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Toxicity Measurement of Imidazolium Ionic Liquids using Acute Toxicity Test

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Abstract

Ionic liquids (ILs) can be considered as environmentally friendly solvent, but they have the ability to dissolve in water and accumulate in the environment. Therefore, the toxicity of ILs should be assessed in order to prevent their harm to human and environment. This study was carried out to investigate the toxicity of ILs towards marine and freshwater fish. Three ILs have been tested, which are *1-Butyl-3-methylimidazolium hydrogen sulfate* and *1-Butyl-3-methylimidazolium bis (trifluoromethylsulfonyl) imide* toward marine fish and *1-Hexyl-3-methylimidazolium bis (trifluoromethylsulfonyl) imide* toward freshwater fish. Two different marine fish were employed, which are: *Cephalopholis cruentata* (grouper) and *Lates calcarifer* (barramundi). For freshwater fish, male *Poecilia reticulate* (guppy) was employed. The toxicity tests were conducted according to OECD (Organisation for Economic Cooperation and Development) guideline 203. For *1-Butyl-3-methylimidazolium hydrogen sulphate* [BMIM][HSO4], the median lethal concentration (LC_{50}) estimated toward *Cephalopholis cruentata*to be 199.98 $mg.L^{-1}$. For *1-Butyl-3-methylimidazolium bis (trifluoromethylsulfonyl) imide* [BMIM][TFSI], LC_{50} estimated toward *LatesCalcarifer*to be 374.11 $mg.L^{-1}$. While, for *1-Hexyl-3-methylimidazolium bis (trifluoromethylsulfonyl) imide*, [HMIM][NTf2], LC_{50} estimated toward *Poecilia Reticulate* to be 207.49 $mg.L^{-1}$. All the LC_{50} values obtained can be identified as practically nontoxic liquids based on Acute Toxicity Rating Scale by Fish and Wildlife Service (FWS). As to our knowledge, there is no previous reported toxicity studies of [BMIM][HSO4] and [BMIM][TFSI] on marine fish and [HMIM] [NTf2] on freshwater fish. Thus, this paper can be used as a benchmark for researchers who are dealing with these three ILs.

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Keywords: Ionic Liquds; Acute toxicity test, LC_{50}

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Nomenclature

Y	Probit value
x	Log Concentration (mg/L)

1. Introduction

The world of Ionic Liquids (ILs) started in 1914 by Walden [1] who synthesized ethylammonium nitrate $[EtNH_3][NO_3]$. ILs are composed of ions only and they are fluid below 100°C due to asymmetry of one of the ions. ILs thermally stable with liquid range up to 300°C compared to 100°C for water and show very low vapour pressure, being of great interest due to these properties. ILs'typically consist of bulky organic cations, such as imidazolium, pyridinium, ammonium, phosphonium, etc. paired with various anions, such as hexaflurophosphate (PF_4^-), bromide (Br^-), etc. Due to ILs unique properties, they are used as media for catalysts, material synthesis, liquid crystals and extraction in electrochemistry and separation processes [2-6].

There are several imidazolium, ammonium, pyridinium, and phosphonium based ILs which have been synthesized in the past and are commercially available. Although intensive information and data regarding ILs' physical and thermodynamic properties have been reported and continuously published, only limited data with regard to the toxicity and ecotoxicity of ILs' were reported [7-10]. Even though ILs are known to be environmental friendly as they do not contribute to the air pollution, somehow ILs are soluble in water [11], which make them to have the ability to dissolve in the water and accumulate in the environment.

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 [12]. Aquatic toxicity acts as an indicator to show the level of toxicity of chemical that exists in the water. OECDGuideline 203 [13] has recommended some test species, which are zebrafish (*Danio rerio*), fathead minnow (*Pimephales promelas*), common carp (*Cyprinus carpio),* ricefish (medaka) *(Oryzias latipes),* guppy (*Poecilia reticulata*), bluegill (*Lepomis macrochirus*), and rainbow trout (*Oncorhynchus mykiss*).

Acute toxicity test of several ILs have also been conducted using several types of aquatic organism and fish such as ILs toxicity on the freshwater crustacean *Daphnia magna*[14-16], zebrafish *Danio rerio*[17], and guppy fish *Poecilia reticulata*[18-19].

In this study, the acute fish toxicity study was conducted according to the prescribed guideline OECD 203 using marine fish [*Cephalopholis cruentata* (grouper) and *Latescalcarifer* (barramundi)] and freshwater fish [male *Poecilia reticulate* (guppy)] to estimate the toxicity of threeImidazoliumILs. To our knowledge there is no published literature regarding the toxicity (LC_{50}) of these ILs.

