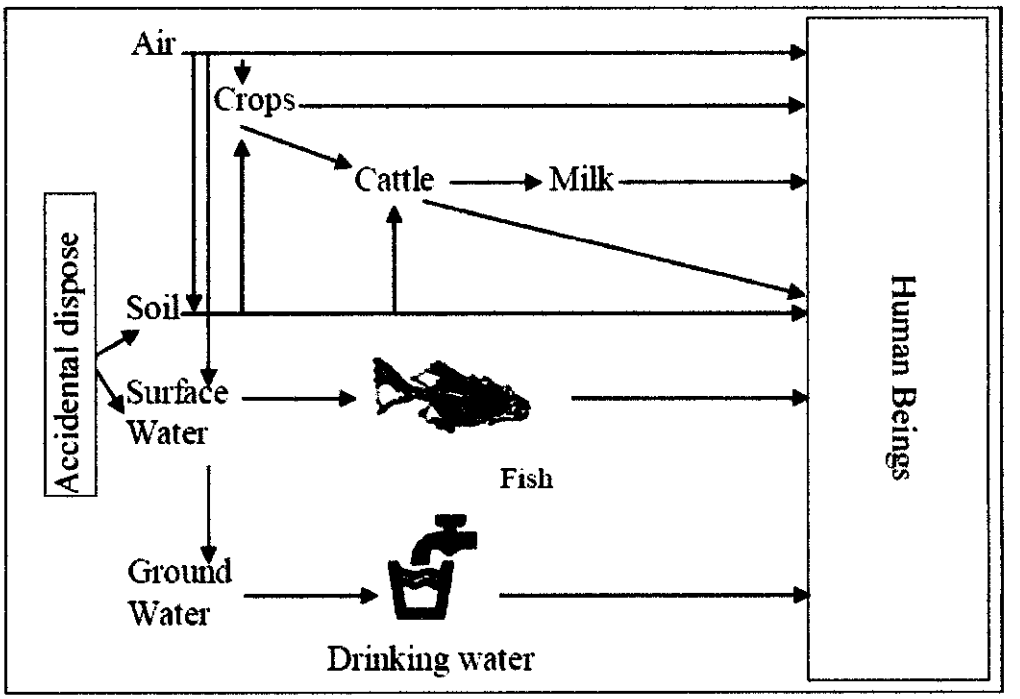


Figure 4: potential routes in assessing the chemical exposure to the public

Aquatic toxicity assessment is the study of the effects of the chemicals and other anthropogenic and natural materials and activities on aquatic organisms at various levels of organization and ecosystems. Generally, most toxic substances generated by industrial, agricultural, and domestic activities are released often into the aquatic environment. Thus, aquatic organisms are the first recipient of most of these toxic substances. As ILs are soluble in water, they cause a large impact on aquatic bodies. The management of chemical compounds entering into water resources is difficult because contaminants can enter into the aquatic system from a multiple of diffuse sources. Although aquatic ecosystems are pliable with different physical, chemical and biological mechanisms by which the chemicals may be assimilated without serious implications for endemic biota, when contaminants reach levels in excess of the assimilative capacity of the receiving waters, they may affect the survival, development, growth, reproduction or behavior of organisms (Pieraccini et al., 2007).

2.2.1.1 Experimental studies of ILs Toxicity

A series of experiment that study the toxicity of ILs towards aquatic organisms have been performed previously by the researcher. (Docherty and Kulpa, 2005) reported on the toxicity of ILs towards *Vibrio fischeri* marine bacteria where the experimental result shown that an increase in alkyl group chain length as well as an increase in the number of alkyl groups substituted on the cation ring corresponded with an increase in toxicity. The study of the toxicity of ILs were conducted towards many types of species such as marine bacterium *Vibrio fischeri* (Ranke et al., 2004), algae (Cho et al., 2008), the freshwater crustacean Daphnia magna (Bernot et al., 2005a), the freshwater snail Physa acuta (Bernot et al., 2005b) and many more. Recently, (Ventura et al., 2012) reported about the toxicity of five-guadinium, six-phosphonium, and six-imidazolium based ILs towards *Vibrio fischeri*. The experimental results have shown that guadinium, unlike the imidazolium and phosphonium based ILs, do not follow the trend of increasing toxicity with the increase in the alkyl chain length.

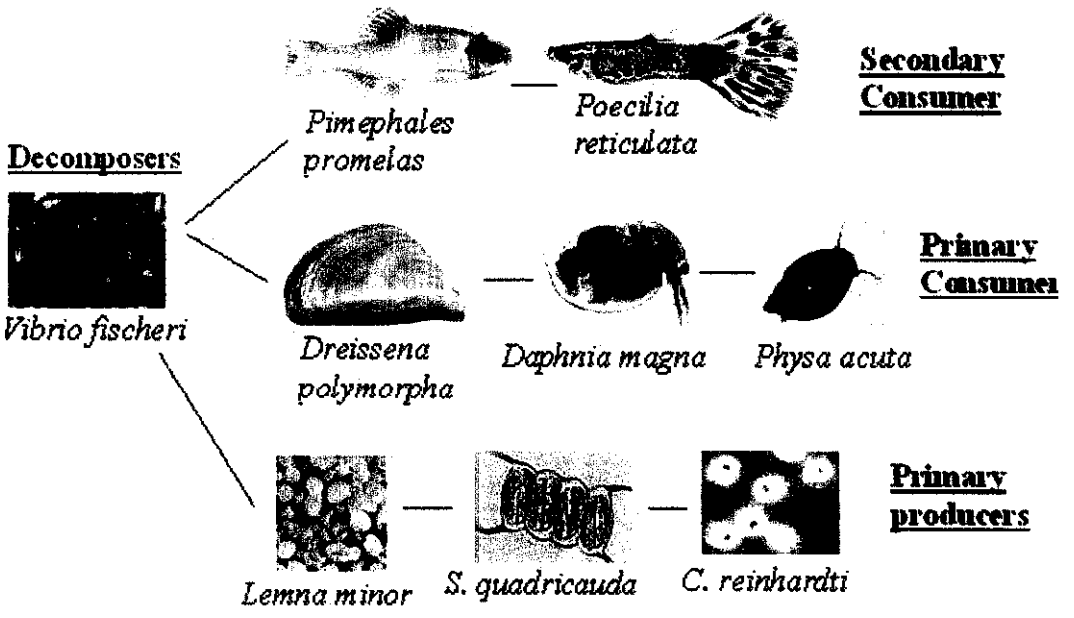


Figure 5: A simple aquatic food web with example members whose ILs toxicity is studied (Kulacki et al., 2007).

It is proved that experimental study can give the value of EC50 (effective concentration where 50% of maximum effect detected) for every single ionic liquid

tested. However, such experimental investigations are time consuming and require some chemical and physical resource. Therefore, there is a need to provide a faster and more cost effective approach to evaluate the toxicity of ILs.

2.2.1.2 Quantitative Structure Activity Relationship (QSAR)

Developing a mathematical model to predict the toxicity of ILs by means of Quantitative Structure Activity Relationship (QSAR) is one of the best alternatives to the experimental assessment. Basically, QSAR method was first developed in nineteenth century by E. J. Mills (Mills,1884), who proposed a QSPR models to predict melting points and boiling points in homologous series. Previously, several QSAR models have been developed by other researcher for prediction of several physical properties such as melting point, density, viscosity. This method was then applied to predict the toxicity of ILs by the several researchers. For example, Couling et al. (2006) proposed QSAR models for two aquatic organism *Vibrio fischeri* and *Daphnia magna*. They were capable to prove their models can follow the relevance between side chain length and toxicity for imidazolium, pyridinium and quarternary ammonium compounds, the anion plays a secondary role regarding to the toxicity. The models can predict the toxicity with an accuracy $R^2 = 0.78$-0.88.

Luis et al., (2007) introduced the group contribution method for estimating the toxicity of ILs by means of a QSAR model on *Vibrio fischeri*. They divided the ILs molecules into nine different fragments (three cations, three anions and three substituents) and applied the contributions to establish the model using the Polymath 5.0 software. The model can predict the toxicity with an accuracy of $R^2 = 0.925$ for 43 ILs. The observed and calculated dimensionless ecotoxicity data has also been plotted.

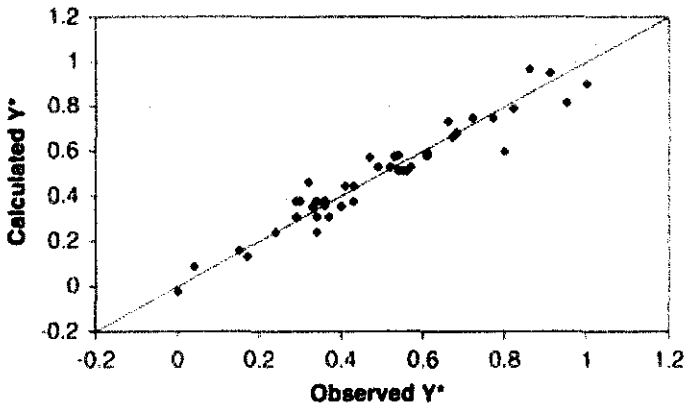


Figure 6: Observed and calculated dimensionless ecotoxicity data plot (Luis et al, 2005)

Later on, Luis et al., (2010) extended the list of ILs for 96 ILs and applied similarly to divide them into fifteen different fragments. The model was developed by using Polymath 5.0 software and can predict the toxicity with accuracy $R^2 = 0.924$.

Garcia-Lorenzo et al. (2008) developed a QSAR model using the topological sub-structural molecular design (TOPS-MODE) approach on the CaCo-2 Carcinoma colon cell line for imidazolium based ILs to predict the effect intensity of side chain and anion.

Torrecilla et al. (2009) designed four mathematical approaches to estimate the toxicity of ammonium, imidazolium, morpholinium, phosphonium, piperidinium, pyridinium, pyrrolidinium and quinolinium ILs in Leukemia rat cell line and acetylcholinestearase enzyme by multiple linear regression (MLR), radial basis network (RB), multilayer perception (MLP) and neural network (NN) models. The empirical formula and molecular weights of 153 ILs were calculated and used for their toxic prediction using MATLAB software.

Katritzky et al. (2001) developed QSAR models for the prediction of aqueous toxicities for *Poecilia reticulata* (guppy) using the CODESSA treatment. Experimental LC_{50} (and log P) values from the literature for 293 compounds were divided into 4 groups based on the structure and a QSAR model was obtained for each.

Other than that, Guerra and Irabien, (2011) have reported on the new approach for estimating the ecotoxicity of ILs, based on the standardised assay with bacterium

Vibrio fischeri by means of the application of Partial Least Squares-Discriminant Analysis (PLS-DA). This model makes it possible to discriminate ILs, formed by combinations of 30 anions and 64 cations, on the basis of their expected toxicity with respect to conventional solvents that they may replace. This model is a technical approach that can be used for screening purposes during early stages of development of ILs for specific tasks.

Hossain et al., (2011) had also reported a QSAR model that had been developed to estimate the toxicity of ILs towards daphnia magna aquatic species. Instead of using Polymath 5.0, this model employed Multiple Linear Regression (MLR) analysis with polynomial model, developed by using MATLAB software. The contributions of substituents, cations, and anions towards the toxicity percentage have also been observed. This model has achieved high value of R^2 which are comparable to other QSAR models developed by other researcher.

