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*Risk assessment*

# ACUTE TOXICITY ASSESSMENT OF SEVERAL CATIONIC AND AMPHOTERIC SURFACTANTS ON AQUATIC ORGANISMS

ST. GHEORGHE*, I. LUCACIU, R. GRUMAZ, C. STOICA

*National Research and Development Institute for Industrial Ecology – INCD ECOIND, 90–92 Panduri Avenue, 050 663 Bucharest-5, Romania*
*E-mail*: *ecoind@incdecoind.ro*

**Abstract**. Due to their favourable physicochemical properties, the surfactants are the essential ingredients in the most household laundry products and industrial cleaners as well as in personal care and cosmetic products. After utilisation, they are mainly discharged into the environment compartments by the wastewater pathway. In accordance with the Europeans norms concerning surfactants and chemicals and OECD/ISO/ASTM testing methodology, the present work intended to assess the aquatic acute toxicity of 2 surfactants raw materials used frequently in detergent products (cationic – dialkyl hydroxyethyl ammonium methasulphate, and amphoteric – cocoamidopropyl betaine). The tests were performed on different aquatic organisms as: fish (*Cyprinus carpio* sp.), crustacean (*Daphnia magna* sp.), marine luminescent bacteria (*Vibrio fischeri* sp.), gram-negative and positive bacteria (11 species – MARA test) and algae (*Selenastrum capricornutum* sp.). The results of research were the LC50/EC50/NOEC and LOEC values and the toxicity effects correlation for the cationic and amphoteric surfactants, in these classes of aquatic organisms. The testing procedure has followed the biological parameters: mortality, behaviour and physiological modifications (in case of fish); immobilisation and reproduction inhibition (at crustacean); inhibition and stimulation (in case of bacteria). This research represents one part of a complex research concerning the risk assessment of cationic and amphoteric surfactants on aquatic organisms.

*Keywords*: environment protection, cationic and amphoteric surfactants, acute toxicity, aquatic organisms.

## AIMS AND BACKGROUND

Surfactants industry is a competitive industry, with a large opening to innovation and economical development. Although very good for sanitation, the significant use of domestic and industrial detergents contribute to surfactants concentrations increase in municipal sewage and implicit to surface water contamination. The freshwater and marine ecosystems are most affected by this pollution because the aquatic organisms are very sensitive to physicochemical variations of their environment.

* For correspondence.

At the European level, detergents and cleaning products have a especial place in the legislative framework of the European Community, because they are manufactured in big amounts and may affect the environment through discharges in environment: water, air, soil and especially by their use, through wastewater discharges with and without treatment in specific installations. The most important European legislative regulation concerning detergents and cleaning products is European Regulation No CE 648/2004 amended through Regulation No CE 907/2006 which establishes strict rules to insure the free circulation of detergents on the EU market, so that the human health and environmental protection be guaranteed at high level. Another legislative program at international level, equally important[1,2] is Regulation No 1907/2006/CE (REACH), which assures a high level of human health and environmental protection as well as the free circulation of the qualitative and environmental friendly chemicals on the national and international market and the increase of innovation and competitiveness[3,4].

The paper will confer special attention to cationic surfactants, frequently use in laundry and dish detergent and balm manufacture, as well as amphoteric surfactants used in personal care products (hair shampoo and balm, liquid soap and cleaning lotion) and for industrial cleaning agents, because scientific literature is relatively poor concerning ecotoxicological characterisation of this type of surfactants[5–7].

The need for toxicity studies comes especially as a result of increase of consumer use of cleaning products based on cationic and amphoteric surfactants designed to combine good foaming properties with low skin irritancy. The basis for evaluating the environmental compatibility of the surfactant products is knowledge of the ecological properties of their raw materials. Biodegradability and the ecotoxicological properties of individual ingredients is part of ecological safety assessment[8].

The main criterion for the environmental protection against chemicals is the ecotoxicological behaviour (assessment of toxicity on aquatic organisms) and because it is impossible to evaluate all potential effects, researchers have agreed with toxicity evaluation on distinct aquatic food chain organisms, starting with plants, bacteria, to up waterfleas and fish[8].

