CHAPTER 1
INTRODUCTION

1.1 BACKGROUND OF STUDY

Ionic liquids, used to known as molten salts, form a big attention to the green chemistry and engineering field these days. The first report of room temperature molten salts was made in 1914 (Paul, 1914). The papers of ionic liquids recentlyranging from organic synthesis to nuclear chemistry, from catalysis to atomistic simulation, from archival history to physical properties, represent an exciting cross-section of the research being carried out, both in industry and academic at the moment (Seddon, 2002).

Ionic liquids are liquids that consist exclusively of ions. Therefore, it exhibit ions conductivity. It has been known to have melting points below 100^{o}C. They are typically organic salts or eutectic mixtures of an organic salt and inorganic salt. The common names which had been used to describe ionic liquids are (Freemantle, 2009):

- room-temperature ionic liquid (RTIL)
- non-aqueous ionic liquid (NAIL)
- molten organic salt
- fused organic salt
- low melting salt
- designer solvent

In recent times, the harmful environmental issues done by the volatile organic compounds (VOCs) used by the industry make them a potential hazard. Ionic liquids on the other way have melting points below 100^{o}C which can directly replacing VOCs because of their negligible vapor pressure. Although ionic liquids can lessen the risk of air pollution, they do have significant solubility in water (Anthony *et. al*, 2001).

Thus, the risk of ionic liquids to contaminate the water source is also high. Over the past 10 years ago, research regarding the new chemicals which might be able to substitute for many 'environmental unfriendly' compounds has grown widely especially in the area of green chemistry and engineering. In particular, ionic liquids have received considerable attention as potential green solvents for a wide range of applications.

Few researches involving phosphonium based ionic liquid had been done. Despite the major development and study done on ionic liquids, the deficiency of information about the impact to the environment especially water will lead to potential problem in future.

Initial efforts had been made to overcome this drawback and offer the preliminary insight into the behaviors of ionic liquids in the aqueous environment The studies gave extensive data sets on (eco)toxicity, biodegradability, bioaccumulation and distribution of ionic liquids in different compartments (Thuy *et al.*, 2009).

1.2 PROBLEM STATEMENT

Ionic liquids become one of the promising technologies due to its thermal stability and negligible vapour pressure. However, those advantages also can give cons during the disposal activities where it may lead to contamination to the water system. The study of toxicity effect had been done by the researchers for many years to evaluate the effect of ionic liquids on survivorship of the aquatic life. The studies include invertebrates and vertebrates species.

The focus of this study will be on the behaviour and toxicity of phosphonium based ionic liquids. Many of ionic liquids had been developed everyday and it is important that the study of toxicology other than characterization need to be done to see the impact of those chemicals towards the environment.

1.3 OBJECTIVE OF STUDY

The main objectives of this study are:

i) To synthesis some phosphonium based ionic liquids

ii) To study the properties of each liquid synthesized such as melting point, molecular weight and solubility in water.

iii) To conduct the toxicity testing of this ionic liquids and measure the toxicity of these ionic liquids using female guppy fish.

iv) To develop new empirical probit equations to be used for estimating the toxicity of phosphonium based ionic liquid.

1.4 SCOPE OF STUDY

The scope of work started with synthesis the ionic liquids and determined related the physical properties such as melting points, molecular weight and solubility of water. The Fish Acute Toxicity Test have been conducted by using female guppy fish. The data recorded from the experiment works have been used incorporating with probit analysis to determine LC_{50} for each type of phosphonium based ionic liquids which have been synthesized.

1.5 THE RELEVANCY OF THE PROJECT

Female guppy fish *(Poecilia Reticulata)* can be found in many rivers in Malaysia. It is suitable for the experiment because according to the methodology (OECD Guidelines for Testing of Chemicals), the species chosen must be available throughout the year, ease of maintenance, convenience for testing and any relevant economic, biological or ecological factors.

Generally, phosphonium ionic liquids have some advantages over imidazolium and pyridinium ionic liquids. They are thermally more stable and the kinetics of the salt formation is faster. Phosphonium based ionic liquids have also no acidic proton, which makes them stable towards nucleophilic and basic conditions, and they have a lower density than water, which provides potential benefits for some applications.

CHAPTER 2
LITERATURE REVIEW AND THEORY

2.1 PHOSPHONIUM BASED IONIC LIQUID

The phosphonium is a positively charged polyatomic with a formula, PH_4. It can be produced from protonation of phosphine. Phosphonium salts are salts which contain alkyl or aryl groups attached to positive phosphorus.

Phosphonium salts are thermally more stable than the corresponding ammonium salts and imidazolium salts which it will be very important for processes which operate at temperatures higher than 100 °C. Phosphonium cations lack acidic protons and thus they are stable under basic conditions which can be problematic for several imidazolium-based Ionic Liquids (carbene formation). Phosphonium salts are, in general, less dense than water, which might be beneficial in product work-up steps (Bradaric, 2003).

In addition, the phosphonium salts, especially halides, have been available commercially for many years. The main contributions of this type of salts are being used as phase transfer catalysts, in resin curing or as biocides. They are typically made by quaternizing a tertiary alkylphosphine with a chloro, bromo or iodo alkane. The resulting phosphonium halides can be used as ionic liquids or as usually is the case, they are converted by various metatheses to non-halogen phosphonium ionic liquids. Alternatively, tertiary alkylphosphines can directly react with active esters such as tosylates, sulfates, phosphates or mesylates to form "non-halogenated salts" directly (Del Sesto *et al.*, 2004).

The research and application regarding phosphonium based ionic liquid had been improve from time to time. Some researchers published numerous papers and patents on petrochemical applications using "molten phosphonium salts" as solvents. The phosphonium based ionic liquids are widely used in many industrial and pharmaceutical applications (Knifton, 1987):

- Extractants for sulfur containing compounds
- Solvents/catalysts for olefin oligermerization, carbonylation, hydroformlation, hydrogenation, esterification and alkylation
- Entrainers and electrolytes
- Media for metal deposition
- Paramagnetic fluids

Phosphonium salts have been successfully used for Heck, Suzuki and esterification reactions (Kaufmann et *al.,* 2008). The Heck reaction (also called the Mizoroki-Heck reaction) is the chemical reaction of an unsaturated halide (or triflate) with an alkene and a strong base and palladium catalyst to form a substituted alkene. The ligand that they used is triphenylphosphine, the cation for phosphonium based ionic liquid. In this way the reaction proceeds in water and the catalyst is re-usable (Gerritsma, 2004).

In Suzuki reaction, the reaction relies on a phosphonium based ionic liquid as the catalyst such as tetrakis (triphenylphosphine) palladium to effect part of the transformation. Esterification is the general name for a reversible chemical reaction in which two reactants (typically an alcohol and an acid) form an ester as the reaction product (McNalty *et al.,* 2007).