CHAPTER 3

METHODOLOGY

3.1 Material and Methods

3.1.1 Data Collection

A bioluminescent bacterium, *Vibrio fischeri*, has been used to develop the QSAR model using MATLAB software. The toxicity data used to develop the model can be gathered from published literature. These data can be found in the work of Kaiser and Palabrica (1991), Ranke et al. (2004), Docherty and Kulpa (2005), Garcia et al. (2005), Couling et al. (2006), Matzke et al. (2007), Stolte et al.(2007), Luis et al. (2007), Samori et al. (2007), Romero et al. (2008), Luis et al. (2010), Ventura et al. (2010), Papaiconomou et al. (2010), Alvarez & Irabien (2011), Sylvie et al. (2012) and from the "UFT/Merck Ionic Liquids Biological Effects Database" (http://www.il-eco.uft.uni-bremen.de/). The list of 217 ILs toxicities studied in this work covers a wide range of alkyl-substituted cations based on imidazolium, pyridinium, pyrollidinium and so on with anions including hexafluorophosphate, tetrafluoroborate, dicyanamide and so forth.

3.1.2 Dataset preparation

The first step in preparing the dataset is to design the 'ILs structure table' based on the molecular fragments (groups) (description of molecular structure of ILs compounds). Next is to find the molecular structures of all these pure compounds. This dataset was prepared manually. Table 3 shows the dataset that have been prepared. The ILs chemical structure was fragmented according to its anion, cation, and alkyl substituents. ILs structure table will be used as a dataset 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. Figure 7 shows some of the descriptors used in the

dataset while figure 7 shows some of the chemical structures of the ILs. Table 1 describes example on how the ILs structure was fragmented.

Fragments name	Structure	Fragments name	Structure
Substituents			
$-CH_3$	Methyl	-Bz	Benzyl group
$>CH_2$	Methylene	-OH	hydroxyl
$\equiv CH$	Methyne	-CN	nitrile
$-NCH_3$	Methyl group attached with nitrogen atom	$-SCH_3$	Methyl group attached with sulfur atom
$-NCH_2-$	Methylene group attached with nitrogen atom	$-SCH_2-$	Methylene group attached with sulfur atom
$-OCH_3$	Methyl group attached with oxygen atom	$-PCH_2-$	Methylene group attached with phosphorous atom
$-OCH_2-$	Methylene group attached with oxygen atom		
IL families			
Imida	Imidazolium	Morp	Morpholinium
Pyrid	Pyridinium	Phos	Phosphonium
Ammo	Ammonium	Pip	Piperidinium
Pyrrol	Pyrrolidinium	Quin	Quinolinium
Thio	Thiophenium		
Anions			
Br^-	Bromide	SCN	Thiocyanide
Cl^-	Chloride	TFO/CF_3SO_3	Trifluoromethylsulfonate
I^-	Iodide	For	Formate
BF_4^-	Tetrafluoroborate	RSO_4	Alkyl sulfate
PF_6^-	Hexafluorophosphate	$N(CF_3SO_2)_2^-$	Alkanoate
TF_2N^-	Bis(trifluoromethylsulfonyl) imide. $(CF_3SO_2)_2N$	R in anion	Alkyl group in anion (R=0 indicates only H)
$N(CN)_2$	Dicyanamide	$FeCl_4^-$	Tetrachloroferrate(III)
Ace-	Acetate	TOS	Tosylate
Cap-	Caprylate	$(CN)_4B^-$	Tetracyanidoborate

Figure 7: Some of descriptor used in dataset

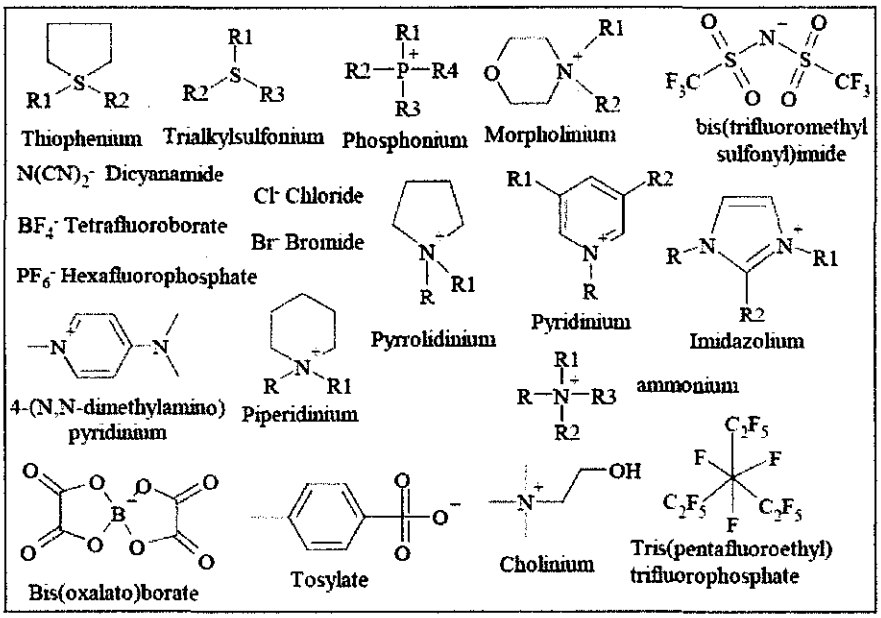
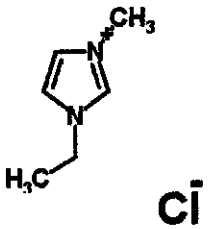
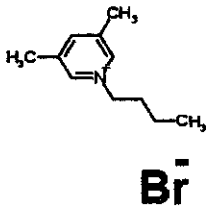


Figure 8: Some of chemical structures of ILs together with substitution groups used in the group contribution method

Table 1: Example of ILs fragment

No	Full Name of Compound	Compound	Structure	Fragment
1	Ethylmethylimidazolium Chloride	$[C_2MIM][Cl]$		$-CH_3$ =1 $-NCH_3$=1 $-NCH_2$=1 imida=1 Cl^-=1
2	Butyldimethylpyridinium Bromide	[C4MMPy][Br]		$-CH_3$ =3 $-NCH_2$=1 -CH2=2 Pyrid=1 Br^-=1

3.1.3 Model Development

In this work, the dataset that has been developed was utilized to develop a new model 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 MRL 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 Fig. 9. The accuracy of the develop model is confirm using the average relative deviation (ARD) (Eq. 5), average absolute relative deviation (AARD) (Eq. 6), and average absolute error (AAE) (Eq. 7) between the predicted values of toxicities and the experimental data.

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

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

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

where,

N is the number of the substances,

Φ_{Cal} is the calculated values, and

Φ_{Exp} is the experiment values.

The selection of significant coefficients based on the descriptors, which construct a relationship between the toxicity of each IL and its molecular structures, is an important step in QSAR modelling. For this purpose, multiple linear regression method plus polynomial model were used to determine the important coefficients based on functional group contributions as given by the Eq. 4 and the calculations were performed using MATLAB programming language. The code was written using a 80%-20% training-predicting split. In the dataset, 174 from 217 data were used for training process; while the rest of the data were used for prediction process. In the training process, the code trained the data to obtain the optimal coefficients based on the minimum square error between the toxicities predicted and experimental values. In order to compress the number of coefficients without decreasing the accuracy of the model, R^2, hypothesis testing was implemented in the code. The most significant coefficients have been used in Eq. 4 to predict the toxicities.

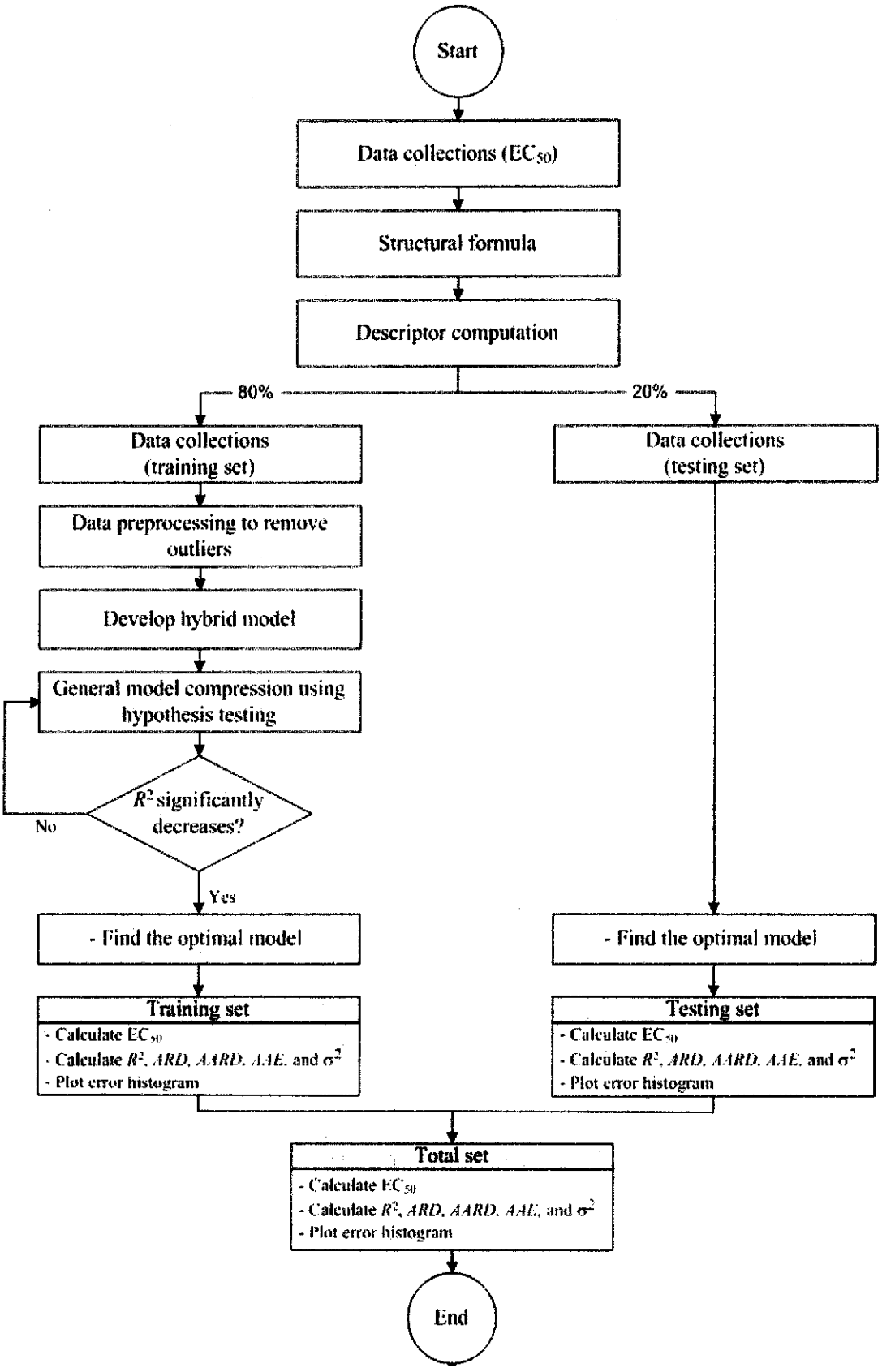


Figure 9: Algorithm for estimation of IL densities using MATLAB 7.8.0.347

3.2 Gantt chart and key milestones

Gantt chart for activities planned along for this final year projects first semester is shown in Figure 8 and suggested milestones