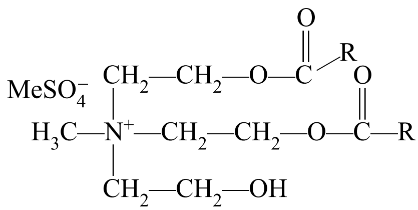
In accordance with the Europeans norms concerning surfactants and chemicals and OECD/ ISO/ ASTM testing methodology[9,10], the present work proposes to assess the aquatic acute toxicity (on fish, crustacean, algae and bacteria) of 2 surfactant raw materials used frequently in detergent products and a cationic standard.

## EXPERIMENTAL

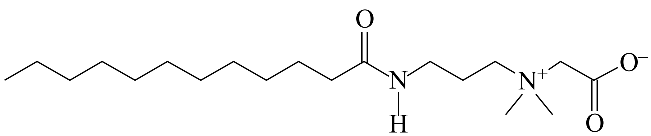
### CHEMICALS

*Two detergent raw materials*. Cationic surfactant – CAS: 93334-15-7, dialkyl hydroxyethyl ammonium methasulphate (TEAQ), triethanolamine C16-C18, commercial name Tetranyl AT-7590, active substance 1.017 meq/g, and amphoteric surfactant – CAS: 4292-10-8, IUPAC: 2-[3-(dodecanoylamino)propyl-imethylazaniumyl] acetate (laurylamidopropyl betaine/cocoamidopropil betaine – CAPB), $C_{19}H_{38}N_2O_3$, commercial name Amfodac LB, active substance 34.6%.

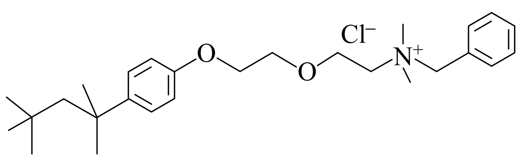
*Standard cationic surfactant.* CAS: 121-54-0, IUPAC:-N-benzyl-N, N-dimethyl-2-{2-[4-(2,4,4-trimethylpentan-2-yl)phenoxy]ethoxy}ethanaminium chloride (benzenthonium chloride monohydrate), $C_{27}H_{42}ClNO_2$,commercial name Hyamine 1622, active substance >96%, Fluka (53752).



TEAQ



CAPB



Hyamine 1622

METHODOLOGY

The acute lethal toxicity was assessed in experiments in static and semi-static conditions with distinct organisms from food chain, as followed:

• Acute lethal toxicity test with freshwater fish (1-year juvenil carp – *Cyprinus carpio* sp.) performed for determination of the mean lethal concentration (LC) which induces the death of half from the test organisms (fish) – $LC_{50}$, according with SR 13216:1994. The aquatic organisms – fish – are exposed to testing chemicals, in different concentrations (0.5–100 mg/l surfactant), for 96 h. The effect (mortality) is registered at every 24 h and the concentration which kills 50% of fish is calculated at the final of test period (Fig. 1).

**Fig. 1**. Test vessels for acute toxicity test with fish

• Acute toxicity test with waterfleas *Daphnia magna status* (Cladocera crustacea) performed for determination of effective concentration ($EC_{50}$) which have a 50% impact on test organisms using Daphtoxkit F™ magna microbiotest containing all the materials (including the Daphnia magna sp. ephippia batch No DM080609) necessary to perfom the test. The tests are performed in accordance with test procedures normed by national and national organisations – OECD 202 (similar to European method C.2. Annex Part C at Regulation No EC 2008/440). The 24- to 48-hour $EC_{50}$ bioassays was performed in disposable multiwell test plates starting from neonates of *Daphnia magna*, uniform in size and age, hatched from ephippia and exposed to different concentration of surfactant (0.05–50 mg/l) at 20°C, in darkness.

• Green algae growth inhibition test performed for determination of inhibitory/stimulatory concentration ($EC_{50}$) with 50% effect on algae – *Selenastrum capricornutum* (*Raphidocelis subcapitata* or *Pseudokirchneriella subcapitata*) in accordance with OECD 201 method and International Guideline ISO/DIS 8692. This toxicity test was performed with Algaltoxkit F™ microbiotest which supposes the mesurement of the algal growth (at 670 nm) in the long cells (Fig. 2) after 24-, 48- and 72-hour incubation (23°C) and calculation of inhibitory concentration in the test concentrations (0.05–10 mg/l surfactant) versus the growth in the control.