The Wilkes group (2005) demonstrated the unusual ability of alkylphosphonium cations to "liquefy" very high molecular weight salt. It also stated that since the low temperature molten composition of this alkylphosphonium are ionically conductive, it is very useful as electrolytes in electrochemical cells, electrolysis, electrowinning and electrorefining processes. Ionic liquids conduct,stable and remain liquid over a wide electrochemical window, and thus become an excellent electrolytes for capacitors, fuel cells and photovoltaic cells.

Furthermore, Clyburne et *al.* (2004) verified the surprising stability of phosphonium salts toward strong bases such as Grignard reagents and borane. In many cases, Grignard reagents can be used in the synthesis of alcohols and carboxylic acids. The

inventors have determined that Grignard reagents and other strong bases are also persistent and reactive in phosphonium-based ionic liquids.

Moreover, some basic compounds, such as nucleophilic carbenes, can be generated in the phosphonium-based ionic liquids. Unpredictably, highly basic or nucleophilic reagents do not result in appreciable deprotonation of phosphonium-based ionic liquids to form phosphoranes.

Thus, the present invention shown there are possibilities to replace volatile and flammable solvents typically used for Grignard chemistry with more environmental friendly and recyclable alternatives. The invention also logically proves the usefulness of phosphonium-based ionic liquids as reliable solvents in which to produce strongly basic nucleophiles, such as nucleophilic carbenes, and also to dissolve and handle highly reactive molecules such as borane (BH_3) (Clyburne *et al.*, 2004).

Pernak *et al.* (2005) has prepared and investigated electrochemical properties of several series of phosphonium salts. The great advantage of ionic liquids is their ionic nature in the absence of solvent, enabling the effective dimensions of the ions. Therefore, it will contribute to the formation of the electrical double layer (EDL) to be reasonably evaluated.

Hence, ionic liquids can be used as in situ or replace probe to explore directly the accessibility of the carbon porous texture during operation of a capacitor. For this purpose, we have prepared phosphonium-derived solvent-free ionic liquids of increasing alkyl chain length. The phosphonium-based ionic liquids, $[(C_6H_{13})_3P(ROCH_2)]$ $[Tf_2N]$, were synthesized following the procedure described elsewhere (Pernak *et al.*, 2005).

There are several reasons why one might consider a phosphonium ionic liquid. The most important one for those contemplating an industrial process is availability and cost.

2.2 PROPERTIES

The basic properties of general ionic liquids can be summarized in Table 1:

Table 1: Modern Ionic Liquids Properties (Pernak *et al.*, 2006)

General Modern Ionic Liquid Properties	
Properties	Description
A salt	cation and or anion with a quite large structure
Freezing point	preferably below 100^{o}C
Liquid range	often > 200 ^{o}C
Thermal stability	Usually high
Viscosity	Normally < 100cP, workable
Polarity	Moderate
Specific conductivity	Usually < 10 mScm^{-1}, "Good"
Molar conductivity	< 10 Scm^{2} mol^{-1}
Electrochemical window	> 2V, even 4.5V, except for Bronsted acidic systems
Solvent and/or catalyst	Excellent for many reactions
Vapor pressure	Usually negligible

Ionic liquids tend to have low vapour pressure in most cases which is a very helpful to solve the volatile organic solvents that will pollute the environment. Lower vapour pressure make ionic liquids are very stable compound in liquid state. The vapor pressure of the "good" ionic liquid is then essentially very low because the Madelung energy as well as the dipole-dipole interaction between ions pairs must be overcome before an ion pair can pass into the vapor state.The vapour pressure of the ionic liquid usually will be determined using Knudsen Effusion Mass Spectrometry method (Pernak *et al.*, 2006).

The specific conductivity on the other hand is good measure of the concentration of total dissolved solids (TDS) and salinity. For ionic liquids, the specific conductivities are moderate, normally in the same range as those of aqueous electrolytes. This is an advantage because it will provide an excellent solvents or catalysts for organic reaction. Ionic liquids' specific conductivity is measured in mScm^{-1} (Knifton , 1987).

Ionic liquids are generally non-flammable and many remain thermally stable at temperature higher than conventional organic molecular solvents. Ionic liquids have wider range of solubilities and miscibilities. For example, some ionic liquids are hydrophilic while others are hydrophobic. The physical, chemical and biological properties of ionic liquids be 'tuned' or 'tailored' by (Freemantle, 2009):

- switching anions or cations
- by designing specific functionalities into the cations and/or anions
- by mixing two or more simple ionic liquids

Therefore, ionic liquids could contribute significantly to the development of green chemistry and technology by replacing toxic, flammable volatile organic solvents that may lead to ozone depleting scenario, reducing or preventing chemical wastage and pollution or by improving the safety of chemical processes and products (Freemantle, 2009).

2.3 TOXICOLOGY STUDIES

Ionic liquids 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). Generally ionic liquids possess a number of favourable characteristics, including negligible vapour pressure, high thermal, chemical, and electrochemical stabilities, and excellent solvent abilities for a wide range of inorganic and organic materials (Suarez *et al.*, 1998).

For toxicity evaluation, aquatic toxicity is one of the methods that can be demonstrated to determine the ionic liquids are toxic or not. The 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. Effects can cause both positive and negative alteration from previously existing circumstances, but aquatic toxicology focuses primarily on the

deviations that are considered to be adverse in nature and on recovery processes in biota that may occur when exposures diminish (Ismail *et al.*, 2010).

Adverse effects at the organism level include both the short-term (acute) and long-term (chronic) lethality (expressed as mortality or survival) and sub-lethal effects such as changes in behaviour, growth, development, reproduction, and detoxification activity, and tissue structure. The serious effects at the sub-organism level include induction or inhibition of enzymes and/or phenotype, as well as changes in the number, relative abundance, and physiological condition of species typically found in a given community type (Rand *et al.*, 1995).

Because exposure to toxic agents may be via the water, sediment, and food in the aquatic environment, the quantities, concentrations, and bioavailability of toxic agents in these compartments are of primary concern. Thus, the study of the sources, transport, distribution, inorganic and organic transformation, and ultimate fate of toxic agents in the aquatic environment is a vital component of aquatic toxicology (Rand *et al.*, 1995).

A working knowledge of aquatic ecology, one or more biological sub disciplines such as physiology, biochemistry, histology, and behaviour, and environmental chemistry is needed to understand the effects of toxic agents on aquatic organisms (Kulacki *et al.*, 2008). Logically, aquatic organisms are the first recipient of most toxic substances generated by industrial, agricultural and domestic activities and released into the environment. As ionic liquids are soluble in water, it causes large impact on aquatic creatures. The management of chemical compounds entering water resources is difficult because contaminants often enter in an aquatic system from multiple of diffuse sources.

Although aquatic ecosystems are adaptable, but with a variety of physical, chemical and biological mechanisms by which chemical compounds 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 survival, development, growth, reproduction or behaviour of organisms (Rand *et al.*, 1995).

