The effects of cation-anion interaction on the toxic properties of ionic liquids

Dissertation

by

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Dissertation submitted in partial fulfilment of

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CERTIFICATION OF APPROVAL

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A project dissertation submitted to the
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Approved by,

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(Dr. Mohanad El-Harbawi)

UNIVERSITI TEKNOLOGI PETRONAS
TRONOH, PERAK
DECEMBER 2010

CERTIFICATION OF ORIGINALITY

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

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(Muhammad Nabil Hakim bin Nasaruddin)

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ABSTRACT

This report is about the study on the toxicity of ionic liquids. Ionic liquids are low melting point organic salts that have become increasingly attractive as green solvents for industrial applications. The reputation of these solvents as “environmentally friendly” chemicals is based primarily on their negligible vapour pressure. However, it is important to remember that not all ionic liquids are environmentally benign and less toxic. Some of the ionic liquids might be dangerous to deal with it and can cause adverse effects. Fish acute toxicity test can be used to identify and prove that ionic liquids are actually toxic. In this project, the scope is mainly about the test (fish acute toxicity test), which is conducted to identify ionic liquids that are considered as toxic, and measure it’s toxicity towards Guppy fish (*Poecilia reticulata*). This test is based on OECD guideline 203. The fish are exposed to the test substance (ionic liquids) preferably for a period of 96 hours. Mortalities are recorded at 24, 48, 72 and 96 hours and the concentrations which kill 50 per cent of the fish (LC_{50}) are determined. At the same time, the interaction between the cation and anion that could really affects the toxicity of ionic liquids are also studied in this project. From the test, some ionic liquids are proven to be toxic to aquatic life (guppy fish) and the results clearly reveal that ionic liquids may cause a completely different effect on guppy fish according to their chemical structure which is the interaction between the cation and anion of the ionic liquids.

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

Ionic liquids are a group of organic salts that result from the combination of several organic cations and inorganic anions, and they may be liquid at room temperature. The most commonly studied ionic liquids contain the imidazolium cation with varying heteroatom functionality. Ionic liquids are considered advantageous not only because of their versatility but also for their "green" credentials (Reichert, 2005).

Ionic liquids have been considered as an interesting solvent because it has the ability to replace any organic solvent. It is because ionic liquids are a low vapour pressure solvent which leads ionic liquids to become low flammability solvent (less toxic). It is because low vapour pressure of ionic liquids minimise the release of chemical in the atmosphere when they are used as a solvents.

A cation anion interaction plays a big role on determining the properties of ionic liquids. In the other mean, the properties of ionic liquids such as physical and solvent properties as well as toxic properties are governed by cation anion interactions (Reichert, 2005). Variations in cation and anion can produce a large number of ionic liquids. Because of that reason, the properties of ionic liquids are totally depends on the structure of ions.

It is important to remember that not all ionic liquids are environmentally benign and less toxic. Some of the ionic liquids might be dangerous to deal with it and can cause adverse effects. The interactions of the cation-anion plays a big role on determining the toxic properties of the ionic liquids as the properties of the ionic liquids totally depends on the structure of ions. A less toxic solvent can make ionic liquids as a green solvent whereby it will be able to replace any classical (volatile) organic solvent to act as a solvent to any chemical substance.

The toxicity of ionic liquids can be measured by using fish acute toxicity test. This test is based on the OECD guideline 203 (OECD, 1992).

1.2 PROBLEM STATEMENT

The dramatic growth in ionic liquid research over the past decade has resulted in the development of a huge number of novel ionic liquids. The perceived environmentally friendly nature of ionic liquids, which results from their negligible vapour pressure, is now under scrutiny. It is because, although ionic liquids will not evaporate into the air, there is no guarantee that they will never enter the environment.

Toxicity research studies towards ionic liquids including ecotoxicity, cytotoxicity, and phytotoxicity, have recently received broad attention as the commonly accepted notion that ionic liquids have low toxicity has shown to be incorrect. Fish acute toxicity test can be used to identify and prove that the ionic liquids are actually dangerous to deal with it and can harm aquatic life.

1.3 OBJECTIVE

The main objectives of this research are:

- To briefly study about the nature of ionic liquids as well as its toxicities
- To measure toxicity of ionic liquids by using fish acute toxicity test
- To study on how the interactions between the cation and anion affects the toxicity of ionic liquids

1.4 SCOPE OF STUDY

In this project, the scope of study is mainly about the test (fish acute toxicity test), to identify and measure the toxicity of ionic liquids towards Guppy fish (*Poecilia reticulata*). In this test, five imidazolium based ionic liquids is used in this test and ten Guppy fish (*Poecilia reticulata*) are tested on one concentration of each imidazolium based ionic liquids. The Guppy fish (*Poecilia reticulata*) are exposed to the test substance (ionic liquids) preferably for a period of 96 hours. Mortalities are recorded at 24, 48, 72 and 96 hours and the concentrations which kill 50 per cent of the fish (LC_{50}) are based on the outcomes from the test. At the same time, the interactions between the cation and anion that could really affect the toxicity of ionic liquids are also investigated in this project.

1.5 RELEVANCE OF THE PROJECT

This project (the effect of cation-anion interaction on toxic properties of ionic liquids) is relevant to Chemical Engineering academic syllabus of Universiti Teknologi PETRONAS (UTP) which is Organic chemistry subject. It incorporates knowledge and enhance the understanding of the ionic liquids itself and also its application in chemical industry. The knowledge of ionic liquids is not useful only during university life, but also during working life. A chemical engineer definitely will be dealing with all these things during their working life. In addition, this project also enhances the project management and communication skills.

1.6 FEASIBILITY OF THE PROJECT WITHIN SCOPE AND TIME FRAME

For this project, the first semester will cover formulation of methodology and conceptualization of this experimental based project based on the experimental methods done by other researchers. It is because this final year project will be evaluated up to methodology only. For the second semester, it will be concentrated on the detail methodology and also the experiment will be running based on the proposed methodology. At the end of the semester, the conclusion of this experimental based project will be come out and will be evaluated whether it is following the theory or not. Based on the draft Gantt chart in Appendix 2 and 3, the project's objectives are considered achievable within the given time frame.

CHAPTER 2

LITERATURE REVIEW

2.1 IONIC LIQUIDS

Ionic liquid (IL) is a liquid that contains only ions. The first research in ionic media consisted of pure inorganic salts or eutectic mixtures of inorganic salts heated above their melting point which is usually several hundred degrees Celsius (Reichert, 2005). However, today, the term "ionic liquid" is used to designate a group of salts that are liquid at low temperatures which is by definition below 100°C (Rodrigues et al., 2010). In particular, the salts that melt at room temperature are called "room temperature ionic liquids" (RTILs) (Beritchte, 2005). Ionic liquids normally constituted by a large of organic cation with long carbonic chains and a weekly coordinating anion (Wassercheid et al., 2000). Room temperature ionic liquids consist of bulky and asymmetric organic cations such as imidazolium, pyrindium, phosponium or ammonium ions. The cations are paired with a wide range of anions which are from simple halides to inorganic anions such as tetrafluoroborate and hexafluorophosphate and to large organic anions like bis (trifluorosulfonyl) amide, triflate or tosylate (Laus et al., 2005).

2.1.1 Synthetic concepts of ionic liquids

The principal synthetic concepts for ionic liquids involves the quaternisation reactions to generate the cation and followed by the anion metathesis, either by the addition of metal salts in order to precipitate the undesired anion, addition of strong Bronsted acids to release the unwanted anion as the volatile corresponding acid, with the use of ion exchange resins, or treatment with Lewis acids in order to form the complex anions like chloroaluminates (Wassercheid et al., 2005). Besides that, the halide ions or acids that remain in the product should often being traced. Halide-free ionic liquids can be produced by using alkylcarbonates as alkylating reagents and removing the carbonate as gaseous CO_2 or unsoluble metal salt (Laus et al., 2005).

2.1.2 Structure of ionic liquids

Typically, ionic liquids consist of an organic cation with delocalized charges and a small inorganic anion, most often halogen anions weakly coordinating (Romero et al., 2008). An ionic liquid can be thought of as “designer” solvent so it should be possible to design, or tailor, a solvent for a certain reaction (Stefan et al., 2006). Therefore, many cation and anion combinations are possible, changing properties as polarity, hydrophobicity and solvent miscibility behaviour.

Among these possibilities, the l-alkyl-3-methylimidazolium is one of the most used because it is non-volatile, non-flammable, presents high thermal stability, and is an excellent solvent for a wide range of inorganic and organic materials. The possibility to modify structural elements in order to optimize technological features like salvation properties, viscosity, conductivity and thermal as well as electrochemical stability is ideal in terms of technical applicability (Stefan et al., 2006). Several structures for ionic liquids can be referred to Appendix 1.

2.1.3 Cation and Anion

Many recent studies have shown that, toxicity of ionic liquids towards *Daphnia magna* and *Pseudokirchneriella subcapitata,* is strongly affected by the cationic head group (Pretti et al., 2009). It decreases on going from aromatic heterocyclic nitrogen-containing compounds (pyridinium and imidazolium) to non-aromatic cyclic and acyclic compounds (pyrrolidinium, ammonium and morpholinium).