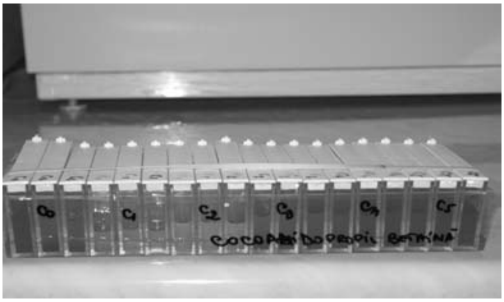


**Fig. 2**. Test long cells for acute toxicity test with algae

• Acute toxicity test with bacteria to estimate the toxic effect of test chemicals on luminescent bacteria *Vibrio fischeri* sp, using the 'BioFix Lumi' equipment (Fig. 3) which respects criteria of DIN EN ISO 11348-3 method. The principle of method is: marine bacteria release luminescence as a metabolic product which can be affected by chemicals. With the help of 'BioFix Lumi' system was measured the light intensity produced by bacteria, before and after 15 or 30 min of incubation, in the presence of pollutant and against the control. The intensity difference between sample and control was associated with the effect of pollutants on microorganisms: inhibition or stimulation. The test concentrations of cationic surfactants were in the interval 0.05–10 mg/l and for amphoteric surfactant 3–80 mg/l.

**Fig. 3**. BioFix Lumi system

• Microbial assay for risk assessment (MARA) test – a multi-species toxicity test based on responses of 11 microorganisms (prokaryote and eukaryote bacteria) to toxic compounds. The microbial growth is determined by a redox dye reduction which induces insoluble reaction products (red) which precipitate and form a pellet in the plate. The plate is scanned and the image is analysed by MARA software for toxicity determination. The test was performed for 0.021–5 mg/l cationic standard solutions and 0.041–10 mg/l cationic raw material solutions

The analytic control of the test solutions was making through spectrometric methods: DIN/EN 38409/1989-07 (for cationic surfactants) and Orange II method – Boiteux 1984 (for amphoteric surfactants).

The conclusion of tests is drawn in accordance with international regulations EPA (Ref. 4) and national legislative program (H.G. 1408/2008) (Ref. 11), as followed: highly toxic – $LC_{50} < 1$ mg/l; toxic –1 mg/l $< LC_{50} \leq 10$ mg/l; harmful/hazardous for aquatic environment – 10 mg/l $< LC_{50} \leq 100$ mg/l; very low toxic, non-toxic – $LC_{50} > 100$ mg/l.

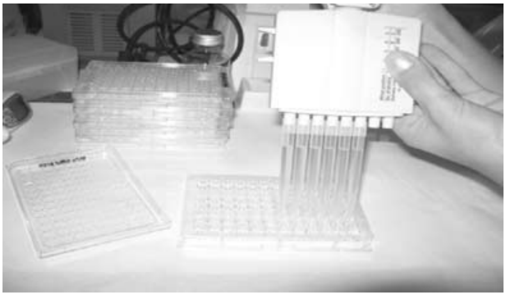


**Fig. 4**. Test plate for microbial toxicity test (MARA)

## RESULTS AND DISCUSSION

In Table 1 and Fig. 5 are presented the $LC_{50}$(96 h) values in static and semi-static tests and toxicity evaluation according to national and international norms. The estimated toxicity values obtained both after static and semi-static tests are comparable or identical, which means that chemicals (cationic and amphoteric surfactants) are stable and remain in tests solutions for the experimental period.

**Table 1**. $LC_{50}$(96h) values in static and semi-static tests

| Test compound | $LC_{50}$ (96 h) | | Toxicity according to national and international norms |
|---|---|---|---|
| | static test | semi-static test | |
| *Cyprinus carpio* sp. | | | |
| Cationic standard (Hyamine 1622) | 4.57 mg/l<br>'–' 1.94 mg/l<br>'+' 9.77 mg/l | 4.57 mg/l<br>'–' 3.31 mg/l<br>'+' 5.62 mg/l | Toxic:<br>1 mg/l < $LC_{50}$ ≤ 10 mg/l<br>risk phrases R51 and R53 |
| Cationic raw material (Tetranyl) | 22.38 mg/l<br>'–' 11.22 mg/l<br>'+' 43.65 mg/l | 22.90mg/l<br>'–'12.58 mg/l<br>'+' 38.90 mg/l | harmful/hazardous for aquatic environment 10 mg/l < $LC_{50}$ ≤ 100 mg/l<br>risk phrases R51 and R53 |
| Amphoteric raw material (Amfodac) | 5.12 mg/l<br>'–' 2.81 mg/l<br>'+' 11.74 mg/l | 6.16 mg/l<br>'–'3.38 mg/l<br>'+' 8.91 mg/l | Toxic:<br>1 mg/l < $LC_{50}$ ≤ 10 mg/l<br>risk phrases R51 and R53 |