2. Materials and Methods

2.1 Materials

The ILs used in this study were obtained from the Ionic Liquid Research Centre located at Universiti Teknologi Petronas, Perak Malaysia. The ILs which were tested on marine fish are *1-Butyl-3-Methylimidazolium hydrogen sulphate* [BMIM][HSO4] and *1-Butyl-3-Methylimidazolium bis (trifluoromethylsulfonyl) imide* [BMIM][TFSI]. The IL tested on freshwater fish, *Hexyl Methylimidazoliumbis (trifluoromethylsulfonyl) imide* [HMIM][NTf2] and it's also obtained from the same research centre. The three ILs were prepared and synthesized using the Metathesis Process.

2.2 Test organism

The two species of marine fish employed in this study are *Cephalopholis cruentata* (grouper) and *Lates calcarifer* (barramundi/siakap). The fish were obtained from the area close to Kumpung Setiawan in Lumut, Perak, Malaysia. The length of the fish is about 1 cm (the weight ranged from 0.1 to 0.2 g) (Fig. 1).

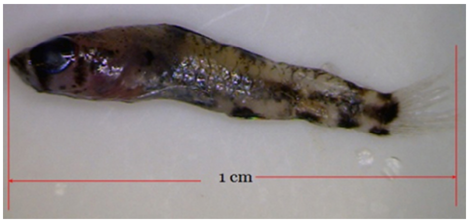

Fig. 1: Grouper baby fish.

The freshwater fish, male *Poecilia reticulate* (guppy) (Fig. 2) used in the test were purchased from a freshwater tackle shop located in Perak, Malaysia.

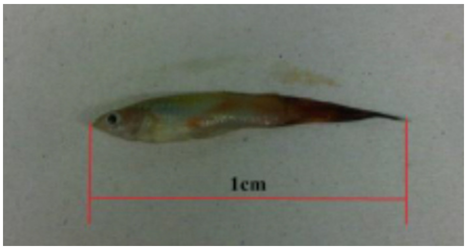

Figure 2: Male guppy fish.

The fish were brought to the laboratory provided with sufficient amount of water and oxygen.

2.3 Fish acute toxicity test

The water used for marine fish toxicity assessmentwere obtained from the same location that the fish were collected from. For freshwater fish, the lake water was used instead of tap water. This is to avoid chlorine contamination that exists in the tap water.The fish were kept under normal laboratory illumination with a daily photoperiod of 12-16 hours. 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 complete; a group of 10 healthy fish were selected randomly and placed into 6.5 litter 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. Each tank was equipped with 5 litters of 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 [20]. Each IL was tested for at least four concentrations. Different concentrations were added to each testing tank. Behaviours 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 hours. Then the median lethal concentration (LC_{50}) value (that is the concentration required to kill 50% of test fish in 96 hours) was 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 Nontoxic	100-1000
Relatively Harmless	>1000

2.3.1 Screening test concentrations

In the screening test, a wide range of IL concentrations were used to determine the limit concentrations. The screening test for [BMIM][HSO4] was conducted with the following concentrations: 200, 400, 500 and 600 ppm. Whereas, the screening test for [BMIM][TFSI] was conducted at the following concentrations: 100, 200, 400 and 500 ppm. While, the screening test for [HMIM][NTf2] was conducted at the concentrations: 50, 250 and 500 ppm. For this IL, only three concentrations were considered due to the limited available amount of the IL.

2.3.2 Definitive test concentrations

Based on the mortality observed in the screening tests, the definitive test concentrations were conducted using smaller concentration ranges. The [BMIM][HSO4] was tested for the following four concentrations 200, 250, 300, and 350 ppm. For [BMIM][TFSI], the five concentrations tested were 200, 250, 300, 350 and 400 ppm. Whereas, for [HMIM][NTf2], the test concentrations were 70, 100, 125, 150, 200 and 250 ppm.

2.3.3 Controls and number of replicates/groups

During the test, 10 fish were placed in a 6.5 litters aquarium that filled with 5 litters of water equipped with electric air pump. Three different groups of tanks (same size/volume) were used and each tank filled with the same volume of water (5 litters).