Many researches had been done performing the ecotoxicity and biodegradation tests in laboratories to understand the fate and behaviour of ionic liquids under real conditions. It is a good initiative because it can also contribute to creating database of environmentally benign structure moieties of ionic liquids based upon their toxicological and biodegradation information for future references (Thuy *et al*., 2010). Table 2 shows some of the researches that had been done to the organisms from various literatures.

Table 2: Aquatic Organisms Tested

Organisms	Type	Reference
Daphnia magna	freshwater crustacean	Pretti C. *et al.,* 2009, Miao *et al.,* 2009, Luo *et al.,* 2008, Garcia *et al*.,2005
Physa acuta	snail	Bernot *et al.,* 2005
Dania rerio	zebra fish	Pretti C. *et al., 2005*
Folsomia candida	springtail	Matzke *et al.,* 2007
Caenorhabditis elegans	soil roundworm	Swatloski *et al.,* 2007
Dreissena polymorpha	zebra mussel	Costello *et al*., 2004
Rana nigromachulata	frog embryos	Li *et al.,* 2009
Cyclotella meneghiniana	diatom	Adam *et al.*,2005
Pseudokirchneriella subcapitata	green algae	Thuy *et al.,* 2008, Pretti C. *et al.,* 2009
Oocystis submarina	green algae	Adam *et al.*,2005
S.obliquus	green algae	Jian *et al.,* 2010
C.ellipsoidea	green algae	Jian *et al.,* 2010
Scenedesmus vacuolatus	green algae	Stolte *et al.,* 2007

Based on Table 2, it shows that *Daphnia magna* is the main focus in the ecotoxicological studies of ionic liquids. Daphnia is believed to be an important link between microbial and higher trophic levels (McQueen *et al.,* 1986). It has been the subject of hundreds of intensive ecological studies. *Physa acuta* on the other hand

showed a different grazing pattern, which it grazed less in higher concentration of ionic liquid (Bernot *et al.*, 2005).

In another research, Li *et al.,* (2009) used amphibian model, frog (*Rana nigromaculata*) for toxicity testing. This is because the amphibians are normally exposed to aquatic systems because their larvae growing in the water (Lahr, 1997). They used 1-ethyl-3-octyl-1H-imidazolium bromide (IM18 Br) on the early embryonic development of the frog. The LC_{50} were recorded in Table 3.

Table 3: Lethal effect for frog (*Rana nigromaculata*) using IM18 Br

Stages	Lethal effect (LC_{50}) (mg/L)
Neural Plate	42.4
Early Gastrula	43.4
Early Cleavage	85.1

The number of dead embryos kept increasing with increasing concentration of IM18 Br. The same ionic liquid also had been used to be tested to freshwater crustacean (*Daphnia magna*) by Luo *et al.,* (2008). The development impact of IM18 Br is more likely the same to the result that they tested on the amphibians.

The organisms most frequently used in freshwater ecotoxicological tests are green algae. [EMIM][BF4], ([BMIM][BF4], [BzMIM][BF4] and [HMIM][BF4] ionic liquids have been used in the test. It were carried out using modified versions of the methods recommended in the European Committee for Standardization's guidelines (Adam *et al.*, 2005).

The green alga *Oocystis submarina* and the diatom *Cyclotella meneghiniana* inhabiting the southern Baltic Sea have been used in the toxicity study. Standard algal testing procedures revealed significant differences in the responses of the two species. *O.submarina* appeared familiarize with the lower concentrations used. After for about 5 days, their ability to grow recovered, and initial densities were eventually restored. In the case of *C.meneghiniana*, growth in batch cultures was effectively inhibited throughout the experiment regardless of the ionic liquid concentration applied (Adam *et al.,* 2005).

In ecotoxicology of vertebrate, zebrafish, *Dania rerio* plays a significant role as prominent model. The effects of ionic liquids on vertebrates will be discussed further in the next section.

2.1.1 Effects of Ionic Liquids on Vertebrates

The toxicity test had been done to zebra fish (*Dania rerio*), by Pretti *et al.*, (2006) stated that ionic liquids will affect differently according to their chemical structures. Imidazolium, pyridinium and pyrrolidinium showed that they can be considered as non-highly lethal towards zebra fish, which their lethal effect (LC_{50}) is higher than 100mg/L. The test was conducted according to OECD Guideline No. 203 (OECD, 1992). The results are illustrated in Table 4.

Table 4: LC_{50} for three types of ionic liquids (Pretti et al., (2006))

Ionic liquids	Limit test/ full test LC_{50} 48 hr	
	mg/L	μM
$[C_2Clmim]Cl$	> 100	> 552
$[C_2Clmim][TF_2N]$	> 100	> 234
$[C_2OHmim]$ $[TF_2N]$	> 100	> 246
$[C_3OHmim]Cl$	> 100	> 566
[TMSiMmim]Br	> 100	> 402
[Hmim]Cl	> 100	> 844
[HC2Clim]Cl	> 100	> 599
$[C_2(Him)_2]_2Cl$	> 100	> 425
$[Chol][PF_6]$	> 100	> 401
[emmor]Br	> 100	> 476
[ebmor]Br	> 100	> 397
[ETHT]Br	> 100	> 508
$[C_2C_2C_2S]Br$	> 100	>505

The same procedure of test had been used by Jadwiga et al., (2010) by using didecyl dimethylammonium saccharinate in rats. LC_{50} values tested on six human cell lines

varied from 1.44 μM to 5.47 μM. Reduced body weight gain and slightly reduced food consumption was observed particularly in high-dose rats. Under the condition of this study the lowest-observed-adverse-effect level of dimethylammonium saccharinate was considered to be 10 mg/kg/day.

CHAPTER 3
METHODOLOGY

3.1 PROJECT WORKFLOW

The methodology of this study is divided to two parts: materials and methods. The workflow of this project will be discussed in Figure 1.

