
BIODEGRADATION - LIFE OF SCIENCE

Edited by **Rolando Chamy**
and **Francisca Rosenkranz**

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Ecotoxicological Behavior of some Cationic and Amphoteric Surfactants (Biodegradation, Toxicity and Risk Assessment)

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Additional information is available at the end of the chapter

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1. Introduction

Detergents industry is a competitive industry, with a large opening to innovation and economical development. Although very good for sanitation, the big domestic and industrial detergents consumption has a significant contribution to surfactants concentrations increase in towns' sewage and implicit to surface water and groundwater contamination [1] (Figure 1). The negative effects manifested by the presence of surfactants in surface water are mostly due to superficial – active proprieties – detergents surfactants characteristic, indifferently of class type. In accordance with molecule charge, the surfactants are grouped in four categories: anionic, cationic, nonionic and amphoteric [2].

This chapter is focus on cationic and amphoteric surfactants frequently used in laundry and dishes detergents, fabric softeners, personal care products and biocides. Cationic and amphoteric surfactants control was not required until 2004, when the European Detergents Regulation no. 648 entered into force, especially because there were no standard methods for quantitative determination of these types of surfactants [3]. Also, the biodegradation assessing was not requested and there is no European standard method for this testing. These surfactants are not currently limited by national or international norms relating to waste waters and surface waters quality. Literature references concerning ecotoxicological characteristics and risk assessment of cationic and amphoteric surfactants are relatively reduced.

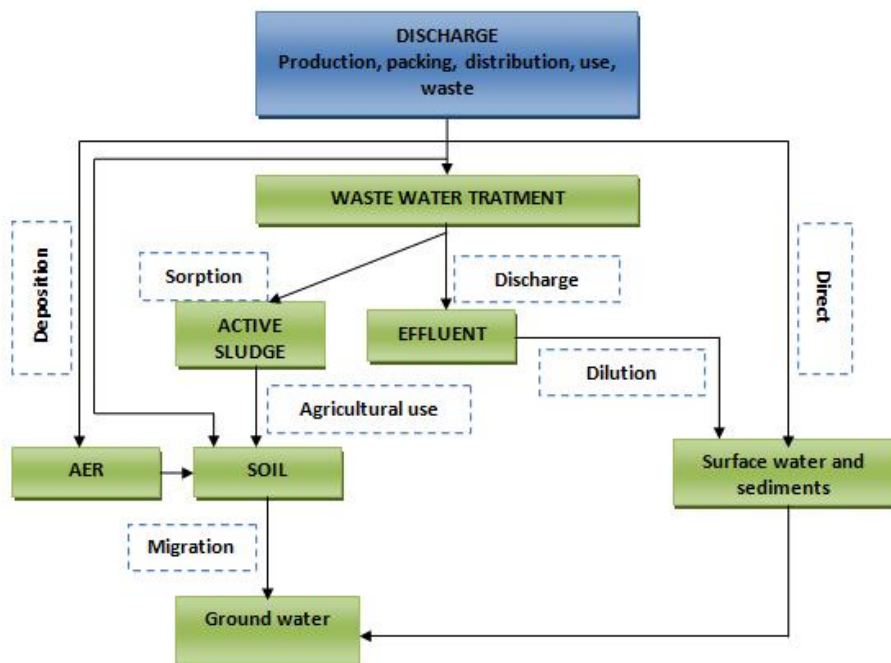


Figure 1. Surfactants environmental contamination [1]

2. Detergents legislative framework

At European level, detergents and cleaning products have a special place in legislative framework of European Community because are manufactured in big quantity and they may affect the environment during both manufacture and using processes. In the last years surfactants biodegradability was the most significant problem.

The chapter broaches a new and important actuality theme at international level, namely *the implementation of the most important European legislative regulations concerning detergents and cleaning products* – Regulation (EC) no. 648/2004 and its amendments. The present Regulation establishes strict rules to assure the free circulation of detergents – products for consumers and industrial and institutional products and implicit of surfactants on the UE market, so that the human health and environmental protection to be guaranteed at a high level. A significant request of Regulation is that each producer/ importer / distributor to attest *ultimate aerobic biodegradability of surfactants used in detergents* [3].

In 2006 have become applicable Regulation (EC) no. 907/2006 – through that is follow the assurance of a environmental higher level protection(impose for detergents (a) *biodegradability* and (b) *conformity with at least one ultimate biodegradability tests* specified in Annex III) and human health (impose requests concerning the information's which must be written on the detergents packages) [4].The last important amendment of Detergents Regulation, is the norm (EU) no. 259/2012 which standardized the use of phosphates and other phosphorous compounds in household laundry detergents and automatic dishwashing for consumers.

Also at European level is applicable the Technical Guidance for stratified approach of Regulation (EC) no. 648/2004, emitted in 2005, which provide that the use of surfactants in detergents is allowable unless that surfactants fulfills the aerobe degradation criteria even if are subject to direct testing as individual substance (mineralization) or through interpolation. For the surfactants which not success to pass one between these mineralization tests, but which respect the primary biodegradability criteria may request a derogation for its utilization in industrial and institutional detergents. These derogations are obtained, in base of environment safety concerning the assessments for the metabolites which may result at the surfactant biodegradation. All assessments will be stratified performed (Figure 2), in accordance with a phased process which will provide all the information's concerning the environmental risks of the recalcitrant metabolites resulted after biodegradation. For passing the complementary risk assessment it is necessary to show that the PEC does not surpass the PNEC of the metabolites [5].

Environmental European legislation showed that only anionic and non-ionic surfactants have set limit values, while the cationic and amphoteric surfactants have not imposed limits in waste waters or surface water, even though they have a frequent use in cleaning products and biocides.

At international level exist some actions to encourage the producers to obtain safe cleaning products, transposed in Regulation (EC) no. 66/2010 concerning UE ecological label. The ecological labeling of products is facultative and promotes the security of detergents on the entire life cycle: from the raw materials, production process, packing, distribution, use, recycling and elimination. Through ecological labeling is trying the reduction of hazardous chemicals use, with effects on water, air and soil and of carcinogenic and allergic risks. The detergents with the European Ecolabel contain no hazardous substances to the aquatic environment; have a increased biodegradability, and an efficient use that does not cause damage to the environment [6].

Beginning with February 2009, the most representative European associations (AISE, CESIO, CEFIC) have informed about their initiative to undertake further researches in order to: establish the surfactants ecotoxicity and assess the potential environmental risk; develop an improved method for measuring the anaerobic biodegradability under sludge digester conditions; and to evaluate the biodegradation of the main organic non-surfactant ingredients from detergents [7, 8].

