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Evaluation of Toxity of Ionic Liquids on *Paramecium*

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ABSTRACT

Volatility of the conventional organic solvents has led to environmental pollution by increasing volatile organic compounds (VOC) in the environment. Steps should be taken to minimize the exposure to VOC's. Control measures would include elimination and substitution for less toxic solvents. R& D scientist can work in direction of controlling these levels by developing newer substituents and one example is of ionic liquids (ILs).Data on toxicity of these ionic liquids when disposed in water has been studied to a limited extent. In present study, two ionic liquids, Triethyl ammonium dibutyl phosphate and 1-Ethyl-3-methyl imidazolium dibutyl phosphate have been selected to study their toxicity on *Paramecium.* The effect of ionic liquid shave been examined on unicellular ciliated protozoa, *Paramecium* and no adverse effects have been found. The *Paramecium* were found to be healthy, moving and dividing at1% concentration of ionic liquid proving that these ionic liquids are non-toxic. This study has provided a simple, inexpensive and rapid method to determine the toxicity of ionic liquids. The method can be adopted at the undergraduate level to perform the assay as it does not require any specialized training and only rudimentary skills are required.
Key words: inflammability, ionic liquids (ILs), *Paramecium,* toxicity, solvent, volatility

INTRODUCTION

Organic solvents are usually clear and colorless liquids and most of them have a characteristic smell. These solvents find various applications as diluents, paint industry, as solvents for chemical synthesis, for dissolution, for separation technology in the manufacture of drugs to mention a few. The major disadvantage of these is their volatility and inflammability. These are highly volatile and are classified into the category of volatile organic carbon or VOC's and thus are potential hazards for the environment. Industrialist and R&D scientists are on look for suitable alternatives. It was nineteen twenties that ionic liquids were reported and suggested as suitable as a solvent. Scientists have been developing newer ionic liquids with tailorable properties like polarity, conductivity, etc.

Ionic liquids (ILs) are organic salts, consisting of organic cations, such as imidazolium, pyridinium, pyrrolidinium, ammonium, phosphonium, and inorganic anions like halides, tetrachloroaluminate,hexaflurophosphate,tetrafluoroborate&bis(trifluoromethylsulfonyl)imide, with low melting points. Ionic liquids have been regarded as "green" solvents because of their negligible vapor pressure, which reduces their evaporation into the air[1].Because of their excellent properties of non-volatility, non-flammability, and high chemical and thermal stability, ILs have been used in many applications, including organic synthesis [2], electrochemistry [3], liquid/liquid extraction [4], clean technology [5] and polymerization processes [6,7].Figure I exhibits various applications of ionic liquids.

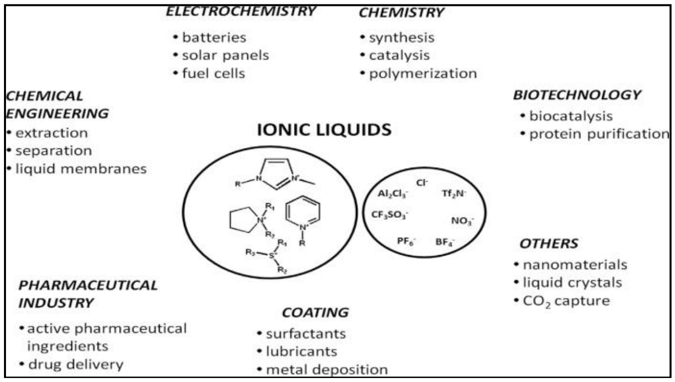


Figure-I Possible applications of Ionic Liquids [34]

However, they should be regarded as successful green solvents only if an acceptable level of risk to various organisms and human beings can be confirmed. Ionic Liquids have been finding increased applications in quantities and types of Ionic Liquids for various industrial and commercial applications, they will therefore be inevitably released into the environment and the aquatic environment in particular. The general fate of ILs in the industry is presented in Figure II.