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

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

For anion, an experiment of ionic liquids with different anions (Cl, Br, PF6 and BF4) has been done whereby the influence of anions on the toxicity of ionic liquids was studied. From the experiment, they have concluded algae are far less sensitive to anion changes in the ionic liquid structure (Adam Latala et al., 2009) and anion has a low effect on toxicity (Romero et al., 2008). On the other hand, a formula to calculate the anion effect ratio (AR) for imidazolium ionic liquids has been developed (Stefan et al., 2006). The formula is:

$$AR = \frac{EC_{50}(R_x MIMCl)}{EC_{50}(R_x MIMY)}$$

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

2.2 PROPERTIES OF IONIC LIQUIDS

Ionic liquids properties depend on both anion and cation characteristics, as the carbonic chain and attached functional groups, what makes them possible to design them to have the best performance for a desired application (Rodrigues et al., 2010). Some ionic liquids can exhibit unusual and advantageous properties like low vapour pressure (stable in large vacuum), non flammability, high thermal stability (up to 300 – 400 Celsius), wide liquid temperature range, high solvency, high thermal conductivity and immiscibility with many organic solvents (Reichert, 2005).

Their physical and solvent properties can be tailor-designed by tuning the pairing and structure of the cations and anions (Wasserscheid et al., 2000). The relations between the properties and the structure of ionic liquids is that the density and melting point is related to the R groups of the organic cation, the viscosity is directly related to the H-bonding and van der wals interactions, and also the acidity and the coordination properties of ionic liquids are determined by the nature of its anion. The properties of a modern ionic liquid are summarized in Appendix 4.

2.4 IONIC LIQUIDS AS GREEN SOLVENT

The green character of ionic liquids is mainly attributed to their negligible vapor pressure (Romero et al., 2007). In the other mean, ionic liquids have very low vapor pressure that minimises the release of chemical in the atmosphere when they are using as a solvents. Negligible of vapor pressure which results in reduced air emission, non flammability, and non explosiveness (Seddon, 2002). Thus, room-temperature ionic liquids could provide environmentally friendly solvents for the chemical and pharmaceutical industries ((Romero et al., 2007).

Although, the low vapor pressure of ionic liquids may reduce the air pollution with respect to the typical volatile organic solvents, this is not enough to justify calling a "green" process (Romero et al., 2007). It must be considered that a release of ionic liquids from industrial processes into aquatic environments may lead to water pollution, because of their high solubilities in water.

2.5 APPLICATIONS OF IONIC LIQUIDS

Ionic liquids are solvents that can be designed for special applications either by paring different cations and anions to fine-tune the properties of a particular ionic liquid in order to fulfil certain applications (Laus et al., 2005). Ionic liquids with the appropriate properties should be selected for each particular application as their importance has increased in recent years, in which they show better performance than water or molecular organic solvents (Rodrigues et al., 2010).

The most common application of ionic liquids has been their use as chemical reaction solvents. Others are like they have been used in liquid-liquid extraction (LLE), applied in BASIL process and one of the most exciting and impressive potential industrial applications of ionic liquids is their use for the storage and delivery of gases that are highly toxic, flammable, and/or reactive (Laus et al., 2005).

In electrochemistry, ionic liquids have clearly potential as conducting media with large electrochemical windows. For example, reversible electrochemical deposition of magnesium on a silver surface was realised in the ionic liquid [BuImMe][BF4] and also the hydrophobic ionic liquids [BuImMe] [PF6] and [Tf2N] were used in developing a highly efficient anode system for lithium/seawater batteries (Laus et al., 2005).

2.5 TOXICITY OF IONIC LIQUIDS

2.5.1 Toxicity of ionic liquids to algae

Several groups have focused their attention on the use of algae primary producers to assess the effects of ionic liquids to aquatic environment (Cho et al., 2007). For example, Cho and co-workers have done their research by using *pseudokirchneriella subcapitata* in order to study the effect of different head groups, side chains, and anions of ionic liquids on algae growth rate and photosynthetic activity. Based on the data obtained, it revealed that the toxic influence of ionic liquids on growth rates were more significant than those of photosynthetic performance and the toxicity increase with the increasing of alkyl chain length (Cho et al., 2007; Pham et al., 2008b).

Latala et al. (2005), who selected two marine algae *Oocystis submarina* (green algae) and *Cyclotella meneghiniana* (diatom) as testing organisms, found that the two species differed dramatically in their ability to recover from IL exposure. Additionally, it was discovered that IL toxicity declined with increasing salinity. The lower toxicity of IL in that case is probably due to the reduced permeability of IL cations through the algal cell walls (Pham et al., 2009).

2.5.2 Phytotoxicity of ionic liquids

The studies on phytotoxicity activity of ionic liquids were conducted mostly on the duckweed, *Lemma minor*, a common aquatic vascular plant (Larson et al., 2008). In general, 1-alkyl-3-methylimidazolium compounds with longer alkyl chains were more toxic to *Lemma minor* than those with the short alkyl chain lengths (Pham et al., 2009). On the other hands, based on Stolte et al. (2007a), the anion had no or even a positive influence on the observed effects on *Lemma minor*.

2.5.3 Toxicity of ionic liquids to invertebrates

Toxicity of ionic liquids to invertebrates mainly focuses on the use of *Daphnia magna* as test organism (Bernot et al., 2005a). The results of all studies again observed the well-established link between toxicity and alkyl chain length of the tested ILs containing imidazolium, pyridinium or quaternary ammonium as counter cations. The most toxic compound towards *D. magna* was found to be IM18 Br. Also, the nature of the anion was suggested to have smaller effects compared to those of the cation (Pham et al., 2009).

In a recent study, Luo et al. (2008) investigated the developmental toxicity of IM18 Br on *Daphnia magna* is investigated. It was found that this compound exhibited toxicity on the development of three generation of *Daphnia magna* with the decrease of number of offspring and average brood size correlated to increasing IM18 Br concentrations. This indicated that IM18 Br could cause deleterious effect to the population of *Daphnia magna* and indirectly disturb freshwater food webs (Pham et al., 2009).

2.5.3 Toxicity of ionic liquids to fish

Ionic liquids today had been found to be toxic to fish. In the research of Pretti et al. (2005), the toxicities of ionic liquids towards zebra fish *(Danio rerio)* had been studied. The aim of that present work was therefore to evaluate the acute toxicity of 15 widely used ionic liquids (bearing different anions and cations) to zebra fish *(Danio rerio)*. Each of ionic liquid was tested at five concentrations (1.25, 2.5, 5.0, 10.0, and 20.0 mg/L)

The number of fish dead was recorded after 1, 12, 24, 48, 72, and 96 hours. The LC50 value which is the concentration in water which 50% of the test batch within a continuous period of exposure of 96 hours is determined based on the outcomes from the test. The results presented by Pretti et al. (2005) clearly reveal that ionic liquids may cause a completely different effect on fish according to their chemical structure.

CHAPTER 3

METHODOLOGY

3.1 INTRODUCTION

The methodology for the experiment (fish acute toxicity test) is formulated based on the OECD guideline 203. This new version of the guideline, originally adopted in 1981 and first updated in 1984, is based on a proposal from the United Kingdom to reduce the numbers of fish in tests of acute aquatic toxicity. The main differences in comparison with the earlier versions are the reduction in group-size allowing the use of seven fish per group. The principle of the test is whereby the fish are exposed to the test substance preferably for a period of 96 hours. Mortalities are recorded at 24, 48, 72 and 96 hours and the concentrations which kill 50 per cent of the fish (LC_{50}) are determined where possible (OECD, 1992).

3.2 MATERIALS

3.2.1 Chemicals

The ionic liquids used in this study are 1-ethyl-3-methylimidazolium chloride ([C_2mim] CL), 1-butyl-3-methylimidazolium chloride ([C_4mim] CL), 1-hexyl-3-methylimidazolium chloride ([C_6mim] CL), 1-octyl-3-methylimidazolium chloride ([C_8mim] CL), and 1-butyl-3-methylimidazolium iodide ([C_4mim] I). All these ionic liquids are synthesized in laboratory conditions. Regarding the concentration of ionic liquid, at least four or five concentrations in a geometric series and with a factor preferably not exceeding 2.2. A range-finding test properly conducted before the definitive test enables the choice of the appropriate concentration range. The concentration range for this test is 6.25, 12.5, 25, 50 and 100 mg/L. For limit test, the concentration used is 100 mg/L. The amount of ionic liquids used in the test is shown in Appendix 5.

3.2.2 Guppy fish

Examples of the fish recommended for this test are given in the Appendix. For this project, guppy fish (*Poecilia reticulata*) has been chosen. The fish should be in good health and free from any apparent malformation. To do that, the fish can be bred and cultivated either in fish farms or in the laboratory, and put it under disease- and parasite-controlled conditions.

All the Guppy fish (*Poecilia reticulata*) must be obtained and held in the laboratory for at least 7 until 12 days before they are used for testing. They must be held in water of the quality to be used in the test for at least seven days immediately before testing and under the following conditions:

- Light: 12 to 16 hours photoperiod daily
- Temperature: appropriate to the species (for Guppy fish, 21 – 25 Celsius)
- Oxygen concentration : at least 80 per cent of air saturation value
- Feeding: three times per week or daily until 24 hours before the test is started

3.3 METHODS

3.3.1 Apparatus

i) Petri dish

ii) PH meter

iii) Dissolved oxygen meter

iv) Aquarium and other fish equipments

3.3.2 Validity of the test

For this test to be valid, following conditions should be fulfilled (OECD, 1992):

i) The mortality in the control(s) should not exceed 10 per cent (or one fish if less than ten are used) at the end of the test.

ii) Constant conditions should be maintained as far as possible throughout the test.

iii) The dissolved oxygen concentration must have been at least 60 per cent of the air saturation value throughout the test.

iv) There must be evidence that the concentration of the substance being tested has been satisfactorily maintained, and preferably it should be at least 80 per cent of the nominal concentration throughout the test. If the deviation from the nominal concentration is greater than 20 per cent, results should be based on the measured concentration.