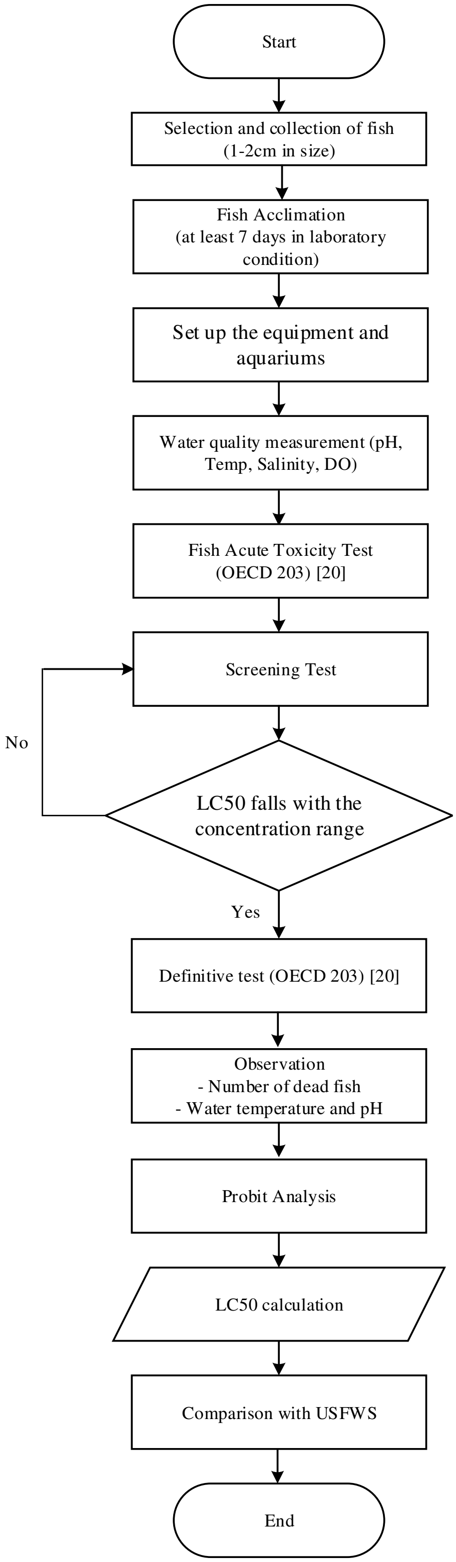

Figure 3: Flow chart describing the fish toxicity assessment steps.

3. Results and Discussion

3.1 Fish Acute Toxicity Results

3.1.1 1-butyl-3-methylimidazolium hydrogen sulfate [BMIM][HSO4]

Prior ofconducting the test, dissolved oxygen, pH, and temperature of theused waters were measured in each tested tanks to ensure that the parameters were within the acceptable limits [13]. The IL was then immediately dissolved into the sea-water and the solution became slightly turbid compared to the control tank.The fish responded immediately after exposed to the [BMIM][HSO4] solution in the screening test. Complete mortality occurred within 3 hours after the test has been started for 500 and 600 ppm and 24 hours later for 400 ppm. Survival of the laboratory control was 100 percent. These results suggest an expected LC_{50} less than 400 ppm. Based on the results obtained from the screening tests, the concentration series for the definitive test were chosen as follows: 200, 250, 300, and 350 ppm. Two replicates of each concentration were run. 50% and 60% mortality was observed for 200 ppm and between 70% and 90% mortality in the range of 250 to 300 ppm. 100% mortality was recorded in 350 ppm concentration. Survival of the laboratory control was 100%. The results are presented in Table 2.

Table 2. Mortalities of acute toxicity test for 1-butyl-3-methylimidazolium hydrogen sulfate [BMIM][HSO4]

[BMIM][HSO4] fish toxicity results			
Replicate A			
Test solution Conc. (ppm, mg/L)	Live organisms at the beginning of the test	Cumulative mortality at 96 hours	Mortality %
0	10	0	0
200	10	5	50
250	10	9	90
300	10	9	90
350	10	10	100
Replicate B			
Test solution Conc. (ppm, mg/L)	Live organisms at the beginning of the test	Cumulative mortality at 96 hours	Mortality %
200	10	6	60
250	10	7	70
300	10	9	90
350	10	10	100

It should be noted that all recorded mortality occurred within 96 hours from the beginning of the test. The fish in the control group as well as the survival species show normal behaviour during the test period. Indeed, less general activity of the fish was recorded compared with the control group. The responses of loss of equilibrium and hanging vertically in the water were observed.