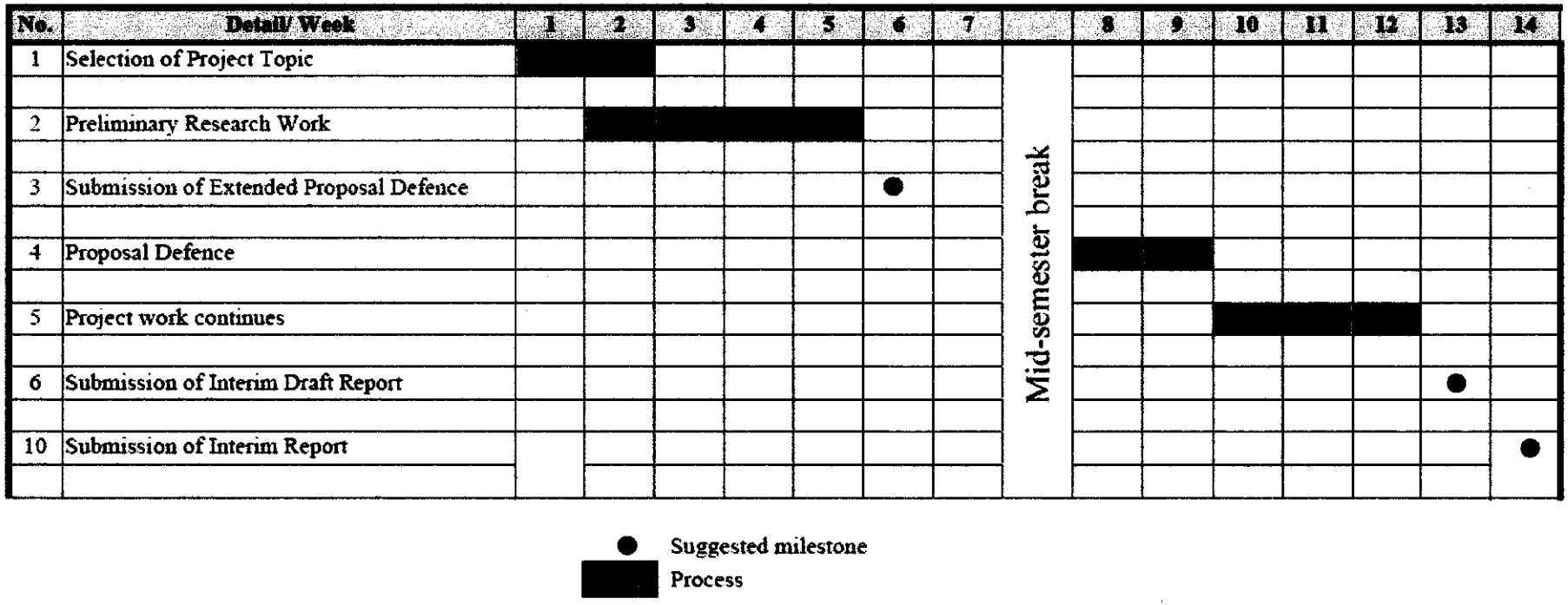


Figure 10: Gantt chart for final year project first semester (FYP 1)

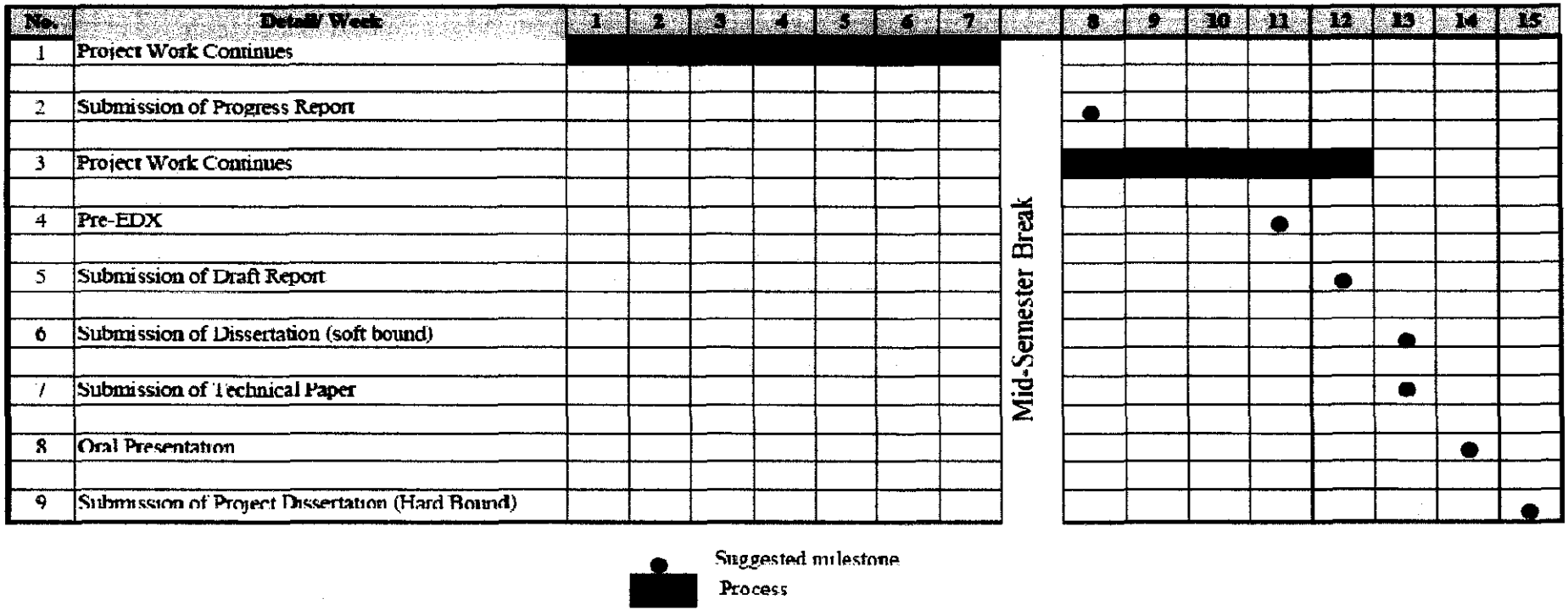


Figure 11: Gantt chart for final year project second semester (FYP 2)

CHAPTER 4

RESULT AND DISCUSSION

The developed models were initially trained with the training data set using least square error method. MATLAB code utilized expermintal data (80% of the whole dataset) for training the code and then to calculate the coefficients of the proposed model ($\alpha_i, \gamma_i, \delta_i, \lambda_{i,j}$) in Eq. (5). Then MATLAB code used the remaning data (20% of the whole dataset) with the obtained coefficients from the training data set to test how good is the model has been trained and then how accurate the model can predict the results. It should be noted that the test set is not used during training process, it is only used to compare the predicted results. For the development of toxicity model, 174 components were utilized for training set and 43 were used for testing set.

The data set of ILs toxicities towards *Vibrio fischeri* marine bacteria is shown in Figure 4.1. Listed anions were Trifluoroacetate (TFA^-), Acetate (Ace^-), Caprylate (Cap^-), Sulfate ($SO4^-$), Bis[1,2-benzenediolato(2-)]borate [(2-OPhO)2B^-], and many more. Cations included imidazolium, pyridinium, ammonium, phosphonium, pyrrolidinium, and many more. Substituent groups included –CH3, –CH2–, –NCH3, –NCH2–, and many more. Table 3 shows the dataset developed.

Table 2: Dataset of IL toxicities on *Vibrio fischeri*.

No.	Compound	Full name of compound	Exp. Log EC50 (µmol/L)	Pred. Log EC50 (µmol/L)	References
1	[IM][TFA]	Imidazolium Trifluoroacetate	4.07	3.18	Luis et al., (2010)
2	[IM][Ace]	Imidazolium Acetate	3.61	3.61	Luis et al., (2010)
3	[IM][TfO]	Imidazolium Trifluoromethane sulfonate	3.81	3.81	Luis et al., (2010)
4	[IM][Cap]	Imidazolium Caprylate	2.29	3.18	Luis et al., (2010)
5	[C1IM][TFA]	Methylimidazolium Trifluoroacetate	3.57	3.57	Luis et al., (2010)

Table 2 (cont)

6	[C1IM][Ace]	Methylimidazolium Acetate	4.07	4.07	Luis et al., (2010)
7	[C1IM][TfO]	Methylimidazolium Trifluoromethanesulfonate	4.35	3.24	Luis et al., (2010)
8	[C1IM][Cap]	Methylimidazolium Caprylate	2.13	3.24	Luis et al., (2010)
9	[C1IM][For]	Methylimidazolium Formate	2.83	2.83	Luis et al., (2010)
10	[MIM]	Methylimidazolium	0.16	0.16	Docherty and Kulpa, (2005)
11	[C1MIM][MetSO4]	Methylmethylimidazolium Methylsulfate	4.76	4.76	Romero et al., (2008)
12	[C2MIM][EtSO4]	Ethylmethylimidazolium Ethylsulfate	4.02	4.02	Romero et al., (2008)
13	[C2MIM][(2-OPhO)2B]	Ethylmethylimidazolium Bis[1,2-benzenediolato(2-)]borate	3	3	Matzke et al., (2007)
14	[C2MIM][Cl]	Ethylmethylimidazolium Chloride	4.55	4.4503	Luis et al., (2007)
15	[C2MIM][Cl]	Ethylmethylimidazolium Chloride	4.33	4.4503	Stolte et al., (2007)
16	[C3MIM][BF4]	Propylmethylimidazolium Tetrafluoroborate	3.94	3.5091	Ranke et al., (2004)
17	[C4IM][TFA]	Butylimidazolium Trifluoroacetate	3.28	3.28	Luis et al., (2010)
18	[C4IM][Ace]	Butylimidazolium Acetate	3.32	3.32	Luis et al., (2010)
19	[C4IM][TfO]	Butylimidazolium Trifluoromethanesulfonate	2.77	2.77	Luis et al., (2010)
20	[C4IM][Cap]	Butylimidazolium Caprylate	2	2	Luis et al., (2010)
21	[C4IM][For]	Butylimidazolium Formate	3.19	3.19	Luis et al., (2010)
22	[C4MIM][PF6]	Butylmethylimidazolium Hexafluorophosphate	3.07	3.07	Garcia et al., (2005)
23	[C4MIM][BF4]	Butylmethylimidazolium Tetrafluoroborate	3.55	3.5091	Ranke et al., (2004)
24	[C4MIM][BF4]	Butylmethylimidazolium Tetrafluoroborate	3.1	3.5091	Garcia et al., (2005)
25	[C4MIM][BF4]	Butylmethylimidazolium Tetrafluoroborate	3.54	3.5091	Matzke et al., (2007)
26	[C4MIM][Br]	Butylmethylimidazolium Bromide	3.07	3.5512	Ranke et al., (2004)
27	[C4MIM][Br]	Butylmethylimidazolium Bromide	4.01	3.5512	(Couling et al., (2006)
28	[C4MIM][Br]	Butylmethylimidazolium Bromide	3.27	3.5512	Garcia et al., (2005)
29	[C4MIM][Cl]	Butylmethylimidazolium Chloride	3.71	3.44	Couling et al., (2006)
30	[C4MIM][Cl]	Butylmethylimidazolium Chloride	3.39	3.44	Romero et al., (2008)
31	[C4MIM][Cl]	Butylmethylimidazolium Chloride	3.4	3.44	Matzke et al., (2007)
32	[C4MIM][Cl]	Butylmethylimidazolium Chloride	3.47	3.44	Stolte et al., (2007)