3.3.3 Procedures

i) The chemicals (ionic liquids) are prepared based on the guideline. Five imidazolium based ionic liquids are used in this fish acute toxicity test. The pictures of these experimental procedures are shown in Appendix 6.

ii) Good quality water for the Guppy fish is prepared based on the guideline. (Waters with a pH 6.0 to 8.5 are preferable). The oxygen dissolved in the water and temperature is measured.

iii) Limit test is conducted on the five imidazolium based ionic liquids to determine the ionic liquids that are relatively non-toxic and toxic to aquatic life.

iv) Experiment (fish acute toxicity test) started. Ten Guppy fish are tested on each concentration of one imidazolium based ionic liquids. Conditions of exposure are checked in order to get accurate results based on the guideline. The conditions are:

- Duration: preferably 96 hours.
- Light: 12 to 16 hours photoperiod daily
- Temperature: 21 – 25 Celsius and constant within a range of 2°C
- Feeding: none.

v) The fish are inspected at least after 24, 48, 72 and 96 hours.

vi) After 96 hours, the result is checked and evaluated in terms of lethality, mortalities of the guppy fish, feeding rate, fish movement and its survival rate. Dead fish are removed when observed and mortalities are recorded. Fish are considered dead if there is no visible movement (e.g. gill movements) and if touching of the caudal peduncle, its produces no reaction.

vii) Theoretical work (probit analysis) is conducted based on the observations and the results after four days (96 hours)

3.3.4 Data treatment

The LC_{50} values for the guppy fish ((*Poecilia reticulata*) were determined by probit analysis. For limit test, the ionic liquids tested will be categorized from practically harmless to highly toxic by using LC_{50} value according to an acute toxicity rating developed by Knight and Thomas, (2003). For the acute toxicity test using ionic liquids that considered toxic to aquatic life, the LC_{50} values is compared in order to see and study on how the interaction between the cation and anion affects the toxicity of ionic liquids.

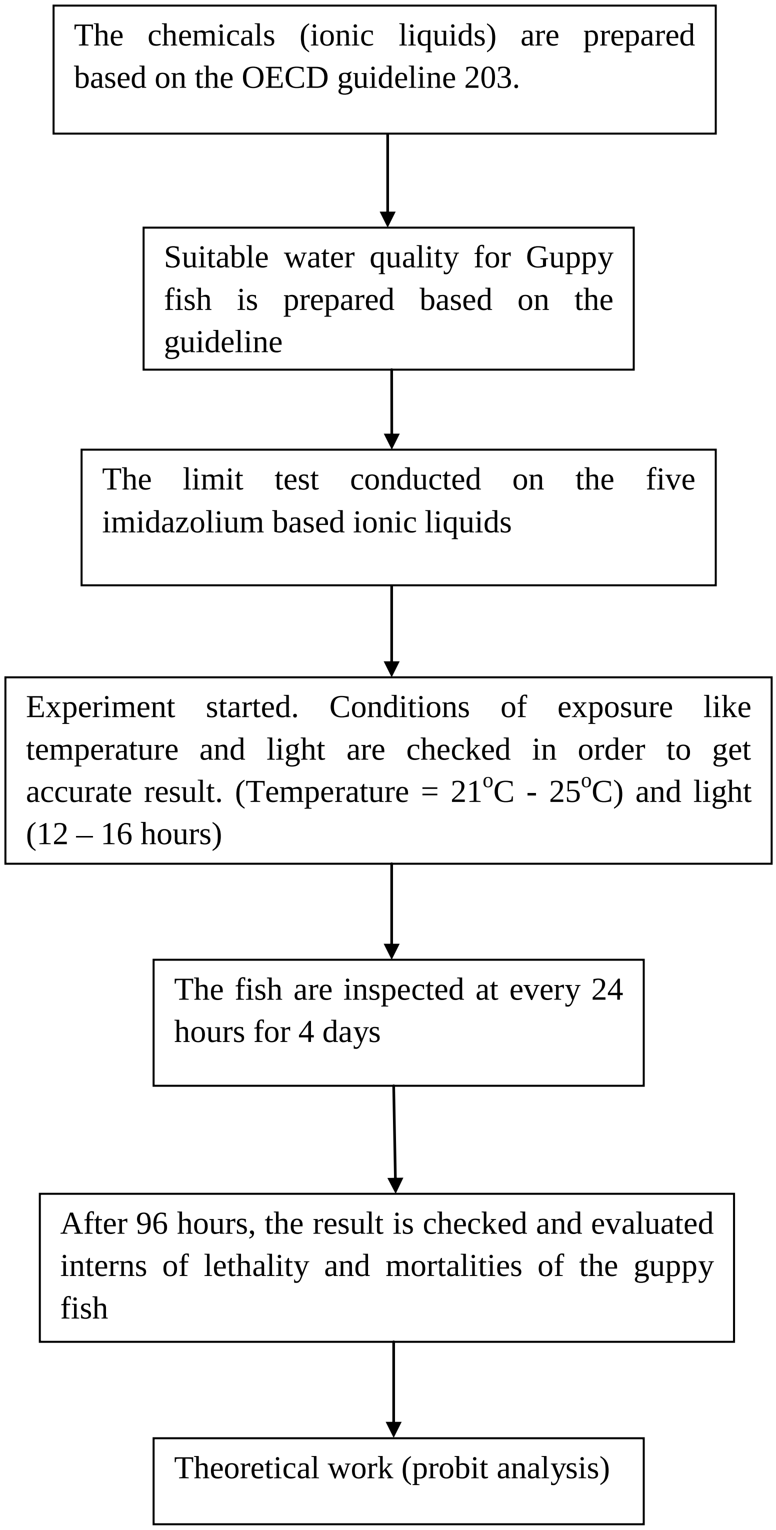


Figure 1: Flowchart of the experiment procedures

3.4 THEORETICAL WORK (PROBIT ANALYSIS)

3.4.1 Manual calculation

Below are the steps involved to do the probit analysis by using manual calculation.

i) Setup a probit calculation table. For this experiment, three types of ionic liquids had been carried out by using 5 concentrations (mg/L). Ten guppy fish were exposed and the numbers of fish died were observed after 96 hours of exposure. The concentration need to be converted into $\log_{10}$ and the percentage of mortality is determined based on how many fish died.

Table 1: Probit calculation table

Conc. (mg/L)	Log Conc.	Total No.	No. dead	% Mortality

ii) The mortality (%) vs. Log_{10} concentration is plotted in figure 2. The graph will be showing a normal sigmoid

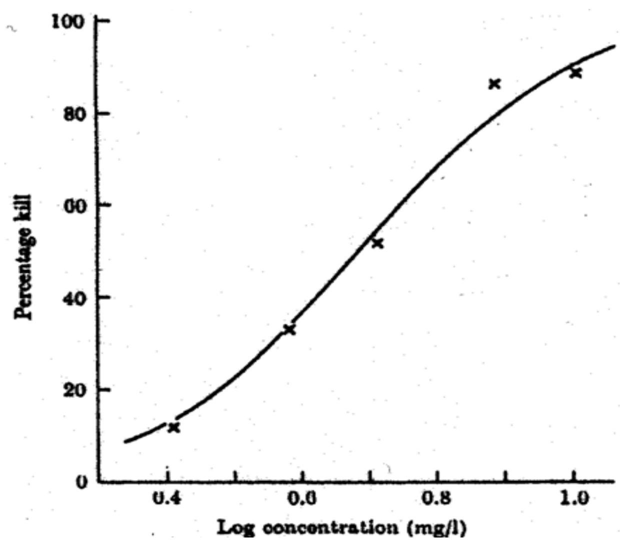


Figure 2: The mortality (%) vs. Log10 concentration

The figure 2 shows that the relation between the % mortality against the $\log_{10}$ of is not a straight line. Therefore, the value of % mortality needs to be transformed into probit. The purpose of the probit transformation is to straighten the line so that we can estimate the value of LC_{50}.

iii) Transformation of % mortality into probit. This can be done by referring to a table 3.

Table 2: Transformation of percentages to probits

%	0	1	2	3	4	5	6	7	8	9
0	—	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33
—	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
99	7.33	7.37	7.41	7.46	7.51	7.58	7.65	7.75	7.88	8.09

88% mortality

For example, let's say the % mortality is 88% and we want to transform it into probit. The value of probit will be 6.18.

iv) A straight line of best line through plotted points is drawn. That line is used to estimate the log10 concentration associated with a probit or Y =5.

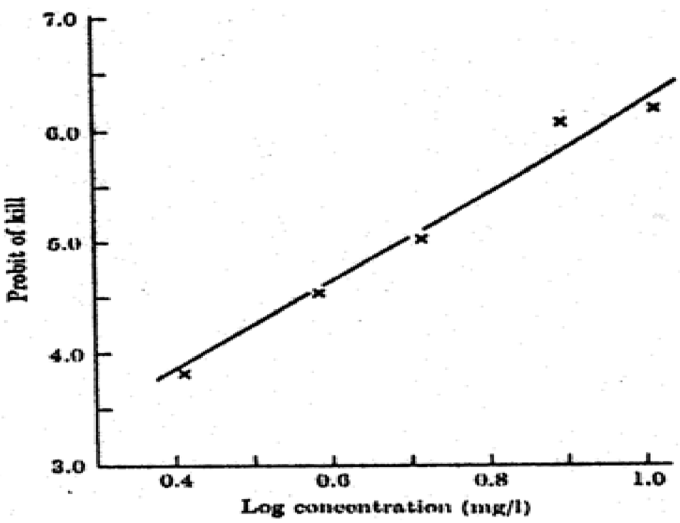


Figure 3: Probit vs. Log10 Concentration

v) A straight line will produce a regression equation. The LC_{50} value is determined from this equation. For example:

Equation: Y = 4.2267X – 3.5333

LC_{50} is at 50% of mortalities and probit at 5.

