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Effects of anionic surfactants on Daphnia Magna

The acute toxicity at 48 h of various linear alkylbenzene sulfonates (LAS), alkyl sulphates (AS) and alkyl ethoxy sulphates (AES) to Daphnia Magna was determined. Also chronic tests were carried out with the same test species. The study was carried out under PRISTINE activities in order to obtain a valid set of data of the above chemicals for environmental classification and labelling according to European Legislation. The results (LC_{50} > 1 mg/L) indicate, that neither commercial LAS nor the alcohol derivatives, which are used in household products, should be classified as dangerous for the environment with respect to their effects to Daphnia Magna.

Es wurde die akute Toxizität nach 48 Stunden von verschiedenen Linearen Alkylbenzensulfonaten (LAS), Alkylsulfaten (AS) und Alkylethoxysulfaten (AES) für Daphnia Magna bestimmt. Auch chronische Tests wurden mit derselben Spezies durchgeführt. Die Studie wurde im Rahmen der PRISTINE-Aktivitäten durchgeführt, um valide Daten für die o.g. Chemikalien zur Klassifizierung und Bewertung ihres Umweltverhaltens entsprechend der europäischen Gesetzgebung zu erhalten. Aus den Ergebnissen (LC_{50} > 1 mg/L) geht hervor, dass weder das handelsübliche LAS noch die Alkoholderivate entsprechend ihrer Wirkung auf Daphnia Magna als gefährlich für die Umwelt eingestuft werden sollten.

1 Introduction

Cladoceran crustaceans, particularly the daphnids, have been accepted as one of the key invertebrate groups for standard tests on ecotoxicological assessment. Tests with *Daphnia* are included in ecotoxicity test methods of international organisations such as OECD, US EPA, EU, and are required for regulatory purposes. There are scientific and practical reasons for the selection of *Daphnia* for ecotoxicity tests [1]; these organisms are widely found in fresh water systems and they represent an important link in the aquatic food chain. On the other hand, they have a short life-cycle (important for reproduction tests) and are relatively easy to reproduce in the laboratory. They are among the most sensitive species to chemicals for assessment purposes.

Surfactants are an important class of chemicals which were extensively tested with daphnids because of their considerable consumption in daily-life products, their discharge to effluents after use and, consequently, their potential environmental impact. Although there is abundant information supporting the low risk of surfactants for the environment [2], it is interesting to further document the existing database in order to get better documentation and support for the no need for environmental classification of this group of chemicals.

The purpose of this work was to provide additional information on the effects of LAS, AS and AES on *Daphnia magna*.

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2 Materials and methods

2.1 LAS

Commercial LAS used in Europe (CAS No: 68411-30-3) as well as other commercial blends and single cut homologues have been studied in our assessment. Single cut products were prepared from distilled fractions of commercial LAB (Linear Alkylbenzene) and sulfonation with SO_3 in a laboratory scale unit. Sulfonation conditions used in our study were:

SO_3/LAB molar ratio	: 1.07
SO_3 in N_2	: 4.34 wt%
Temperature	: 40 °C – 42 °C
Reaction time	: 1 h

The molar ratio of 1.07 that was used in laboratory conditions is equivalent to 1.02 that is normally accepted as optimum in industrial sulfonation.

After sulfonation reaction, an ageing step at 42 – 45 °C for 45 min took place, then the anhydrides were hydrolyzed with 0.5 % water.

The sulfonic acids were neutralised with caustic soda to obtain the sodium salt derivative. One magnesium derivative was also prepared in order to evaluate its toxicity as compared to the standard Na salt. The characteristics of the raw materials (LAB) used in the preparation of the various LAS products are given in Tables 1 and 2. All LAB derivatives used in our study, commercial blends and single cuts, were produced by HF alkylation at Petresa's plant in San Roque (Cadiz, Spain)

Table 1. Composition of the commercial LAB blends of this paper

Sample/content (% w/w)	A	B	C*	D	E	F	G	H	I	J
<Ph. C_{10}	1.2	1.0	0.6	0.1	1.5	1.2	0.5	0.4	–	–
Ph. C_{10}	24.7	16.5	10.4	0.4	29.0	17.5	15.0	8.9	5.0	1.0
Ph. C_{11}	48.4	49.4	36.5	2.4	39.0	41.5	45.0	33.7	21.0	3.5
Ph. C_{12}	24.8	31.7	33.1	24.5	28.5	38.5	35.0	31.0	26.0	17.8
Ph. C_{13}	0.9	1.4	19.0	67.0	2.0	1.3	4.5	24.0	29.0	37.3
Ph. C_{14}	–	–	0.4	5.6	–	–	–	2.0	19.0	40.4
2 Ph. Alk.	18.3	16.7	17.0	14.5						
Molec. weight	231.8	234.2	240.2	256.6	232	235	236	242	250	261.6