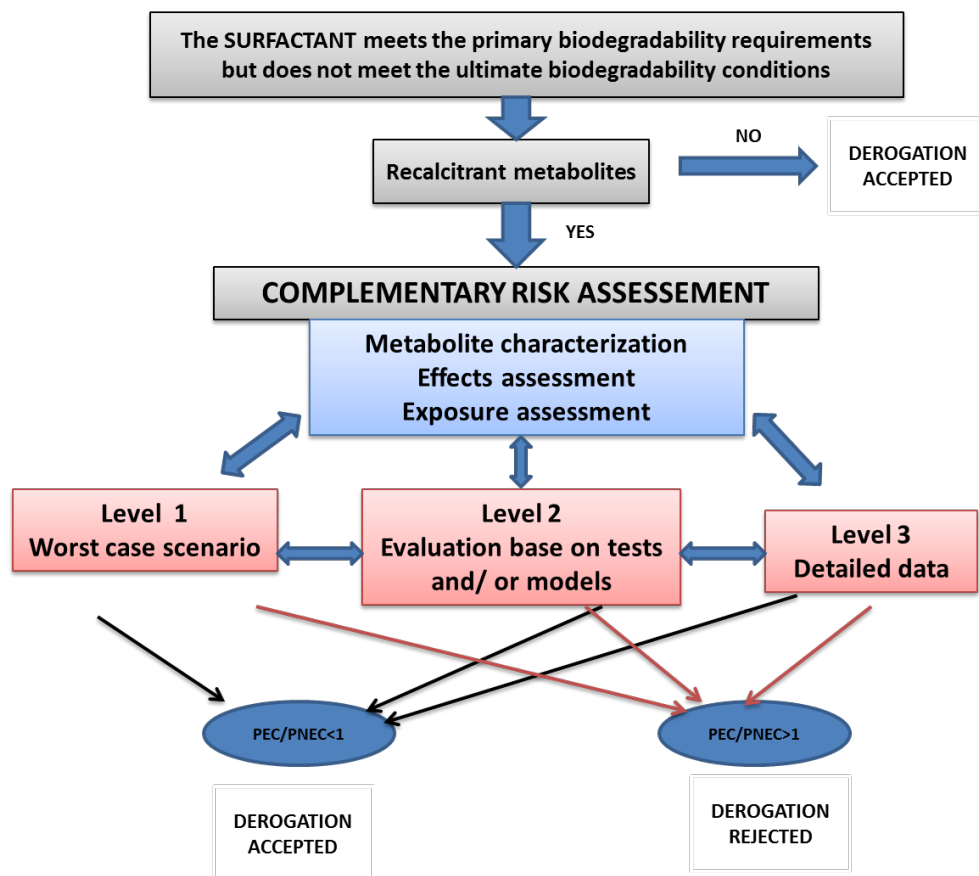


Figure 2. Complementary risk assessment, adapted from CESIO and AISE

3. Surfactants ecotoxicity

In literature there are many studies to evaluate the ecotoxicity of anionic and non-ionic surfactants, and therefore future research should be directed especially to elucidate the toxic effects of cationic and amphoteric surfactants whose ecotoxicological profile is unknown, and their physical and chemical properties can significantly interfere in the results of the toxicological studies.

According to CESIO reports, half of detergents consumption has been used in domestic applications and other half in cosmetic industry, metal processing, paper and leather industry. In 2007 the most used surfactants were anionic and non-ionic surfactants covering half of

produced surfactants [9]. In 2008 it was estimated that in Eastern Europe were used annually > 4.2 million tons of detergents and 1.2 million tons of softeners, up to 2006 [10].

It was found that during 1990-2010, in the international waste waters were identified following surfactants concentrations: anionic 330 - 9450 µg/L; nonionic 5 - 395 µg/L; cationic 0.1 - 325 µg/L (even 6000 µg/L in hospitals waste waters) [1,10-12]. No data on amphoteric surfactants were identified.

In surface water were estimated following concentrations: anionic <4 - 81 µg/L; nonionic <0.002 - 31 µg/L; amphoteric <0.01 to 3.8 µg/L; cationic <0.1 - 34 µg/L [13, 14].

According to our research studies, in Romania, in the last 10 years, the concentrations of surfactants in waste waters and surface waters were: anionic 0.3-9 mg/L; non ionic 0.05 - 4 mg/L; cationic 0.03 - 0.35 mg/L; amphoteric 0.02 -0.05 mg/L.

According with international regulations, the first criteria in environmental risk assessment of surfactants is to assess their biodegradation. Biological degradation of surfactants could be performed by a several tests which ones decrease in order of stringency as followed: Ultimate / Readily biodegradability tests, Inherent biodegradability tests, Rapidly biodegradability tests and Primary biodegradability tests [15].

The ultimate biodegradability tests are recommended to assess the biodegradation of surfactants, because by using them, we can control whether surfactants are degraded in the presence of microorganisms to the metabolites (non-surfactants), mineral salts, biomass and CO₂ (the measured parameters).

Biodegradability testing methodology is required by Detergents Regulation no. 648/2004 (Annexes no. III and VIII), which provides degradation limits of surfactants used in cleaning products [16]. All data concerning biodegradability, use informations, consumption and current conditions of environmental exposure of the substance, make it possible to *PEC* (Predict Environment Concentration) of the substance.

Legislation, in force requires a primary biodegradability of cationic and amphoteric surfactants greater than 80%. In terms of ultimate biodegradability (Table 1), these compounds are finally degraded under aerobic (>60%-100%) and anaerobic conditions (64-100%). Some problems are highlighted for quaternary cationic surfactants and amphoteric alkyl betaines in both conditions.

Surfactants products have some negative effects on surface waters as: decreasing of air / water oxygen transfer, water quality damage because of foam, sorption on solid particles preventing the sedimentation, reduction of river self-cleaning capacity, affecting the gases transfer between the microorganism cells and have a great toxicity on the aquatic organisms in trophic level.

Toxicological behavior is the second criterion in environmental risk assessment. Detergents show toxic effects for all aquatic organisms if there are present in sufficient amounts and that include biodegradation products. Most fish die when the detergent concentration in water is about 15 mg/L and also, at concentrations above 5 mg/L cause the

death of eggs and affecting the fish reproduction [17]. Another study reported that 0.4 to 40 mg/L of detergents induce toxic effects by damaging the gills, growth delay, alteration of feeding process and the inhibition of the organs chemoreceptors in vertebrates. In case of invertebrates at detergent concentrations below that 0.01 mg/L, the reproduction, growth and development are disturbed [18].

Generally, the toxicity of surfactants is influenced by a range of abiotic and biotic factors. The abiotic factors, eg. physico-chemical properties of water (pH, hardness, other polar substances, dissolved oxygen, suspended matters) lead to a low bioavailability of the compound to aquatic organisms. Also, the physico-chemical properties of the surfactant (the size of aliphatic chain [26, 32], type of surfactant, absorption capacity and concentration) have a great influence of the toxicity level. Biotic factors generally refer to: age of organisms, tested species [33, 34], sensitivity between species [35] and acclimatization at very low concentrations of detergent [10, 18].

As long as, it is practically impossible to perform bioassays on all aquatic food chain, in order to assess the ecotoxicological effects of chemicals (Figure 3), at international level, certain representative aquatic food chain species were established, as follows: microorganisms, algae, crustaceans (benthic and planktonic) and fish. With the REACH Regulation implementation [36], the eco-toxicity tests were diversified by applying of microbiotests [37] as an alternative to conventional methods, in order to reduce or replace animal testing (highlighted in the OECD, ISO and EPA methodology – Table 2).