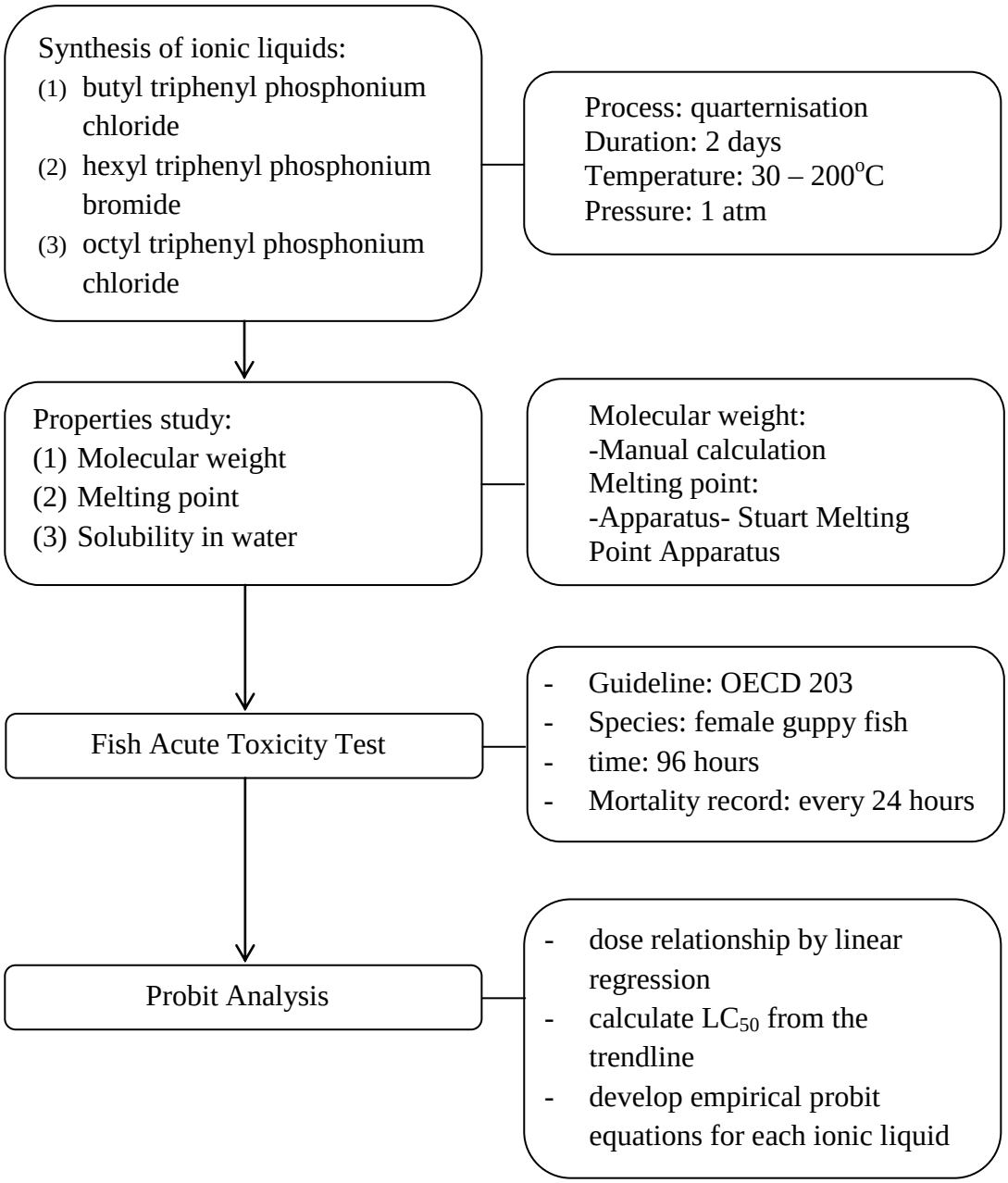

Figure 1: Project Workflow

3.2 MATERIALS AND METHODS

3.2.1 Materials

3.2.1.1 Ionic liquids

The phosphonium based ionic liquids used in this study are butyl triphenyl phosphonium chloride, hexyl triphenyl phosphonium bromide, octyl triphenyl phosphonium chloride. The ionic liquids will be synthesized in Ionic Liquid Laboratory. All the chemicals, reagents and solvent used are obtained from commercial sources.

3.2.1.2 Preparation of ionic liquids

The general synthesis procedure starts with equivalent tertiary phosphine was added dropwise to the appropriate alkylating reagent under nitrogen and stirred at a temperature ranging from 30 to 200^{o}C, depending on the reactivity of the alkylating reagent. Normally, short-chain alkylating reagents were more reactive than the long chain analogues.

Equipment required for the experiment:

1) Set of glassware for synthesis (eg: condenser, round bottom flask, guard tube, etc.) (see Figure 2)
2) Mixer, heater and thermometer for the synthesis

Time taken for the reactions normally varies from 10 to 29 hours. The speed of adding the tertiary phosphine to the alkylating reagent can be adjusted until no free tertiary phosphine could be observed in the mixture. After addition was complete, and the mixture had been stirred for 2 to 3 hours at the initial temperature, higher temperatures can be used to increase the reaction (Bradaric, 2002).

For the synthesis of tri-*n*-hexyl phosphonium halides, the general procedure started by using a round bottom flask with side arm adaptor and septum, which was dried over high vacuum and purged three times with nitrogen. Then, it was charged with tri-*n*-hexylphosphine 6 (2 mL, 5.79 mmol). The flask was subsequently immersed into an oil bath (50 °C), the appropriate haloalkane was added *via* syringe and the

mixture was stirred at 50 °C, before the product was dried over night at 50 °C over high vacuum (Atefi,2009).

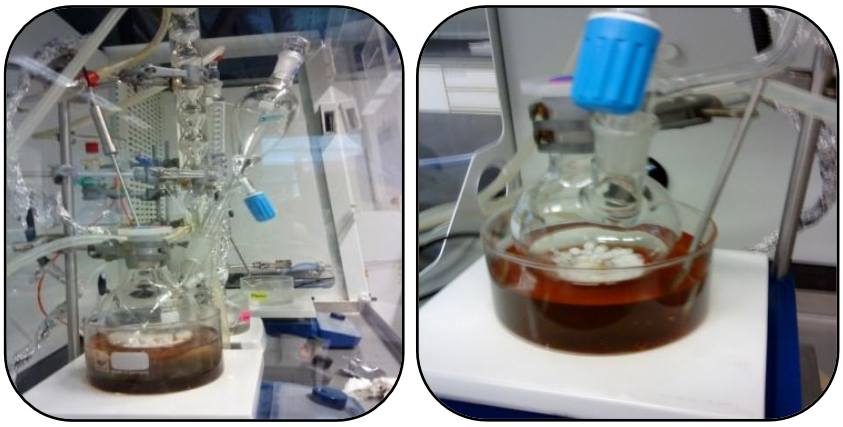

Figure 2: Synthesis of Ionic Liquid.

3.2.2 Methods

3.2.2.1 Properties Study

Molecular Weight

Molecular mass or frequently referred to molecular weight is the ratio of the mass of that molecule to 1/12 of the mass of carbon-12. Molecular weight of each ionic liquid used has been calculated manually according to the molecular formula.

Melting Point

The melting point is a fundamental physical property of compounds, which has been found wide use in chemical identification, as a criterion of purity and for the calculation of other physicochemical properties such as vapor pressure, aqueous solubility and phase equilibrium properties (Katritzky, 2001). Melting point is also one of the most important thermal properties required for heat transfer applications for ionic liquids. This study handled by using the Stuart Melting Point Equipment (see Figure 3).

The sample is prepared by placing a small amount in the end of a glass capillary tube. A suitable plateau temperature is set, approximately 10°C below the expected

melting point of the sample. The desired plateau temperature may now be set using the arrow keys to scroll the display up or down as required. An appropriate ramp rate Is between 1ºC per minute and 10ºC per min. Results obtained at 1ºC per minute will be the most accurate, while more approximate results can be obtained faster at higher ramp rates. The tube is inserted into the side of the heating block via the holes provided. The sample is observed until the melt occurs and record the temperature from the digital display.

