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Original Research

Toxicity of Imidazolium Chlorides to Aquatic Organisms

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Abstract

The toxicity of 5 new cationic surface active substances (CSAS) – imidazolium chlorides against freshwater organisms – was evaluated. Tests were conducted with the green algae *Scenedesmus quadricauda*, the fish *Lebistes reticulatus* and the crustacean *Daphnia magna*. European Classification was used for the evaluation of adverse effects of investigated preparations. Two of them were classified as very toxic, the others were toxic. For genotoxic effect evaluation *Bacillus subtilis* rec-assay was performed. It was shown that none of the examined surfactants possessed genototoxic properties.

Keywords: cationic surface active substances, toxicity, aquatic organisms, genotoxicity

Introduction

Cationic surface active substances (CSAS) are an important class of pollutants of surface waters. CSAS are frequently used as factors against lumping in artificial fertilizers [1], corrosion protectors [2, 3], and as factors delivering softening effect and static control in washing powders and liquids [2, 4-6]. Frequently they are components in cosmetics [7], pharmaceuticals [8], and antiseptic and sanitary products [5, 9]. CSAS exhibit strong bactericidal and fungicidal activity [1,10-12]. Directive 76/464/EEC [13] includes biocides and their derivatives among dangerous substances. It could be assumed that CSAS are also dangerous substances due to their properties and applications. Assessment of toxicity and biodegradability of new CSAS will help to avoid chemical contamination of water and soil. According to the new regulation project REACH, ecotoxicological research is important for maintaining a competitive and innovative chemical industry in Europe [14].The objective of the authors was to determinate the effects of substituent groups in the imidazole rings on the toxicity of preparations.

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Materials and Methods

Examined Substances

Five imidazolium chlorides were selected for research:
K-1 – 1-benzil-3(hexyloxymethyl)imidazolium chloride,
K-2 – 1-benzil-3-(2-methyl-(heksyloxymethyl)imidazolium chloride,
K-3 – 1-benzil-3-(2-ethylbuthyloxymethyl)imidazolium chloride, substances mentioned above were pure chemical individuals with molecular formulae $C_{17}H_{26}ON_2Cl$ and molecular weight 274; they were straw-coloured liquids.
K-4 – 1,3-di(heksyltiomethyl)imidazolium chloride (molecular weight 329 and molecular formulae $C_{17}H_{33}N_2S_2Cl$),
K-5 – 1-benzil-3-heksyltiomethyl imidazolium chloride (molecular formulae $C_{17}H_{33}N_2SCl$ and molecular weight 289).

Examined substances were readily water-soluble (in examined concentrations). Methods of synthesis have been described in a few earlier papers [15, 16]. All examined substances possessed antimicrobial and antifungal properties [15, 17].

Toxicity Tests

IC_{50}, LC_{50} and EC_{50} values were calculated on the ground of results of toxicity tests with: the algae *Scenedesmus quadricauda*, the fish *Lebistes reticulatus* and the crustaceans *Daphnia magna*. Organisms were obtained from the Institute of Organic Industry in Pszczyna, Poland. Tests were performed according to OECD methods 201, 202 and 203 [18]. Tests were carried out in three parallel series with a minimum of five different concentrations (concentration spacing 1.5).

Algae growth inhibition test was carried out over 72 h at 25°C and continuous illumination of 7000 lux (OECD procedure specifies 8000 lux, but test results were acceptable as the number of algae in the control increased 16 times during 72 hours). Initial concentration of algae was 1×10^4 cells/cm^3. At the end of the experiment $IC_{50}$was calculated at the base of graphical interpolation method outlined in OECD Guideline 201 [18].

An acute toxicity test with fish was performed in 500 cm^3 containers with 10 organisms introduced to each replicate (length of organisms 8-12 mm, before sex dimorphism occurrence). A test was carried out at 22°C, over 96h. LC_{50} was calculated using the log-probit method [19].

Acute toxicity test with crustacean *Daphnia magna* was performed in 20°C, during 48 h with 16h/8h day and night cycle. 10 organisms, younger than 24h, were introduced into each replicate. EC_{50} was calculated with the log-probit method [20].

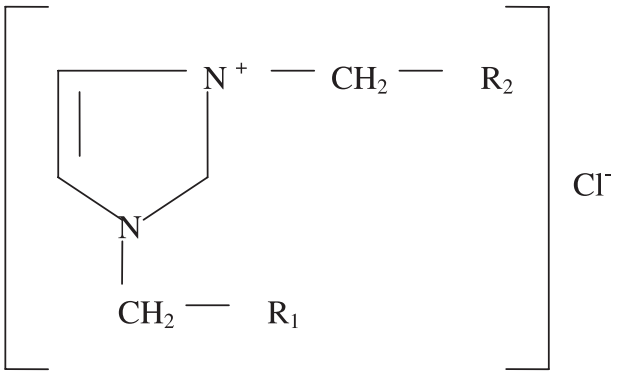

Fig. 1. Structural formula of investigated preparations.