Therefore, Y = 5

5 = 4.2267X – 3.5333

X = 2.02

LC_{50} = antilog (2.02)

= **<u>104.7 mg/L</u>**

For this approach, the value of X is determined form the regression equation, not from the graph.

vi) Another approach to determine LC_{50} is as per below:-

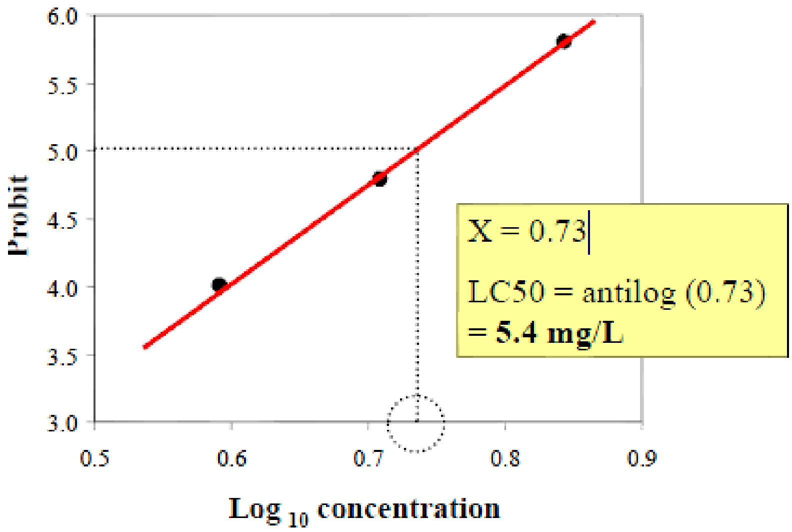


Figure 4: Estimation of LC_{50} value

The value is determined by taking the dosage (log_{10} of concentration) associated with a probit or Y =5. For example, at Y = 5, the value of X which is the log_{10} concentration is equal to 0.73. So, the LC_{50} for this types of ionic liquids will be antilog (0.73) = 5.4 mg/L

3.4.2 Software

Due to the time constraint, the probit analysis also can be done by using a statistical package such as SPSS, SAS, R, or S. Below are some steps for the probit analysis using the software.

i) The percentage of mortality of the fish is converted to probit. Computer software such as SPSS, SAS, R, or S convert the percent responded to probit automatically. The concentrations of ionic liquids are taken as the log of concentration.

ii) The probit versus the log of the concentrations are plotted and fit a line of regression. Both least squares and maximum likelihood are acceptable techniques to fitting the regression, but maximum likelihood is preferred because it gives more precise estimation of necessary parameters for correct evaluation of the results. For the computer software like SPSS, it uses maximum likelihood to estimate the linear regression. To run it in the SPSS, simply input a minimum of three columns into the Data Editor. The columns are the number of individuals per container that responded, total of individuals per container and concentrations.

iii) The LC_{50} is determined by searching the probit list for a probit of 5.00 and then taking the inverse log of the concentration it is associated with.

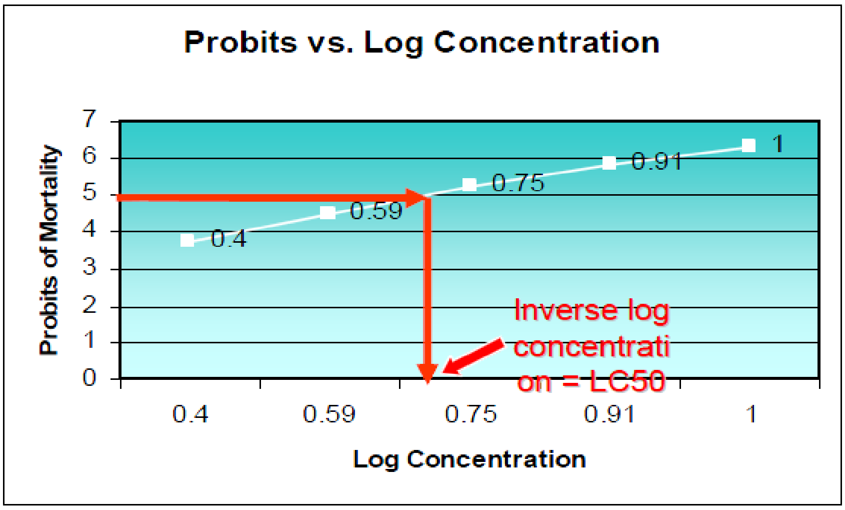


Figure 5: Probit vs. Log10 Concentration

CHAPTER 4

RESULTS AND DISCUSSION

4.1 LIMIT TEST

The limit test of five kinds of 1-Alkyl-3-methylimidazolium ionic liquids is summarized in the table below. The results shows that for 1-ethyl-3-methylimidazolium chloride ([C_2mim] CL), and 1-butyl-3-methylimidazolium iodide ([C_4mim] I), there are no mortalities occurs after four days. While for 1-butyl-3-methylimidazolium chloride ([C_4mim] CL), 1-hexyl-3-methylimidazolium chloride ([C_6mim] CL) and 1-octyl-3-methylimidazolium chloride ([C_8mim] CL), there are mortalities occurs after four days. Two fish dead for 1-butyl-3-methylimidazolium chloride ([C_4mim]CL) and 1-hexyl-3-methylimidazolium chloride ([C_6mim] CL) while three fish dead for 1-octyl-3-methylimidazolium chloride ([C_8mim] CL).

Table 3: Limit test

Types of ionic liquid	No. of fish	Concentration (mg/L)	No. of fish dead (96 hours)
1-ethyl-3-methylimidazolium chloride ([C_2mim]CL)	10	100	None
1-butyl-3-methylimidazolium chloride ([C_4mim]CL)	10	100	2
1-butyl-3-methylimidazolium iodide ([C_4mim]I)	10	100	None
1-hexyl-3-methylimidazolium chloride ([C_6mim]CL)	10	100	2
1-octyl-3-methylimidazolium chloride ([C_8mim]CL)	10	100	3

Based on OECD guideline 203, this limit test is performed at 100 mg (active ingredient)/l in order to demonstrate that the LC_{50} is greater than this concentration. The limit test is performed by using a minimum of 7 fish and maximum 10 fish. For this experiment, 100 mg/L of each of ionic liquids has been used and put it in the aquarium containing 10 numbers of fish. Binomial theory dictates that when 10 fish are used with zero mortality, there is a 99.9 % confidence that the LC_{50} is greater than 100 mg/l (OECD, 1992).

With 7, 8 or 9 fish, the absence of mortality provides at least 99% confidence that the LC_{50} is greater than the concentration used in the limit test (OECD, 1992). For 1-ethyl-3-methylimidazolium chloride ([C_2mim] CL) and 1-butyl-3-methylimidazolium iodide ([C_4mim] I), there is 99.9 % confidence that the LC_{50} for these types of ionic liquids is greater than 100 mg/l (LC_{50} > 100 mg/L) as they produce zero mortality of guppy fish. Thus, they are categorized as non-toxic to aquatic life.

For 1-butyl-3-methylimidazolium chloride ([C_4mim] CL), 1-hexyl-3-methylimidazolium chloride ([C_6mim] CL) and 1-octyl-3-methylimidazolium chloride ([C_8mim] CL), mortalities occurs. As mortalities occur, there is 99.9 % confidence that the LC_{50} is below the concentration, 100 mg/L (LC_{50} < 100mg/L). Thus, they are categorized as toxic to aquatic life and further study should be conducted to measure the toxicity of both ionic liquids. Table below shows how they are being categorized as toxic and non-toxic to aquatic life.

Table 4: Classification of chemicals as dangerous to environment (Knight and Thomas, 2003)

Classification of chemicals as dangerous to the environment	
Rating	**LC_{50}**
Highly toxic to aquatic life	< 1 mg/l
Toxic to aquatic life	> 1 < 10 mg/l
Moderately toxic to aquatic life	> 10 < 100 mg/l
Relatively non-toxic	> 100 mg/l

Based on the results from limit test, three kinds of 1-Alkyl-3-methylimidazolium ionic liquids have been identified as toxic to aquatic life which are 1-butyl-3-methylimidazolium chloride ([C_4mim]CL), 1-hexyl-3-methylimidazolium chloride ([C_6mim]CL) and 1-octyl-3-methylimidazolium chloride ([C_8mim]CL). Therefore, next experiment is continued with these three kinds of 1-Alkyl-3-methylimidazolium ionic liquids to measure their toxicity towards aquatic life (guppy fish).