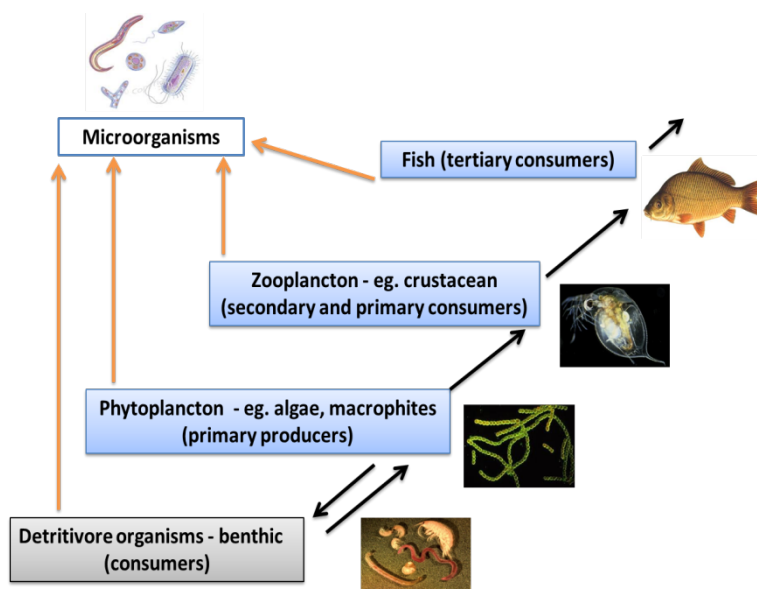


Figure 3. Aquatic food chain

Surfactant type	Biodegradability
CATIONIC SURFACTANTS	
Esterquats (DEEDMAC, HEQ, TEAQ, etc.)	79% (ISO 10708) [1] 80->85% (OECD 301B) [11, 19] 90% (OECD 301F) [20] 75% (OECD 302B) [11] >60% (OECD 301D) [21] 64-100% (ECETOC) [9] 73-100% (ECETOC)) [11, 20]
Diesterquats	92% (OECD 301A) [22] 90% (OECD 301B) [23]
Ammonium quaternary compounds (eg. DSDMAC, DTDMAC, ATMAC)	>5% (OECD 301D) [1] 0 -24%(ECETOC) [9] 40-81% (OECD 301F, 301B, 302A) [24]
Other cationic surfactants (hydrogenated chain)	63% (OECD 301A) [22]
AMPHOTERIC SURFACTANTS	
Alkyl betaines (dimethylaminebetaines / alkyl amidobetaines)	99% (OECD 301A) [22] 0% - >60%(ECETOC) [9] >60%(ThOD) (OECD301D) [25] 60 – 100% (ISO 14593) [26]
Hidroxisulfobetaines	40-47% [25, 27]
Imidazoline derivatives (cocoamphoacetates / alkyl amphoacetates/ alkylamino propionates)	>60% (ECETOC) [9] >60% [25] 80-90% (OECD 301E) [1] 79.8% (ECETOC) [9] 80-100%(ISO 14593) [26] 60-79% (OECD 301D, 301E) [27] 2.5% ThGP (ISO 11734) [27]
Cocamidopropylbetaine / Coco alkyl derivatives	82% (ThOD), 95% (COD) (OECD 301C) [28] 90- 100% [29,30] 97% (OECD 301A) [22] 57 – 84% (ThOD) (OECD 301D) [25] 45 – 75% ThGP (ISO 11734) [27]
Other amphoteric surfactants	97% (DOC) (OECD 303A) [31] 60% (ThCO ₂), 70% (DOC)(OECD 301B) [31]

Table 1. Cationic and amphoteric surfactants biodegradability

Usually, it is recommended to perform the standardized OECD and ISO methodology for assessing of ecotoxicity. The toxicity level of surfactants is assessed using tests batteries covering all trophic level of aquatic environment, and it is recommended the acute and chronic tests in classical or alternative system. Both cationic and amphoteric surfactants cause high or moderate acute toxicity on fish, crustaceans, algae and bacteria. It is noted that the ranges of toxicity values are very large and diversified, even for the same aquatic organism or test method and for this reason the literature is very permissive (Table no.3).

No.	Toxicity tests	OECD	ISO/EPA	Microbiotests
<i>Vertebrates (tertiary consumers)</i>				
1.	Fish acute toxicity test, (static test/ semi static test/ dynamic test)	203	ISO 7346: 1,2,3 ISO 13216 ISO 10229 ISO/CD 15088-1 EPA 2000.0; 2004.0; 2006.0	(Q)SAR and ECOSAR methods Fish cell cytotoxicity tests Genetic tests
2.	Fish, prolonged toxicity test- 14 days study	204	-	Endocrine tests [approved by ICCVAM, ECVAM, OECD, EPA, SETAC, ECETOC in order to reduce / replace the animals used in toxicity tests according to REACH]
3.	Fish juvenile growth tests	215	-	
4.	Fish, early-life stage toxicity test	210	-	
5.	Fish, Short term toxicity test on embryonic stages	212	ISO 12890 EPA 1000.0	
6.	Bioaccumulation in fish	305	-	
7.	Fish sex development test	234	-	
<i>Zooplankton (primary and secondary consumers)</i>				
8.	<i>Daphnia magna</i> , acute immobilization test (static and semi static test)	202	ISO 6341 ISO 14669 EPA 2002.0; 2021.0	Daphtoxkit F, <i>Daphnia</i> IQ Test (<i>Daphnia magna</i> , <i>Daphnia pulex</i>) Thamnotoxkit F (<i>Thamnocephalus</i> <i>platyrus</i>)
9.	<i>Daphnia magna</i> , reproduction test	211	ISO 10706 EPA 1002.0	Rotoxkit F (<i>Brachinous calyciflorus</i>) Rotoxkit M (<i>Brachinous splicatilis</i>)
10.	<i>Daphnia magna</i> , chronic toxicity test	-	ISO 10706 ISO/DIS 20665 ISO/WD 20266	Ceriodaphtoxkit K (<i>Ceriodaphnia dubia</i>) Ostracodtoxkit (<i>Heterocypris incongruens</i>)
<i>Phytoplankton (primary producers)</i>				
11.	Fresh algal growth inhibition test, <i>Pseudokirchnerilla subcapitata</i>	201	ISO 8692 SR 13328 EPA 1003.0	Algaltoxkit F (<i>Raphidocelis subcapitata</i> , <i>Selenastrum capricornutum</i> , <i>Chlorella</i> <i>vulgaris</i>)

No.	Toxicity tests	OECD	ISO/EPA	Microbiotests
<i>Benthic organisms</i>				
12.	Water - Sediment toxicity,	218,	-	Ostracodtokit(<i>Heterocypris incongruens</i>) Microtox (<i>Vibrio fischeri</i>)
	<i>Chironomus riparius</i>	219		
<i>Aquatic floating plants</i>				
13.	Growth inhibition tests, <i>Lemna minor</i>	221	ISO 20079	-
<i>Microorganisms</i>				
14.	Inhibition of oxygen consumption by active sludge	209	ISO 8192	Microtox (<i>Vibrio fischeri</i> , <i>Photobacterium phosphoreum</i>)
15.	Inhibition of nitrification of active sludge microorganisms	-	ISO 9509	Test ECHA (<i>Bacillus stearothermophilis</i>) Toxi-chromotest PAD, MetPAD, MetPLATE, FluoroMetPLATE, SOS –
16.	Bioluminescent bacteria inhibition test, <i>Vibrio fischeri</i>	-	ISO 11348-1,2,3	Chromotest, Toxi-chromotest (<i>Escherichia coli</i>)
17.	Bacteria growth inhibition test, <i>Pseudomonas aeruginosa</i>	-	ISO 10712	Muta- ChromoPlate (<i>Salmonella typhimurium mutant</i>) MARA test (with 11 bacteria sp.)

Table 2. OECD / ISO / EPA and microbiotests methods generally used in UE for aquatic toxicity assessment of chemicals / environmental samples

4. Laboratory experiments

4.1. Chemicals

To assess the ecotoxicity and risk assessment of cationic and amphoteric surfactants, seven compounds were selected:

- *cationic surfactants*: dialkylhydroxyethyl ammonium methasulphate (TEAQ) C16-C18, commercial name TETRANYL AT 7590, CAS: 93334-15-7, 1.017 meq/g, Kao Corporation S.A; Cetylpyridinium bromide, CAS: 140-72-7; benzethonium chloride monohydrate, commercial name HYAMINE 1622, CAS: 121-54-0, >96% (Sigma-Aldrich). tow softeners base on TEA esterquats CAS 91995-81-2 and CAS 157905-74-3;
- *amphoteric surfactants*: laurilamidopropylbetaine / cocamidopropylbetaine – CAPB, commercial name AMFODAC LB, CAS: 4292-10-8, 34.6 %, Sasol Italy S.P.A; and a commercial toilet detergent base on CAPB.

The ecotoxicity experiments were performed for individual surfactants, mixtures of the cationic with amphoteric surfactants and different products base on cationic and amphoteric compounds, in order to obtain a complex response of the surfactants toxicity.