3.1.21-butyl-3-methylimidazolium bis (trifluoromethylsulfonyl) imide [BMIM][TFSI]

The concentration series for the screening test were 100, 200, 400 ppm. Some of the tested IL was settled at the bottom of the tank and gradually dispersed into the sea-water over a period of approximately 24 hours. Fish were observed periodically for signs of mortality or abnormality. No mortalities were observed in the 100 ppm concentration. Between 10% and 60% mortality was observed in the range of 200 to 400 ppm. The results show that the expected LC_{50} is within the range of the screening test concentrations. Based on the results obtained from the screening test, the concentration series for the definitive test were chosen as follows: 200, 250, 300, 350, and 400 ppm. Replicates of the test could not be done due to the limited quantity of [BMIM][TFSI]. No mortality was observed in the 200, 250 and 300 ppm concentrations. 40% mortality was observed in 350 ppm and 60% mortality in 400 ppm concentration. The results are presented in Table 3.

3.1.3 1-hexyl-3-methylimidazolium bis (trifluoromethylsulfonyl) imide [HMIM][NTf2]

For [HMIM][NTf2], the 96-hour acute toxicity test was performed using freshwater fish, male *Poecilia Reticulate* (known as guppy fish). The concentration series for the screening test were: 50, 250, and 500 ppm. The IL was immediately dissolved into the lake water and similar to *BMIM][HSO4*,the solution became slightly turbid compared to the control tank. The fish responded immediately after exposed to the [HMIM][NTf2] test solution. The IL caused 100 percent mortality for 250 and 500 ppm concentrations. Complete mortality occurred 24 hours after the test has been started for 500 ppm and 48 hours later for 250 ppm concentration. No mortality was recorded for 50 ppm. Survival of the laboratory control was 100 percent. These results suggest an expected LC_{50}between 50 ppm and 250 ppm. The concentration series for the definitive test were chosen as follows: 70, 100, 125, 150, 200 and 250 ppm. Three replicates of each concentration were run. No mortality was observed in 70, 100 and 125 ppm. Between 10% and 40% mortality were observed in the range of 150 to 200 ppm and complete mortality occurred for 250 ppm concentration. Survival of the laboratory control was 100%. The results are presented in Table 4.

Table 3. Mortalities of acute toxicity test for 1-butyl-3-methylimidazolium bis (trifluoromethylsulfonyl) imide [BMIM][TFSI]

[BMIM][TFSI] fish toxicity results			
Replicate A			
Test solution Conc. (ppm, mg/L)	Live organisms at the beginning of the test	Cumulative mortality at 96 hours	Mortality %
0	10	0	0
200	10	0	0
250	10	0	0
300	10	0	0
350	10	4	40

Table 4: Mortalities of acute toxicity test for 1-hexyl-3-methylimidazolium bis(trifluoromethylsulfonyl) imide [HMIM] [NTf2]

[HMIM] [NTf2]fish toxicity results			
Replicate A			
Test solution Conc. (ppm, mg/L)	Live organisms at the beginning of the test	Cumulative mortality at 96 hours	Mortality %
0	10	0	0
70	10	0	0
100	10	0	0
125	10	0	0
150	10	0	0
200	10	2	20
250	10	10	100
Replicate B			
Test solution Conc. (ppm, mg/L)	Live organisms at the beginning of the test	Cumulative mortality at 96 hours	Mortality %
70	10	0	0
100	10	1	10
125	10	0	0
150	10	3	30
200	10	7	70
250	10	10	100
Replicate C			
Test solution Conc. (ppm, mg/L)	Live organisms at the beginning of the test	Cumulative mortality at 96 hours	Mortality %
70	10	0	0
100	10	0	0
125	10	1	10
150	10	1	10
200	10	4	40
250	10	10	100