Table 2 (cont)

33	[C4MIM][Cl]	Butylmethylimidazolium Chloride	3.34	3.44	Garcia et al., (2005)
34	[C4MIM][N(CN2)2]	Butylmethylimidazolium Dicyanamide	3.67	3.67	Couling et al., (2006)
35	[C4MIM][8OSO3]	Butylmethylimidazolium *O*-Octylsulfate	1.85	1.85	Matzke et al., (2007)
36	[C4MIM][N(CF3SO2)2]	Butylmethylimidazolium Bis(trifluoromethanesulfonyl)imide	3.39	2.8617	Couling et al.,(2006)
37	[C4MIM][N(CF3SO2)2]	Butylmethylimidazolium Bis(trifluoromethanesulfonyl)imide	2.48	2.8617	Matzke et al., (2007)
38	[C4MIM][N(CF3)2]	Butylmethylimidazolium Bis(trifluoromethyl)amide	3.48	3.48	Matzke et al., (2007)
39	[C4MIM][pTS]	Butylmethylimidazolium p-Toluenesulfonate/methylbenzenesulfonate	3.52	3.52	Ranke et al., (2004)
40	[C4EIM][BF4]	Butylethylimidazolium Tetrafluoroborate	2.8	2.8	Ranke et al., (2004)
41	[C5MIM][BF4]	Pentylmethylimidazolium Tetrafluoroborate	3.14	3.4198	Ranke et al., (2004)
42	[C6MIM][Br]	Hexylmethylimidazolium Bromide	1.42	2.2685	Couling et al., (2006)
43	[C6MIM][Cl]	Hexylmethylimidazolium Chloride	1.94	2.308	Luis et al., (2007)
44	[C6MIM][Cl]	Hexylmethylimidazolium Chloride	2.18	2.308	Romero et al., (2008)
45	[C6MIM][Cl]	Hexylmethylimidazolium Chloride	2.91	2.308	Stolte et al., (2007)
46	[C6MIM][Cl]	Hexylmethylimidazolium Chloride	2.32	2.308	Garcia et al., (2005)
47	[C6MMIM][Cl]	Hexyldimethylimidazolium Chloride	1.74	1.74	Luis et al., (2007)
48	[C6MIM][PF6]	Hexylmethylimidazolium Hexafluorophosphate	2.17	2.14	Garcia et al., (2005)
49	[C6MIM][PF6]	Hexylmethylimidazolium Hexafluorophosphate	2.11	2.14	Romero et al., (2008)
50	[C6MIM][BF4]	Hexylmethylimidazolium Tetrafluoroborate	3.18	2.9948	Ranke et al., (2004)
51	[C6EIM][BF4]	Hexylethylimidazolium Tetrafluoroborate	2.15	2.15	Ranke et al., (2004)
52	[C7MIM][BF4]	Heptylmethylimidazolium Tetrafluoroborate	2.44	2.3293	Ranke et al., (2004)
53	[C8MIM][Br]	Octylmethylimidazolium Bromide	0.63	1.3486	Docherty and Kulpa, (2005)
54	[C8MIM][Cl]	Octylmethylimidazolium Chloride	1.19	1.1721	Garcia et al., (2005)
55	[C8MIM][Cl]	Octylmethylimidazolium Chloride	0.94	1.1721	Romero et al., (2008)
56	[C8MIM][Cl]	Octylmethylimidazolium Chloride	1.01	1.01	Stolte et al., (2007)
57	[C8MIM][PF6]	Octylmethylimidazolium Hexafluorophosphate	0.95	0.825	Garcia et al., (2005)

Table 2 (cont)

58	[C8MIM][PF6]	Octylmethylimidazolium Hexafluorophosphate	0.7	0.825	Romero et al., (2008)
59	[C8MIM][BF4]	Octylmethylimidazolium Tetrafluoroborate	1.41	1.5365	Ranke et al., (2004)
60	[C9MIM][BF4]	Nonylmethylimidazolium Tetrafluoroborate	0.72	0.7033	Ranke et al., (2004)
61	[C10MIM][Cl]	Decylmethylimidazolium Chloride	0.5	0.135	Ranke et al., (2004)
62	[C10MIM][Cl]	Decylmethylimidazolium Chloride	−0.23	0.135	Stolte et al., (2007)
63	[C10MIM][BF4]	Decylmethylimidazolium Tetrafluoroborate	−0.18	-0.18	Ranke et al., (2004)
64	[C14MIM][Cl]	Tetradecylmethylimidazolium Chloride	−0.15	-0.167	Stolte et al., (2007)
65	[C16MIM][Cl]	Hexadecylmethylimidazolium Chloride	0.23	0.2431	Stolte et al., (2007)
66	[C18MIM][Cl]	Octadecylmethylimidazolium Chloride	1.45	1.447	Stolte et al., (2007)
67	[MPy]	Methylpyridinium	3.07	3.07	Couling et al., (2006)
68	[C4Py][Br]	Butylpyridinium Bromide	3.4	3.5086	Couling et al., (2006)
69	[C4MPy][Br]	Butylmethylpyridinium Bromide	2.75	2.7527	Couling et al., (2006)
70	[C4MMPy][Br]	Butyldimethylpyridinium Bromide	2.69	2.7237	Couling et al., (2006)
71	[C4Py][Cl]	Butylpyridinium Chloride	3.41	3.3296	Couling et al., (2006)
72	[C4Py][Cl]	Butylpyridinium Chloride	3.18	3.3296	Stolte et al., (2007)
73	[C4Py][N(CN2)]	Butylpyridinium Dicyanamide	3.31	3.3695	Couling et al., (2006)
74	[C4MPy][N(CN2)]	Butylmethylpyridinium Dicyanamide	2.66	2.541	Couling et al., (2006)
75	[C4MMPy][N(CN2)]	Butyldimethylpyridinium Dicyanamide	2.38	2.4395	Couling et al., (2006)
76	[C6MPy][Br]	Hexylmethylpyridinium Bromide	2.06	1.9917	Couling et al., (2006)
77	[C6MPy][Cl]	Hexylmethylpyridinium Chloride	1.44	1.44	Luis et al., (2007)
78	[C8MPy][Br]	Octylmethylpyridinium Bromide	0.79	0.9746	Couling et al., (2006)
79	[C4PyRR][Cl]	Butylpyrrolidinium Chloride	4.3	4.3	Stolte et al., (2007)
80	[C4Pyrrol][N(CF3SO2)2]	Butylpyrrolidinium Bis(trifluoromethanesulfonyl)imide	2.54	2.54	Stolte et al., (2007)
81	[C6MPyrrol][Cl]	Hexylmethylpyrrolidinium Chloride	2.99	3.0618	Luis et al., (2007)
82	[C4MMorp][Br]	Butylmethylmorpholinium Bromide	4.3	4.875	Stolte et al., (2007)
83	[C4MMorp][N(CF3SO2)2]	Butylmethylmorpholinium Bis(trifluoromethanesulfonyl)imide	2.49	2.49	Stolte et al., (2007)

Table 2 (cont)

84	[C4MPiper][Br]	Butylmethylpiperidinium Bromide	4.27	4.245	Stolte et al., (2007)
85	[C4MPiper][N(CF3SO 2)2]	Butylmethylpiperidinium Bis(trifluoromethanesulfonyl)im ide	2.56	2.56	Stolte et al., (2007)
86	[C4(CH3)2N-Py][Cl]	Butyl(dimethylamino)pyridiniu m Chloride	2.52	2.52	Stolte et al., (2007)
87	[C4(CH3)2N-Py] [N(CF3SO2)2]	Butyl(dimethylamino)pyridiniu m Bis(trifluoromethanesulfonyl)im ide	1.85	1.615	Stolte et al., (2007)
88	[Choline][Cl]	Choline Chloride	5	5	Couling et al., (2006)
89	[Choline][N(CF3SO2) 2]	Choline Bis(trifluoromethanesulfonyl)im ide	4.15	4.15	Couling et al., (2006)
90	[TMG][TFA]	1,1,3,3-Tetramethylguanidine Trifluoroacetate	0.47	0.47	Luis et al., (2010)
91	[TMG][Ace]	1,1,3,3-Tetramethylguanidine Acetate	3.17	3.17	Luis et al., (2010)
92	[TMG][TfO]	1,1,3,3-Tetramethylguanidine Trifluoromethanesulfonate	3.52	3.52	Luis et al., (2010)
93	[TMG][Cap]	1,1,3,3-Tetramethylguanidine Caprylate	2.29	2.29	Luis et al., (2010)
94	[Melamine][TFA]	1,3,5-Triazine-2,4,6-triamine Trifluoroacetate	2.24	2.24	Luis et al., (2010)
95	[Melamine][Ace]	1,3,5-Triazine-2,4,6-triamine Acetate	2.39	2.39	Luis et al., (2010)
96	[Melamine][TfO]	1,3,5-Triazine-2,4,6-triamine Trifluoromethanesulfonate	2.45	2.45	Luis et al., (2010)
97	[C1MIM][CH3SO4]	Methylmethylimidazolium methylsulfate	4.76	4.76	Santos personal communicati on
98	[C2MIM][C2H5SO4]	Ethylmethylimidazolium Ethylsulfate	4.02	4.02	Santos personal communicati on
99	[TMGC4]I	di-butyl-tetramethyl-guanidinium iodide	1.94	1.94	Ventura et al., (2012)
100	[TMGC7]I	di-heptyl-tetramethyl-guanidinium iodide	0.88	0.88	Ventura et al., (2012)
101	[TMGC12]I	di-dodecyl-tetramethyl-guanidinium iodide	1.43	1.43	Ventura et al., (2012)
102	[(C3O)4DMG]Cl	N"N"-dimethyl-N,N,N',N'-tetra-(2-methoxyethyl)-guanidinium chloride	2.46	2.46	Ventura et al., (2012)
103	[(di-h)2DMG]Cl	tetrahexyl-dimethyl-guanidiniumchloride	0.92	0.92	Ventura et al., (2012)
104	[C5O2mim]Cl	1-(2-(2-methoxyethoxy)ethyl)-3-methylimidazolium chloride	2.26	2.26	Ventura et al., (2012)
105	C10C(O)OEtmim]Br	3-methyl-1-(ethoxycarbonyloctyl)imidazoli um bromide	1.08	1.08	Ventura et al., (2012)

Table 2 (cont)