Aquatic toxicity of surfactants
L(E)C50/ NOEC [mg/L]
CATIONIC SURFACTANTS
<i>Esterquats</i> [1, 11, 12, 21, 23, 25, 38 - 47]
Fish: 0.63-42 mg/L / 3.5 mg/L
Crustacean: 0.38 – 45 mg/L / 1 -3 mg/L
Bacteria: 10->130 mg/L / 0.9 -2.7 mg/L
Algae: 0.06 – 11 mg/L / 0.16 – 4.8 mg/L
<i>Ammonium quaternary compounds</i> [1, 10, 25, 48-51]
Fish: 0.62 -4.5 mg/L / 0.58 mg/L
Crustacean: 0.13 – 18 mg/L /0.18 -1.34 mg/L
Bacteria: 0.15- 6.9 mg/L
Algae: 0.05 – 18 mg/L / 0.12 mg/L
<i>Alkyl dimethyl benzyl ammonium chloride</i> [25, 52]
Fish: 0.28-2 mg/L / 0.004 – 0.03 mg/L
Crustacean: 0.004 – 0.006 mg/L
Algae: 0.67 – 1.8 mg/L
<i>Alkyl trimethyl ammonium salts</i> [25, 53 - 56]
Fish: 0.36 - 8.6 mg/L
Crustacean: 0.1 – 24 mg/L /0.43 -0.05 mg/L
Algae: 0.03 – 0.38 mg/L
<i>Other cationic surfactants</i> [51]
Fish: 0.07 – 24 mg/L
Crustacean: 0.07 - > 5 mg/L
AMPHOTERIC SURFACTANTS
<i>Imidazoline derivatives</i> [1]
Fish: 8.1 mg/L
Crustacean: 41 - 520 mg/L
Bacteria: 22 -900 mg/L
<i>Coco alkyl derivatives</i> [10, 25, 26, 30, 31, 57, 58]
Fish: 2 - 31 mg/L / 0.16 – 1.7 mg/L
Crustacean: 2.15 - 48 mg/L / 0.9 -1.6 mg/L
Bacteria: 5.2 - 78 mg/L
Algae: 0.09 – 48 mg/L / 0.09 - 10 mg/L

Table 3. Aquatic toxicity data for cationic and amphoteric surfactants

4.2. Analytical control

The methods used for qualitative and quantitative analytical control of surfactants are spectrometric, titrimetric and chromatographic [16, 59-64].

In our studies, the analytical control of cationic and amphoteric surfactants in the synthetic solutions used in ecotoxicity tests and also in the environmental samples (waste water and surface water) was performed according to the standard methods specified in Annex II of

Detergents Regulation (spectrometric methods: DIN/EN 38409/1989-20 for cationic surfactants and Orange II method - Boiteux 1984 for amphoteric surfactants). The performance parameters are shown in Table 4. According to other scientific studies [2, 65], our results were comparable.

Performance parameters of the methods	DIN 38409:1989, part 20 Cationic surfactants	Boiteux 1984 method, Amphoteric surfactants
	Hyamine 1622 (99.99%)	Cocamidopropylbetaine (34.6%)
Wavelength	628 nm	485 nm
Accuracy	96.8%	99%
Fidelity [CV (RSD)]	6.508 %	3.392 %
Repeatability (r)	0.088 mg/L	0.1128 mg/L
Intern reproducibility (R _i)	0.0115 mg/L	0.5161 mg/L
Calibration curve equation	$x = 6.9108y - 0.0069$	$x = 5.7837y - 0.0925$
Detection limits (LoD)	0.003 mg/L	0.002 mg/L
Quantification limits (LoQ)	0.035 mg/L	0.032 mg/L
Concentrations domain	0.003 - 4 mg/L	0.002 - 2 mg/L
Recuperation	80 % - 110 %	80 % - 110 %
Interferences	Small concentrations of anionic surfactants. This interference may be eliminated through the use of the ions exchange resins column.	Small concentration of cationic and anionic surfactants; this interference could be remove by pH adjustment at alkaline values or the use of the ions exchange resins column.

Table 4. Methods performance parameters for quantitative determination of cationic and amphoteric surfactants

Detection limits of spectrometric methods are 0.003 mg/L for cationic surfactants and 0.002 mg/L for amphoteric surfactants. The methods interferences are determined by the presence of other types of surfactants (anionic, cationic) and/or other organic substances, which react with the surfactant or with the color reagent to form stable compounds. These problems can be eliminated by using of ion exchange resins to separate the target surfactants.

Methods selectivity was ensured by using of standard curves performed for the main studied substances. For the selective detection of cationic compounds in environmental samples is recommended the use of standard HPLC techniques.

5. Biodegradability assessment

A significant request of Detergents Regulation is that each producer / distributor must to attest ultimate aerobic biodegradability of surfactants used in detergents. Our experiments target

was to assess the primary and ultimate biodegradability for 2 surfactant raw materials (cationic - ammonium quaternary compounds and amphoteric - alkyl betaines), their mixture and 2 commercial cleaning products based on this type of surfactant.

For the compliance of the first criterion of aquatic risk assessment (biodegradability), was used OECD methodology specified in Annex III of Detergents Regulation (OECD 303A – similar with ISO 11733) –Simulation Test – Aerobic Sewage Treatment for primary biodegradability [3, 66]; OECD 301A (similar with ISO 7827) - DOC Die – Away Test [67] and OECD 301D (similar with ISO 10707) – Closed Bottle Test [68])

5.1. Primary biodegradability

OECD confirmatory test for primary biodegradability assessment of surfactants describes a small – activated sludge plant in continued flow (Figure 4), consisting in a vessel for synthetic sewage, an aeration vessel, a settling vessel, air-lift pumps to recycle the activated sludge and vessel for collecting the treated effluent. The degradation test was performed at 19-24°C and the duration of experiments was about 60 days. The monitored parameters of experimental equipment were the surfactants concentrations and chemical oxygen demand (COD) in influents and effluents, the content of dry mater in the activated sludge and oxygen concentration from aeration tank vessel.



Figure 4. Laboratory simulation of aerobic sewage treatment

The efficiency of the biodegradation process (COD removal) and the percentage of biodegradability (surfactants degradation) were calculated (Table 5). Surfactants biodegradability was calculated as an arithmetic mean of daily removal efficiency values of surfactants, obtained in effective biodegradation period, during which degradation has been regular and the operation of the equipment trouble-free (Figure 5).

Result type	Tetranyl AT 7590	Hyamine 1622	CAPB	Hyamine 1622 + CAPB	Tetranyl AT 7590 + CAPB	Cetylpyridinium bromide
Test time (days)		36			30	36
Lag time (days)		10			12	10
Effective biodegradation time (days)		26			18	26
COD removal (%)	70 - 89	90	90	61	68	50
Cationic surfactant removal (%)	90	84	-	-	-	77 - 80
Amphoteric surfactant removal (%)	-	-	99	-	-	-
Total surfactants removal (cationic + amphoteric) (%)	-	-	-	80	90	-
Biodegradation (%)	91	84	97	90.7	97.6	80

Table 5. Primary biodegradability of cationic and amphoteric surfactants

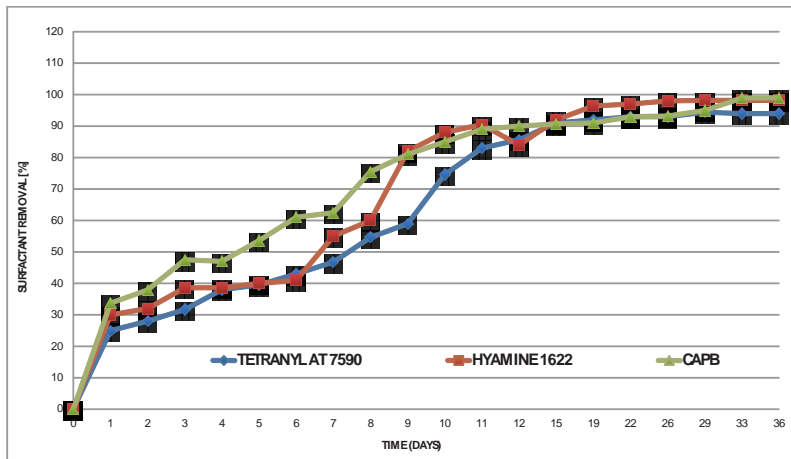


Figure 5. Primary biodegradability - individual surfactants removal

Considering the OECD confirmatory test, the level of primary biodegradability must be at least 80% in 21 days after the biological system initiation, for that the surfactant can be accepted as biodegradable and used as basic compound in commercial products. Our results showed a primary biodegradation > 90% for the cationic surfactant (TETRANYL AT 7590), amphoteric surfactant (cocamidopropylbetaine) and mixtures (cationic + amphoteric), while for the HYAMINE 1622 (cationic standard) and commercial product (cationic biocide - cetylpyridinium bromide) the primary biodegradation is in limit of 80-84%.