**Product C corresponds to the typical commercial European LAS (CAS: 68411-30-3)*

Table 2. Composition of the single cut LAB homologues of this paper

Sample/content (% w/w)	Phenyl C_{10}	Phenyl C_{11}	Phenyl C_{12}	Phenyl C_{14}
<Phenyl C_{10}	0.5	–	–	–
Phenyl C_{10}	96.8	5.5	0.4	–
Phenyl C_{11}	2.7	93.7	13.9	0.6
Phenyl C_{12}	–	0.8	84.5	1.0
Phenyl C_{13}	–	–	1.2	15.4
Phenyl C_{14}	–	–	–	82.1
>Phenyl C_{14}	–	–	–	0.9
Molec. weight	222.7	231.7	244.4	271.7

2.2 *Alkyl sulphates and alkyl ethoxy sulphates*

The compositions of the two commercial alkyl sulphates and of the alkyl ethoxy sulphate supplied by Marchon, Barcelona (Spain) which were used in our study are shown in Table 3.

2.3 *Methods*

Ecotoxicity studies were carried out according to the OECD–202 guidelines [3] for both acute and chronic toxicity tests. In acute tests, the LC_{50} (Lethal concentration for 50% of population) was determined after 48 h of exposure. The test conditions used are summarised in Table 4.

In chronic tests, young female daphnids were exposed to surfactants in either semi-static or static renewal systems for a period of three weeks. NOEC (No Observed Effect Concentration) was calculated by comparison of the number of offspring per surviving organism in the surfactant test solutions with those in the controls. In this case the technique used to calculate the final NOEC result was the Dunnett method.

3 Results

3.1 *LAS single cut homologues*

The results of the LC_{50} determination are summarised in Table 5. It can be seen that with increasing molecular weight (longer alkyl chains) the LC_{50} shifts to lower values (higher toxicity). The results of the LC_{50} at 48 h of the various homologues versus their corresponding molecular weight can be adjusted by linear regression according to the following equation:

$$\text{Log}LC_{50} \text{ at } 48\text{ h} = 5.32 - 0.0188 \text{ LAB Molecular weight } (r = 0.953) \quad (1)$$

This equation can be used to assess the LC_{50} for other homologues.

Table 3. Composition of the alcohol derivatives of this paper

Sample/content (%w/w)	AS $C_{12}-C_{14}$	AS $C_{16}-C_{18}$	AS $C_{12}-C_{14}$ 2.35EO
C_{10}	1.5	–	2–4
C_{12}	51–57	–	63–71
C_{14}	41–47	–	22–28
C_{16}	1.5	61	8
C_{18}	–	37	–
C_{20}	–	2	--
Molec. weight	296	354.7	403

Table 4. Conditions of the acute toxicity tests

Number of concentrations	5 + control
Test organisms	*Daphnia magna* less than 24 h old
Temperature	20 °C
Photoperiod	Light: 12 h, Darkness: 12 h
Number of test organisms	20 per concentration
LC_{50} calculation method	Probit

Table 5. LC_{50} of LAS homologues after 48 h

Sample	LC_{50} (mg/L)	95 % Confidence limits	
		Lower	Upper
Ph.C_{10} LAS Na	13.9	11.7	17.2
Ph.C_{11} LAS Na	8.7	6.8	9.8
Ph.C_{12} LAS Na	8.1	7.2	8.9
Ph.C_{14} LAS Na	1.22	1.18	1.26

3.2 *Commercial products*

Commercial sulfonic acids

The toxicity results obtained for the sulfonic acids (HLAS) derived from the commercial LAB products A, B, C and D in Table 1, are summarised in Table 6.

Similar to the LAB single cuts there is a relation between the LC_{50} and molecular weight of commercial sulfonic acids which is given in equation (2)

$$\text{Log}LC_{50} \text{ at } 48\,h = 23.2 - 9.4 \log \text{LAB Molecular weight } (r = 0.984) \quad (2)$$

Commercial Na LAS derivatives

The acute toxicity of NaLAS derivatives is of the same order as that of sulfonic acids. This fact indicates that the toxicity mechanism is more related to the alkylchain length than to the hydrophilic group. The results obtained for the various LAS tested are summarised in Table 7.

The independence of toxicity of the hydrophilic group is also confirmed when comparing the toxicity of Na and Mg salts of the same LAB (Table 8).