106	[P6,6,6,14]Br	trihexyltetradecylphosphonium bromide	1.05	1.05	Ventura et al., (2012)
107	[P6,6,6,14][CH3SO3]	trihexyltetradecylphosphonium methanosulfonate	1.11	1.11	Ventura et al., (2012)
108	[P6,6,6,14]Cl	trihexyltetradecylphosphonium chloride	1.14	1.14	Ventura et al., (2012)
109	[Pi(4,4,4)1][TOS]	triisobutyl(methyl)phosphonium tosylate	2.65	2.65	Ventura et al., (2012)
110	[P4,4,4,1][CH3SO4]	tributyl(methyl)phosphonium methylsulfate	2.84	2.84	Ventura et al., (2012)
111	[P4,4,4,4]Br	tetrabutylphosphonium bromide	2.71	2.71	Ventura et al., (2012)
112	[C4MIM]Br		3.53	3.5512	Ventura et al., (2012)
113	[C4mim][CH3SO3]	1-butyl-3-methylimidazolium methanosulfonate	3.56	3.56	Ventura et al., (2012)
114	[C4mim][TOS]	1-butyl-3-methylimidazoliumtosylate	3.32	3.32	Ventura et al., (2012)
115	[CNC1MIM][N(CF3SO2)2]	(Cyanomethyl)methylimidazolium Bis(trifluoromethanesulfonyl)imide	3.81	3.81	Stolte et al., (2007)
116	[EOC1MIM][N(CF3SO2)2]	(Ethoxymethyl)methylimidazolium Bis(trifluoromethanesulfonyl)imide	3	3	The UFT/Merk ionic liquids effects database
117	[C2MIM][(C2F5)2PO2]	Ethylmethylimidazolium Bis(pentafluoroethyl)phosphinate	3.05	3.05	The UFT/Merk ionic liquids effects database
118	[C2MIM][(CN)4B]	Ethylmethylimidazolium Tetracyanidoborate	3.56	3.56	The UFT/Merk ionic liquids effects database
119	[C2MIM][SCN]	Ethylmethylimidazolium Thiocyanate	4.15	4.15	The UFT/Merk ionic liquids effects database
120	[C2MIM][TFA]	Ethylmethylimidazolium Trifluoroacetate	4.28	4.28	The UFT/Merk ionic liquids effects database
121	[C2MIM][FeCl4]	Ethylmethylimidazolium Tetrachloroferrate(III)	1.51	1.51	Alvarez & Irabien (2011)

Table 2 (cont)

122	[EOC2MIM][N(CF3SO2)2]	(Ethoxyethyl)methylimidazolium Bis(trifluoromethanesulfonyl)imide	2.96	2.96	The UFT/Merk ionic liquids effects database
123	[OHC2MIM][N(CF3SO2)2]	(Hydroxyethyl)methylimidaziliu m Bis(trifluoromethanesulfonyl)imide	4.08	4.08	The UFT/Merk ionic liquids effects database
124	[OHC2MIM][I]	(Hydroxyethyl)methylimidaziliu m Iodide	3.89	3.89	Stolte et al., (2007)
125	[MOC2MIM][N(CF3SO2)2]	(Methoxyethyl)methylimidazoli um Bis(trifluoromethanesulfonyl)imide	2.84	2.84	The UFT/Merk ionic liquids effects database
126	[MOC2MIM][Cl]	(Methoxyethyl)methylimidazoli um Chloride	4.18	4.18	The UFT/Merk ionic liquids effects database
127	[MOC2MIM][BF4]	(Methoxyethyl)methylimidazoli um Tetrafluoroborate	1.15	1.15	Samori et.al (2007)
128	[MOC2MIM][N(CN)2]	(Methoxyethyl)methylimidazoli um Dicyanamide	1.07	1.07	Samori et.al (2007)
129	[C3MIM][N(CF3SO2)2]	Propylmethylimidazolium Bis(trifluoromethanesulfonyl)imide	2.77	2.77	Ventura et.al (2010)
130	[OHC3MIM][N(CF3SO2)2]	(Hydroxypropyl)methylimidazol ium Bis(trifluoromethanesulfonyl)imide	3.89	3.89	The UFT/Merk ionic liquids effects database
131	[MOC3MIM][N(CF3SO2)2]	(Methoxypropyl)methylimidazo lium Bis(trifluoromethanesulfonyl)imide	3.24	3.24	The UFT/Merk ionic liquids effects database
132	[C4MIM][I]	Butylmethylimidazolium Iodide	3.59	3.59	The UFT/Merk ionic liquids effects database
133	[C4MIM][FeCl4]	Butylmethylimidazolium Tetrachloroferrate(III)	1.51	1.51	Alvarez & Irabien (2011)
134	[C6MIM][N(CF3SO2)2]	Hexylmethylimidazolium Bis(trifluoromethanesulfonyl)imide	2.05	2.1965	The UFT/Merk ionic liquids effects database

Table 2 (cont)

135	[C6MIM][P(C2F5)3F3]	Hexylmethylimidazolium Trifluoridotris(pentafluoroethyl) phosphate	2.65	2.65	The UFT/Merk ionic liquids effects database
136	[C6MIM][2-SO2PhCO)N]	Hexylmethylimidazolium 1,1-Dioxo-1,2-dihydrobenzo[d]isothiazol-3-onate	2.67	2.67	The UFT/Merk ionic liquids effects database
137	[C8MIM][N(CF3SO2)2]	Octylmethylimidazolium Bis(trifluoromethanesulfonyl)imide	0.83	0.83	The UFT/Merk ionic liquids effects database
138	[C10MIM][FeCl4]	Decylmethylimidazolium Tetrachloroferrate(III)	-0.43	-0.43	Alvarez & Irabien (2011)
139	[OHC3Py][N(CF3SO2)2]	Hydroxypropylpyridinium Bis(trifluoromethanesulfonyl)imide	4.01	4.01	The UFT/Merk ionic liquids effects database
140	[C4Py][Al2Cl7]	Butylpyridinium μ-Chlorohexachloridodialuminate	3.01	3.01	The UFT/Merk ionic liquids effects database
141	[C4MPy][BF4]	Butylmethylpyridinium Tetrafluoroborate	3.02	3.02	The UFT/Merk ionic liquids effects database
142	[C8Py][Cl]	Octylpyridinium Chloride	1.69	1.6207	The UFT/Merk ionic liquids effects database
143	[C8MMPy][Br]	Octyldimethylpyridinium Bromide	1.12	0.9473	Papaiconomou et.al., (2010)
144	[C8MMPy][N(CF3SO2)2]	Octyldimethylpyridinium Bis(trifluoromethanesulfonyl)imide	0.36	0.36	Papaiconomou et.al., (2010)
145	[EOC2MPyRR][N(CF3SO2)2]	(Ethoxyethyl)methylpyrrolidinium Bis(trifluoromethanesulfonyl)imide	2.97	2.97	The UFT/Merk ionic liquids effects database
146	[OHC3MPyRR][N(CF3SO2)2]	(Hydroxypropyl)methylpyrrolidinium Bis(trifluoromethanesulfonyl)imide	3.91	3.91	The UFT/Merk ionic liquids effects database

Table 2 (cont)

147	[C4MPyRR][N(CF3SO2)2]	Butylmethylpyrrolidinium Bis(trifluoromethanesulfonyl)imide	2.54	2.6865	Stolte et al., (2007)
148	[C4MPyRR][P(C2F5)3F3]	Butylmethylpyrrolidinium Trifluoridotris(pentafluoroethyl)phosphate	1.7	1.7	The UFT/Merk ionic liquids effects database
149	[C6MPyRR][Cl]	Hexylmethylpyrrolidinium Chloride	3	3.0618	Luis. P et.al., (2007)
150	[C6MPyRR][N(CF3SO2)2]	Hexylmethylpyrrolidinium Chloride	2.4	2.2535	The UFT/Merk ionic liquids effects database
151	[EOC1MMorp][N(CF3SO2)2]	(Ethoxymethyl)methylmorpholinium Bis(trifluoromethanesulfonyl)imide	3.38	3.38	The UFT/Merk ionic liquids effects database
152	[C4(CH3)2*N*-Py][Br]	Butyl(dimethylamino)pyridinium Bromide	2.32	2.32	Couling et.al (2006)
153	[C6(CH3)2*N*-Py][N(CF3SO2)2]	Hexyl(dimethylamino)pyridinium Bis(trifluoromethanesulfonyl)imide	1.38	1.615	The UFT/Merk ionic liquids effects database
154	[C16MMMN][Br]	Cethyltrimethylammonium Bromide	0.04	0.04	Kaiser and Palabrica (1991)
155	[C16MMMN][Cl]	Cethyltrimethylammonium Chloride	0.4	0.4	Kaiser and Palabrica (1991)
156	[C16BnMMN][Cl]	Hexadecylbenzyldimethylammonium Chloride	-0.31	-0.31	Kaiser and Palabrica (1991)
157	[C12BnMMN][Cl]	Benzyldimethyldodecylammonium Chloride	-0.23	-0.23	Kaiser and Palabrica (1991)
158	[C4N][Cl]	Tetrabutylammonium Chloride	2.69	2.69	Kaiser and Palabrica (1991)
159	[C4N][Br]	Tetrabutylammonium Bromide	3.27	3.27	Couling et.al., (2006)
160	[C6EEEN][Br]	Hexyltriethylammonium Bromide	2.46	2.46	Couling et.al., (2006)
161	[C4EMMN][N(CF3SO2)2]	Butylethyldimethylammonium Bis(trifluoromethanesulfonyl)imide	3.21	3.21	Stolte et al., (2007)
162	[EOCOC1EMMN][N(CF3SO2)2]	(Ethoxycarbonylmethyl)ethyldimethylammonium Bis(trifluoromethanesulfonyl)imide	3.32	3.32	The UFT/Merk ionic liquids effects database

Table 2 (cont)

163	[OHC2MMN][Ace]	(Hydroxyethyl)dimethylammonium Acetate	4.29	4.29	The UFT/Merk ionic liquids effects database
164	[MOC2EMMN][N(CF3SO2)2]	(Methoxyethyl)ethyldimethylammonium Bis(trifluoromethanesulfonyl)imide	3.35	3.35	The UFT/Merk ionic liquids effects database
165	[C4P][Br]	tetrabutylphosphonium bromide	2.71	2.71	Stolte et al., (2007)
166	[(C4)3EP][(CH3CH2)2PO4]	Tributylethylphosphonium Diethylphosphate	3.07	3.07	Stolte et al., (2007)
167	[BMMOR][N(CN)2]	1-Butyl-1-methylmorpholinium dicyanamide	4.89	4.89	Sylvie Viboud et.al (2012)
168	[BMPYRRO][N(CN)2]	1-Butyl-1-methylpyrrolidinium dicyanamide	4.26	4.26	Sylvie Viboud et.al (2012)
169	[OQUINU][N(CN)2]	1-Octylquinuclidinium dicyanamide	2.26	2.26	Sylvie Viboud et.al (2012)
170	[OTROP][N(CN)2]	1-Octyltropinium dicyanamide	2.52	2.52	Sylvie Viboud et.al (2012)
171	[BMMOR][CF3SO3]	1-Butyl-1-methylmorpholinium triflate	4.4	4.4	Sylvie Viboud et.al (2012)
172	[BMPYRRO][CF3SO3]	1-Butyl-1-methylpyrrolidinium triflate	3.98	3.98	Sylvie Viboud et.al (2012)
173	[B2M3MPYR][CF3SO3]	1-Butyl-2,3-dimethylpyridinium triflate	2.84	2.84	Sylvie Viboud et.al (2012)
174	[BTROP][SCN]	1-Butyltropinium thiocyanate	4.07	4.07	Sylvie Viboud et.al (2012)
175	[B3M5MPYR][SCN]	1-butyl-2,3-dimethylpyridinium thiocyanate	2.25	2.25	Sylvie Viboud et.al (2012)
176	[OEPIP][SCN]	1-Octyl-1-ethylpiperidinium thiocyanate	1.5	1.5	Sylvie Viboud et.al (2012)
177	[BTROP][NTf2]	1-Butyl-1-methyltropinium Bis(trifluoromethylsulfonyl)imide	2.61	2.61	Sylvie Viboud et.al (2012)
178	[OTROP][NTf2]	1-Octyl-1-methyltropinium Bis(trifluoromethylsulfonyl)imide	2.17	2.17	Sylvie Viboud et.al (2012)
179	[BMPYRRO][NTf2]	1-Butyl-1-methylpyrrolidinium Bis(trifluoromethylsulfonyl)imide	2.72	2.72	Sylvie Viboud et.al (2012)
180	[OMPYRRO][NTf2]	1-Octyl-1-methylpyrrolidinium Bis(trifluoromethylsulfonyl)imide	1.58	1.58	Sylvie Viboud et.al (2012)