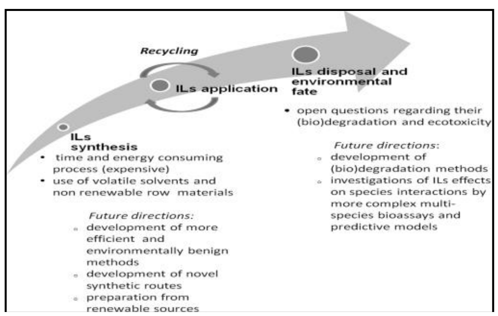


Figure-II Ionic liquids (ILs): From preparation to disposal [34]

Moreover, most ILs have shown high solubility and poor biodegradation [8,9]. For these reasons, pyridinium, imidazolium, and pyrrolidinium ILs have been proposed for toxicological testing by the United States National Toxicology Program in the year 2005[10].It has become imperative to perform the aquatic environmental risk assessment of several critical ILs associated with eco-toxicological hazards.

The possible effects of ILs on aquatic organisms have been investigated in many studies [11], and a few in vivo studies have shown that most ILs exhibit varying toxic impacts on aquatic organisms. The toxic effects of ILs include decreased fertility [12, 13], oxidative stress [14, 15], animal behavioural changes [16,17], increased mortality (17), embryonic malformations and injuries [18,19] as well as the inhibition of the growth, primary productivity, and photosynthetic activity of algae [20-24].

It has been reported that environmental toxicants could cause oxidative damage to plants either directly or indirectly by triggering reactive oxygen species (ROS) production[25].To avoid the harmful effects of ROS, plants have developed an effective scavenging system composed of enzymatic antioxidants, such as superoxide dismutase, catalase, and peroxidase andnonenzymatic antioxidants such as ascorbate, carotenoids, and glutathione[26,27].

Several toxicity studies on the effects of ionic liquids toward organisms, including *Vibrio fischeri* [10], *Daphnia magna* [12], animal cells [28], algae [29], *Escherichia coli* [30], lactic acid producing bacteria have recently been documented [31-32]. The ILs has revealed to be more harmful to aquatic systems than the tested organic solvents, reaffirming the need to analyze carefully the (eco) toxicological impact of these compounds (35).

In this paper efforts have been made to study the toxicity of ionic liquids on *Paramecium* in aquatic ponds as we have got good population of Paramecium in our culture. The analysis of the *Paramecium* in the cultured ponds was studied at different concentration of ionic liquids. The developed analysis could be exploited as a technique for the testing of toxicity of ionic liquids on ciliated protozoa, *Paramecium*.

METHODOLOGY

Culturing

Three liters of natural pond water was taken in a trough, and examined under the microscope which showed the presence of various microorganisms, as *Paramecium*, *Euglena*, *Rotifers* and various others which remained unidentified.

A trough of diameter 12 in and 3 in depth was maintained to culture these microorganisms; tap water was added in trough with the sample. The trough with 3 liter of water was kept near the window to provide almost natural conditions till the experiments were carried out.

The culturing was carried by feeding the organisms with boiled cabbage. Because of the presence of *Paramecium*, cabbage is added as a food for all the organisms. After every week the remnants of cabbage were removed and fresh boiled cabbage was added to the medium.

The level of water was maintained as there was loss of water every week, lowering of water level was observed due to overcrowding of organism. To maintain the culture fresh tap water was added every week so that organisms do not die because of lack of food and oxygen. The culture was maintained for 3 months with proper growth and good population of the organism.

The culture was set open initially to provide natural conditions but due to the appearance of mosquito larvae that was not of interest as per the experiment, it was covered by a net sheet so that proper air ventilation could occur and restricted the entry of mosquito for laying their eggs.