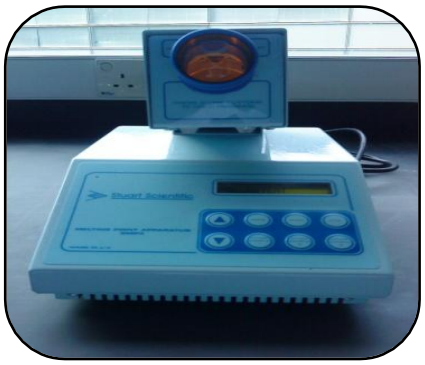

Figure 3: Stuart Melting Point Equipment.

3.2.2.3 Fish Acute Toxicity Testing

For this testing, the Organisation for Economic Cooperation and Development (OECD) Guideline for testing Chemical 203 (OECD 203) will be followed. This is the new version of the guideline, originally adapted in 1981 and first updated in 1984, is based on the proposal from the United Kingdom to reduce numbers of fish in tests of acute aquatic toxicity (OECD, 1992).

The principle of the test is the fishes 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 percent of the fish (LC_{50}) are later determined by probit analysis.

Equipment required:

1) Sets of aquariums: for guppy fishes
2) DO meter: to check DO in water
3) pH meter: to check pH of the water

4) Air pump: supply oxygen to the aquariums
5) Food pellets: to feed the fishes

For selection of the fish, the species of the fish are ready availability throughout the year, ease of maintenance, convenience for testing and any relevant economic, biological and ecological factors. The fishes should be in good health and free from any apparent malformation. Therefore, the testing for this study is using female guppy fish (*Poecilia reticulate*).

For the condition of exposure, preferably the test will be done in 96 hours non-stop, with 12 to 16 hours photoperiod daily. According to the guideline, temperature appropriate to the species should be within a range of 2^{o}C. Any disturbance that may change the behaviour of the fish should be avoided if possible during the test.

For the observation, the fish are inspected at least after 24, 48, 72 and 96 hours. Observations at three and six hours after the start of the test are desirable. Fish are considered dead if there is no visible movement and if touching of the caudal peduncle (tail fin) produces no reaction.

Records are kept of visible abnormalities such as loss of equilibrium, swimming behaviour, respiratory function etc. Dead fish are removed after observed and mortalities are recorded. The dissolved oxygen (DO) and pH are advisable to check everyday to maintain the balance of dissolved oxygen and pH in the water used during the experiment.

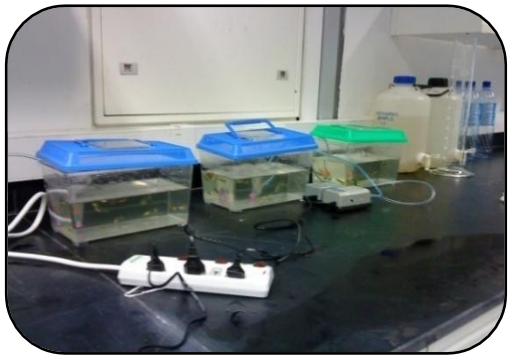

Figure 4: Fish Acute Toxicity Test.

3.2.2.3 Probit Analysis

The method was originally published in Science by Chester Ittner Bliss in 1934. He was an entomologist in Connecticut agricultural experiment station. This method had been developed while he was concern in finding an effective pesticide control for insects that fed on grape leaves. He developed the idea in understanding dose-response relationship by linear regression (Greenberg, 1980).

Probit analysis had been used widely in toxicity studies, for example in predicting the dose of the compound that cause 50% mortality in population (LC_{50}) of dieldrin in rats was found to be 50.8 mg/kg (Barnes and Heath,1964). The susceptibility of the Adult toads to Endosulfan and Diazinon pesticides were determined by using the probit method of analysis for median lethal concentration at 96 hours (4 days). The confidence interval of mortality rate computation was also obtained from the probit analysis in order to determine the LC_{50} (Lawrence and Isioma, 2010).

This kind of method is actually a type of regression used to analyze binomial response variables. The sigmoid dose-response curve will be transformed to a straight line and will be analyzed by regression. Three techniques can be used to conduct this probit analysis.

The first one is by using tables to estimate the probits. The second method is by manual calculation of the probits, regression coefficient and confidence intervals in the analysis. The third method is by using the statistical package such as SPSS (Vincent, 2008).

The regression will fit a line to your data to compare the relation between the response variable or dependent variable (Y) with the independent variable (X) (refer Eq. 1).

$$Y = a + b X + e \quad [1]$$

where:

Y is equal to y-intercept

b is equal to the slope of the line

e is a term error.

Binomial response variable refers to a response which only has two outcomes. For example, for toxicity testing, the result is death or no death and for beauty product testing, is either rash or no rash (Vincent, 2008).

The first step is to convert percentage mortality to probits, there is two methods that might be considered. The first method is by using Table 5 and the second method is by manual calculation.

Table 5: Transformation of percentages to probits (Finney, 1952)

%	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	4.10	4.23	4.26	4.20	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	5.74	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

To read Table 5, the percentage is located at the left side of the table and the corresponding probits are the rest of it. For example, for a 12% response, the corresponding probit would be 3.82.

The manual calculations by using Eq. 2 also can be used as second method (Finney, 1952). The probit Y, of the proportion is defined by:

$$P = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{Y-5} \exp -\frac{1}{2} u^2 \, du \qquad [2]$$

where:

P is probability in normal distribution

Y is response metameter

u is dose concentration

The standard method of analysis is using the maximum and minimum working probits as illustrated by Eq. 3 and Eq. 4:

$$Y_{max} = Y + \frac{Q}{Z} \quad [3]$$

$$Y_{min} = Y - \frac{P}{Z} \quad [4]$$

where:

Q is the maximum value

P is the minimum value

The second step is to calculate the log of the concentration. This can be done by manual calculations or by using any suitable computer program such as SPSS or Polymath.

The graph of probits versus the log of concentrations needs to be plotted and a line of regression can be fit by several ways. The first one is by hand fit line bye eye. This technique can minimize the space between the line and the data. Despite this way is accurate, but manual calculations and computer program will be more reliable and precise. Furthermore, both manual calculations and computer program can provide the confidence intervals values (Vincent, 2008).

Eq. 5 can be used to calculate the linear regression:

$$Y = \frac{(x - \mu)}{\sigma} \quad [5]$$

where:

x is the concentration

μ is the error

σ is the standard deviation

The proportion responding to be equal to p= r/n and the complement is equal to q = 1-p. The working probit can be calculated from either Eq. 6 or Eq. 7.

$$y = Y + \frac{Q}{Z} - \frac{q}{Z} \quad [6]$$

$$y = Y + \frac{P}{Z} - \frac{p}{Z} \quad [7]$$

Now we can find the LC_{50} by manual calculations, we can find by searching the probit list of 5.00 (50%) and then taking the inverse log of the concentration captured.

Probit analysis is one of the useful tools to determine the LC_{50} by calculation or computer program. The error of the result also can be calculated by this analysis (Vincent, 2008).

