Toxicity of quaternary ammonium ionic liquids to aquatic organisms

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Introduction

Ionic liquids are simple mixtures of cations and anions. These compounds are identified by the acronym ILs. ILs are liquids in low temperature, and their melting point is below 100°C [1]. Quaternary ammonium ionic liquids (QAILs) are composed of cation with positive charged nitrogen atom with long alkyl chains and organic or inorganic anions. It was found that cation have large impact on toxicity of ionic liquids [2], while anion have impact only on physical properties of these compounds, like melting point or viscosity [1]. Quaternary ammonium ionic liquids are widely used in industry and agriculture as pesticides, fungicides, corrosion inhibitors, disinfectants, emulsifiers, surfactants and they are also used in synthesis, and catalysis [3].

Presence of ammonium compounds in environment are confirmed in many analyses. For example, Quaternary Ammonium Compounds were detected in low concentrations in the North Sea (120 – 270 ng/dm^3) and in the Austrian rivers (1.9 μg/dm^3) [4, 5]. These compounds are present in environment as a result of waste water treatment plants effluents, accidental spills and being washed out of the site of application (protection of wood against fungi) [3].

Toxicity of ionic liquids were being analyse for many years. Research showed that QAILs have antibacterial properties, which are closely dependent on the structure of these compounds. Positively charged quaternary nitrogen atoms interact with negatively charged phospholipids belonging to the bacterial membrane. QAILs may also cause denaturation of cellular proteins and enzymes [6]. Quaternary ammonium ionic liquids also showed toxicity to algae. The trend of increasing toxicity with increasing alkyl chain length was observed [7]. There were also studies about the impact of QAILs on the intensity of photosynthesis of algae *Pseudokirchneriella subcapitata*. The results indicate that if the substituents were longer, the inhibition of photosynthesis was better [8, 9]. Ionic liquids are highly toxic toward invertebrates. The most frequently examined test organism from that group is *Daphnia magna*. It was observed that with increasing alkyl chain length of the cation, toxicity of the compound also increase [10, 11]. Research showed that ionic liquids may also cause adverse effects with respect to vertebrates. ILs can cause negative effects on growing of embryonic frog *Rana nigromaculata*. With increasing concentration of the ionic liquid, the mortality rate of frog embryos also increase [12]. Studies on mice and rats indicate that some ionic liquids can be a teratogen [13]. ILs can also be cytotoxic [14÷16] and genotoxic [17].

The correct prediction of the toxicity of the newly-synthesized compounds is very significant, especially due to the ability to easily design properties of ionic liquids by changing the structure of the anion or cation.

The main goal of this project was to define toxicity of three newly-synthesized QAILs towards aquatic organisms.

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Materials and methods

Compounds

In the present study the toxic effects of three newly-synthesized QAILs were presented. The compounds were synthesized by the group of Juliusz Pernak from Poznań University of Technology in Poland. All tested compounds were composed of didecyldimethylammonium cations and different anions: nitrite, nitrate or dihydrocitrate. Structures of the tested compounds are listed in Table 1.

Table 1

Structures of the tested ionic liquids.

Cation	Anion	Formula	Mole weight, g/mol
didecyldimethy-lammonium	nitrite	[DDA][NO_2]	372.62
	nitrate	[DDA][NO_3]	388.62
	dihydrocitrate	[DDA][H_2Cytr]	517.73

Ecotoxicity tests

Three test organisms were selected to the tests. *Tetrahymena thermophila* – freshwater protozoan ciliate and two sea organisms: bacteria *Vibrio fischeri* and crustaceans *Artemia franciscana*. Test organisms represent different species commonly present in the environment and they are very sensitive to harmful substances. Moreover bacteria are potentially very sensitive organisms on action of ionic liquids because of their bactericidal activity. It is very important to examine other bioindicators than those the most often used in ecotoxicology tests (for example *Daphnia magna*) because of the differences of sensitivity between different species found in the environment. The use of selected test organisms may provide new information about the toxicity of analysed compounds.

The results were analysed statistically using t-Student test with significance level $\alpha = 0.05$ to find out if there were any statistically significant differences between measurements and the control. According to the EU Directive (93/67/EEC) all the tested compounds were categorized to one of the defined in Directive classes.

Artoxkit M™

Aquatic ecotoxicity acute test relies on determination of mortality of aquatic crustaceans *Artemia franciscana*. Test was performed according to the ASTM E1440–91 norm. The test was based on the exposure of the test organism to the harmful compound to determine the concentration causing lethal effects (LC – Lethal Concentration) after 24 hours of exposition. Two type of test were performed: preliminary and confirmation test. Pre-test was intended to determine range of concentrations

to confirmation test. Tested concentrations were ranged from 0.01 to 100 mg/dm^3. According to the results of pre-test, the concentrations to confirmation test were selected. To dilution 0.9 % NaCl was used. Based on the results, using log-probit method, the LC_{50} (concentration that cause death of 50 % of test organisms) value was appointed.