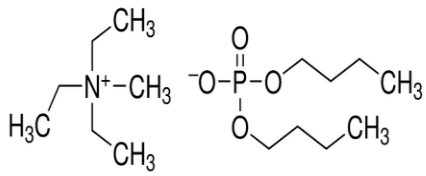
A regular examination of culture was done to see their growth and their increase in population. Regular examination was one of the important steps adopted for maintaining the culture. The obtained culture was named as the stock culture.

After culturing of the microorganisms, a single microorganism was isolated by the help of serial dilution method. With the help of serial dilution method *Paramecium* was isolated and cultured in a separate trough.

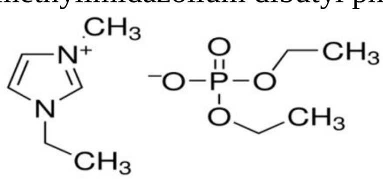
Serial dilution method: The sample was prepared by diluting1ml of stock culture and with 9ml of tap water. The diluted culture was examined and the culture with *Paramecium* only was added to a new culture medium.

This new culture was maintained in the trough with three liters of water. Boiled cabbage as a food was added every week and fresh water added as per requirement. The culture was examined at frequent intervals of 3 hours initially to see that *Paramecium* had adopted the new conditions and had started reproducing.
The *Paramecium* was cultured till we had a good population.
Exposure to ionic liquids: The effects of ionic liquid were examined on *Paramecium* by directly adding different concentration of ionic liquids as per Table1 to the culture medium. This allows direct contact of organism with the ionic liquid to be tested.
The ionic liquids selected for the study are Triethyl methyl ammonium dibutyl phosphate and 1-Ethyl-3-methylimidazolium dibutyl phosphate; ILs were purchased from Sigma-Aldrich with purity ≥ 97.0%.Their chemical structures are given below:

(1) Triethyl methyl ammonium dibutyl phosphate



(2) 1-Ethyl-3-methylimidazolium dibutyl phosphate



From stock culture 10 ml of *Paramecium* culture was taken in each of six different test tubes and are labeled. In the six test tubes different concentrations of ionic liquid was taken as given in Table 1

Table-I Concentration of ionic liquid used to evaluate the toxicity on Paramecium

S.No	Different concentrations of ionic liquid (v/ v %)
0	No ionic liquid
1	0.02%
2	0.04%
3.	0.06%
4.	0.08%
5.	0.1%
6.	1%

In each test tube 2 ml of ionic liquid with different concentrations is added to the 10 ml of culture already present in it. After the addition procedure the test tubes were shaken to mix ionic liquid with culture completely. The culture and ionic liquid in the test tubes were provided similar conditions as done for culturing.
The next part was to examine the effect ILs on *Paramecium* with respect to time. After the addition and mixing of ionic liquid with *Paramecium* culture, the culture was examined under microscope at 0 hours stage and successive observations were taken after 1 hour.

After every one hour the culture was examined and observations were noted.
A control was run in which 10 ml of culture is added with 2 ml of distilled water, the control is also examined at 0 hour and successively after 1 hour each

RESULTS

The observations made with Triethyl methyl ammonium dibutyl phosphate as ionic liquid are shown in Table-II.

Table-II Effect of various concentrations of Triethyl methyl ammonium dibutyl phosphate on *Paramecium*

