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Toxicity Assessment of Phosphonium Based Ionic Liquids Towards Female Guppy Fish

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In this study, two phosphonium based ionic liquids (butyl triphenyl phosphonium chloride and hexyl triphenyl phosphonium bromide) have been synthesized using quarternization process. The toxicities of these ionic liquids are unknown and may be harmful to humans and the environment. Therefore, the toxicity assessment of these ionic liquids was carried out according to the Organization for Economic Cooperation and Development (OECD) guideline 203 using female guppy fish (*Poecilia reticulata*). The median lethal concentrations (LC_{50}) have been estimated for butyl triphenyl phosphonium chloride and for hexyl triphenyl phosphonium bromide to be 73.35 mg/L and 61.36 mg/L respectively. Both LC_{50} obtained can be identified as slightly toxic ionic liquids based on Acute Toxicity Rating Scale by by the United States Fish and Wildlife Service (USFWS). The findings from this study can be used for better design of phosphonium-based ionic liquids with consideration of their aquatic toxicities.

Keywords: Ionic liquids, Fish, Toxicity, Lethal concentration.

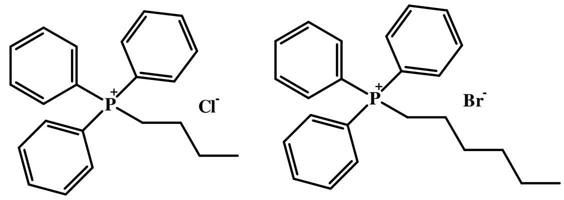
INTRODUCTION

The world of ionic liquids (ILs) started in 1914 by Walden[1] who synthesized ethylammonium nitrate [$EtNH_3$][NO_3]. Later, after several years, ionic liquids began to gain more of the attention of scientists when first room temperature ionic liquid with a 1-alkyl-3-methylimidazolium cation was reported[2]. Ionic liquids are composed of ions only and they are fluid below 100 °C due to asymmetry of one of the ions. They are thermally stable with liquid range up to 300 °C compared to 100 °C for water and show very low vapour pressure, which makes them of great interest due to these properties. 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*.[3,4]. They are currently considered as a remarkable class of solvents due to their large liquidous range and non-volatile behaviour and can be used for a broad range of industrial applications. The research on this area is rapidly increasing due to their exciting applications and wide usages. The reason for the increasing interest is clearly due to the realization that these materials have a great utility as solvents for reactions and material processing, media for extraction and working fluids for mechanical applications. Volatile organic compounds (VOCs) were previously used as solvents (such as, methanol, ethanol, benzene, toluene, ether *etc*.) in industrial applications causing a major concern in the current chemical processing industry. Although intensive information and data regarding ionic liquids' physical and thermodynamic properties have been reported and continuously published, only limited data with regard to the toxicity and ecotoxicity were reported[5-12]. The toxic effects of many solvents combined with serious environmental issues, such as atmospheric emissions and contamination of aqueous effluents are making them harmful to use. Thus, many researchers have emphasized on the improvement of green engineering which represents research aimed at finding environmentally benign alternatives to harmful chemicals. Among the neoteric solvents applicable in green technologies, ionic liquids have garnered increasing attention over the others due to their numerous applications in recent years. In general, the toxicity of chemicals can be measured using (a) mammalian acute toxicity test (b) bacteria acute toxicity test (c) fish acute toxicity test and (d) biological dissociation test[13]. 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. The 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 the exposure diminishes. Adverse effects at the organismal 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, detoxification activities and tissue structure. Adverse effects at the sub-organismal level include induction or inhibition of enzymes and/or phenotypes, as well as changes in the number, relative abundance and physiological condition of species typically found in a given community type[14]. There are several test species that are recommended by the Organization for Economic Cooperation and Development (OECD) guidelines, which are rainbow trout (*Oncorhynchus mykiss*), fathead minnow (*Pimephales promelas*), bluegill (*Lepomis macrochirus*), zebrafish (*Danio rerio*), medaka (*Oryzias latipes*), guppy (*Poecilia reticulata*) and common carp (*Cyprinus carpio*). A number of acute toxicity studies have been performed using a variety of fish. For instance, McKim[15] used Chinook salmon (*Oncorhynchus tshawytscha*) for chronic toxicity evaluation. Baser *et al*.[16] investigated the acute toxicity of permethrin (a synthetic pyrethroid pesticide) on guppy fish (*Poecilia reticulata*). Pretti *et al*.[8] assessed the acute toxicity of several ionic liquids' towards zebrafish (*Danio rerio*). Sarikaya *et al*.[17] used guppy fish to estimate the toxicity of Fenitrothion (O,O-dimethyl-O-(3-methyl-4-nitrophenyl) phosphorothioate). Wang *et al*.[18] estimated the toxicity of 1-methyl-3-octylimidazolium bromide ([C_8mim][Br]) using goldfish (*Carassius auratus*). Dumitrescu *et al*.[19] evaluated the acute toxicity of tetrabuthylammonium bromide using zebrafish. Li *et al*.[20] employed goldfish to evaluate the toxicity of 1-methyl-3-octylimidazolium bromide ([C_8 mim][Br]). Most researchers in recent years have carried out acute toxicity tests for at least 48 h and usually 96 h. For more information regarding toxicity assessment using guppy fish (*Poecilia reticulata*), readers can refer to PAN (Pesticide Action Network) Pesticides Database (http://www.pesticideinfo.org) and ECOTOX database (http://cfpub.epa.gov/ecotox/). The recent searching (in 2012) with "Guppy (*Poecilia reticulata*) toxicity studies" gave around 4104 individual studies on this species with different chemicals, which is even greater than with goldfish, *Carassius auratus* (3290). Other studies can be found with zebra fish, *Danio rerio* (4658), bluegill, *Lepomis macrochirus* (9677), rainbow trout, *Oncorhynchus mykiss* (26662) and fathead minnow, *pimephales promelas* (15128). For more detailed information on the acute toxicity tests of different fish species, readers are directed to the summary tables provided by the U.S. fish and wildlife service reports[21,22], the handbook of acute toxicity of chemicals to fish and aquatic invertebrates[23] and work of Baser *et al*.[16].