Protoxkit F™

Protoxkit F™ is a chronic test that use ciliates *Tetrahymena thermophila* to determinate ecotoxicity of selected compounds (according to the standard protocol Microbiotests Inc. [18]). After 24 hours of incubation of test organisms (in dark, temperature 30°C) with tested compounds, the IC_{50} (inhibitory concentration – concentration that cause inhibition of growth of 50% of test organisms) value was calculated. Two type of test were performed: preliminary and confirmation test. According to the results of pre-test (ranged from 0.01 to 100 mg/dm^3) the concentrations to confirmation test were selected. The assay Protoxkit F™ was based on the measurement of the turnover of food substrate into *Tetrahymena thermophil*a biomass by optical density readings. Reduce of the amount of intake food indicated on inhibition of growth of test organisms.

Microtox®

Test Microtox® is an acute toxicity test that use bioluminescent bacteria. Test was performed according to the European Union norms EN-ISO 11348–3:2007 and DIN 38412–34:1997ASTM E1440–91 norm. In the test, the bacteria *Vibrio fischeri* were used as test organism. The exposure of the bacteria to toxic substances can cause disruption of cell metabolism processes, causing inhibition of luminescence in comparison to the control. The change of the bioluminescence is defined as a measure of toxicity of tested compound. Two type of test were performed. Preliminary test was based on Screening Test protocol and it was intended to determine range of concentrations to confirmation test. The output concentration was 10 mg/dm^3. The serial dilution of tested compound was performed to receive the following concentrations: 10 mg/dm^3, 5 mg/dm^3, 2.5 mg/dm^3, 1.25 mg/dm^3, 0.625 mg/dm^3, 0.313 mg/dm^3, 0.156 mg/dm^3, 0.078 mg/dm^3, 0.039 mg/dm^3. To dilution 0.9% NaCl was used. The confirmation test was based on Whole Effluent Toxicity (WET) protocol in two time intervals: 5 and 15 minutes of exposure and IC_{50} value was determined.

Results

Artoxkit M™

According to the results of the test, the LC_{50} value was determined (Fig. 1). The most toxic compound was [DDA][H_2Cytr] (the lowest LC_{50} value equal 12.29 mg/dm^3), but the standard deviation for this compound was the highest (3.34 mg/dm^3). The least toxic compound toward *Artemia franciscana* was [DDA][NO_2]. The LC_{50} value for this compound was 24.57 mg/dm^3. The concentration that cause lethal effect of 50 % of test organisms for [DDA][NO_3] was 12.90 mg/dm^3.

Protoxkit F™

According to the results of Protoxkit F™ test, the IC_{50} value of tested ionic liquids was determined (Fig. 2). The highest toxicity showed [DDA][NO_2], for which the IC_{50} value was equal 0.35 mg/dm^3. The least toxic compound to *Tetrahymena thermophila* was [DDA][H_2Cytr]. IC_{50} value for this test organism was 5.20 mg/dm^3. Determined IC_{50} value for [DDA][NO_3] was 3.11 mg/dm^3.

Microtox®

According to the results, the IC_{50} value for tested ionic liquids was determined (Fig. 3). The most toxic compound was [DDA][NO_2] both after 5 and 15 minutes of incubation (IC_{50} value equal 0.431 mg/dm^3 and 0.293 mg/dm^3 respectively). The least toxic compound toward bacteria *Vibrio fischeri* was [DDA][H_2Cytr] ($IC_{50,5\ min}$ = 0.841 mg/dm^3; $IC_{50,15\ min}$ = 0.642 mg/dm^3). Determined IC_{50} values for [DDA][NO_3] were 0.443 mg/dm^3 after 5 minutes and 0.331 mg/dm^3 after 15 minutes of exposure.

After a longer incubation time each of the liquid ionic exhibit a higher inhibition of processes (lower IC_{50} value). Inhibiting properties of the ionic liquids [DDA][NO_2] and [DDA][NO_3] were similar, but compound [DDA][H_2Cytr] exhibited significantly lower toxicity against bacteria *Vibrio fischeri*.