4.2 FISH ACUTE TOXICITY TEST

4.2.1 1-butyl-3-methylimidazolium chloride ([C_4mim] CL)

Table 5: Probit calculation table for BMIM Chloride

Concentration (mg/L)	Log_{10} concentration	Total No. fish	Total No. fish dead (96hours)	% mortality	Probit
6.25	0.8	10	0	0	0
12.5	1.1	10	0	0	0
25	1.4	10	1	10	3.72
50	1.7	10	1	10	3.72
100	2	10	3	30	4.48

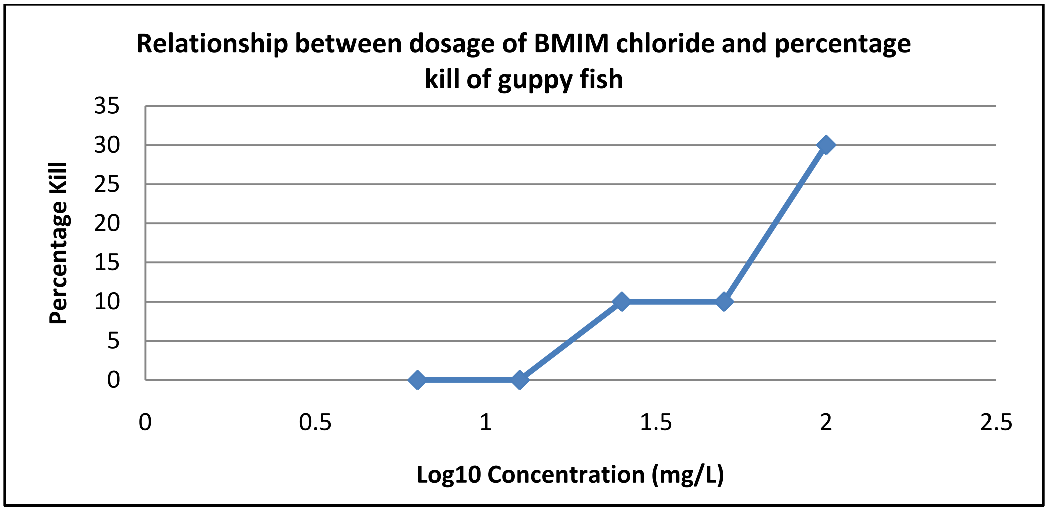


Figure 6: Relationship between dosage and percentage of kill

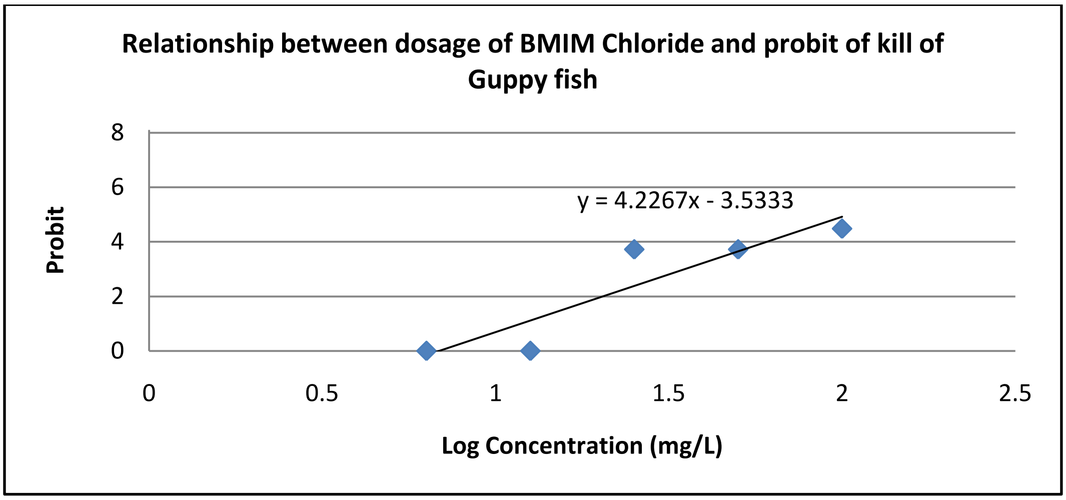


Figure 7: Relationship between dosage and probit of kill

4.2.2) 1-hexyl-3-methylimidazolium chloride ([C_6mim] CL)

Table 6: Probit calculation table for HMIM Chloride

Concentration (mg/L)	Log10 concentration	Total No. fish	Total No. fish dead (96hours)	% mortality	Probit
6.25	0.8	10	0	0	0
12.5	1.1	10	1	10	3.72
25	1.4	10	1	10	3.72
50	1.7	10	2	20	4.16
100	2	10	4	40	4.75

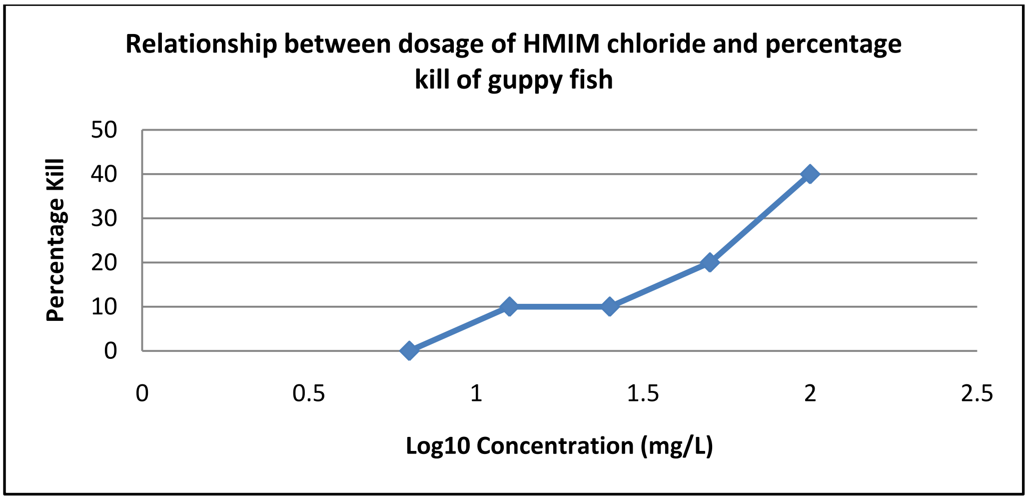


Figure 8: Relationship between dosage and percentage of kill

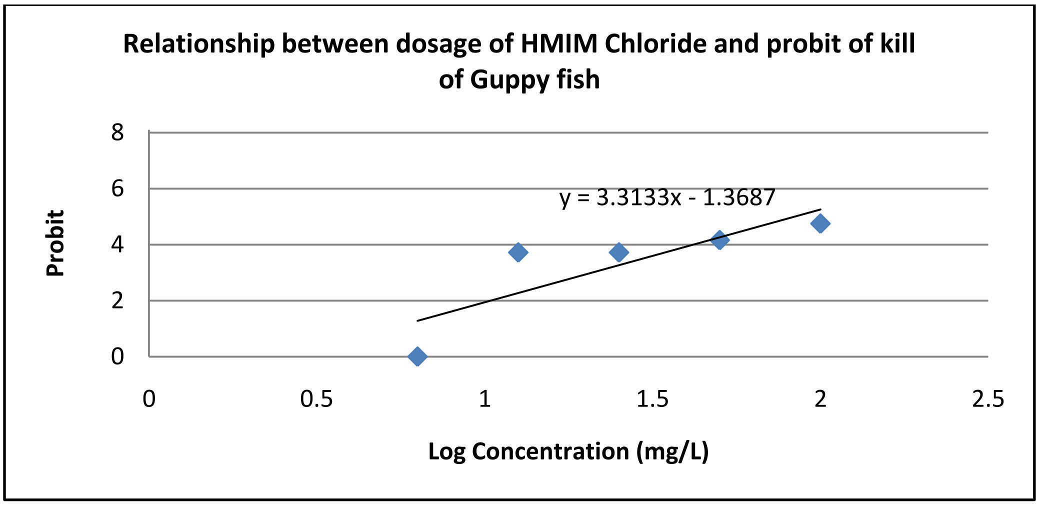


Figure 9: Figure 9: Relationship between dosage and probit of kill

4.2.3) 1-octyl-3-methylimidazolium ([C_8mim] CL)

Table 7: Probit calculation table for OMIM Chloride

Concentration (mg/L)	Log10 concentration	Total No. fish	Total No. fish dead (96 hours)	% mortality	Probit
6.25	0.8	10	0	0	0
12.5	1.1	10	1	10	3.72
25	1.4	10	2	20	4.16
50	1.7	10	3	30	4.48
100	2	10	5	50	5