Concentrations of IL (%)	Time (in hours)							
	0	1	2	3	4	5	6	24
No IL	Alive (6-7)	Alive (2-4)	Alive (3-5)	Alive (3-4)	Alive (5-7)	Alive (6-7)	Alive (6-7)	Alive (4-5)
0.02%	Alive (5-7)	Alive (4-6)	Alive (6-8)	Alive (5-7)	Alive (3-5)	Alive (5-7)	Alive (6-8)	Alive (2-4)
0.04%	Alive (6-8)	Alive (3-5)	Alive (3-5)	Alive (2-4)	Alive (4-6)	Alive (6-8)	Alive (5-7)	Alive (5-7)
0.06%	Alive (3-5)	Alive (2-4)	Alive (4-6)	Alive (6-8)	Alive (5-7)	Alive (3-5)	Alive (2-4)	Alive (4-6)
0.08%	Alive (4-6)	Alive (5-7)	Alive (5-7)	Alive (3-5)	Alive (2-4)	Alive (6-8)	Alive (6-8)	Alive (6-8)
0.1%	Alive (5-7)	Alive (6-8)	Alive (2-4)	Alive (6-8)	Alive (5-7)	Alive (4-6)	Alive (3-5)	Alive (3-5)
1%	Alive (4-6)	Alive (3-5)	Alive (5-7)	Alive (4-6)	Alive (6-8)	Alive (2-4)	Alive (4-6)	Alive (5-7)

*In brackets ( ) number of paramecium in one drop.

At the 0 hours stage, ionic liquid had no effect on *paramecium*, the culture was examined till 6 hours and it showed no effect on *Paramecium*. Another observation was taken after 24 hours which also showed no effect. All the *Paramecium* were found moving. In control, all the *Paramecium* remain alive during the whole experiment time.

The observations made with1-Ethyl-3-methylimidazolium dibutyl phosphate are shown in Table-III.

Table-III Effect of various concentrations of 1-Ethyl-3-methylimidazolium dibutyl phosphate on *Paramecium*

Concentrations of IL (%)	Time(in hours)							
	0	1	2	3	4	5	6	24
No IL	Alive (6-8)	Alive (5-7)	Alive (7-8)	Alive (3-4)	Alive (6-7)	Alive (3-5)	Alive (4-6)	Alive (7-8)
0.02%	Alive (5-8)	Alive (2-4)	Alive (3-5)	Alive (6-8)	Alive (5-8)	Alive (4-6)	Alive (6-8)	Alive (6-8)
0.04%	Alive (2-4)	Alive (4-6)	Alive (4-6)	Alive (6-8)	Alive (3-5)	Alive (6-8)	Alive (2-4)	Alive (4-6)
0.06%	Alive (4-6)	Alive (6-8)	Alive (6-8)	Alive (4-6)	Alive (4-6)	Alive (5-8)	Alive (3-5)	Alive (5-8)
0.08%	Alive (2-4)	Alive (5-8)	Alive (2-4)	Alive (3-5)	Alive (6-8)	Alive (2-4)	Alive (6-8)	Alive (2-4)
0.1%	Alive (3-5)	Alive (6-8)	Alive (6-8)	Alive (5-8)	Alive (6-8)	Alive (3-5)	Alive (5-8)	Alive (6-8)
1%	Alive (6-8)	Alive (3-5)	Alive (5-8)	Alive (2-4)	Alive (2-4)	Alive (6-8)	Alive (4-6)	Alive (3-5)

*In brackets ( ) number of paramecium in one drop.

At the 0 hours stage, ionic liquid had no effect on *Paramecium*, the culture was examined till 6 hours and it showed no effect on *Paramecium*. Another observation was taken after 24 hours which also showed no effect.

DISCUSSION

All the *Paramecium* were found moving i.e., all are alive and not affected by the concentrations of ionic liquid studied. In control (without IL), all the *Paramecium* remained alive during the experiment. Triethyl methyl ammonium dibutyl phosphate and 1-Ethyl-3-methylimidazolium dibutyl phosphate have no effect on *Paramecium* for all the concentrations of ILs studied. Even the maximum concentration of1% ionic liquid is not killing the organism under study. Therefore the ILs studied are not toxic to the aquatic organism *Paramecium* at the concentrations studied.

CONCLUSIONS

The ionic liquids Triethyl methyl ammonium dibutyl phosphate and 1-Ethyl-3-methylimidazolium dibutyl phosphate have been found to have no adverse effects on *Paramecium*, these two ionic liquid can be further examined on various other aquatic organism to determine the complete toxicity on an aquatic system.

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