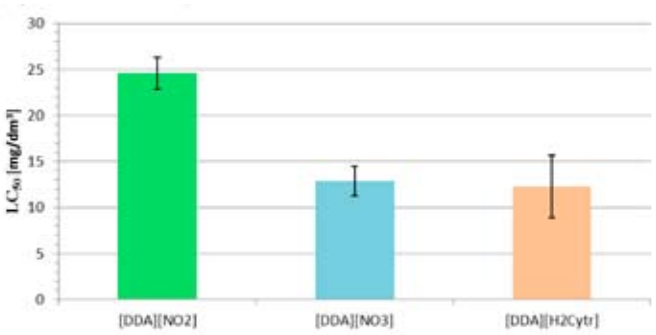

Fig. 1. LC_{50} values of tested QAILs in *Artemia franciscana*

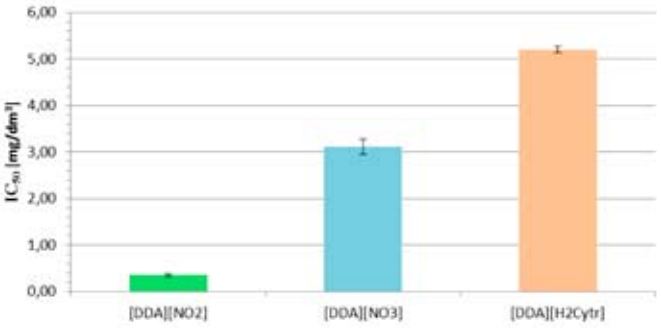

Fig. 2. IC_{50} values of tested QAILs in *Tetrahymena thermophila*

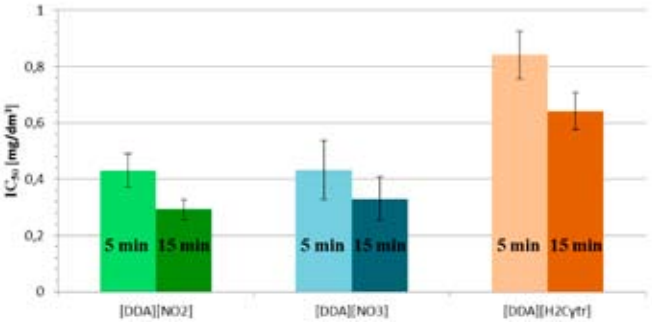

Fig. 3. IC_{50} values of tested QAILs in *Vibrio fischeri*

For every tested ionic liquids it was observed increase of the toxicity with increase of used concentration, which was confirmed by performed statistical analyses (statistically significant differences in comparison to the control was observed).

The results of performed tests are presented in Table 2.

According to the EU Directive (93/67/EEC) all the tested compounds were classified to one of the toxicity classes proposed by European Union. The classification of tested ionic liquids are presented in Table 3.

Table 2

The results of the performed tests

Microbiotest	Duration of the test	LC_{50} or IC_{50} value for selected ionic liquids, mg/dm^3		
		[DDA][NO_2]	[DDA][NO_3]	[DDA][H_2Cytr]
Artoxkit M™	24 h	24.57	12.90	12.29
Protoxkit F™	24 h	0.35	3.11	5.20
Microtox®	5 min	0.431	0.443	0.841
	15 min	0.293	0.331	0.642

Table 3

Classification of tested QAILs according to the European Union Directive (93/67/EEC)

Microbiotest	Test organism	Duration of the test	Toxicity classification		
			[DDA][NO_2]	[DDA][NO_3]	[DDA][H_2Cytr]
Artoxkit M™	*Artemia franciscana*	24 h	harmful	harmful	harmful
Protoxkit F™	*Tetrahymena thermophila*	24 h	very toxic	toxic	toxic
Microtox®	*Vibrio fischeri*	5 min	very toxic	very toxic	very toxic
		15 min	very toxic	very toxic	very toxic

Discussion

In the present study the toxicity of three newly synthesized ionic liquids belong to quaternary ammonium ionic liquids towards aquatic organisms was evaluated. In tests three test organisms were selected: *Artemia franciscana*, *Tetrahymena thermophila* and *Vibrio fischeri*.

According to the presented classification (Tab. 3) all tested compounds were classified as very toxic to aquatic organisms. The most toxic compound was [DDA][NO_2], which was very toxic to bacteria *Vibrio fischeri* and protozoa *Tetrahymena thermophila*.

The increase of the toxicity of ionic liquids after a longer incubation of bacteria *Vibrio fischeri* showed that there were a correlation between exposure time and toxicity. The longer the test organisms had contact with the harmful substances, the lower concentration caused toxic effects. Moreover, tested bacteria were the most sensitive test organisms with terms to analysed quaternary ammonium ionic liquids. It can be caused by the strong bactericidal activity of tested compounds.

Studies carried out by the different authors indicate that the toxicity of ionic liquids is varied and dependent on the structure of the compound and the used test organism. In the case of crustaceans *Daphnia magna* they had a higher sensitivity than tested in this study crustaceans *Artemia franciscana*. In the case of compound [DDA][NO_2] LC_{50} value for *Artemia franciscana* was equal 24.57 mg/dm^3 while for *Daphnia magna* was only 0.105 mg/dm^3 [19]. Such a large difference shows on high resistance of tested crustaceans in Artoxkit M™ test, so the selection of the bioindicators for tests is very important in toxicological studies.