In this study, the acute fish toxicity study was conducted according to the prescribed guideline OECD 203[24] elaboration done one time before using fresh-water Guppy fish (*Poecillia reticulata*) to estimate the toxicity of two phosphonium ionic liquids. To our best of knowledge there is no published literature regarding the toxicity (LC_{50}) of these ionic liquids.

EXPERIMENTAL

Chemicals used in this study were obtained from commercial sources and had been used without drying and further purification. All ionic liquids' were synthesized according to established methods[25,26]. To an equivalent amountof triphenylphosphine (2 mL, 5.79 mmol) contained in a round-bottomed flash equipped with a reflux condenser, a magnetic stirrer and an inlet and outlet of nitogen gas, the appropriate alkylating reagent (6.00 mmol, in excess) was added drop-wise *via* syringe and the mixture was stirred at a temperature rising from 30 to 200 °C.The reaction time normally varies from 10 to 29 h. After the addition was complete, the mixturewas stirred for 2 to 3 h at the highest temperature reached (200 °C). The progress of the reaction was monitored by NMR spectroscopy, and thus the solid product obtained was dried at high vacuum overnight. The general structures of these ionic liquids' are shown in Fig. 1.



Butyltriphenylphosphonium chloride **Hexyltriphenylphosphonium bromide**

Fig. 1. Structures of ionic liquids used in this study

Fish acute toxicity test: The species used for this study is female guppy fish (*Poecilia reticulata*). The fish were purchased from a fish tackling shop in Perak, Malaysia, and brought to the laboratory within 15 min of purchase in plastic bags containing sufficient amounts of water for sustainability. Upon arrival at the test site, the fish were placed in plastic tanks for acclimation. Prior to the toxicity test, the fish were held in the laboratory for 12 days. The fish were fed twice daily, once in the morning and once in the evening. The fish were kept under normal laboratory illumination with a daily photoperiod of 12-16 h. The temperature of the water was between 23 ± 2 °C. The dissolved oxygen and pH of the water were 5-7 ppm and 7, respectively. The fish were observed carefully every day for signs of disease, stress, physical damage and mortality. Dead and abnormal specimens were removed immediately upon observation. After the adaptation period was completed, a group of 10 healthy fishes were selected randomly and placed into 6.5 L plastic tanks provided with electric air pumps. Healthy fish can be recognized by their normal swimming style. Fish swimming abnormally should not be selected as they may have a weak resistance to the chemicals used in testing. Initially the experiments were conducted using tap water. Unfortunately, the results were rejected due to an abnormal death rate of the fish. Therefore, lake water was then used to avoid the chlorine contamination of tap water. Each tank was equipped with 5 L of lake water. No food was provided for the fish during the test. The weight of each fish is approximately 0.5 g. The acute fish toxicity test was performed according to the OECD standard methods[24]. Each ionic liquid was tested for four concentrations. Different concentrations were added to each testing tank. Behaviors of the fish were monitored closely and dead fish were removed immediately. The number of dead fish for each concentration was recorded after 24, 48, 72 and 96 h. The median lethal concentration (LC_{50}) that is the ionic liquids' concentration in water which kills 50 % of the test batch of fish within a continuous period of exposure of 24, 48, 72 and 96 h were calculated. The results from the experiment were compared with acute toxicity rating scale provided by U.S. Fish and Wildlife Service (USFWS) (Table-1).