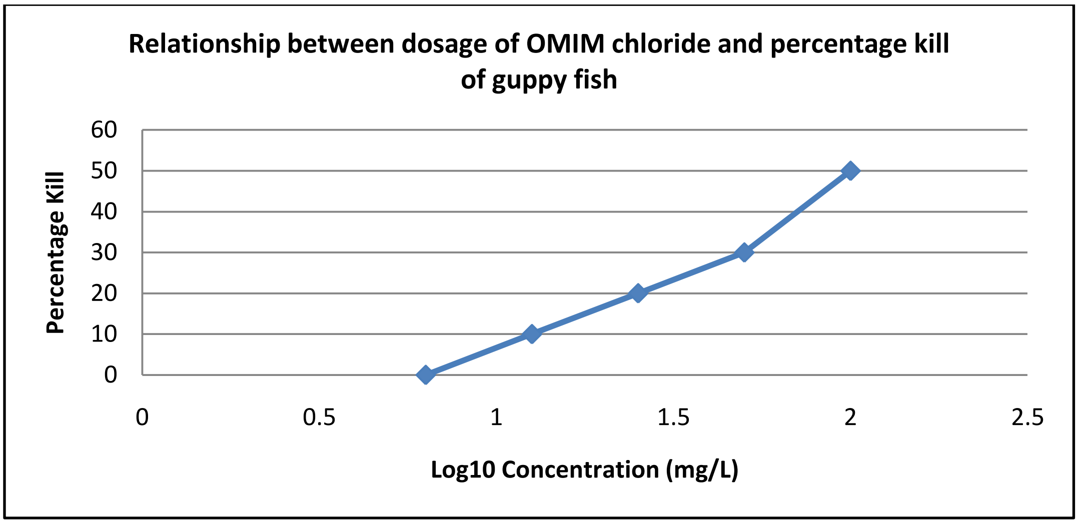


Figure 10: Relationship between dosage and percentage of kill

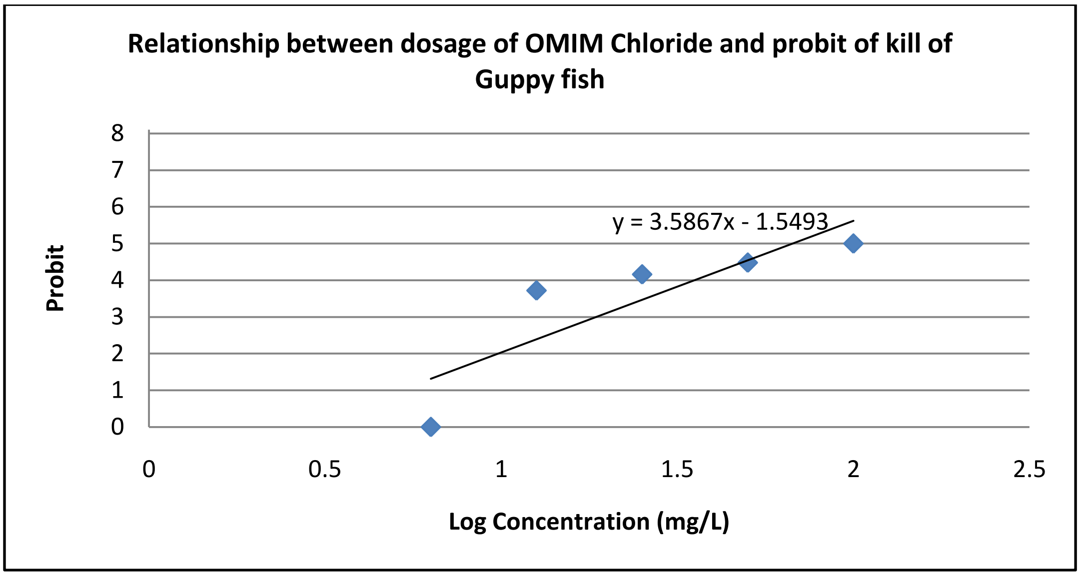


Figure 11: Relationship between dosage and probit of kill

4.3 MEDIAN LETHAL CONCENTRATION (LC_{50})

4.3.1 1-butyl-3-methylimidazolium chloride ([C_4mim] CL)

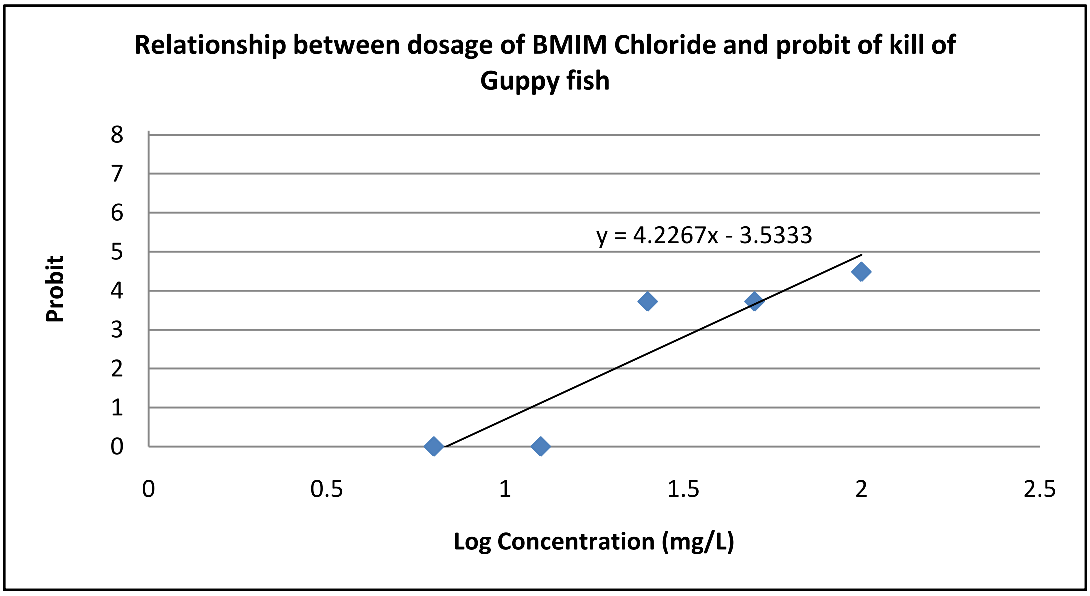


Figure 12: Estimation of LC_{50} value for BMIM Chloride

Equation: Y = 4.2267X – 3.5333

LC_{50} is at 50% of mortalities and probit at 5. Therefore, Y = 5

5 = 4.2267X – 3.5333

X = 2.02

LC_{50} = antilog (2.02)

= **104.7 mg/L**

The LC_{50} for 1-butyl-3-methylimidazolium chloride ([C_4mim] CL) is 104.7 mg/L. This means that, at 104.7 mg/L of concentration, it will kill 50 per cent of the guppy fish out of 10 fish. Next experiment is conducted with 1-hexyl-3-methylimidazolium chloride ([C_6mim] CL). We want to see the impact on the LC_{50} value when we increase the alkyl chain length in cation of the 1-Alkyl-3-methylimidazolium ionic liquids.

4.3.2) 1-hexyl-3-methylimidazolium chloride ([C_6mim] CL)

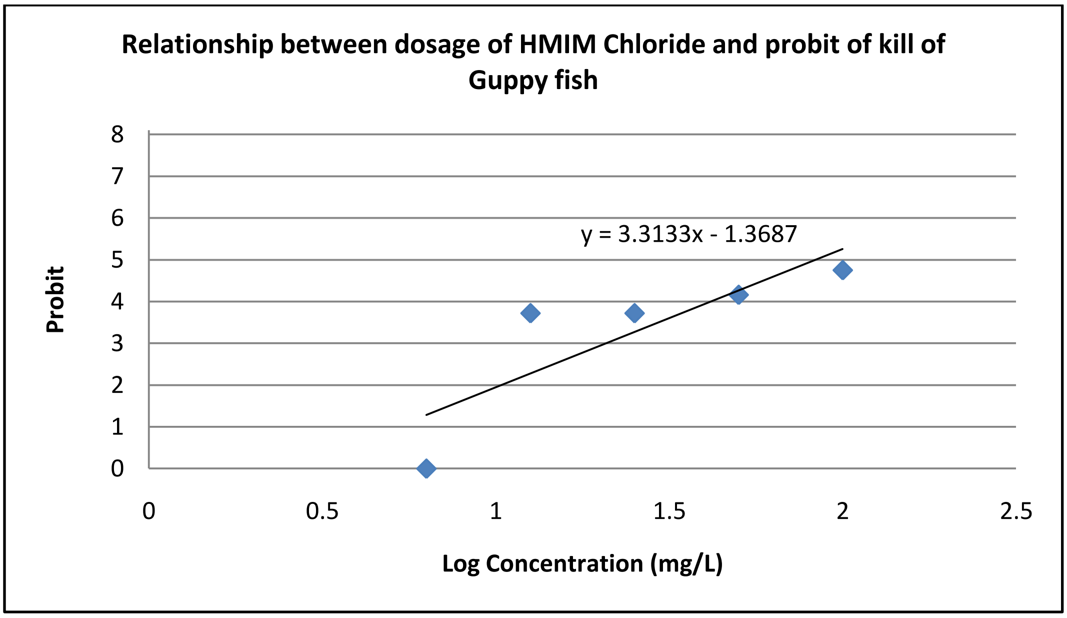


Figure 13: Estimation of LC_{50} value for HMIM Chloride

Equation: Y = 3.3133X – 1.3687

LC_{50} is at 50% of mortalities and probit at 5. Therefore, Y = 5

5 = 3.3133X – 1.3687

X = 1.92

LC_{50} = antilog (1.92)

= **83.2 mg/L**

The LC_{50} for 1-hexyl-3-methylimidazolium chloride ([C_6mim] CL) is 83.2 mg/L. This means that, at 83.2 mg/L of concentration, it will kill 50 per cent of the guppy fish out of 10 fish. Next experiment is conducted with 1-octyl-3-methylimidazolium chloride ([C_8mim] CL). We want to see the impact on the LC_{50} value when we keep increasing the alkyl chain length in cation of the 1-Alkyl-3-methylimidazolium ionic liquids.