It was found that structure of the cation have large impact on toxicity of ionic liquids. According to the results of this study, anion can also have impact on toxicity of tested compounds. In two tests (Protoxkit F™ and Microtox®) it was observed that if the structure of anion was bigger (higher molar weight), the toxicity of tested compound was lower. Moreover ionic liquid composed of organic anion (dihydrocitrate) in these two tests showed the lowest toxicity. In the case of Artoxkit M™ test this relationship was reversed. It can be causes by the different mechanism of action of ionic liquids against that organism.

Tested compounds are used as fungicides, so the comparison of toxicity of these compounds with commonly used preparations for protection against fungi is very important. The agent used to protect the grain from fungi is PRO-PROCHLORAZ 450 EC, for which the LC_{50} value for *Artemia franciscana* equals 40.7 mg/dm^3 [20]. Comparing literature data with the results of the study, it can be concluded that the analysed ionic liquids are much more toxic to the organisms *Artemia franciscana*. Another frequently used compound for protection against fungi is Thiophanate-methyl 70 WP, for which the IC_{50} value for *Vibrio fischeri* equals 267 mg/dm^3 after 5 and 117 mg/dm^3 after 15 minutes of incubation [21]. Analysed fungicides show a lower toxicity to aquatic organisms than tested ionic liquids.

The results of the study indicated that the analysed ionic liquids are harmful or toxic toward aquatic organisms, however in the environment there are detected only very low levels of quaternary ammonium compounds, in μg/dm^3 [5], that indicates a low risk to the aquatic environment. However the growing interest of ILs can lead to an increase of quantity of ionic liquids discharged into the surface waters, therefore the presence of these compounds in the environment should be monitored.

Knowledge obtained in toxicological tests is very important source of information, which are valid for producers of those compounds. Because of the ease of entry of these compounds into the environment, it is very important issue to determine their effects on organisms representing different trophic levels. The information obtained in the toxicological tests should be taken into account at the design stage of ionic liquids, so they should have certain physical properties, whereas toxicity to aquatic organisms should be minimalized.

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Centrum Nanotechnologii w Gdańsku

26 czerwca br. na Politechnice Gdańskiej otwarto Centrum Nanotechnologii wyposażone w 11 pracowni ze sprzętem służącym głównie do badań właściwości mechanicznych materiałów. W tym samym obiekcie stworzono też Centrum Nauczania Matematyki i Kształcenia na Odległość. Centrum Nanotechnologii jest największą inwestycją Politechniki Gdańskiej ostatnich lat. Wybudowano je kosztem ponad 73,6 mln PLN, z czego 85% stanowiła dotacja Unii Europejskiej, a pozostałe 15% pochodziło z Ministerstwa Nauki i Szkolnictwa Wyższego. *(kk)*

(http://naukawpolsce.pap.pl/, 27.06.2015)

Laboratoria i budynki uczelni mogą być lepiej wykorzystane

Dobra wiadomość dla uczelni, których budynki i laboratoria zostały sfinansowane w latach 2007–2013 ze środków europejskich. Będzie można wykorzystywać je do celów komercyjnych. To oznacza, że uczelnie będą mogły współpracować z firmami, samorządami i NGO'sami w dużo większym zakresie niż dotychczas. Od 2007 r. Polska zainwestowała ponad 30 mld PLN w uczelnianą i instytutową infrastrukturę. Dotychczasowe przepisy w zakresie pomocy publicznej nie zezwalały na wykorzystanie do celów komercyjnych budynków, laboratoriów ani sal wykładowych. MNiSW od dłuższego czasu przekonywało unijnych komisarzy do wyrażenia zgody na zastosowanie nowych przepisów dotyczących pomocy publicznej także do zakończonych już inwestycji. Chodziło przede wszystkim o dopuszczenie komercyjnego wykorzystania infrastruktury badawczej, która powstała z Programu Operacyjnego Infrastruktura i Środowisko. MNiSW wspólnie z Ministerstwem Infrastruktury i Rozwoju opracowują teraz szczegółową procedurę, która pozwoli na komercyjne wykorzystywanie tej infrastruktury. Wśród najważniejszych warunków, które będą musieli spełnić beneficjenci będzie zapewnienie, iż dana infrastruktura nie zmieni swojego podstawowego przeznaczenia, a komercyjne wykorzystanie będzie dotyczyło co najwyżej 20% wydajności badawczej danej infrastruktury. *(kk)*

(http://www.nauka.gov.pl/, 30.06.2015)

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