Table 1. Characteristic of investigated preparations in dependence on substituents in imidazole ring.

Preparation/ Substituent	R_1	R_2
K-1	$-C_6H_6$	$-O-C_6H_{13}$
K-2	$-C_6H_6$	$-O-(CH_2)_2-CH(CH_3)-CH_2-CH_3$
K-3	$-C_6H_6$	$-O-CH_2-CH(C_2H_5)-CH_2-CH_3$
K-4	$-SC_6H_{13}$	$-SC_6H_{13}$
K-5	$-C_6H_6$	$-SC_6H_{13}$

Genotoxicity Tests

Genotoxicity tests were carried out according to the method described by Kada et al. [21]. *Bacillus subtilis* strains H17 Rec$^+$ and M45 Rec$^-$ were cultivated during 24 h with B-2 bouillon. After this period microorganisms were transferred to Petri dish with the same nutrient medium and at the centre of the dish the investigated substance was applied. After 48h incubation time (24h in 4°C, and 24h in 37°C) differences between strain growth were measured and estimated as:

(-) not genotoxic properties – difference between strain growth ≤ 2 mm,

(±) potentially genotoxic properties – difference between strain growth > 2mm and ≤ 4 mm,

(+) genotoxic properties – difference between strain growth >4 mm and ≤ 6 mm.

Results and Discussion

Results of algae growth inhibition test are presented in Fig. 2.

The highest toxicity was caused by substances indicated as K-4 and K-5, IC_{50} was 0.21 mg/dm^3 and 0.33 mg/dm^3 respectively. Toxicity of substances K-1 to K-3 was much lower, IC_{50} values ranged from 1.32 to 1.84 mg/

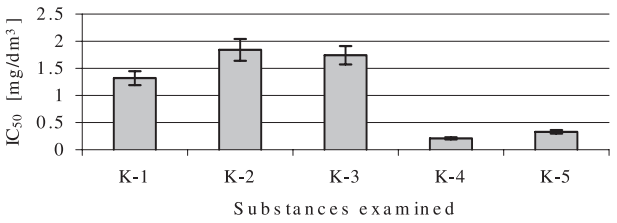

Fig. 2. Results of acute toxicity tests with *Scenedesmus quadricauda.*

Table 2. Statistical analyses of the results of algae growth inhibition test (t-Student test; p>0.95).

	K-1	K-2	K-3	K-4	K-5
K-1		+	+	+	+
K-2			+	+	+
K-3				+	+
K-4					+
K-5					

"+" – important differences, "-" – not important differences.

dm^3. Statistical analysis of IC_{50} values, performed with t-Student's test, showed that differences between preparations were important with a probability of 95% (table 2). Therefore it was concluded that the toxicity of examined imidazolium chlorides was connected with their chemical structure. The strongest algaecide action was shown by K-4, containing 2 atoms of sulphur in the molecule. Replacement of one of the alkyl groups with sulphur by benzyl ring in K-5, or by oxygen in K-1, decreased toxicity of preparations K5 and K-1.

Results of acute toxicity tests with *Lebistes reticulatus* are shown in Fig. 3.

Substances indicated as K-1, K-2 and K-3 exhibited low toxicity in relation to fish. Calculated values LC_{50} approached one another and were within the range 29.4 mg/dm^3 for K-5 to 7.5 mg/dm^3 for the most toxic K-4. Differences between values LC_{50} were statistically irrelevant (Table 3).

An experiment performed with crustacean *Daphnia magna* allowed us to point out that examined substances were more toxic to crustaceans than to fish, as effect concentrations (EC_{50}) ranged from 0.74 to 4.68 mg/dm^3. Differences between EC_{50} values were statistically important (Fig. 4, Table 4). Results obtained in those tests corresponded to literature data [22, 23], where also *Daphnia magna* exhibited a sensitivity to CSAS higher than fish. However, algae appeared to be the most sensitive bioindicator, which had also been observed in earlier works [24].

It is reported that the mechanism of algaecide action of CSAS is connected with the absorption of preparation on a negatively charged cellular membrane and slow diffusion into the interior of the cell [24]. As a result of disturbances in enzymatic processes and ATP production, inhibition of growth and photosyntesis is observed [25, 26].

Despite the kind of tested organism, K-4 containing atom of sulphur in the molecule was more toxic than the other substances. The molecular weight of K-4 was also the highest. It corresponds with literature data where fungicidal properties of CSAS were also increased with the increase of molecular weight [27].

High correlation of toxic effects in different tests was also observed (Fig. 5-7). Differences in sensitivity of bioindicators revealed the necessity of conducting multispecies toxicity studies in experimental ponds, streams and also *in situ* [28, 29].

In order to assess ecological risk, the lowest value IC_{50}/LC_{50}/EC_{50} was taken under consideration [30]. These values were related to the European Union Classification [31].

According to this classification, substances K-1 to K-3 appeared to be toxic and substances K-4 and K-5 very toxic to the aquatic environment.