According to biodegradability criteria imposed by Regulation 648/2004, all the testing surfactants meet the conditions; the levels of biodegradation obtained were $\geq 80\%$ [69-71]. The results were in line with the literature data on primary biodegradability of cationic and amphoteric surfactants (Table 5) [11, 22, 27].

5.2. Ultimate biodegradability

OECD 301A (ISO 7827) - *DOC Die – Away Test*, allowed the ultimate biodegradability assessment of substances / chemical products, in a given concentration, in a synthetic media, subject to aerobic microorganisms. According to this method, cleaning product – toilet detergent solution based on amphoteric surfactant was tested. The concentrations of DOC and amphoteric surfactant were determined and the percentage DOC / surfactant removal were calculated (Table 6). The obtained remove percentages were graphically represented in Figure 6.

Result type	Toilet detergent based on CAPB
Experimental period (days)	30
Maximum level of biodegradation (%)	91.43 – removal of DOC
Lag time (days)	3
Biodegradation time (days)	20
Amphoteric surfactant removal after 30 days (%)	72.85
Abiotic removal for DOC (%)	14

Table 6. Ultimate biodegradability test results for an amphoteric product

Biodegradability test performed considers that a substance is biodegradable if no significant abiotic removal was observed, the curves shows a typical form with lag and degradation phase and the DOC removal can be attributed to the biodegradation process of the substance. In conclusion, our results considered that:

- The total removal of dissolved organic carbon (DOC %) for the testing product (toilet cleaner based amphoteric surfactant - cocamidopropyl betaine) is $\sim 92\%$, with an abiotic elimination of 14% ;
- Effective biodegradation (the interval between the end of the lag time and the necessary time for the 90% DOC removal) is 20 days;
- Toilet cleaner commercial product base on amphoteric surfactant is biodegradable.

In line with the literature we estimated that 91.43% of ultimate biodegradability obtained for cocamidopropylbetaine is within the range of 57% - 100% specified for the same method or different methods recommended by OECD for ultimate biodegradability testing of amphoteric surfactants (see Table 1).

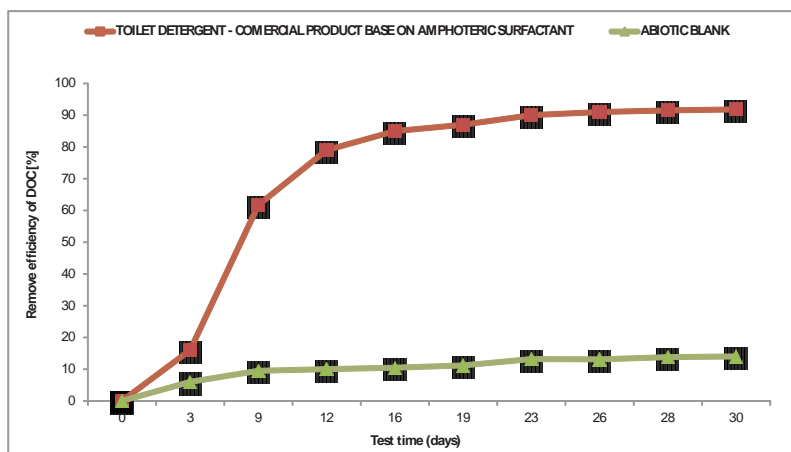


Figure 6. Ultimate biodegradability efficiency chart for the amphoteric product

OECD 301D (ISO 10707) – Closed Bottle Test, allows the ultimate biodegradability assessment of organic compounds present in a given concentration, subject to aerobic microorganisms, through biochemical oxygen demand (BOD) analyze. The test substance solution (which is the single source of carbon and energy), is inoculated with a little number of mixed aerobic microorganisms, incubated at dark, in closed and well filled recipients. The biodegradability indicator is dissolved oxygen concentration, parameter that is measured at regular intervals in a standard 28-days period. At the end of each time interval was calculated the oxygen removal, as a difference between the oxygen concentration of test substance and the control (Table 7). A biodegradability curve with the testing time on abscises and the biodegradability mean percentages on ordinate were plotted, for each time moment (Figure 7).

According with OECD 301D methodologies, an organic compound is biodegradable, when the biodegradation percentage is $\geq 60\%$, after 28 days of testing.

The experimental results obtained, have shown that the studied cationic surfactants and the cleaning product – laundry softeners are finally biodegraded with $>70\%$. Considering other biodegradability studies for similar cationic compounds, our experimental results for TET-RANYL AT 7590 (78%) and fabric softeners based on it (77%, 85%) can be compared with the ultimate biodegradability values of esterquat and diesterquat cationic compounds, ranging from $>60\%$ - 79% using the same method and 75% - 92% using other OECD methods.

Regarding the ultimate biodegradability for the benzethonium chloride, the literature, specify a range of 0-81% biodegradability using various OECD methods and $>5\%$ using the OECD 301D method (Table 1). Therefore, the percentage of biodegradation – 67% after 28 days, obtained in our laboratory experiments can be correlated with existing data.

Result type	Tetranyl AT 7590	Hyamine 1622	Laundry balm I	Laundry balm II	Aniline
Test time (days)			28		
Lag time (days)	7-10	7-10	7-10	7	7
Effective biodegradation time (days)	14	14	14	21	12-14
Remove of surfactants (%)	83.86	73.15	84.70	99.16	-
Biodegradability after 28 days (%)	78.37	67.23	77.34	85.80	95.23

Table 7. Ultimate biodegradability results for cationic surfactants

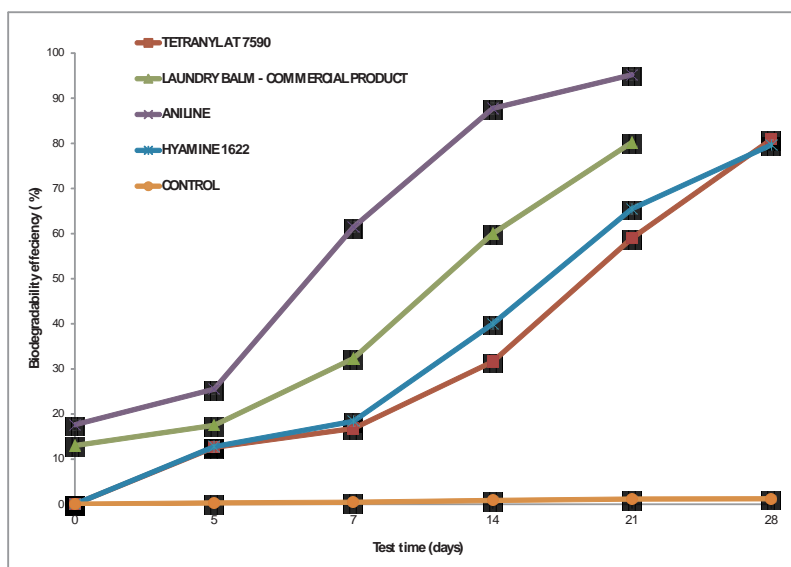


Figure 7. Ultimate biodegradation of cationic surfactants

6. Acute toxicity assessment

Considering the second criterion of ecotoxicological characterization / aquatic risk assessment, this chapter part aimed to evaluate the aquatic toxicity of surfactants on the most representative species of the Romanian surface waters. In accordance with the European norms concerning surfactants and chemicals and OECD/ ISO/ ASTM testing methodology [72 - 75], the present study want to highlight the direct and indirect effects of cationic and amphoteric surfactants (benzethonium chloride, dialkylhydroxyethyl ammonium methasulphate and cocamidopropylbetaine).