3.2 Statistical analysis and LC_{50} determination

Probit analysis [21] is one of the methods to observe the dose relationship by linear regression. The data obtained from fish acute toxicity test (Table 2, 3 and 4) were used to calculate and plot the cumulative percentage mortalities after 96 hr of exposure time against the logarithm of concentrations(Table 5, 6 and 7). Thenprobit analysis was employed to determine the lethal concentration values in which 50% of organism died (LC_{50}) (Figure 4, 5 and 6). Figure 4 shows the percentage of the average mortality for two replicates at different concentrations of [BMIM][HSO4]. It can be concluded that the log concentration which gave 50% of mortality is 2.301 (LC_{50}=199.98 mg/L).While Figure 5 shows the percentage of the average mortality at different concentrations of [BMIM][TFSI]. It can be clearly seen that the log concentration that gave 50% of mortality is 2.573(LC_{50}=374.11 mg/L).Whereas, Figure 6illustrates the percentage of the average mortality for three replicates at different concentrations of [HMIM][NTf2] [log $LC_{50}$is 2.317(LC_{50}=207.49 mg/L)].Probit equations for the results obtained are indicated in the inset of Figures 7, 8 and 9. The correlation coefficient, R^2 obtained were 88.55%, 71.10% and 81.94% respectively. Transformation of the toxicity curve to a straight line indicated that the lethal threshold concentration is estimated correctly. This fact was confirmed by Burdick [20].This confirms the accuracy of the results obtained from the experimental work. Probit equations obtained from this work (Table 8) can be used to predict the lethal concentration (ex. LC_n, where n can be 1 to 100) for the same ILs without the need for the experimental work. It should be noted that these three equations are only applicable to estimate the LC_n for the same aquatic organism.

Table 5: Log Concentration and percentage of mortality for 1-butyl-3-methylimidazolium hydrogen sulfate [BMIM][HSO4]

Log Concentration and percentage of mortality for [BMIM][HSO4]					
Replicate A					
Test solution Conc. (ppm, mg/L)	Log Conc.	Live organisms at start of test	Cumulative mortality at 96 hours	Percent mortality	Probit Variable
200	2.30103	10	5	50	5
250	2.39794	10	9	90	6.28
300	2.47712	10	9	90	6.28
350	2.54407	10	10	100	8.09
Replicate B					
Test solution Conc. (ppm, mg/L)	Log Conc.	Live organisms at start of test	Cumulative mortality at 96 hours	Percent mortality	Probit Variable
200	2.30103	10	6	60	5.25
250	2.39794	10	7	70	5.52
300	2.47712	10	9	90	6.28
350	2.54407	10	10	100	8.09
Average Mortality					
Test solution Conc. (ppm, mg/L)	Log Conc.	Live organisms at start of test	Cumulative mortality at 96 hours	Percent mortality	Probit Variable
200	2.30103	10	5	50	5
250	2.39794	10	8	80	5.84
300	2.47712	10	9	90	6.28
350	2.54407	10	10	100	8.09

Table 6: Log concentration and percentage of mortality for 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl) imide [BMIM][TFSI]

Log Concentration and percentage of mortality for [BMIM][TFSI]					
Test solution Conc. (ppm, mg/L)	Log Conc.	Live organisms at start of test	Cumulative mortality at 96 hours	Percent mortality	Probit Variable
200	2.30103	10	0	0	0
250	2.39794	10	0	0	0
300	2.47712	10	0	0	0
350	2.54407	10	4	40	4.75
400	2.60206	10	6	60	5.25

Table 7: Log concentration and percentage of mortality for 1-hexyl-3-methylimidazolium bis(trifluoromethylsulfonyl) imide [HMIM] [NTf2]

Log Concentration and percentage of mortality for [HMIM] [NTf2]					
Replicate A					
Test solution Conc. (ppm, mg/L)	Log Conc.	Live organisms at start of test	Cumulative mortality at 96 hours	Percent mortality	Probit Variable
70	1.8451	10	0	0	0
100	2	10	0	0	0
125	2.0969	10	0	0	0
150	2.17609	10	0	0	0
200	2.30103	10	2	20	4.16
250	2.39794	10	10	100	8.09
Replicate B					
Test solution Conc. (ppm, mg/L)	Log Conc.	Live organisms at start of test	Cumulative mortality at 96 hours	Percent mortality	Probit Variable
70	1.8451	10	0	0	0
100	2	10	1	10	3.72
125	2.0969	10	0	0	0
150	2.17609	10	3	30	4.48
200	2.30103	10	7	70	5.52
70	1.8451	10	0	0	0
Average Mortality					
Test solution Conc. (ppm, mg/L)	Log Conc.	Live organisms at start of test	Cumulative mortality at 96 hours	Percent mortality	Probit Variable
200	2.30103	10	5	50	5
250	2.39794	10	8	80	5.84
300	2.47712	10	9	90	6.28
350	2.54407	10	10	100	8.09

According to the acute toxicity rating scale provided by the U.S. Fish and Wildlife Service (USFWS), the obtained LC_{50}values lie within the range of practically nontoxic.