The majority of drinking waters originate from treated surface waters. Examined substances showed toxic activity against freshwater organisms; therefore, genotoxicity of these substances was also examined with *Bacillus subtilis rec-assay.* Within the tested range of concentrations: 0.001–100 mg/dm^3, genotoxic effects were not observed. CSAS were detected in surface waters of Holland and France in concentrations of 5-16 µg/dm^3 and in Germany 0.01-0.03 mg/dm^3 [32, 33]. Therefore, it was decided to not increase the range of concentrations in genotoxicity testing. In a very few earlier works regarding genotoxic properties of quaternary ammonium salts, a lack of mutagenic action was also shown [32, 33]

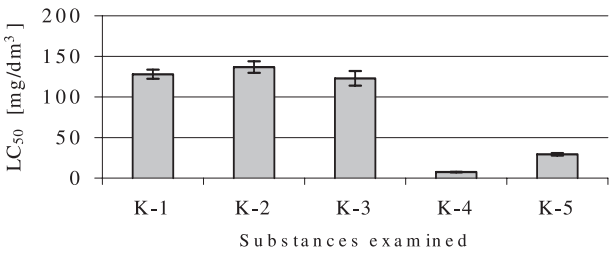

Fig. 3. Toxicity of imidazolium chlorides in relation to fish.

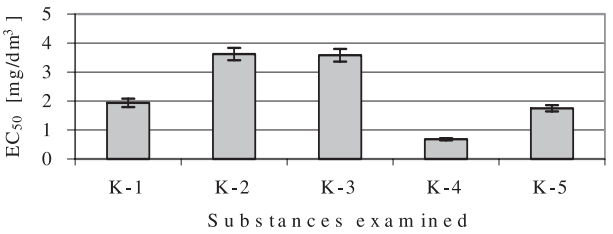

Fig. 4. EC_{50} values for *Daphnia magna.*

Table 3. Results of statistical evaluation of LC_{50} values (t-Students test; p>0.95).

	K-1	K-2	K-3	K-4	K-5
K-1		-	-	+	+
K-2			-	+	+
K-3				+	+
K-4					+
K-5					

"+", "-" – as in Table 2.

Table 4. Results of statistical analyses of EC_{50} values obtained in test with *Daphnia magna* (t-Students test; p>0.95).

	K-1	K-2	K-3	K-4	K-5
K-1		+	+	+	+
K-2			-	+	+
K-3				+	+
K-4					+
K-5					

"+", "-" – as in Table 2

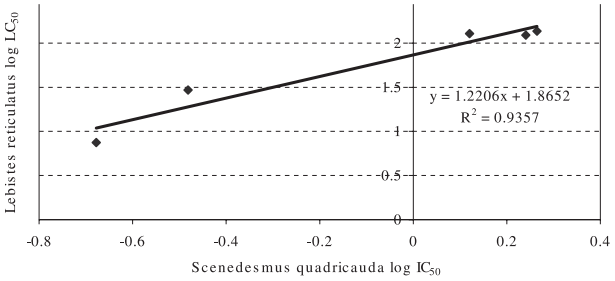

Fig 5 Correlation of results obtained in tests with algae and fish.

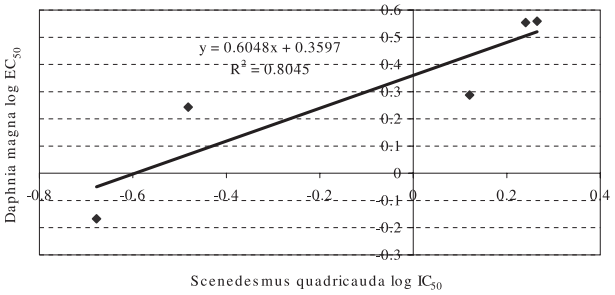

Fig 6. Correlation of results obtained in tests with algae and crustaceans.

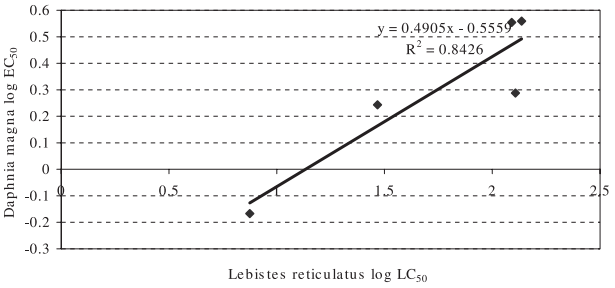

Fig 7. Correlation of results obtained in tests with fish and crustaceans.

Table 5. Classification of chemical substances according to Directive 92/32/EEC [31].

Classification	very toxic	toxic	harmful
Values: $IC_{50}/LC_{50}/EC_{50}$ mg/dm^3	<1	>1-10	>10-100

On the basis of the obtained results and former findings regarding biodegradability of tested substances [34] it could be concluded that preparations K-4 and K-5 could be potentially used in algaecide and fungicide formulations.

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