6.1. Direct toxicity

To evaluate the acute toxic effects of surfactants have conducted laboratory experiments in static and semi-static conditions, with distinct organisms from aquatic food chain, as followed:

- Acute lethal toxicity test with freshwater fish (1 year juvenile carp – *Cyprinus carpio* sp.) performed for determination of the mean Lethal Concentration which induce the death of half from the test organisms (fish) - LC₅₀, according with OECD 203. The fish are exposed to testing surfactants, in different concentrations (0.5 mg/L – 100 mg/L), for 96h. The effect (mortality) is registered at each 24h and the concentration which kills 50% of fish at the final of test period is calculated.
- Acute toxicity test with water fleas *Daphnia magna* Status (Cladocera crustacea) performed for determination of Effective Concentration (EC₅₀) which have a 50% impact on test organisms using Daphtoxkit FTM magna microbiotest, in accordance with OECD 202. The 24h to 48h EC₅₀ 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 mg/L – 50 mg/L) at 20°C, in darkness.
- Green algae growth inhibition test performed for determination of inhibitory / stimulatory concentration (EC₅₀) with 50% effect on algae – *Selenastrum capricornutum* (*Raphidocelis subcapitata* or *Pseudokirchneriella subcapitata*) in accordance with OECD 201 and ISO/DIS 8692. This toxicity test was performed with Algaltoxkit FTM microbiotest which suppose the measurement of the algal growth (at 670 nm) in the long cells after 24h, 48h and 72h incubation (23°C) and calculation of inhibitory concentration in the test concentrations (0.05 – 10 mg/L surfactant) versus the growth in the control.
- Acute toxicity test with luminescent bacteria to estimate the toxic effect of surfactants on *Vibrio fischeri* sp, using the “BioFixLumi” equipment which respects criteria of DIN EN ISO 11348-3. The principle of method is: marine bacteria release luminescence as a metabolic product which can be affected by chemicals. With help of “BioFixLumi” system was measured the light intensity produced by bacteria, before and after 15 or 30 minute 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.
- Microbial Assay for Risk Assessment (MARA) test– is 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 analyzed 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 levels of toxicity class are drawn in accordance with international regulations EPA [72] and national legislative program (H.G. 1408/2008)[76], as followed: Highly toxic - LC₅₀ / EC₅₀ < 1mg/L; Toxic - 1mg/L < LC₅₀ / EC₅₀ ≤ 10 mg/L; Harmful / hazardous for aquatic environment - 10 mg/L < LC₅₀ / EC₅₀ ≤ 100 mg/L; Very low toxic, non-toxic - LC₅₀ / EC₅₀ > 100 mg/L.

The final results concerning the acute effects of individual surfactants are summarized in the Table 8 and Figure 8.

Test organisms	HYAMINE 1622			TETRANYL AT 7590			CAPB		
	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
<i>Cyprinus carpio</i>	4.57 (1.94–9.77)	0.5	1	22.90 (11.22-33.65)	2	7	6.16 (2.81-11.74)	1	2
<i>Daphnia magna</i>	0.39 (0.15-0.48)	0.05	0.1	4.78 (3.05-6.13)	0.05	0.1	9.54 (7.25–11.08)	1	5
<i>Selenastrum capricornutum</i>	0.56 (0.12-1.25)	0.05	0.1	3.48 (1.67-5.12)	0.05	0.1	5.55 (3.59 – 7.21)	0.1	0.5
<i>Vibrio fischeri</i>	1.2	0.3	-	2.89	0.4	-	>100	0.4	-
Microbial toxicity	1.1	-	0.02	1.6	-	0.04	-	-	-
TOXICITY CLASS	HIGHLY TOXIC (for crustacean and algae) / TOXIC (for fish and bacteria)			TOXIC (for crustacean, algae, and bacteria) / HARMFUL/HAZARDOUS (for fish)			NON-TOXIC (for luminescent bacteria) / TOXIC (for fish, crustacean and algae)		
Literature toxicity data according to Table 3	Fish: LC ₅₀ – 0.28 – 42 mg/L; NOEC: 0.004 – 3.5 mg/L Crustacean: EC ₅₀ - 0.0059 – 78 mg/L; NOEC – 0.0041 – 3 mg/L Algae: EC ₅₀ – 0.09 -11 mg/L; NOEC – 0.16 -4.8 mg/L Bacteria: EC ₅₀ -0.5 - >130 mg/L.			Fish: LC ₅₀ - 2 – 31 mg/L; NOEC: 0.16 – 1.7 mg/L Crustacean: EC ₅₀ - 2.15 – >200 mg/L; NOEC – 0.9 – 1.6 mg/L Algae: EC ₅₀ – 0.55 -48mg/L; NOEC – 0.09 -10 mg/L Bacteria: EC ₅₀ – 5.2 - 900 mg/L.					

Table 8. Experimental toxicity data of studied surfactants

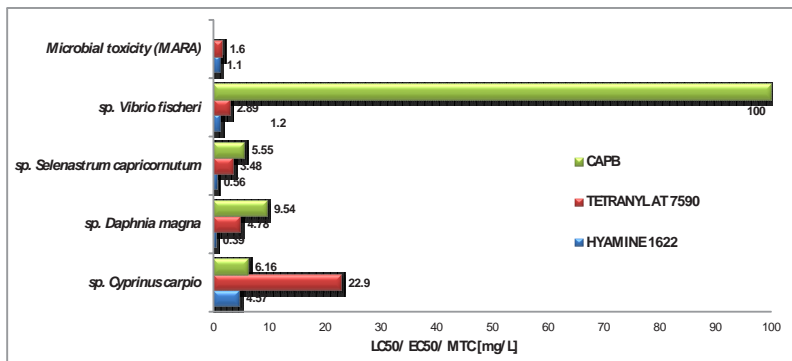


Figure 8. Toxicity quantum of cationic and amphoteric surfactants on aquatic organisms

Compared with other ecotoxicity studies (Table 3), the selected cationic surfactant Hyamine 1622 (benzethonium chloride) with aromatic chains have a great toxic effect, while the TETRANYL AT 7590 (dialkylhydroxyethyl ammonium metasilphate) with linear alkyl chains have a toxic / harmful effect on aquatic organisms, indicating that toxicity was influenced by the chemical structure, which is also indicated in the literature [18, 32].

The highly toxic effect of benzethonium chloride is caused by his biocide proprieties which damage the fish, algae, crustacean and bacteria, whit a great environmental risk potential in the most detrimental scenario. In case of cationic raw material (TETRANYL AT 7590), the acute toxicity effects on the testing organisms were smaller. This effect is due to the presence of the slight ester ties which are easily biodegraded by microorganisms and thus the substance biodisponibility to the target organisms is more reduced.

Because of intense foaming, the amphoteric surfactant, cause the exchange gases blocking in the gills and cell membranes, inducing the mortality / immobilization and growth inhibition in fish, water flea and algae. No toxic effect on bacteria was observed.

In accordance with Table 8 and Globally Harmonized System for Classification and Labelling of Chemicals (GHS) [77], we estimated that the cationic surfactant - benzethonium chloride is classified as "Acute toxic, first class" because caused a highly toxic effect on the crustacean and algae at < 1mg/L. The cationic (diakylhydroxyethyl ammonium metasilphate) and amphoteric (cocamidpropylbetaine) surfactants were classified as "Acute toxic, second class" due to their toxic / harmful effects for the majority of test organisms at 1-10 mg/L.

Our toxicity results (LC50/ EC50 and NOEC) are in line with other toxicity values for this type of pollutants, and therefore we consider being scientifically relevant and can be used in aquatic risk assessment.

6.2. Indirect toxicity

To meet the requirements concerning the complementary aquatic risk of surfactants, toxicity bioassays (with fish, crustaceans, algae and bacteria) of effluents from biodegradation experiments were performed [5].

The toxicity evaluation of cationic surfactants effluents (benzethonium chloride and dialkylhydroxyethyl ammonium metasilphate) resulted after ultimate biodegradability tests was performed according to "Toxicity Classification System for the discharged effluents into the aquatic environment" [37]. The principle is to determine and quantify the acute toxicity of effluents using a microbiotests battery. Effluent toxicity assessment is based on two types of values: an acute toxic value of effluent - transformed in toxicity units $TU = [1/L(E)C_{50}] \times 100$, that can fit in one of the 5 classes of toxicity and value of the weight score for each toxicity class.