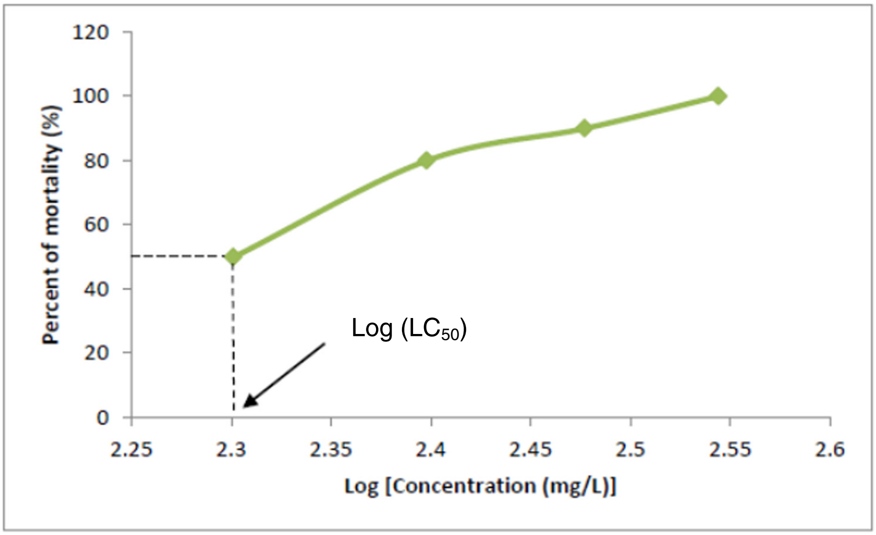

Figure 4: Log Concentration *vs*. mortality curve for [BMIM][HSO4].

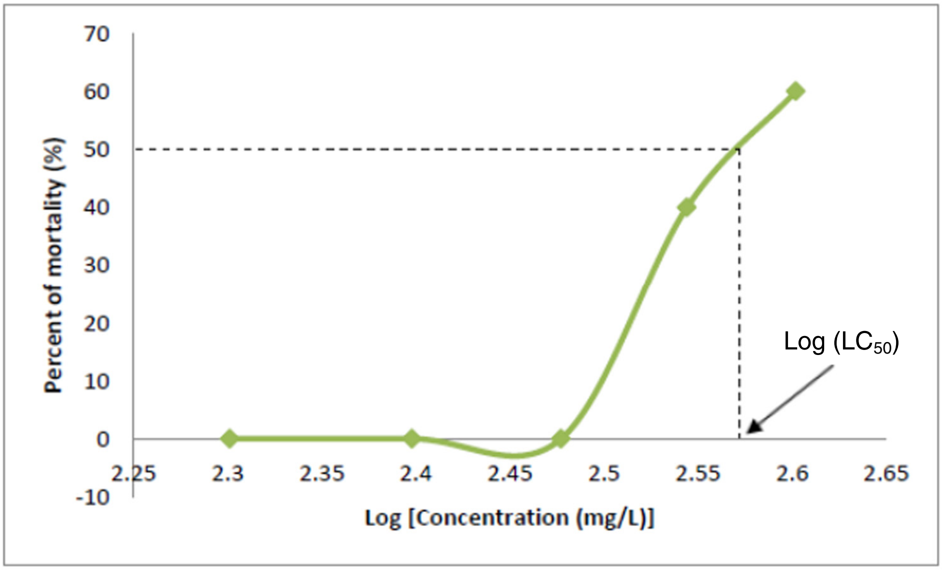

Figure 5: Log Concentration *vs*. mortality curve for [BMIM][TFSI].