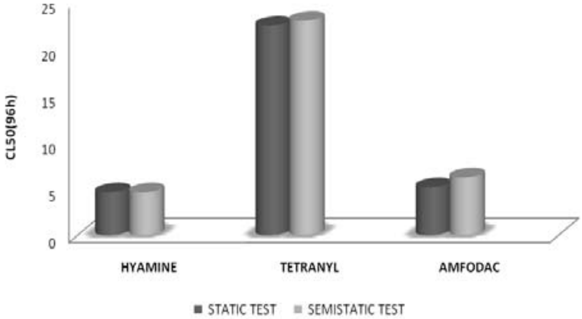



**Fig. 5**. Graphic of static and semi-static tests toxicity values for fish

From the comparative analyses of acute lethal toxicity obtained for cationic surfactants we observed that the standard – Hyamine 1622, had a high toxic effect on organisms and the cationic raw material (Tetranyl), having a smaller content in surfactant active substance and supplementary compounds (KOH, volatile matter, dyes and perfumes), had a harmful impact to aquatic organisms but did not exceed standard surfactant hazard area. From the point of view of visual inspection of physiological aspect of aquatic organisms and their behaviour in toxicity tests we have observed that the fish lose the equilibrium and were inactive to stimulus, without physiological changes of extern organs, concerning the cationic surfactant intoxications. In case of water fleas we have observed immobilisation and lent swimming. The final results are centralised in Table 2 and Fig. 6.

**Table 2**. Toxicity data obtained for test surfactants

| Test organisms | Hyamine (cationic standard) | | | Tetranyl (cationic raw material) | | | Amfodac (amphoteric raw material) | | |
|---|---|---|---|---|---|---|---|---|---|
| | $LC_{50}$ /$EC_{50}$ (mg/l) | NOEC (mg/l) | LOEC (mg/l) | $LC_{50}$ /$EC_{50}$ (mg/l) | NOEC (mg/l) | LOEC (mg/l) | $LC_{50}$ /$EC_{50}$ (mg/l) | NOEC (mg/l) | LOEC (mg/l) |
| *Cyprinus carpio* | 4.57 | 0.5 | 1 | 22.90 | 2 | 7 | 6.16 | 1 | 2 |
| *Daphnia magna* | 0.39 | 0.05 | 0.1 | 4.78 | 0.05 | 0.1 | 9.54 | 1 | 5 |
| *Selenastrum capricornutum* | 0.56 | 0.05 | 0.1 | 3.48 | 0.05 | 0.1 | 5.55 | 0.1 | 0.5 |
| *Vibrio fischeri* | 1.2 | 0.3 | – | 2.89 | 0.4 | – | >100 | 0.4 | – |
| Microbial toxicity (MARA) | 1.1 | – | 0.021 | 1.6 | – | 0.041 | – | – | – |
| Toxicity | highly toxic / toxic | | | toxic / harmful/hazardous | | | non-toxic for *Vibrio fischeri* sp. / toxic for fish, daphnia and algae | | |

Note: $LC_{50}$ – lethal concentration with 50% effect on fish; $EC_{50}$ – lethal or inhibitory effective concentration with 50% effect on crustacean, algae or bacteria; NOEC – no observed effect concentration; LOEC – lowest observed effect concentration.

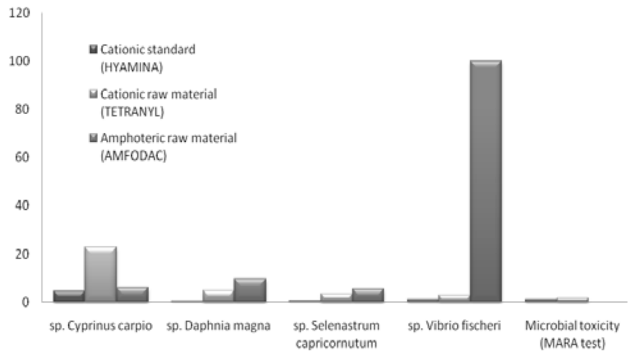



**Fig. 6**. Comparative graph of toxicity values obtained for fish, crustacean, algae and bacteria

Literature data concerning the acute toxicity of cationic surfactants on fish are relatively poor and the existent specify $LC_{50}$ values between 1–42 mg/l and NOEC values (no observed effect concentration) 0.02–0.23 mg/l (Refs 12–14). Comparative with these data the $LC_{50}$ (96 h) experimental values obtained in case of cationic standard (Hyamine 1622) and cationic raw material (Teranyl) were within specified literature data.