The tests showed that biodegradation effluents have a toxic impact on target organisms and the level of toxicity varies depending of species. The algae and bacteria were the most sensible, which can be correlated with the effect caused by the original compounds of these species.

In Table 9 are presented the measured effects of the cationic biodegradation effluents and their toxicity classification. Experimental results have highlighted that benzethonium chloride

(Hyamine 1622) effluent was acutely toxic for all target organisms, while the Teranyl AT 7590 effluent determined a low toxic effect, and also, a greater influence on the algae and bacteria for both tested substances.

Organisms	Toxicity Unit (TU) calculated for each LC ₅₀ / EC ₅₀		Classification System of discharged effluents in to natural aquatic environmental [32]
	Biodegradation effluent of HYAMINE 1622 (0.9 mg/L)	Biodegradation effluent of TETRANYL AT 7590 (0.25 mg/L)	
<i>Cyprinus carpio</i>	0 weight score 0	0 weight score 0	TU < 0.4 Class I – no acute toxicity
<i>Daphnia magna</i>	2 weight score 2	0 weight score 0	0.4<TU<1 Class II – small acute toxicity
<i>Selenastrum capricornutum</i>	3.32 weight score 2	1.2 weight score 2	1<TU<10 Class III – acute toxicity
<i>Vibrio fischeri</i>	4.98 weight score 2	2.24 weight score 2	10<TU<100 Class IV – high acute toxicity
TU for biotests battery / Toxicity Class	2.57 Class III – acute toxicity weight score 2	0.86 Class II – small acute toxicity weight score 1	TU>100 Class V – very high acute toxicity

Table 9. Toxicity classification of biodegradability effluents of the studied cationic surfactants

The toxic effects of surfactants biodegradation solutions, lead us to hypothesize of recalcitrant biodegradation metabolites occurrence with toxic effects potential on aquatic organisms, but their detection is not yet clarified. Other hypothesize is the persistence of testing surfactants, in case of benzenethonium chloride, for which was recorded the lowest ultimate biodegradability (67%), also confirmed by literature data.

For in situ extrapolation (surface water), the experimental toxicity values obtained will be reduced considerably, concerning the rivers dilution (100 fold to 1000 fold). Toxicity behavior of the surfactants depends on physical - chemical factors (pH, temperature, oxygen, microbial charge, climate change, the presence of other chemicals, etc.) that can affect the bioavailability.

Another toxicity experiment was performed in order to reveal the toxic effects of the cationic surfactants used as base ingredient in commercial products (eg. biocide - algicide). In this case was estimated the toxicological behavior of this compound mixed with other ingredients. An acute growth inhibition test with algae was performed for a biocide product containing 50% of alkylbenzyl dimethyl ammonium chloride C12-C16 (CAS 68424-85-1). In mixture with other ingredients (eg. ethylene glycol 2% and water 48%), cationic surfactants maintain his initial toxicity, but the level of effects depend of purpose of use and proportion of ingredients. According to international norms the product was highly toxic / very toxic to freshwater algae *Selenastrum capricornutum*, the estimated CE_{r50} value was <1 mg/L.

7. Aquatic risk assessment – Case study

The aim of surfactants aquatic risk assessment methodology was to establish the maximum allowable cationic and amphoteric surfactants (HYAMINE 1622 CAS 121-54-00 and cocamidopropyl betaine CAS 4292-10-8) concentrations in surface water in order to avoid their negative impact on aquatic ecosystem and to assure the health of aquatic organisms in trophic chain.

The aquatic risk assessment involve the collection of literature data and laboratory testing results to estimate the predicted exposure concentrations of cationic and amphoteric surfactants in the water (PEC aquatic) and the no-effect concentration on organisms (PNEC aquatic). Comparison of these data allowed us to determine whether the studied substances have adverse effects in the aquatic environment, using the PEC / PNEC ratio, where the PEC value must be lower than the PNEC, so that the compounds not present risk to aquatic life. For individual substances PEC / PNEC must be <1, which indicates that there will not be necessary further researches to identify potentially risk.

Given the international methodologies, environmental risk studies [11, 52, 78-83] and the laboratory informations obtained in this work, the important steps of aquatic risk assessment strategy are presented in Figure 9.

In Table 10 and 11 are summarized the most important data of risk coefficients (PEC/PNEC ratio) for each studied surfactant class. We have selected several scenarios, considering the minimum and maximum of aquatic PEC values and the lowest acute (LC/EC₅₀) and chronic (NOEC) toxicity values, identified in the relevant literature studies and from our studies. In order to obtain the PNEC values, we used the lowest toxicity values and different application factors recommended at international level for risk assessment (OECD, EC and ECETOC).

The risk coefficients calculated for the studied surfactants were different. In case of benzenethonium chloride (cationic surfactant class) from 15 scenarios of risk coefficients, the PEC/PNEC rapport was <1 (Table 10), in the range of 2.56 - 512. The results suggest that this compound and its class homologues could have negative impact on the aquatic environment. This conclusion is sustained by hypothesis of complementary effects concerning the persistence or recalcitrant metabolites occurrence. As a result of risk data analysis and taking into consideration that monitoring and control of cationic surfactants concentrations are not imposed within national and international regulations on surface water quality, we recommend the value of benzenethonium chloride ≤0.002 mg/L as maximum allowed concentration in surface water (MATC), so that aquatic ecosystem life is not affected.

The risk coefficients of the amphoteric surfactant (cocamidopropylbetaine) were >1 and 10 different scenarios were analyzed in range of 0.036 – 0.38. In this case the studied amphoteric surfactant and its homologue class were safety for aquatic environment. Considering that amphoteric surfactants control and monitoring are not imposed, we estimate the value of 0.01 mg/L cocamidopropylbetaine as maximum allowed concentration (MATC) in surface water.