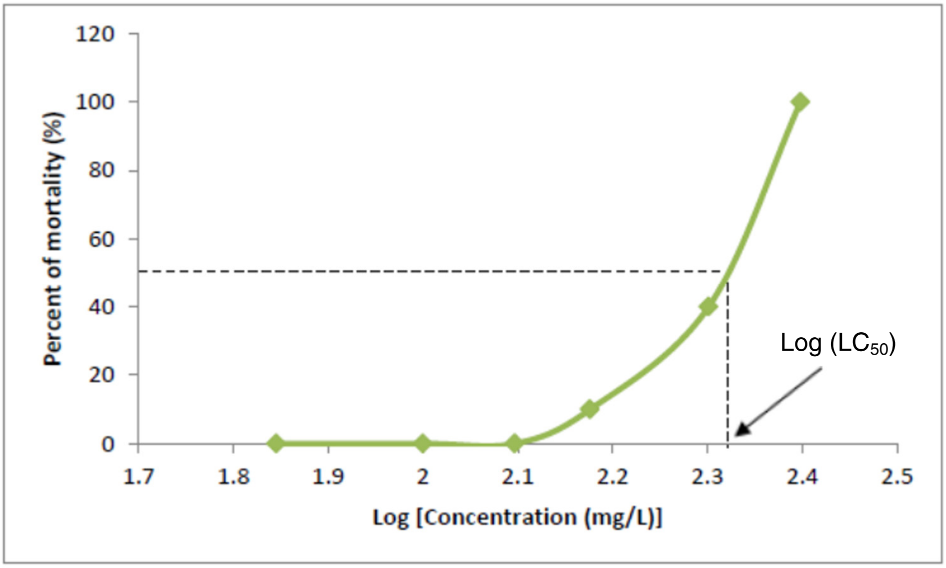

Figure 6: Log Concentration *vs*. mortality curve for [HMIM][NTf2].

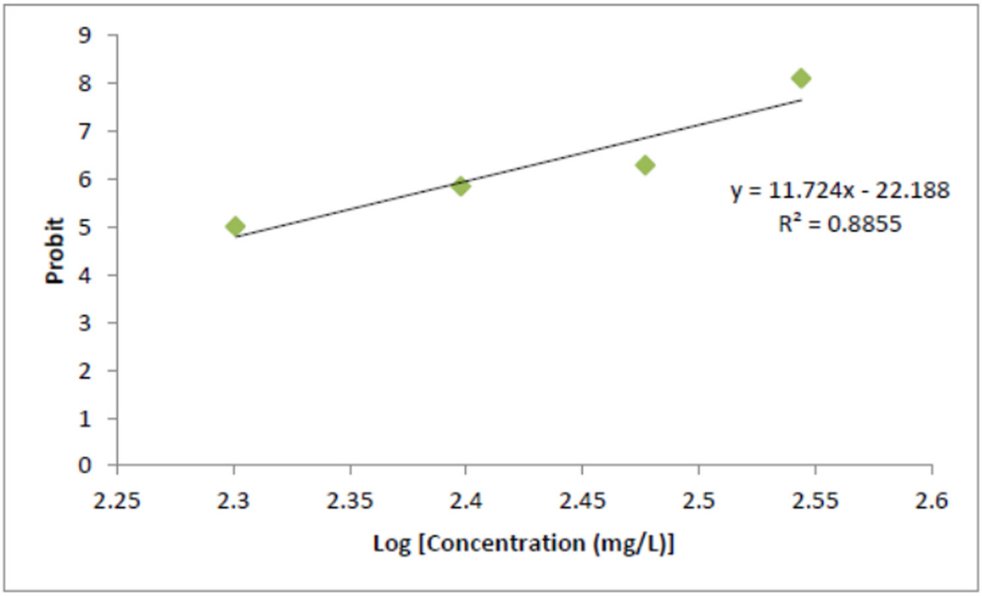

Figure 7: Plot of adjusted probit and predicted regression line for [BMIM][HSO4].

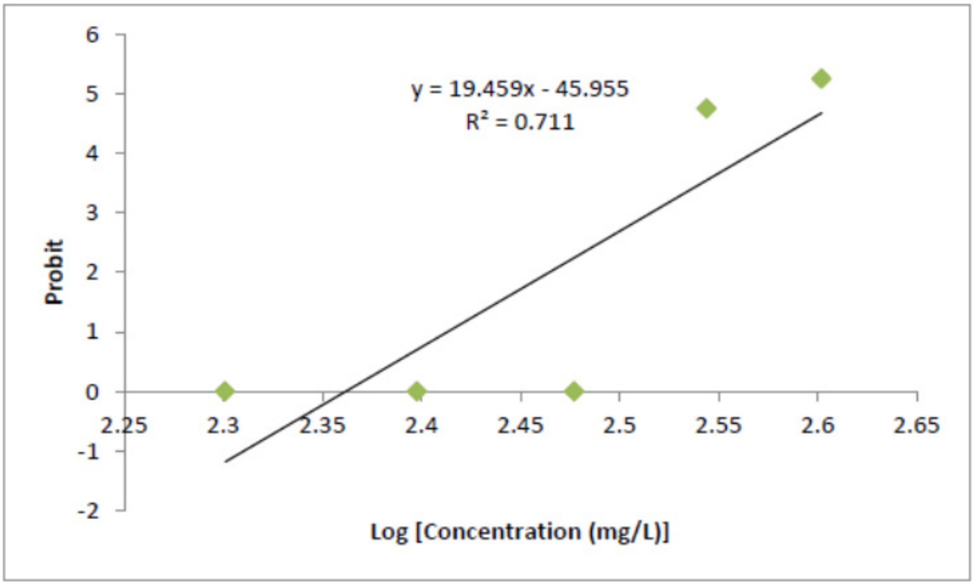

Figure 8: Plot of adjusted probit and predicted regression line for [BMIM][TFSI].