4.3.3 1-octyl-3-methylimidazolium chloride ([C_8mim] CL)

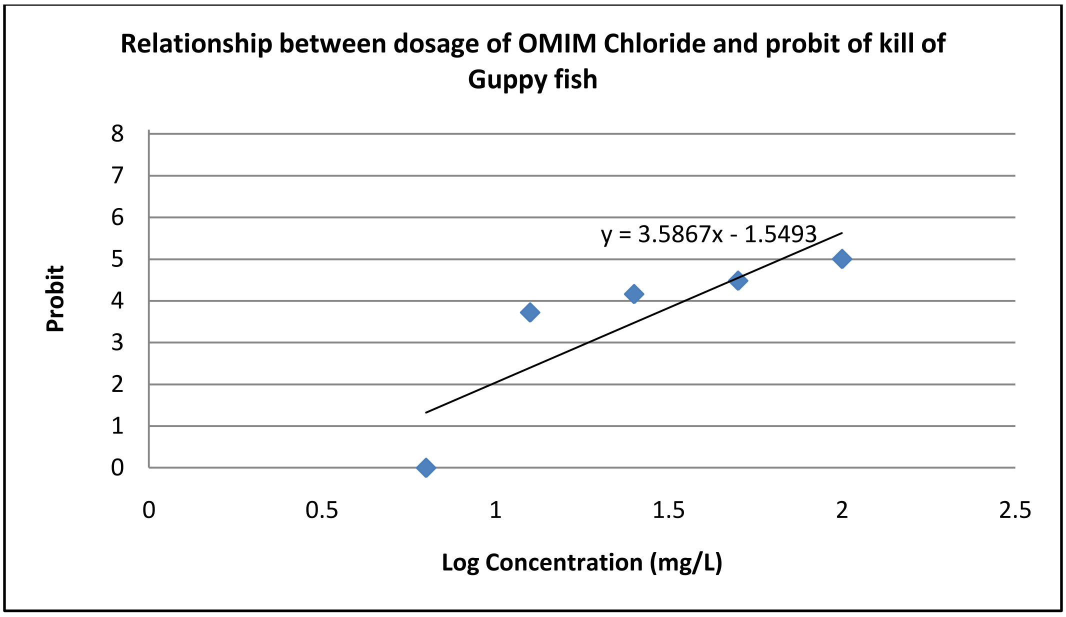


Figure 14: Estimation of LC50 value for OMIM Chloride

Equation: $Y = 3.5867X – 1.5493$

LC_{50} is at 50% of mortalities and probit at 5. Therefore, $Y = 5$

$5 = 3.5867X – 1.5493$

X = 1.83

LC_{50} = antilog (1.83)

= **<u>67.6 mg/L</u>**

The LC_{50} for 1-octyl-3-methylimidazolium chloride ([C_8mim] CL) is 67.6 mg/L. This means that, at 67.6 mg/L of concentration, it will kill 50 per cent of the guppy fish out of 10 fish.

4.4 DISCUSSION

From these three results, we found that ionic liquids toxicity was mainly dependent on their alkyl chain length. After guppy fish was exposed to these three ionic liquids for 96 hours, LC_{50} values decreased from 1-butyl-3-methylimidazolium chloride (104.7 mg/L) to 1-hexyl-3-methylimidazolium chloride (83.2 mg/L) and 1-octyl-3-methylimidazolium chloride (67.6 mg/L). LC_{50} decreased with elongation of the alkyl chain length in cation. Thus, toxicities of ionic liquids increased from 1-butyl-3-metylimidazolium chloride ([C_4mim] CL) to 1-octyl-3-metylimidazolium chloride ([C_8mim] CL), remarkably indicating that their toxicities increased gradually as alkyl chain length became longer (Ma et al., 2009).

In addition, based on Ranke et al. (2004), ionic liquids with longer alkyl chain length tend to possess more lipophilic property and this property enhances membrane permeability. Longer alkyl chain length of ionic liquids are incorporated into lipid-bilayer membrane of the organisms (guppy fish), change their ion permeability and finally, killing those fish. These findings indicated that 1-butyl-3-methylimidazolium chloride ([C_4mim] CL) and 1-hexyl-3-metylimidazolium chloride ([C_6mim] CL) are less toxic than 1-octyl-3-metylimidazolium chloride ([C_8mim] CL). Anion of ionic liquids, which is halide (Chloride), seems to have less influence on the toxicity of ionic liquids compared to changes of alkyl chain length in cation. In a recent study, Latala et al. (2009) investigated toxicity of imidazolium and pyrindium based ionic liquids towards algae, using various types of anion. They have found that algae are far less sensitive to anion changes in the ionic liquids structure compared to changes in alkyl chain length.

Both experiments produce different regression equations. The regression equation obtained in the results can be used to predict the percentage of mortalities occur (number of guppy fish dies) when we use certain concentration of ionic liquids. This can be done by just putting the value of the concentration of ionic liquids at X. Hence, we will know the percentage of mortalities occur (number of guppy fish dies) where Y is the percentage of mortalities occurs.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

This objective of this project is to measure toxicity of ionic liquids by conducting this experimental based project (fish acute toxicity test). From the measurement, we can see how the interaction between cation and anion affects the toxic properties of ionic liquids and thus, determine whether it is the cation or anion with the variation of functionality that give more effects on the toxic properties of ionic liquids. The objective of this project was successfully achieved. Some ionic liquids have been proven to be toxic to aquatic life by conducting the fish acute toxicity test on guppy fish by using five different types of 1-Alkyl-3-methylimidazolium ionic liquids. This fish acute toxicity test proves that the titles of green solvent to ionic liquids are no longer relevant. Toxicities of tested ionic liquids increased with the augment of alkyl chain length in cation and anion seems to have less influence on the toxicity of ionic liquids compared to cation.

5.2 RECOMMENDATION

There is recommendation for this project for future works. In the fish acute toxicity test, run the experiment twice on each concentration of the imidazolium based ionic liquids. More accurate results will be produced in terms of the number of dead fish. It is because the number of dead fish reflects the LC_{50} value of the ionic liquids used in the test. Hence, more accurate results in terms of the LC_{50} value and that value might be less different when compared with the LC_{50} value that produced by probit software.

REFERENCES

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, pp 87-92.

Cho, C.-W., Pham, T.P.T., Jeon, Y.-C, Vijayaraghavan, K., Choe, W.-S., Yun, Y.-S., (2007b). Toxicity of imidazolium salt with anion bromide to a phytoplankton *Selenastrum capricornutum*: effect of alkyl-chain length. Chemosphere, 69 (2007), pp 1003-1007.

Knight, D.J., Thomas, M.B., (2003). *Practical Guide to Chemical Safety Testing*, 1st Edition, Rapra Technology Limited, United Kingdom

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

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

Latala, A., Nedzi, M., Stepnowski, P., (2009). Toxicity of imidazolium and pyrindium based ionic liquids towards algae. *Bacillaria Paxillifer* (a microphytobenthic diatom) and *Geitlerinema amphibium* (a microphytobenthic blue green algae). Advance article, Green Chemistry. 11. pp 1371-1376.

Laus, G., Bentivoglio, G., Schottenberger, H., Kahlenberg, V., Kopacka, H., Roder, T., Sixta, H., (2005), Ionic liquids: Current developments, potential and drawbacks for industrial applications. Lenzinger Beritche, 84, pp 71-85.

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

Ma, J.-M., Cai, L.-L., Zhang, B.-J., Hu, L.-W., Li, X.-Y., Wang, J.-J., (2009). Acute toxicity and effects of 1-alkyl-3-methylimidazolium bromide ionic liquids on green algae, Ecotoxicology and Environmental safety, 73, pp 1465-1469.

OECD *guideline for testing of chemical*, Fish Acute Toxicity Test. (1992, july 17). Retrieved from oecd: www.oecd.com.

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

Pham, T.P.T., Cho, C.-W., Min, J., Yun, Y.-S., (2009). Environmental fate and toxicity of ionic liquids: A review, Water Research. 44, pp 352-372.

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

Ranke, J., Molter, K., Stock, F., Bottin-Webber, U., (2004). Biological effects of imidazolium ionic liquids with varying chain lengths in acute Vibrio Fischeri and WST-1 cell viability assays. Ecotoxical. Environ. Saf. 58, pp 396-404.

Reichert, W.M., (2005). *The effects of cation-anion interactions on the physical and solvents properties of ionic liquids*, Thesis, Department of Chemistry, University of Alabama, US

Rodrigues, S., Santos, P., (2010). Effect of the chain length in the structure of imidazolium ionic liquids and dimethylformamide solutions probed by Raman spectroscopy, Vibrational Spectroscopy, 54, pp 123-126.

Romero, A., Santos, A., Tojo, J., Rodriguez, A., (2007). Toxicity and biodegrability of imidazolium ionic liquids. Journal of hazardous materials, 151 (2008), pp 268-273.

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

Seddon, K.R., Rogers, R., Holbrey, J.D., Reichert, W.M., Swatloski, R.P., Broker, G.A., Pitner, W.R., Green Chem. 4 (2002), pp 407-413.

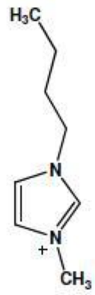
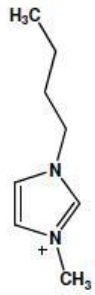
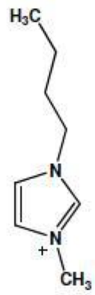
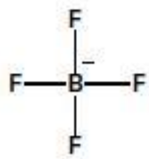
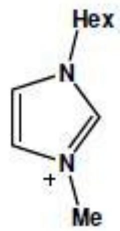
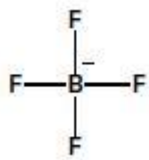
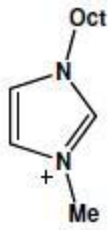
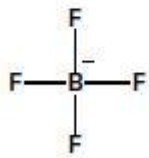
Stock, F., Hoffmann, J., Ranke, J., Stormann, R., Ondruschka, B. and Jastorff, B., (2004), "Effects of ionic liquids on the acetylcholinesterase – a structure-activity relationship consideration," *The Royal Society of Chemistry 2004, Green Chem.*, 2004, 6, pp 286-290.

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

Wasserscheid, P., Keim, W., (2000). Ionic liquids-New "solutions" for transition metal catalysis. Angew. Chem. Int. Ed. 39, pp 3772-3789.