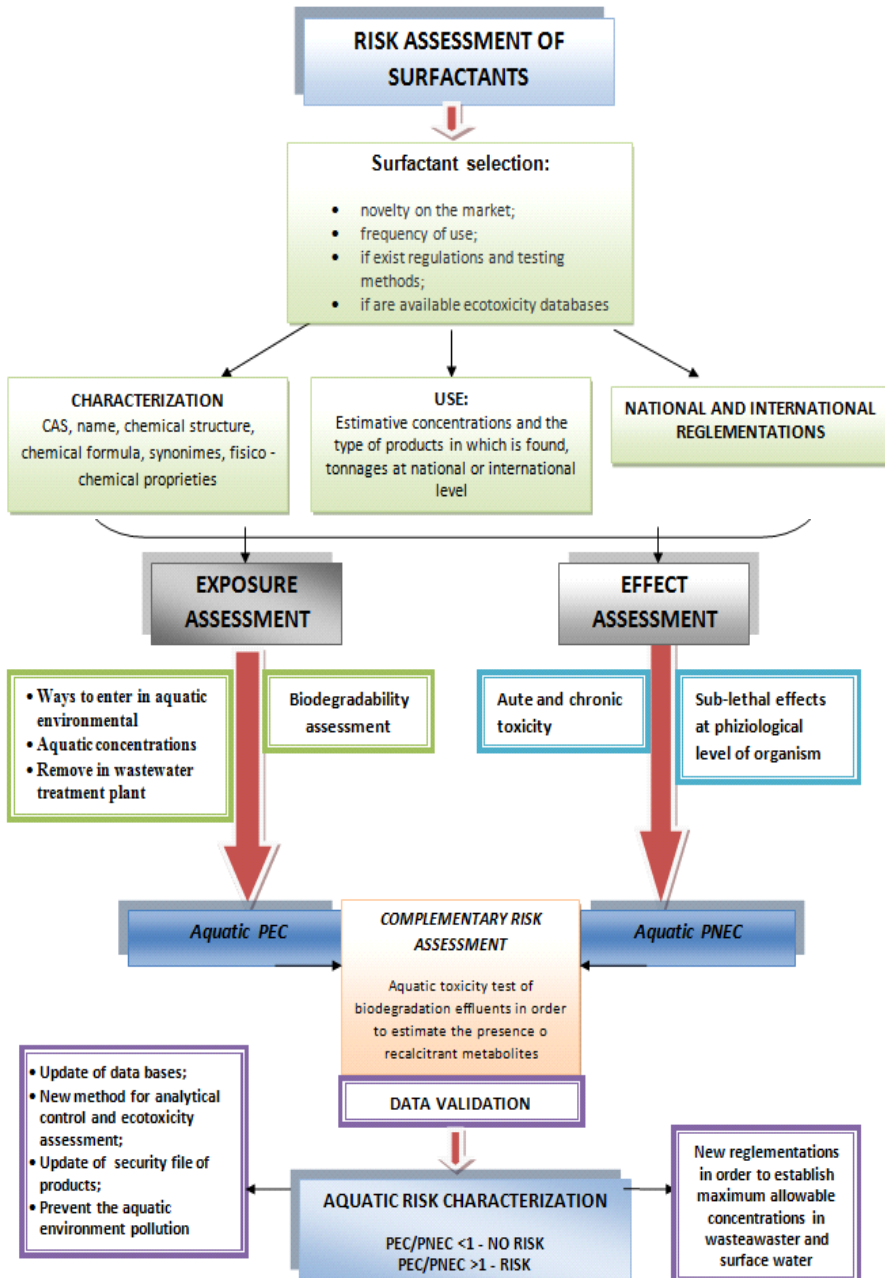


Figure 9. Aquatic risk assessment strategy plan for surfactants

PEC surface water	EC50/NOEC	Application factors for the lowest EC50 /NOEC	PNEC surface water	Risk coefficients (PEC/PNEC)
PEC _{max} =0.2 mg/L [12]	0.39 mg/L (algae) [84]	100 (OECD)	0.0039	51.28
		1000 (EU)	0.00039	512.20
		200 (ECETOC)	0.00195	102.56
	0.004 mg/L (<i>Daphnia</i>) [12]	10 (OECD and EU)	0.0004	500
PEC _{min} =0.002 mg/L [12]	0.39 mg/L (algae) [84]	100 (OECD)	0.0039	0.51
		1000 (EU)	0.00039	5.12
		200 (ECETOC)	0.00195	1.025
	0.004 mg/L (<i>Daphnia</i>) [12]	10 (OECD and EU)	0.0004	5
PEC _{max} =0.01 mg/L (Danube River Romania)	0.39 mg/L (algae) [84]	100 (OECD)	0.0039	2.56
		1000 (EU)	0.00039	25.64
		200 (ECETOC)	0.00195	5.12
	0.004 mg/L (<i>Daphnia</i>) [12]	10 (OECD and EU)	0.0004	25
	0.05 mg/L (algae) [84]	100 (OECD)	0.0005	20

Table 10. Risk coefficients assessment for quaternary ammonium salts (eg. benzethonium chloride / Hyamine 1622, CAS: 121-54-0) according to OECD, EC and ECETOC

PEC surface water	EC50/NOEC	Application factors for the lowest EC50 /NOEC	PNEC surface water	Risk coefficients (PEC/PNEC)
PEC _{max} =0.002 mg/L (Danube River Romania)	5.55 mg/L (algae) [84]	100 (OECD)	0.055	0.036
		1000 (EU)	0.0055	0.36
		200 (ECETOC)	0.027	0.074
	0.5 mg/L (algae) [84]	100 (OECD)	0.005	0.4
PEC _{min} =0,0019 mg/L [13]	5.55 mg/L (algae) [84]	100 (OECD)	0.055	0.034
		1000 (EU)	0.0055	0.34
		200 (ECETOC)	0.027	0.07
	0.5 mg/L (algae) [84]	100 (OECD)	0.005	0.38
	0.09 mg/L (algae) [30]	10 (OECD / EU)	0.009	0.21

Table 11. Risk coefficients assessment for coco alkyl derives (eg. Cocamidopropylbetaine / AMFODAC LB, CAS: 4292-10-8) according to OECD, EC and ECETOC

8. Future challenges

The surfactants ecotoxicology domain remain open for new researches, because this compounds are in a dynamic change of molecular structure which can modified the level of biodegradability and toxicity. It is necessary to develop new control analytical methods for all type of surfactants (HPLC, LC / ELSD, LC-(ESI) MS). Some problems were highlighted concerning the strong absorption capacity of surfactants on the active sludge and also will be interesting to study the impact of cationic and amphoteric surfactants on sludge microorganisms.

There are still gaps in ecotoxicological and risk assessment databases of cationic and amphoteric surfactants, and also for the several nonionic surfactants. Also, an important subject in this research field is the study of biotic and abiotic factors influence on the bioavailability of surfactant compounds.

A great attention should be given to monitoring studies of the surfactants in the national and international surface waters, in order to underline the level of domestic and industrial pollution with this compounds and also to upgrade the current legislation or if is necessary to replace them.

In this field are significant gaps concerning the bioconcentration, bioaccumulation, acute and chronic sub lethal effects and the impact of surfactants on the metabolic pathways, whatever of surfactant type.

Another limitation of this research was the detection of metabolic compounds resulted after biodegradation process, which requires completion of equipment endowment and involve new expenses.

9. Conclusions

The aim of this chapter was the cationic and amphoteric surfactants ecotoxicological characterization according to European Regulation EC no. 648/2004 and risk assessment generated by them on the aquatic environment. Experimental researches were performed to establish the biodegradation level and aquatic toxicity, risk assessment and estimation of the maximum allowable limits in surface water.

Has been pointed that the cationic and amphoteric surfactants have a primary biodegradation >80% and a final removal >60%, noting that the cationic surfactants have registered the lowest values. In terms of acute aquatic toxicity was found that cationic surfactants are toxic for crustaceans, algae and bacteria ("Acute Toxicity, class 1") and amphoteric surfactants are toxic to fish, crustaceans and algae ("Acute Toxicity, class 2").

A complementary risk assessment study was performed for biodegradation liquids of cationic surfactants. The biodegradation effluents maintain the compounds toxicity on algae and bacteria in case of standard surfactant (Hyamine 1622), which means that in the surfactant

biodegradation effluents the active substance was persistent or can arise recalcitrant metabolites.

Based on PEC / PNEC ratios, the aquatic risk assessment of cationic and amphoteric surfactants has been assessed: cationic surfactants PEC / PNEC > 1 - risk to aquatic organisms; amphoteric surfactant PEC / PNEC < 1 - no risk to aquatic organisms.

Were estimated maximum allowable concentrations (MATC) of cationic surfactants (≤ 0.002 mg/L) and amphoteric (0.01 mg/L) in surface waters, so that the aquatic life in trophic chain, will not be affected.

The present study was relevant for the conformity control of market cleanup products to assure the human health and environment protection.

Abbreviations

AISE – International Association for Soaps, Detergents and Maintenance Products;

CEFIC – European Chemical Industry Council;

CESIO – European Committee of Organic Surfactants and their Intermediates;

EC - European Commission;

EC₅₀ – lethal or inhibitory Effective Concentration with 50% effect on crustacean, algae or bacteria;

ECETOC – European Centre for Ecotoxicological & Toxicological Safety Assessment of Chemicals;

ECVAM – The European Centre for the Validation of Alternative Methods;

EPA - US Environmental Protection Agency;

GHS – Globally Harmonized System for Classification and Labeling of Chemicals;

HERA - Human and Environmental Risk Assessment on ingredients of European household cleaning products;

ICCVAM – Interagency Coordinating Committee on the Validation of Alternative Methods;

ISO – The International Standardization Organization;

IUCLID – International Uniform Chemical Information Database;

LC₅₀ - Lethal Concentration with 50% effect on fish;

LOEC – Lowest Observed Effect Concentration

NICNAS – National Industrial Chemicals Notification and Assessment Scheme;

NOEC – No Observed Effect Concentration;

OECD – Organization for Economic Co – operation and Development;

Q(SAR) – Quantitative Structure Activity Relationship;

REACH – Regulation concerning Registration, Evaluation, Authorization and Restriction of Chemicals Substances;

SETAC - Society of Environmental Toxicology and Chemistry;

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