Table 2 (cont)

181	[B3M5MPYR][NTf2]	1-Butyl-3,5-dimethylpyridinium Bis(trifluoromethylsulfonyl)imide	1.86	1.71	Papaiconomou et.al (2010)
182	[B2M3M5MPYR][NTf2]	1-Butyl-2,3,5-trimethylpyridinium Bis(trifluoromethylsulfonyl)imide	1.56	1.71	Papaiconomou et.al (2010)
183	[O2M3M5MPYR][NTf2]	1-Octyl-2,3,5-trimethylpyridinium Bis(trifluoromethylsulfonyl)imide	0.62	0.62	Papaiconomou et.al (2010)
184	[EMIM][B(CN)4]	1-Ethyl-1-methylimidazolium tetracyanoborate	3.62	3.62	Sylvie Viboud et.al (2012)
185	[OTROP][BF4]	1-Octyltropinium tetrafluoroborate	2.77	2.77	Sylvie Viboud et.al (2012)
186	[O3M5MPYR][BF4]	1-Octyl-3,5-dimethylpyridinium tetrafluoroborate	2.77	2.77	Sylvie Viboud et.al (2012)
187	[OEPIP][BPh4]	1-Octyl-1-ethylpiperidinium tetraphenylborate	1.36	1.36	Sylvie Viboud et.al (2012)
188	[Ch][Cl]	Choline chloride	6.17	6.17	Sylvie Viboud et.al (2012)
189	[BMMOR][Br]	1-Butyl-1-methyllmorpholinium bromide	5.45	4.875	Sylvie Viboud et.al (2012)
190	[OMMOR][Br]	1-Octylmorpholinium bromide	2.56	2.56	Sylvie Viboud et.al (2012)
191	[BTROP][Br]	1-Butyltropinium bromide	5.36	5.36	Sylvie Viboud et.al (2012)
192	[BTROP][I]	1-Butyltropinium iodide	4.74	4.74	Sylvie Viboud et.al (2012)
193	[BQUINU][Br]	1-Butylquinuclidinium bromide	4.45	4.45	Sylvie Viboud et.al (2012)
194	[OQUINU][Br]	1-Octylquinuclidinium bromide	2.32	2.32	Sylvie Viboud et.al (2012)
195	[BMPYRRO][Br]	1-Butyl-1-methylpyrrolidinium bromide	4.4	4.2535	Sylvie Viboud et.al (2012)
196	[HMPYRRO][Br]	1-Hexyl-1-methylpyrrolidinium bromide	3.19	3.203	Sylvie Viboud et.al (2012)
197	[OMPYRRO][Br]	1-Octyl-1-methylpyrrolidinium bromide	2.26	2.3936	Sylvie Viboud et.al (2012)
198	[OMPYRRO][Cl]	1-Octyl-1-methylpyrrolidinium chloride	2.17	2.0364	Sylvie Viboud et.al (2012)

Table 2 (cont)

199	[BMPIP][Br]	1-Butyl-1-methylpiperidinium bromide	4.22	4.245	Sylvie Viboud et.al (2012)
200	[HMPIP][Br]	1-Hexyl-1-methylpiperidinium bromide	2.94	2.94	Sylvie Viboud et.al (2012)
201	[OMPIP][Br]	1-Octyl-1-methylpiperidinium bromide	2	2	Sylvie Viboud et.al (2012)
202	[BEPIP][Br]	1-Butyl-1-ethylpiperidinium bromide	3.96	3.96	Sylvie Viboud et.al (2012)
203	[OEPIP][Br]	1-Octyl-1-ethylpiperidinium bromide	1.8	1.8	Sylvie Viboud et.al (2012)
204	[OEPIP][I]	1-Octyl-1-ethylpiperidinium iodide	2.2	2.2	Sylvie Viboud et.al (2012)
205	[BMIM][Br]	1-Butyl-1-methylimidazolium bromide	3.66	3.5512	Sylvie Viboud et.al (2012)
206	[HMIM][Br]	1-Hexyl-1-methylimidazolium bromide	3.13	2.2685	Sylvie Viboud et.al (2012)
207	[OMIM][Br]	1-Octyl-1-methylimidazolium bromide	2.27	1.3486	Sylvie Viboud et.al (2012)
208	[BPYR][Br]	1-Butylpyridinium bromide	3.72	3.5086	Sylvie Viboud et.al (2012)
209	[HPYR][Br]	1-Hexylpyridinium bromide	2.85	2.9183	Sylvie Viboud et.al (2012)
210	[OPYR][Br]	1-Octylpyridinium bromide	1.89	1.865	Sylvie Viboud et.al (2012)
211	[B2M3MPYR][Br]	1-Butyl-2,3-dimethylpyridinium bromide	2.93	2.7237	Sylvie Viboud et.al (2012)
212	[O2M3MPYR][Br]	1-Octyl-2,3-dimethylpyridinium bromide	1.08	0.9473	Papaiconomou et.al (2010)
213	[B3M5MPYR][Br]	1-Butyl-3,5-dimethylpyridinium bromide	2.49	2.7237	Papaiconomou et.al (2010)
214	[O3M5MPYR][Br]	1-Octyl-3,5-dimethylpyridinium bromide	1.12	0.9473	Papaiconomou et.al (2010)
215	[B2M3M5MPYR][Br]	1-Butyl-2,3,5-trimethylpyridinium bromide	2.48	2.4497	Papaiconomou et.al (2010)
216	[O2M3M5MPYR][Br]	1-Octyl-2,3,5-trimethylpyridinium bromide	0.64	0.6703	Papaiconomou et.al (2010)
217	[O2M5EPYR][Br]	1-Octyl-2-methyl-5-ethylpyridinium bromide	0.59	0.9473	Sylvie Viboud et.al (2012)

Table 3: Fragmentation of ILs for the toxicities on *Vibrio fischeri*

No.	CH3	CH2	CH	NH	NCH3	NCH2	OCH3	OCH2	SCH2	CN	COH	C=O	CEN	Benzene	PCH2	PCH3	Imida	Pyrid	Ammo	Pyrrol	thio	Morp	Guanidine	Piper	melamine	Phos	Quinuclidinium	Tropinium	I-	Br-	Cl-	BF4-	BPh4-	PF6-	Tf2N-	N(CN)2	TFA-	Ace-	Cap-	TfO-	For-	SO4-	SO3-	PO4-	R	(2-OPhO)2B-	8OSO3-	N(CF3SO2)2-	CF3SO3-	N(CF3)2-	pTS-	TOS-	(C2F5)2PO2-	(OOCCOO)2B-	(CN)4B-	SCN-	FeCl4-	P(C2F5)3F3-	2-SO2PhCO)N-	Al2Cl7-
1	0	0	0	2	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	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	2	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	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	2	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	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	2	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	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	1	1	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	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	1	1	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	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	1	1	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	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	1	1	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	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	1	1	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	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	1	1	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	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	0	0	0	0	0
11	0	0	0	0	2	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	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	0	0	0	0	0
12	1	1	0	0	1	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	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	1	1	0	0	1	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	0	0	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	0
14	1	1	0	0	1	1	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	0	0	0	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
15	1	1	0	0	1	1	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	0	0	0	0	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
16	1	2	0	0	1	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	1	0	0	0	0	0	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
17	1	2	0	1	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	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
18	1	2	0	1	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	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
19	1	2	0	1	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	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	0	0

Table 3 (cont)

20	1	2	0	1	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	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	1	2	0	1	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	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	0	0	0	0
22	1	2	0	0	1	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	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	1	2	0	0	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	1	2	0	0	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	1	2	0	0	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	1	2	0	0	1	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	1	2	0	0	1	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	1	2	0	0	1	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	1	2	0	0	1	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	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	1	2	0	0	1	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	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	1	2	0	0	1	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	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32	1	2	0	0	1	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	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33	1	2	0	0	1	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	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	1	2	0	0	1	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	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35	1	2	0	0	1	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	0	0	0	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
36	1	2	0	0	1	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	0	0	0	0	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
37	1	2	0	0	1	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	0	0	0	0	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
38	1	2	0	0	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
39	1	2	0	0	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
40	2	2	0	0	0	2	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
41	1	3	0	0	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	1	4	0	0	1	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
43	1	4	0	0	1	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	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 3 (cont)

44	1	4	0	0	1	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	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
45	1	4	0	0	1	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	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
46	1	4	0	0	1	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	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
47	2	4	0	0	1	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	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
48	1	4	0	0	1	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	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
49	1	4	0	0	1	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	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
50	1	4	0	0	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
51	2	4	0	0	0	2	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
52	1	5	0	0	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
53	1	6	0	0	1	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
54	1	6	0	0	1	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	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
55	1	6	0	0	1	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	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
56	1	6	0	0	1	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	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
57	1	6	0	0	1	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	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
58	1	6	0	0	1	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	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
59	1	6	0	0	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
60	1	7	0	0	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
61	1	8	0	0	1	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	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
62	1	8	0	0	1	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	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
63	1	8	0	0	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
64	1	12	0	0	1	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	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
65	1	14	0	0	1	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	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
66	1	16	0	0	1	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	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 3 (cont)

67	0	0	0	0	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
68	1	2	0	0	0	1	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
69	2	2	0	0	0	1	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
70	3	2	0	0	0	1	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
71	1	2	0	0	0	1	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
72	1	2	0	0	0	1	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
73	1	2	0	0	0	1	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	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
74	2	2	0	0	0	1	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	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
75	3	2	0	0	0	1	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	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
76	2	4	0	0	0	1	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
77	2	4	0	0	0	1	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
78	2	6	0	0	0	1	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
79	1	2	0	0	0	1	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
80	1	2	0	0	0	1	0	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	0	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
81	1	4	0	0	1	1	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
82	1	2	0	0	1	1	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
83	1	2	0	0	1	1	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
84	1	2	0	0	1	1	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85	1	2	0	0	1	1	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
86	1	2	0	0	2	1	0	0	0	1	0	0	0	0	0	0	0	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
87	1	2	0	0	2	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	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
88	0	1	0	0	3	1	0	0	0	0	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
89	0	1	0	0	3	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	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
90	0	0	0	0	4	0	0	0	0	0	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	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 3 (cont)