In case of cationic surfactants toxicity on *Daphnia magna*, literature data specify $EC_{50}$ (48 h) values between 0.1–45 mg/l (Refs 12–14). Our experimental data referring to acute toxicity of cationic surfactants on crustacean can be comparable to international scientifical data, we have obtained $EC_{50}$ (48 h) values about 0.39 mg/l in case of Hyamine 1622 and 4.78 mg/l in case of cationic raw material. We have obtained a $EC_{50}$ (48 h) value about 9.54 mg/l for amphoteric raw material (Amfodac LB) – the studied scientifical literature has showed toxicity values between 0.22–>100 mg/l in class of amphoteric surfactants[12,15].

Refer to algae, scientifical data mentioned for cationic surfactants toxicity an $EC_{50}$ (72 h) values interval between 0.06–3 mg/l (Refs 12–14). Our experimental data for acute toxicity of studied cationic surfactants were within range of specified values. We have obtained $EC_{50}$ (72 h) about 0.56 mg/l in case of Hyamine 1622 and 3.48 mg/l in case of Tetranyl. For amphoteric raw material (Amfodac) the toxicity data on algae are reduced, we have obtained $EC_{50}$ (72 h) values of about 5.55 mg/l but we did not identified a rough concentrations in studied literature. Scientific literature specified that some algal species were very sensitive to all detergents used, while others were tolerant to the same detergents and sensitive to others. It was also observed that a third group of algae was resistant to high doses of different detergents. These could be considered as detergent-tolerant species regarding as biological indicators of pollution[16]. In accordance with this research studies we consider that the *Selenastrum capricornutum* sp. is a sensitive species.

Concerning *Vibrio fisheri* sp., we have obtained toxic values (in case of cationic surfactants $EC_{50}$ (30 min) about 1.2 mg/l Hyamine 1622 and 2.89 mg/l

Tetranyl) and non toxic in case of amphoteric raw material ($EC_{50}$ (30 min) >100 mg/l Amfodac).

MARA toxicity test has showed that cationic standard surfactant inhibits the microbial growth of gram-positive and negative bacteria (Figs 7 and 8), while the cationic raw material has showed a selectively low toxicity (Figs 9 and 10). The toxic effect of cationic surfactants on bacteria is associated with biocide activity for this type of surfactants[16].