TABLE-1
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 non-toxic	100-1000
Relatively harmless	>1000

RESULTS AND DISCUSSION

The screening tests for the two ionic liquids were conducted with the concentration of 100 mg/L as limit test, which showed the range of toxicity below 100 mg/L and it lies between 50 to 100 mg/L. The observations of the fish mortality for butyl triphenyl phosphonium chloride and hexyl triphenyl phosphonium bromide are presented in Tables 2 and 3. It can be clearly seen that at higher concentrations, the number of dead fish increased. Numbers of dead fish in the time period prescribed by the test were used to determine the mortality per cent and median lethal concentration (LC_{50}). The cumulative percentage mortality for 96 h exposure time is plotted against a logarithm concentration (Figs. 2 and 3).

Data presented in Tables 4 and 5 were used to determine LC_{50} values employing probit analysis[27]. The best fit dose-response lines for butyl triphenyl phosphonium chloride and hexyl triphenyl phosphonium bromide were plotted in Figs. 4 and 5, respectively. Transformation of the toxicity curve to a straight line indicates that the lethal threshold concentration is estimated correctly. This fact was confirmed by Burdick[28]. Probit equations were derived from the experimental data presented in Tables 4 and 5 for both ionic liquids'; which are Y = 9.95x-13.56 and Y = 3.63x-1.49, respectively. The correlation coefficients, R^2 were obtained 94 and 99 %, respectively. This is confirming the accuracy of the results obtained from the experiment. Probit equations obtained from this work can be used to examine the toxicity of any ionic liquids' from the same family without the need for experimental work. It should be noted that these two equations are only valid to estimate the LC_{50} for the same ionic liquids and aquatic organism (Guppy fish).

TABLE-2
MORTALITIES OF ACUTE TOXICITY TEST FOR BUTYL TRIPHENYL PHOSPHONIUM CHLORIDE

Concentration (mg/L)	24 h	48 h	72 h	96 h	Total fish died
25.5	0	0	0	0	0
52.5	0	1	1	1	3
77.5	0	2	2	2	6
100	1	2	2	3	8

TABLE-3
MORTALITIES OF ACUTE TOXICITY TEST FOR HEXYL TRIPHENYL PHOSPHONIUM BROMIDE

Concentration (mg/L)	24 h	48 h	72 h	96 h	Total fish died
27.5	0	0	0	1	1
50.0	1	1	2	2	4
78.0	2	2	2	2	8
100	2	2	2	2	8

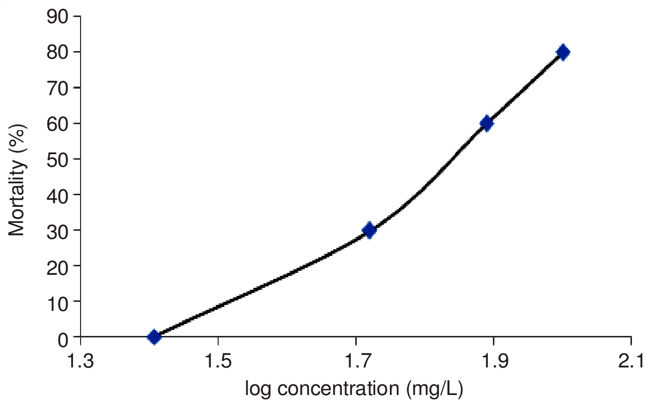


Fig. 2. log_{10} concentration *vs.* percent of mortality for butyl triphenyl phosphonium chloride