91	0	0	0	0	4	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
92	0	0	0	0	4	0	0	0	0	0	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	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
93	0	0	0	0	4	0	0	0	0	0	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	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
94	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
95	0	0	0	0	0	0	0	0	0	0	0	0	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	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
96	0	0	0	0	0	0	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
97	0	0	0	0	2	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	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	0	0	0	0
98	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
99	2	4	0	0	4	2	0	0	0	1	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
100	2	10	0	0	4	2	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
101	0	20	0	0	4	2	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
102	0	4	0	0	2	4	4	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
103	4	16	0	0	2	4	0	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
104	0	2	0	0	1	1	1	1	0	0	0	0	0	0	0	0	1	0	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	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
105	1	9	0	0	1	1	0	1	0	0	0	1	0	0	0	0	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
106	4	24	0	0	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
107	4	24	0	0	0	0	0	0	0	0	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	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
108	4	24	0	0	0	0	0	0	0	0	0	0	0	0	4	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
109	7	0	3	0	0	0	0	0	0	0	0	0	0	0	3	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	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
110	4	6	0	0	0	0	0	0	0	0	0	0	0	0	3	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
111	4	8	0	0	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
112	1	2	0	0	1	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 3 (cont)

113	1	2	0	0	1	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	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
114	1	2	0	0	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
115	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	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
116	1	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	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
117	1	0	0	0	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
118	1	0	0	0	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
119	1	0	0	0	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
120	1	0	0	0	1	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	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
121	1	0	0	0	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
122	1	1	0	0	1	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	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
123	0	1	0	0	1	1	0	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	0	0	0	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
124	0	1	0	0	1	1	0	0	0	0	1	0	0	0	0	0	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
125	0	1	0	0	1	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	0	0	0	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
126	0	1	0	0	1	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	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
127	0	1	0	0	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
128	0	1	0	0	1	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	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
129	1	2	0	1	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	0	0	0	0	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
130	0	2	0	0	1	1	0	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	0	0	0	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
131	0	2	0	0	1	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	0	0	0	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
132	1	2	0	0	1	1	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
133	1	2	0	0	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
134	1	4	0	0	1	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	0	0	0	0	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
135	1	4	0	0	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
136	1	4	0	0	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0

Table 3 (cont)

137	1	6	0	0	1	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	0	0	0	0	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
138	1	8	0	0	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
139	0	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	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
140	1	2	0	0	0	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
141	2	2	0	0	0	1	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	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
142	1	6	0	0	0	1	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
143	3	6	0	0	0	1	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
144	3	6	0	0	0	1	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	0	0	0	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
145	2	1	0	0	0	1	0	1	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	0	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
146	0	1	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	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
147	1	2	0	0	1	1	0	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	0	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
148	1	2	0	0	1	1	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
149	1	4	0	0	1	1	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
150	1	4	0	0	1	1	0	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	0	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
151	1	0	0	0	1	1	0	1	0	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	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
152	1	2	0	0	2	1	0	0	0	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
153	1	2	0	0	2	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	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
154	1	14	0	0	3	1	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
155	1	14	0	0	3	1	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
156	1	14	0	0	2	2	0	0	0	0	0	0	0	1	0	0	0	0	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
157	1	10	0	0	2	2	0	0	0	0	0	0	0	1	0	0	0	0	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
158	4	8	0	0	0	4	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
159	4	8	0	0	0	4	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 3 (cont)

160	1	4	0	0	3	1	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
161	2	2	0	0	2	2	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	0	0	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
162	2	1	0	0	2	2	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	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
163	0	0	0	1	2	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0
164	2	0	0	0	2	2	1	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	0	0	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
165	4	8	0	0	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
166	4	6	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
167	1	2	0	0	1	1	0	0	0	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	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
168	1	2	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0
169	1	6	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
170	1	6	0	0	0	1	0	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	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
171	1	2	0	0	1	1	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
172	1	2	0	0	1	1	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
173	3	2	0	0	0	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
174	1	2	0	0	1	1	0	0	0	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
175	3	2	0	0	0	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
176	2	6	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
177	1	2	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
178	1	6	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
179	1	2	0	0	1	1	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
180	1	6	0	0	1	1	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
181	3	2	0	0	0	1	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	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
182	3	2	0	0	0	1	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	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
183	4	6	0	0	0	1	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	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 3 (cont)

184	2	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
185	1	6	0	0	0	1	0	0	0	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
186	3	6	0	0	0	1	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	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
187	2	6	0	0	0	2	0	0	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
188	0	0	0	0	3	1	0	0	0	0	1	0	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
189	1	2	0	0	1	1	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
190	1	6	0	0	1	1	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
191	1	2	0	0	0	1	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
192	1	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
193	1	2	0	0	0	1	0	0	0	0	1	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
194	1	6	0	0	0	1	0	0	0	0	1	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
195	1	2	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
196	1	4	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
197	1	6	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
198	1	6	0	0	1	1	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
199	1	2	0	0	1	1	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
200	1	4	0	0	1	1	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
201	1	6	0	0	1	1	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
202	2	2	0	0	0	2	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
203	2	6	0	0	0	2	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
204	2	6	0	0	0	2	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
205	1	2	0	0	1	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
206	1	4	0	0	1	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
207	1	6	0	0	1	1	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 3 (cont)

208	1	2	0	0	0	1	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
209	1	4	0	0	0	1	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
210	1	6	0	0	0	1	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
211	3	2	0	0	0	1	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
212	3	6	0	0	0	1	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
213	3	2	0	0	0	1	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
214	3	6	0	0	0	1	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
215	4	2	0	0	0	1	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
216	4	6	0	0	0	1	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
217	3	6	0	0	0	1	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 4 shows the statistical parameters of the training, testing and the total dataset. It can be clearly concluded from this table that the developed models is cabable of predict the toxicity with high accuracy, R^2=97.53% for the traning set, R^2=99.56% for the testing set and R^2=96.83% for the whole dataset. It can also be concluded from this table that the values of R^2, ARD, AARD, AAE and $\hat{\sigma}^2$ between the between the training and testing sets are very close to each other. This indicates that the proposed model is stable for training and testing abilities.

Table 4: The statistical parameters of the developed models

Statistical parameters	Training set	Testing set	The whole dataset
Toxicity			
No. of compounds	174	43	217
R^2	97.53	99.56	96.83
ARD	0.007	-0.0069	-0.0017
AARD	0.0192	0.0285	0.0387
AAE	$-1.01*10^{-13}$	$-2.5*10^{-5}$	$-8.36*10^{-15}$
$\hat{\sigma}^2$	0.0379	0.0075	0.0496

A comparison was made between the results obtained using the proposed model for toxicty and other experimental values. A good fit is achieved with squared correlation coefficient, as illustrated in Figure 11.

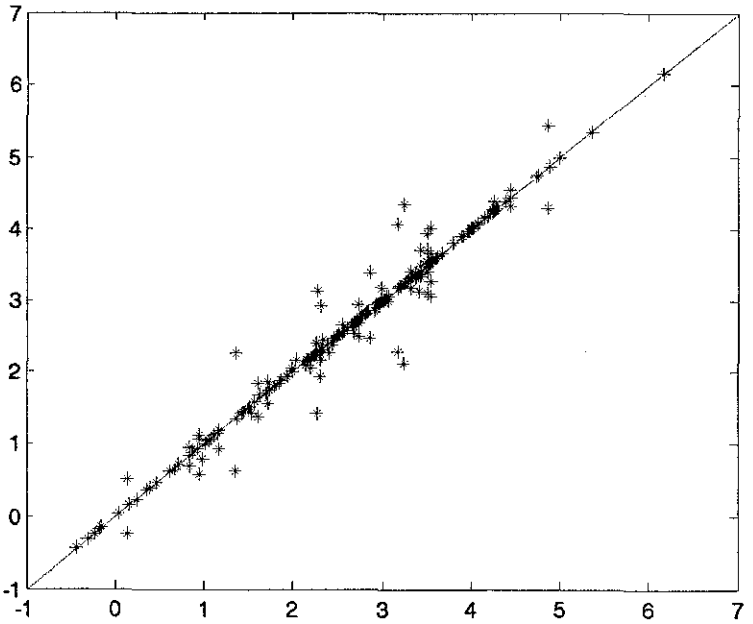


Figure 11: Comparison between predicted and experimental Toxicity values.

The average relative deviation (ARD), average absolute relative deviation (AARD), average absolute error (AAE), and standard deviation were respectively obtained equal to -0.0017, 0.0387, $-8.36*10^{-15}$, and 0.0496. It can be concluded from Figure 12 – 14, that out of 217 components, there are about 170 components with 'zero' error between the predicted toxicity values and those experimental toxicity values. From table 5,it is also proved that the results predicted from the model are the most accurate as compared to other models that predicted toxicity of ILs towards *Vibrio fischeri* marine bacteria.