3.3GANTT CHART

From the Gantt chart (refer to the next page), the project done with updating the current literature review. Some of the literature review specific on the synthesis process of phosphonium based ionic liquid, the probit analysis for analysis work as well as the toxicity test for vertebrate's species (fish).

Next, Progress Report I and II have been submitted to the supervisors to update the work progress specifically on the experimental work. Poster presentation was being held on the ninth week and the oral presentation will be held on week 16 or 17.

GANTT CHART and MILESTONE: FINAL YEAR PROJECT II (SEMESTER JULY 2010)																	
Month	July 2010	August 2010				September 2010				October 2010				November 2010			
Week	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Literature Review (update)																	
Receiving chemicals																	
Progress Report I					○												
Experimental/Analysis work																	
Mid Semester Break																	
Poster presentation																	
Progress Report II																	
Dissertation																	
Oral presentation																	

CHAPTER 4
RESULT AND DISCUSSION

4.1 PROPERTIES STUDY

4.1.1 Molecular Weight

The molecular weights were calculated manually based on the atomic weight which apply to naturally occurring isotopic composition and are based on an atomic mass of Carbon 12. The molecular formula and molecular weights of three types of phosphonium based ionic liquids are presented in the Table 6:

Table 6: Molecular Weight Calculation

Molecular Formula	Atomic Weight	Subtotal	Total (Molecular Weight)
$C_{22}H_{24}ClP$ (Butyl triphenyl phosphonium chloride)	C: 12.011 x 22	264.242	354.861
	H: 1.008 x 24	24.192	
	Cl: 35.453 x 1	35.453	
	P: 30.974 x 1	30.974	
$C_{24}H_{28}BrP$ (Hexyl triphenyl phosphonium bromide)	C: 12.011 x 24	288.264	427.366
	H: 1.008 x 28	28.224	
	Br: 79.904 x 1	79.904	
	P: 30.974 x 1	30.974	
$C_{26}H_{32}ClP$ (Octyl triphenyl phosphonium chloride)	C: 12.011 x 26	312.286	410.969
	H: 1.008 x 32	32.256	
	Cl: 35.453 x 1	35.453	
	P: 30.974 x 1	30.974	

4.1.2 Melting Point

The melting point of each ionic liquid was measured using Stuart Melting Point Equipment, the melting point of each ionic liquid which is in solid form can be determined. The expected melting point is found in literature which will be use to set

the plateau temperature. The melting point of each synthesized ionic liquids determined and show in Table 7:

Table 7: Melting Point of Synthesized Ionic Liquid

Ionic Liquid	Melting Point (^{o}C)
Butyl triphenyl phosphonium chloride ($C_{22}H_{24}ClP$)	222
Hexyl triphenyl phosphonium bromide ($C_{24}H_{28}BrP$)	202
Octyl triphenyl phosphonium chloride ($C_{26}H_{32}ClP$)	62

From Table 7, it can be concluded that the purity of ionic liquids have a good agreement of the one taken from literatures.

4.2 FISH ACUTE TOXICITY TESTING

Fish Acute Toxicity Testing focuses on the fish most likely affected and the route of entry that will cause worst effect. This testing is also known as aquatic toxicity test. Aquatic toxicity normally is an indicator of the relative toxicity of chemical or compound in water (William *et al.,* 2001). The toxicity of this testing will be compared to the standard toxicity reference to determine their potential impact and evaluated on the specific conditions. Several scales are available and in this study the testing will follow Fish and Wildlife Service (FWS) scale (see Table 8).

Table 8: Acute Toxicity Rating Scale by Fish and Wildlife Service (FWS)

Relative Toxicity	Aquatic LC_{50} (mg/L)
Super Toxic	0.01-0.1
Highly Toxic	0.1-1
Moderately Toxic	1-10
Slightly Toxic	10-100
Practically Nontoxic	100-1000
Relatively Harmless	>1000

The tests had been conducted in a well ventilated laboratory. Four aquariums had been used to vary the concentration of each ionic liquid. The experiment started with

the limit test where 100 mg/L of ionic liquid have been dissolved into lake water which will be used in the aquarium consisting of at least 7 female guppy fishes (*Poecilia reticulate*). The experiment takes 96 hours to be completed. The results are shown in Table 9:

Table 9: Limit test results on female guppy fish

Ionic Liquid	24 hrs	48 hrs	72 hrs	96 hrs	Total fish died
$C_{22}H_{24}ClP$	1	2	2	3	8
$C_{24}H_{28}BrP$	2	2	2	2	8
$C_{26}H_{32}ClP$	0	0	0	0	0

Note: $C_{22}H_{24}ClP$: Butyl triphenyl phosphonium chloride; $C_{24}H_{28}BrP$: Hexyl triphenyl phosphonium bromide; $C_{26}H_{32}ClP$: Octyl triphenyl phosphonium chloride.

From Table 9, it can be concluded that the mortality of fish is 8 for butyl triphenyl phosphonium chloride and hexyl triphenyl phosphonium bromide. On the other hand, for octyl triphenyl phosphonium chloride, the limit test shows no mortality occurs for the fish. Therefore, the full test will only applicable for butyl triphenyl phosphonium chloride and hexyl triphenyl phosphonium bromide. The results also indicate that octyl triphenyl phosphonium chloride is harmless according to the Fish and Wildlife Service (FWS) Scale.

Figure 5: Example of aquarium dissolved by ionic liquid.

The experiment for butyl triphenyl phosphonium chloride and hexyl triphenyl phosphonium bromide, 3 different concentrations had been chose to determine the

mortality of the fish. The results of the mortality for 96 hours are illustrated in Table 10 and Table 11.

Table 10: Mortalities for Full Test for butyl triphenyl phosphonium chloride

Concentration (mg/L)	24 hrs	48 hrs	72 hrs	96 hrs	Total fish died
25.5	0	0	0	0	0
52.5	0	1	1	1	3
77.5	0	2	2	2	6

Table 11: Mortalities for Full Test for hexyl triphenyl phosphonium bromide

Concentration (mg/L)	24 hrs	48 hrs	72 hrs	96 hrs	Total fish died
27.5	0	0	0	1	1
50.0	1	1	2	2	4
78.0	2	2	2	2	8

From Table 10 and 11, the mortalities are decreased as the concentrations decreases. It suits the logic where the more concentration of ionic liquid in the water, the more harmful the water to the aquatic. The trends of these mortalities are presented by Figure 6 and 7.