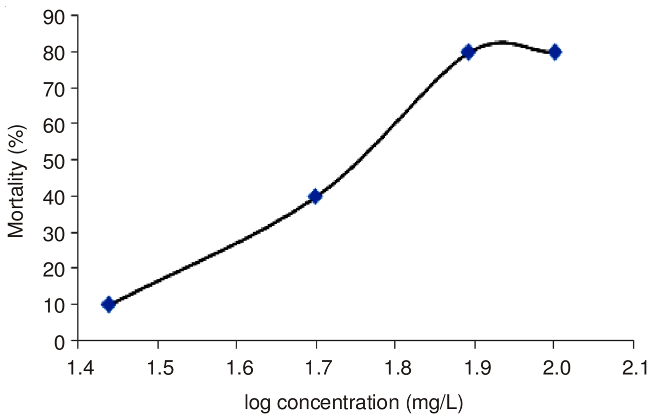


Fig. 3. log_{10} concentration *vs.* percent of mortality for hexyl triphenyl phosphonium bromide

Results presented in Figs. 2 and 3 or in Figs. 4 and 5 were used to obtain the LC_{50} values. These values were obtained to be 67.5 mg/L for butyl triphenyl phosphonium chloride and 56.3 mg/L for hexyl triphenyl phosphonium bromide respectively.

According to the acute toxicity rating scale provided by the U.S. Fish and Wildlife Service (USFWS), these values lie within the range of slightly toxic.

TABLE-4
log DOSE AND PERCENTAGE OF MORTALITY FOR BUTYL TRIPHENYL PHOSPHONIUM CHLORIDE

Dose (mg/L)	log dose (mg/L)	Total fish	Total mortality	% of mortality	Probit variable
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-5
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 variable
27.5	1.439	10	1	10	3.72
50.0	1.699	10	4	40	4.75
78.0	1.892	10	8	80	5.25
100	2.000	10	8	80	5.84

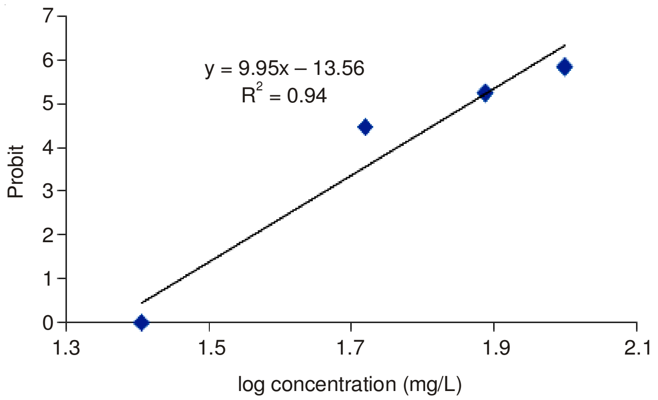


Fig. 4. $\log_{10}$ concentration *vs.* probit for butyl triphenyl phosphonium chloride

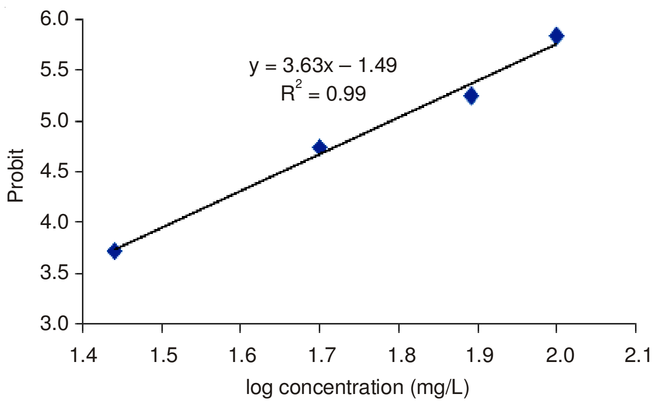


Fig. 5. $\log_{10}$ concentration *vs.* probit for hexyl triphenyl phosphonium bromide

Conclusion

Fish acute toxicity method was used to evaluate the LC_{50} of two phosphonium based ionic liquids. The acute fish toxicity test was performed using Guppy fish (*Poecilia reticulata*) according to the OECD 203 standard methods. Results obtained from this work indicate that the butyl triphenyl phosphonium chloride and hexyl triphenyl phosphonium bromide are slightly toxic. The result from this study can be beneficial to industrial people who use phosphonium based ionic liquids in their industrial processes.

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