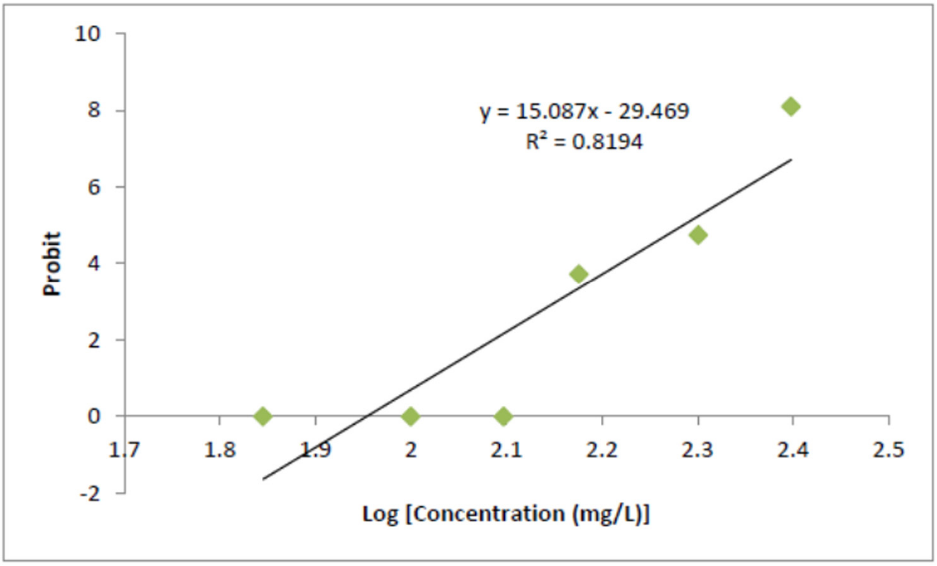

Figure 9: Plot of adjusted probit and predicted regression line for [HMIM][NTf2].

Table 8: Probit equations and correlation coefficient, R^2 of tested ionic liquids

Ionic Liquid	Probit Equations	Correlation coefficient, R^2
[BMIM][HSO4]	*Y=11.724x-22.188*	*88.55*
[BMIM][TFSI]	*Y=19.459x-45.955*	*71.11*
[HMIM][NTf2]	*Y=15.087x-29.469*	*81.94*

4. Conclusion

Acute toxicity test was conducted to evaluate the LC_{50} of three ILs, which are*1-butyl-3-methylimidazolium hydrogen sulfate* [BMIM][HSO4], *1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl) imide* [BMIM][TFSI] and *1-hexyl-methylimidazolium bis(trifluoromethylsulfonyl) imide* [HMIM][NTf2]. The experiment was conducted using marine fish Cephalopholis cruentata (grouper), Lates calcarifer (barramundi) and freshwater fish male Poecilia reticulate (guppy) according to the OECD 203 standard methods. Result obtained shows that the LC_{50} estimated for [BMIM][HSO4] toward Cephalopholis cruentata to be 199.98 mg/L. The estimated LC_{50} for [BMIM][TFSI] toward Lates calcarifer is 374.11 mg/L and the LC_{50} for [HMIM][NTf2] toward Poecilia reticulate is 207.49 mg/L. Results obtained indicated according to the FWS scale that all these three ILs are practically nontoxic. The results from this study can be beneficial to industrial people who use these ILs in their industrial processes.

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Appendix A.

Transformation of probits to percentages (Vílchez*et. al*., [21])

%	0	2	4	6	8
0	-	2.95	3.25	3.45	3.59
10	3.72	3.82	3.92	4.01	4.08
20	4.16	4.23	4.29	4.36	4.42
30	4.48	4.53	4.59	4.64	4.69
40	4.75	4.80	4.85	4.90	4.95
50	5.00	5.05	5.10	5.15	5.20
60	5.25	5.31	5.36	5.41	5.47
70	5.52	5.58	5.64	5.71	5.77
80	5.84	5.92	5.99	6.08	6.18
90	6.28	6.41	6.55	6.75	7.05
99	7.33	7.41	7.46	7.65	7.88