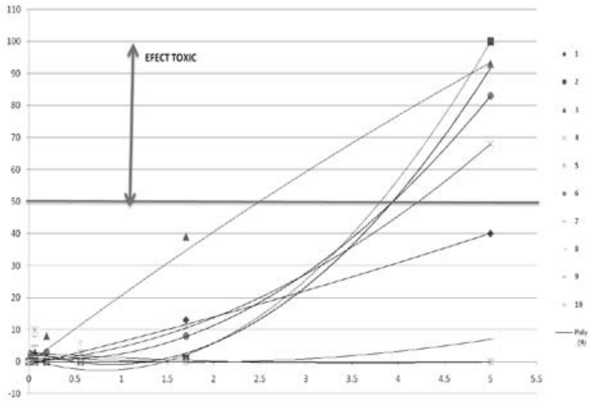



**Fig. 7**. Toxic effect of cationic standard (Hyamine) on the 11 bacteria strains (MARA test)

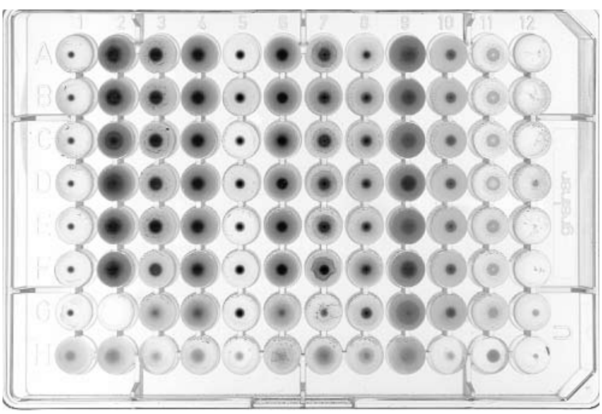


**Fig. 8**. Toxicity MARA plate for cationic standard (Hyamine 1622)

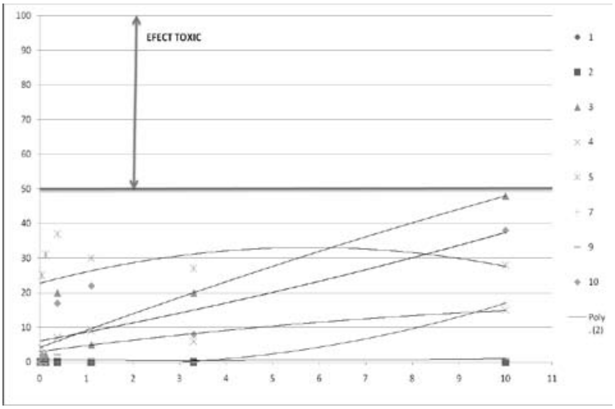



**Fig. 9**. Toxic effect of cationic raw material (Tetranyl) on the 11 bacteria strains (MARA test)

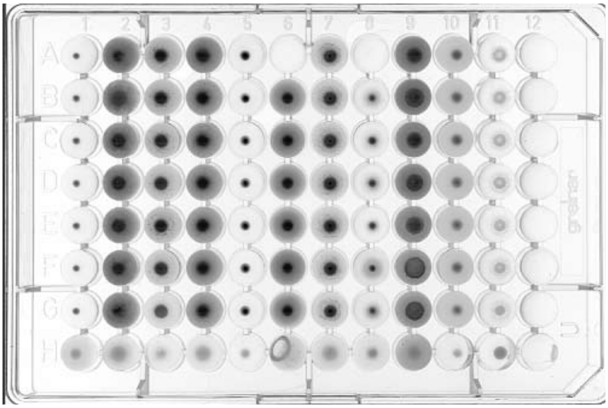


**Fig. 10**. Toxicity MARA plate for raw cationic material (Tetranyl)

## CONCLUSIONS

The performed toxicity tests have showed the followed conclusions:

– cationic standard (Hyamine 1622) has a highly toxic or moderately toxic impact for the all test aquatic organisms. The most affected organisms are crustacean (represented by *Daphnia magna* sp.) and algae (represented by *Selnastrum capricornutum* sp.) for which we have obtained $EC_{50} \leq 1$ mg/l – highly toxic or very toxic for aquatic organisms, with possibility to raise adverse effects for long-term on aquatic environment (risk phrases R50 and R53). In case of fish and bacteria $LC_{50}$/ $EC_{50}$ values were within 1 mg/l – ≤ 10 mg/l – toxic for aquatic organisms, with possibility to raise adverse effects for long-term on aquatic environment (risk phrases R51 and R53);

– cationic raw material (Tetranyl AT-7590) has a toxic or harmful effect for all test aquatic organisms, with a pronounced effect on luminescent bacteria *Vibrio fischeri* sp. The $EC_{50}$ obtained for bacteria, crustacean and algae was within 1 mg/l – ≤ 10 mg/l – toxic for aquatic organisms, with possibility to raise adverse effects for long-term on aquatic environment (risk phrases R51 and R53). Concerning the cationic surfactant toxicity on fish the $LC_{50}$ value was within 10 mg/l – ≤ 100 mg/l – harmful for aquatic organisms, with possibility to raise adverse effects for long term on aquatic environment (risk phrases R52 and R53);

– amphoteric raw material (AMFODAC LB) has a toxic effect on fish, crustacean and algae, the $LC_{50}$/ $EC_{50}$ obtained values were within 1 mg/l – ≤ 10mg/l – toxic for aquatic organisms, with possibility to raise adverse effects on long-term on aquatic environment (risk phrases R51 and R53). In case of luminescent bacteria *Vibrio fisheri* sp. this surfactant has non toxic effect – $EC_{50}$ >100 mg/l.

The future researches will be focused on ecotoxicological assessment of effluents resulted from biodegradability tests of surfactants (especially in case of cationic and amphoteric surfactants) to obtain new information concerning the recalcitrant metabolites and to assess the complementary environmental risk of these compounds.

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