APPENDICES

Appendix 1: Several structures of imidazolium based ionic liquids with several anions

Name	Cation	Anion
1-Butyl-3-methyl-imidazolium chloride		Cl^-
1-Butyl-3-methyl-imidazolium bromide		Br^-
1-Butyl-3-methyl-imidazolium tetrafluoroborate		
1-Hexyl-3-methyl-imidazolium tetrafluoroborate		
1-Methyl-3-octyl-imidazolium tetrafluoroborate		

No	Detail\Week	1	2	3	4	5	6	7	Mid Semester break	8	9	10	11	12	13	14
1	Project Topic Selection															
2	Preliminary research															
3	Submission of progress report															
4	Seminar															
5	Research methodology															
6	Interim report submission															
7	Oral presentation															

Appendix 2: Semester 1 Gantt chart

No	Milestone\Week	1	2	3	4	5	6	7	Mid Semester break	8	9	10	11	12	13	14
1	Limit test	■	■													
2	Fish acute toxicity test		■	■	■	■	■									
3	Progress Report 1									■						
4	Fish Acute toxicity test						■	■		■	■					
5	Pre-EDX evaluation											■	■			
6	Progress Report 2												■	■		
7	Submission of Dissertation (Soft bound)													■	■	
8	Oral Presentation															■
9	Submission of Dissertation (Hard bound)														■	■

Appendix 3: Semester 2 Gantt chart

Appendix 4: Properties of modern ionic liquids

Properties	
Freezing point	Preferably below 100°C.
Liquidus range	Often > 200°C
Thermal stability	Usually high
Viscosity	Normally < 100 cP, workable
Dielectric constant	Implied ≤ 30
Polarity	Moderate
Specific Conductivity	Usually < 10 mScm-1, "Good"
Molar conductivity	< 10 Scm2 mol-1
Electrochemical window	2V, even 4.5 V, except for Brǿnsted acidic systems
Solvent and/or catalyst	Excellent for many organic reactions, inorganic and polymeric materials
Vapor pressure	Usually negligible
Flammability	Non – flammable

Appendix 5: Calculation for the amount of ionic liquids used in the test

1) Limit test

For limit test, 500 mg of each of ionic liquids has been used and put it in the aquarium containing 10 numbers of fish. It is because each aquarium contains 5 litres of water. So, the amount should be 5 x 100 mg = 500 mg. The calculations are as below:-

Test at 100 mg/L

Volume of water in the aquarium = 5L

Amount of ionic liquid used = 5 x 100 mg/L = 500 mg

2) Fish acute toxicity test

For this test, the range of concentration used is 6.25, 12.5, 25, 50, 100 mg/L and each aquarium contains 5 litres of water. So, the amounts of ionic liquids used are:-

6.25 mg/L x 5L = 31.25 mg

12.5 mg/L x 5L = 62.5 mg

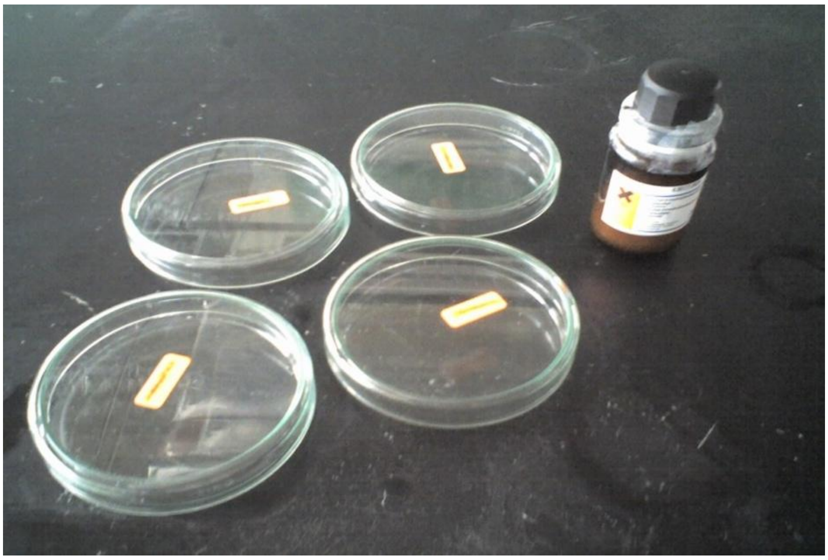
25 mg/L x 5L = 125 mg

50 mg/L x 5L = 250 mg

100 mg/L x 5L = 500 mg

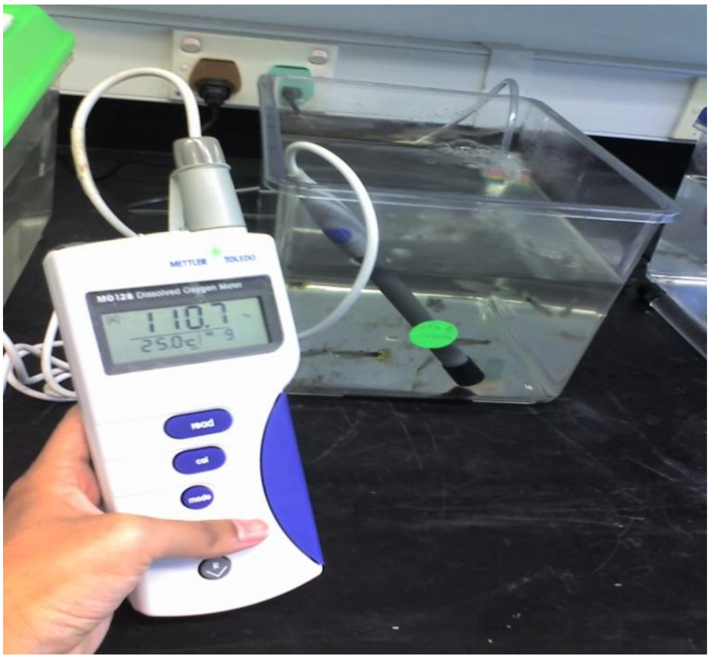
Appendix 6: Pictures of the experimental procedures

1)



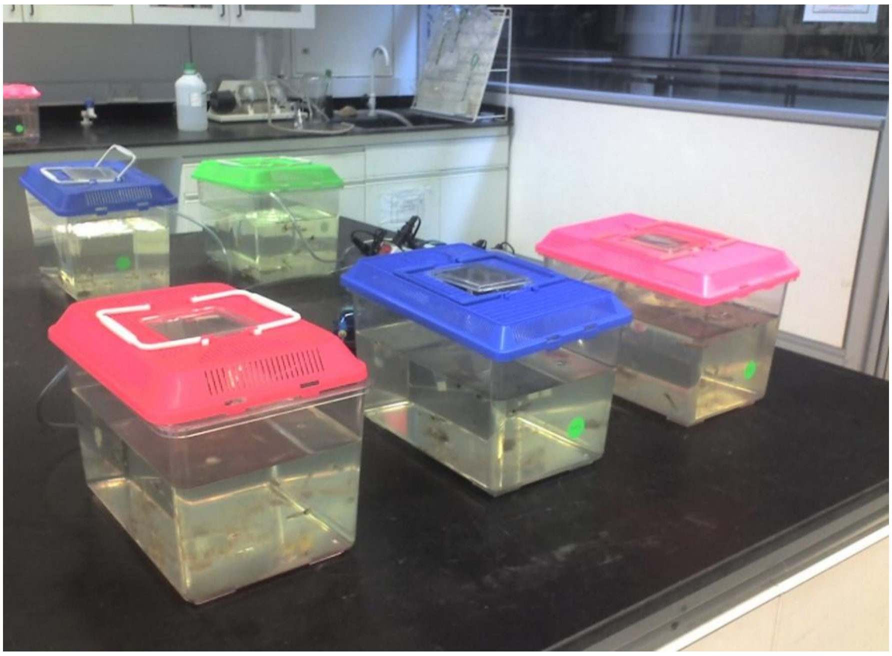
Preparation of the ionic liquids used in the test

2)



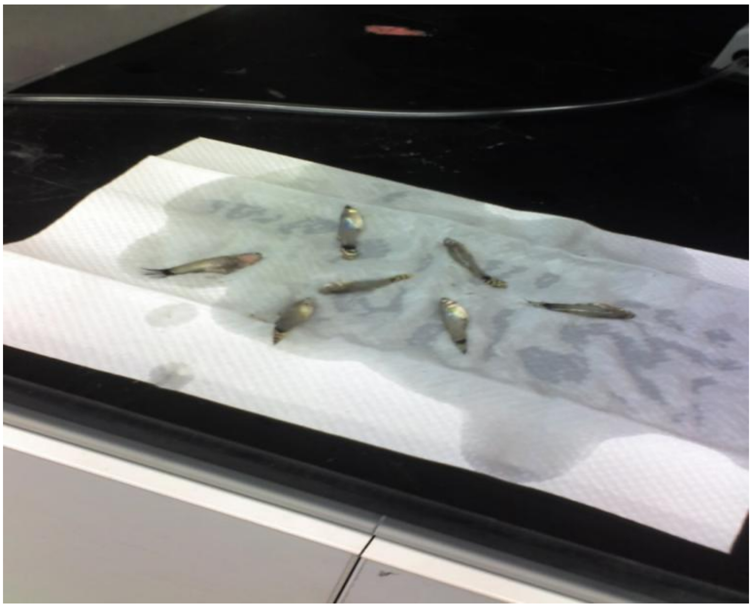
Oxygen dissolved in the water is measured by using dissolved oxygen meter

3)



The conditions during fish acute toxicity test

4)



Dead fish are removed and mortalities are recorded