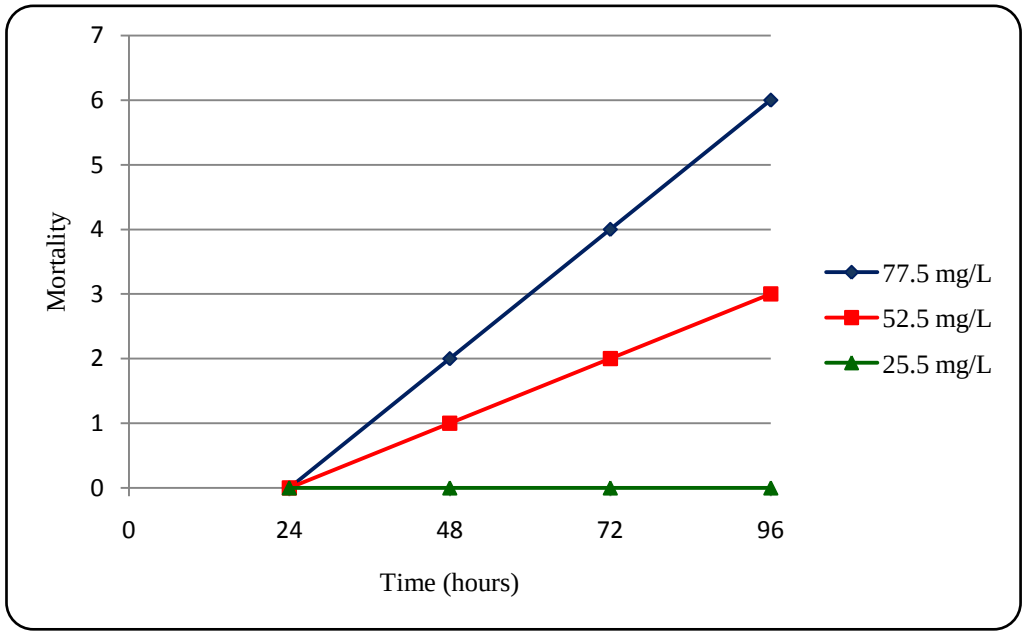

Figure 6: Mortalities vs. time for different concentration of butyl triphenyl phosphonium chloride.

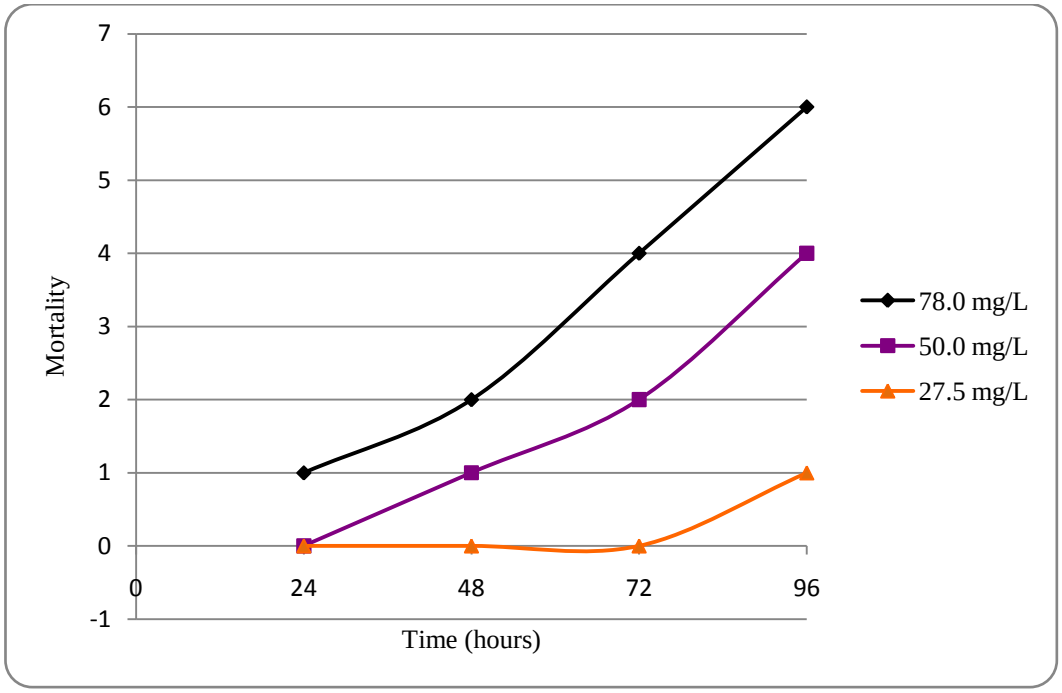

Figure 7: Mortalities vs. time for different concentration of hexyl triphenyl phosphonium bromide.

From the data collection of Limit test and Full Test, we can estimate the median lethal concentration, LC_{50} by using probit analysis.

4.3 PROBIT ANALYSIS

Probit analysis is one of the methods to observe the dose relationship by linear regression. The $\log_{10}$ of concentration have been used to develop the empirical probit equations. Data from Fish Acute Toxicity Test (Table 12 and 13) have been used to plot the dose vs mortality graph. In this study, this method is applicable for two ionic liquids: butyl triphenyl phosphonium chloride and hexyl triphenyl phosphonium bromide. The concentrations have been converted to $\log_{10}$ concentrations. The percentage of the mortality for each concentration is determined. The probit table (Table 5) is referred to find the probits of each concentration.

Table 12: Log dose and percentage of mortality for butyl triphenyl phosphonium chloride

Dose (mg/L)	Log dose (mg/L)	Total Fish	Total mortality	% of mortality	Probits
25.5	1.407	10	0	0	0
52.5	1.720	10	3	30	4.48
77.5	1.889	10	6	60	5.25
100	2.000	10	8	80	5.84

Table 13: Log dose and percentage of mortality for hexyl triphenyl phosphonium bromide

Dose (mg/L)	Log dose (mg/L)	Total Fish	Total mortality	% of mortality	Probits
27.5	1.439	10	1	10	3.72
50.0	1.699	10	4	40	4.75
78.0	1.892	10	6	60	5.25
100	2.000	10	8	80	5.84

From the probits values that we get from probit table (Table 5), we can plot $\log_{10}$ concentration vs probit and determine the trendline to develop the empirical probit equation. The graph can be illustrated in Figure 8 and 9.

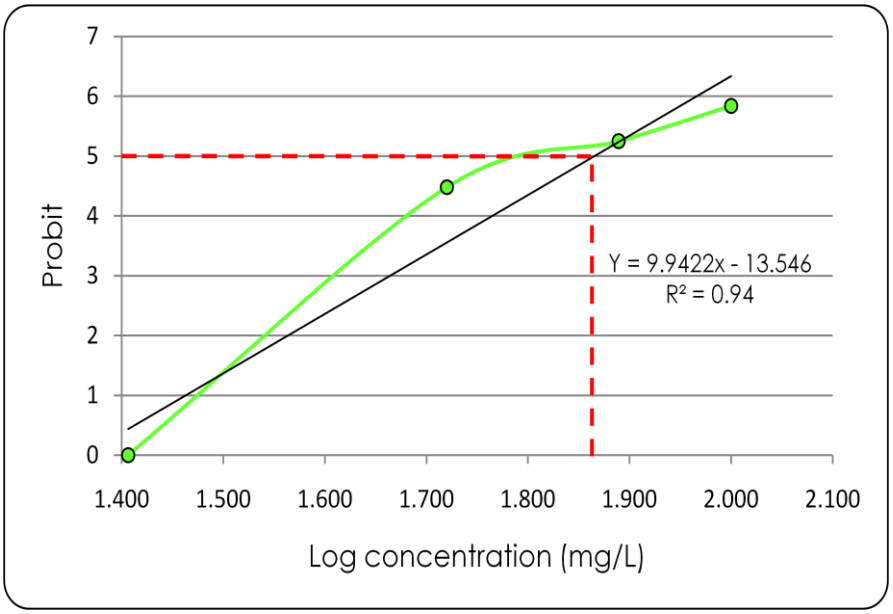

Figure 8: Log_{10} concentration vs probit for butyl triphenyl phosphonium chloride.