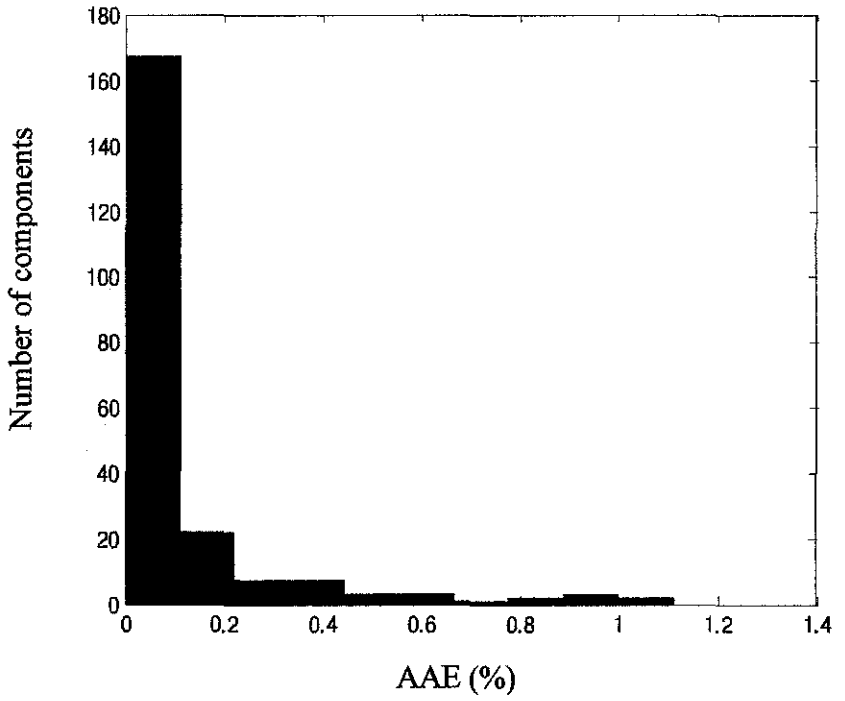


Figure 12: AAE histogram for the toxicity model

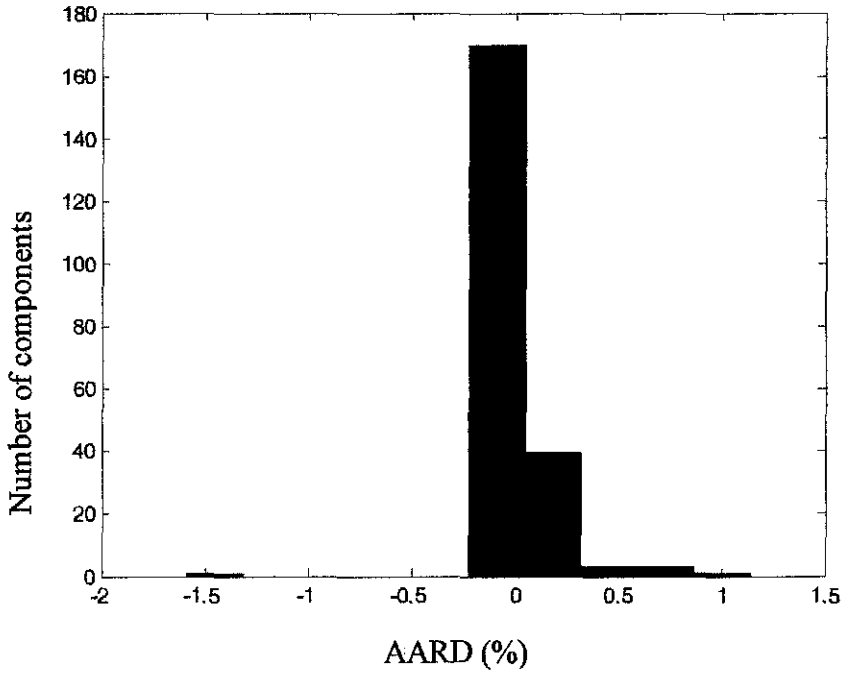


Figure 13: AARD histogram for the Toxicity model.

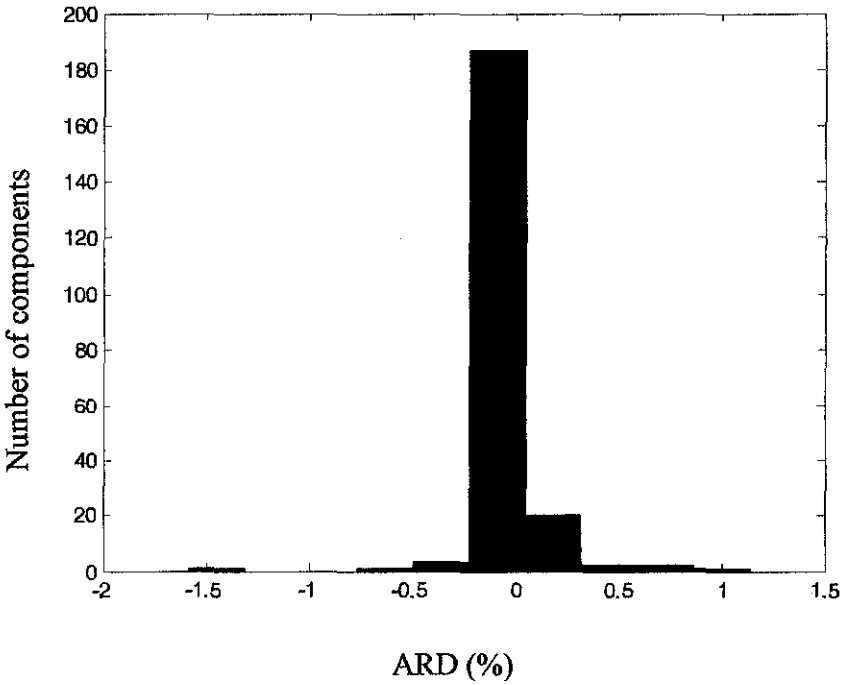


Figure 14: ARD histogram for the Toxicity model

Table 5: Comparison of QSAR model

Method	Organism(s)	Number of ILs	Correlation Coefficient, R^2	Software used	Error	References
Genetic function approximation (GFA)	*Vibrio fischeri; Daphnia magna*	25 and 17	0.78-0.88	MOPAC	na	Couling et al. (2006)
MLR	*Vibrio fischeri*	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 fischeri*	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 et al. (2008)
MLR, Radial Basis (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	0.022 and 0.038	Torrecilla et al. (2009)
MLR	*Vibrio fischeri*	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 Regression- Discriminant Analysis (PLS-DA)	*Vibrio fischeri*	148	na	PLS_Toolbox 5.2 for MATLAB	na	Guerra and Ibáñez (2011)
MLR and polynomial methods	*Vibrio fischeri*	217	0.9683	MATLAB 7.8.0.347	0.0496	This work

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

This study presents a new method for prediction of toxicity of ILs towards *Vibrio fischeri* marine bacteria based on QSAR approach. Developments of this model have been done based on a code written using MATLAB software (version 7.8.0.347) and it is based on the combination of MLR, and polynomial models. For developing this toxicity model, 217 ILs of experimental data were used. The model show very accurate results, where R^2=96.83% with standard deviation=0.0496. Moreover, the accuracy of the developed models was compared with other established models and found to have a higher accuracy than others established models. Therefore, this model is capable and reliable of predicting the toxicity of a variety of ILs towards *Vibrio fischeri* marine bacteria. This model provides a practical, cost-effective, convenient and reliable alternative to experimental measurements of ILs toxicity determination.

5.2 Recommendation and future work

Future studies in this area will need to cover a wider spectrum of ILs with respect to their toxicity towards algae, fish, and other higher-order organisms.

REFERENCES

Alvarez-Guerra, M., & Irabien, A. (2011). Design of ionic liquids: An ecotoxicity (*Vibrio fischeri*) discrimination approach. *Green Chemistry*, 13(6)

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

Angelov, P.P., (2002). *Evolving Rule-Based Models: A Tool for Design of Flexible Adaptive Systems.* Springer-Verlag, London, United Kingdom.

Anthony JL, Brennecke JF, Holbrey JD, Maginn EJ, Mantz RA, Rogers RD, Trulove PC, Visser AE & Welton T (2003) Physicochemiocal properties of ionic liquids. In Wassercheid P & Welton T (Eds) Ionic Liquids in Synthesis, Wiley-WCH, Weinheim: 41-126

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 ionic liquids on the survival, movement, and feeding behavior of the fresh water snail Physa acuta. *Environ. Toxicol. Chem. 24*, 1759–1765.

Chatterjee, S., Hadi, A.S., (2006). *Regression Analysis by Example*, fourth ed. JohnWiley, New York.

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

Cho, C.-W., Phuong Thuy Pham, T., Jeon, Y.-C. and Yun, Y.-S. (2008). Influence of anions on the toxic effects of ionic liquids to a phytoplankton Selenastrum capricornutum, *Green Chemistry*, 10(1), 67-72

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

organisms via quantitative structure–property relationship modeling, *Green Chemistry*, 8(1), 82-90

Docherty, K. M., & Kulpa, J.,Charles F. (2005). Toxicity and antimicrobial activity of imidazolium and pyridinium ionic liquids. *Green Chemistry*, 7(4)

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

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.

Ismail Hossain, M., Samir, B. B., El-Harbawi, M., Masri, A. N., Abdul Mutalib, M. I., Hefter, G., et al. (2011). Development of a novel mathematical model using a group contribution method for prediction of ionic liquid toxicities. *Chemosphere*, 85(6), 990-994.

Ismail Hossain, M. (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

Kaiser K.L.E and Palabrica V.S (1991) Photobacterium phosphoreum toxicity data index Water Poll. Res.J. Canada 26 361-431.

Katritzky, A. R., Tatham, D. B. and Maran, U. (2001). Theoretical Descriptors for the Correlation of Aquatic Toxicity of Environmental Pollutants by Quantitative Structure-Toxicity Relationships, *Journal of Chemical Information and Computer Sciences*, 41(5), 1162-1176..

Kulacki, K. J., Chaloner, D. T., Costello, D. M., Docherty, K. M., Larson, J. H., Bernot, R. J., Brueseke, M. A., Kulpa, C. F. and Lamberti, G. A. (2007).

Aquatic toxicity and biodegradation of ionic liquids: A synthesis, *Chemistry Today*, 25(6), 32-36

Latala, A., Stepnowski, P., Nedzi, M. and Mrozik, W. (2005). Marine toxicity assessment of imidazolium ionic liquids: Acute effects on the Baltic algae *Oocystis submarina* and *Cyclotella meneghiniana*, *Aquatic Toxicology*, 73(1), 91-98.

Luis, P., Ortiz, I., Aldaco, R., & 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., & 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.

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 ionic liquids observed in an (eco)toxicological test battery. *Green Chemistry* **9** (11) 1198 – 1207

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

Pernak, J., Stefaniak, F. and Węglewski, J. (2005), Phosphonium Acesulfamate Based Ionic Liquids. Eur. J. *Org. Chem.*, 2005: 650–652.

Pieraccini Daniela, Chiappe Cinzia, Intorre Luigi, Pretti Carlo, (2007), Thermodynamics, Solubility and Environmental Issues in Ecotoxicity of ionic liquids in an aquatic environmet,pp 259, 276

Plechkova, N. V., & Seddon, K. R. (2008). Applications of ionic liquids in the chemical industry. *Chemical Society Reviews*, 37(1)

Pretti, C., Chiappe, C., Pieraccini, D., Gregori, M., Abramo, F., Monni, G. and Intorre, L. (2006). Acute toxicity of ionic liquids to the zebrafish (*Danio rerio*), *Green Chemistry,* 8(3), 238-240.

Ranke, J. and Jastorff, B. (2000). Multidimensional risk analysis of antifouling biocides, *Environmental Science and Pollution Research,* 7(2), 105-114

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

Stolte, S., Matzke, M., Arning, J., Boschen, A., Pitner, W., Welz-Biermann, U., et al. (2007). Effects of different head groups and functionalised side chains on the aquatic toxicity of ionic liquids. *Green Chemistry*, 9(11), 1170-1179.

Torrecilla, J. S., García, J., Rojo, E. and Rodríguez, F. (2009). Estimation of toxicity of ionic liquids in Leukemia Rat Cell Line and Acetylcholinesterase enzyme by principal component analysis, neural networks and multiple lineal regressions, *Journal of hazardous materials,* 164(1), 182-194

Ventura, S. P. M., Gonçalves, A. M. M., Gonçalves, F., & Coutinho, J. A. P. (2010). Assessing the toxicity on [C3mim][Tf2N] to aquatic organisms of different trophic levels. *Aquatic Toxicology*, 96(4), 290-297.

Ventura, S. P. M., Marques, C. S., Rosatella, A. A., Afonso, C. A. M., Gonçalves, F., & Coutinho, J. A. P. (2012). Toxicity assessment of various ionic liquid families towards *Vibrio fischeri* marine bacteria. *Ecotoxicology and Environmental Safety*, 76(0), 162-168.

Wasserscheid, P. and Welton, T. Ionic Liquids in Synthesis, Vol. 1. Wiley-VCH Verlag GmbH & Co. KGaA, Germany, 2009

Yasri, A., & Hartsough, D. (2001). Toward an optimal procedure for variable selection and QSAR model building. *Journal of Chemical Information and Computer Sciences*, 41(5), 1218-1227.