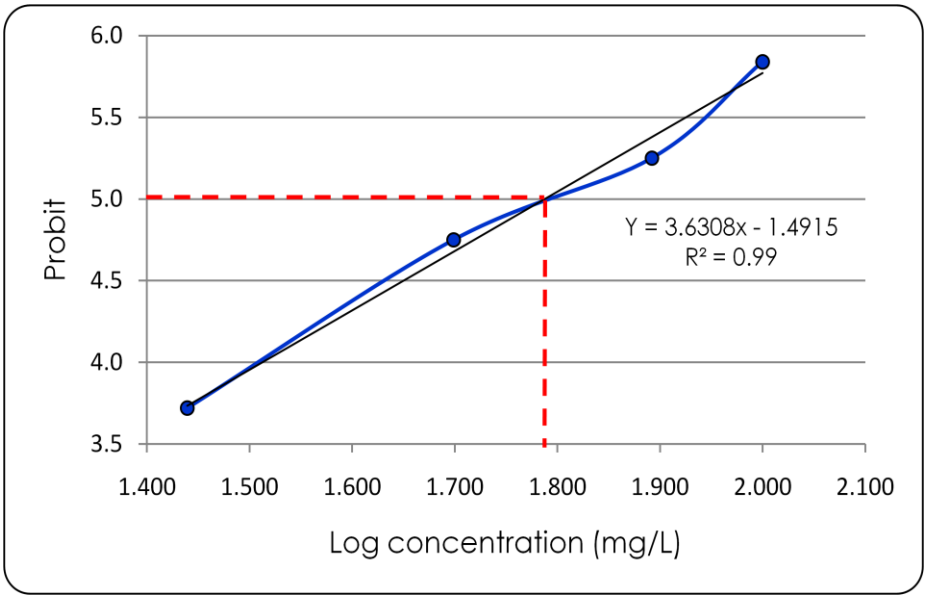

Figure 9: Log_{10} concentration vs probit for hexyl triphenyl phosphonium bromide.

From Figure 8 and 9, we can interpolate the graph to find the median lethal concentration where 50% mortality is equal to probit = 5.0. The median lethal concentration, LC_{50} can be seen in Table 14:

Table 14: Median Lethal Concentration, LC_{50} (mg/L) of tested ionic liquids

Ionic Liquid	Median Lethal Concentration, LC_{50} (mg/L)
Butyl triphenyl phosphonium chloride	73.35
Hexyl triphenyl phosphonium bromide	61.36

The LC_{50} also can be calculated using the empirical probit equation based on the linear regression of the graph plotted. The empirical equations for both ionic liquids used are:

Butyl triphenyl phosphonium chloride:

$$Y = 9.9422x - 13.546 \quad [8]$$

To calculate LC_{50}, substitute Y= 5.0,

$$5.0 = 9.9422x - 13.546$$

$$x = 73.35 \text{ mg/L}$$

Hexyl triphenyl phosphonium bromide:

$$Y = 3.6308x - 1.4915 \quad [9]$$

The same step used to calculate LC_{50},

$$5.0 = 3.6308x - 1.4915$$

$$x = 61.36 \text{ mg/L}$$

From the result of LC_{50} shown, we can conclude that both ionic liquid butyl triphenyl phosphonium chloride and hexyl triphenyl phosphonium bromide are slightly toxic (refer to Table 8). Furthermore, hexyl triphenyl phosphonium bromide is more toxic than butyl triphenyl phosphonium chloride because the LC_{50} value is lower, indicates less concentration needed to kill 50% of the fish population.

Standard deviation is a statistical measure of spread or variability. The standard deviation is the root mean square (RMS) deviation of the values from their arithmetic mean. Table 15 and 16 shows the calculation for standard deviation for butyl triphenyl phosphonium chloride and hexyl triphenyl phosphonium bromide

Table 15: Standard deviation calculation of butyl triphenyl phosphonium chloride

Dose (mg/L)	Total mortality (A)	Mean (B)	(A-B)	$(A-B)^2$
25.5	0	4.25	-4.25	18.0625
52.5	3	4.25	-1.25	1.5625
77.5	6	4.25	1.75	3.0625
100	8	4.25	3.75	14.0625
			Total	36.75

Therefore, standard deviation for butyl triphenyl phosphonium chloride can be calculated by:

$$\text{Standard deviation: } \frac{\sqrt{36.75}}{\sqrt{3}} = 3.5$$

Table 16: Standard deviation calculation of hexyl triphenyl phosphonium bromide

Dose (mg/L)	Total mortality (A)	Mean (B)	(A-B)	$(A-B)^2$
27.5	1	4.75	3.25	10.5625
50.0	4	4.75	1.25	1.5625
78.0	6	4.75	-0.75	0.5625
100	8	4.75	-3.75	14.0625
			Total	26.75

Standard deviation for butyl triphenyl phosphonium chloride can be calculated by:

$$\text{Standard deviation: } \frac{\sqrt{26.75}}{\sqrt{3}} = 2.986$$

CHAPTER 5
CONCLUSION

The experimental work including the preparation of ionic liquids, properties studies and fish acute toxicity testing had been completed. Three phosphonium based ionic liquids have been synthesized; butyl triphenyl phosphonium chloride, hexyl triphenyl phosphonium bromide and octyl triphenyl phosphonium chloride. The molecular weight had been calculated accordingly by manual calculation. The melting point of the synthesized ionic liquid had been measured using Stuart Melting Point Apparatus. The solubility in water have been tested for the these ionic liquids. it is found that the three ionic liquids are soluble in water.

The results from the toxicity testing were being analysed using probit analysis. An empirical probit equation is developed in order to estimate the toxicity of each ionic liquid. Among the three ionic liquids being used, only two of them are slightly toxic according to US Fish and Wildlife Service Scale which are butyl triphenyl phosphonium chloride and hexyl triphenyl phosphonium bromide. The results shown hexyl triphenyl phosphonium bromide gave the least concentration of median lethal concentration which is 61.36 mg/L. This indicates that the hexyl triphenyl phosphonium bromide has the highest toxicity level among the other two ionic liquids. The developed empirical probit equations for each ionic liquid can be used to estimate any percentage of lethal concentration only for the specific type of ionic liquid. This study is useful for people who handling with these ionic liquids in future.

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