Table 6. LC_{50} of commercial HLAS after 48 h

Sample	LC_{50} (mg/L)	95 % Confidence limits	
		Lower	Upper
A-HLAS	11.6	10.5	13.4
B-HLAS	10.8	8.1	14.7
C-HLAS	9.3	8.4	10.2
D-HLAS	4.3	3.5	4.7

Table 7. Acute toxicity of Na LAS

Sample	LC_{50} (mg/L)	95 % Confidence limits	
		Lower	Upper
Na LAS (E)	9.5	8.3	10.4
Na LAS (F)	8.8	7.3	9.7
Na LAS (G)	8.6	7.3	9.7
Na LAS (H)	6.3	5.7	7.3
Na LAS (I)	5.5	5.3	5.9
Na LAS (J)	2.8	2.5	3.9

Table 8. Acute toxicity of a Na and a Mg salt of the same LAS

Na LAS	8.4 mg/L
Mg LAS	8.2 mg/L

In the chronic tests we used both a single homologue cut (C_{10} LAS Na) and a commercial blend product. Their composition is shown in Table 9.

The results after 21 days' exposure are shown in Table 10.

These concentrations are considerably higher, at least one order of magnitude, than those which are usually found in surface waters in environmental compartments.

Alcohol derivatives

The LC_{50} values which were obtained for the various alcohol derivatives tested are in the same order as those of LAB products as is shown in Table 11.

4 Conclusions

LAS

- The LC_{50} values for LAS homologues in the Daphnia test are in the range of 13.9 mg/L for the lightest one (C_{10}) to 1.22 mg/L for the heaviest one present in the commercial product (C_{14}).
- The representative NOEC for LAS (Na salt) was higher than 1.28 mg/L. This concentration is at least one order of magnitude higher than the concentration found in environmental compartments (surface waters). The NOEC for the lower homologue (C_{10}) was > 3.5 mg/L.
- There is a clear relationship between the molecular weight (alkyl chain length) of the LAB and the acute toxicity to

Table 9. Composition of the products used in chronic tests

Sample/content (% w/w)	Na LAS C_{10}	Commercial LAS
Phenyl C_9	2.2	0.4
Phenyl C_{10}	96.8	9.0
Phenyl C_{11}	1.0	33.7
Phenyl C_{12}	–	31.0
Phenyl C_{13}	–	24.0
Phenyl C_{14}	–	1.9

Table 10. No observed effect concentration (NOEC) after 21 days exposure

	Phenyl C_{10} Na LAS	Commercial Na LAS
NOEC (mg/L)	>3.5	>1.28

Table 11. Chronic toxicity of alcohol derivatives (commercial blend)

Sample	LC_{50} (mg/L)	95 % Confidence limits	
		Lower	Upper
AS $C_{12}-C_{14}$	5.7	4.5	6.5
AS $C_{16}-C_{18}$	4.2	1.9	6.2
AES $C_{12}-C_{14}$	7.1	8.9	8.9

Daphnia: The higher the molecular weight the higher the toxicity. This is valid for both the two commercial products and single cut homologues.

- The toxicity values of the commercial LAB sulfonic acids are in the same order as their sodium salts; from 4.3 mg/L up to 11.6 mg/L with sulfonic acids and from 2.8 mg/L up to 9.5 mg/L with Na salts.
- The toxicity effect is basically linked to the hydrophobic part of the molecule more than to the hydrophilic moiety. The same LC_{50} is obtained regardless of the cation present in the sulfonate (H, Na or Mg) for a given alkyl chain.

Alcohol derivatives

- The LC_{50} values obtained for the various commercial alcohol derivatives are in the same order as those of the LAB derivatives.

The results of this study show that these types of surfactant should not be classified as dangerous for the environment as their LC_{50} at 48 h is > 1 mg/L in all cases.

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Rapid and simple calibration method for thermal desorption

Suitable sampling and measuring methods are required to determine organic compound contamination of indoor and outdoor air. Enrichment on thermally desorbable sorbents is a widely-used method, offering the advantage over solid-liquid extraction of allowing the entire sample to be injected for GC analysis at once, which results in low detection limits. It also does not require the use of toxic solvents.

The calibration of measuring process is particularly important. In co-operation with *Reinhard Keller*, Head of the Department at the Institute of Medical Microbiology and Hygiene at the Medical University of Lübeck, Germany, Gerstel has designed its TubeStandard-PreparationSystem (TSPS)–a calibration unit taking six TDS tubes to make this stage faster and simpler.

Using a knurled nut, the TDS tubes can be fixed to the bottom of the unit via a Teflon ferrule, with six septumless sampling heads (SLHs) located on the top for contamination-free injection of the TSPS solution. The entire unit is purged with carrier gas and the volumetric rate of flow can be regulated.

The pre-conditioned TDS tubes were clamped into the calibration unit. In his tests, Keller used analytes which are listed in VDI 4300, Sheet 6 for determining the overall concentration of volatile organic compounds and which also span a relatively broad boiling-point range (66–287°C). Between 10 ng and 2000 ng were selected for the concentrations to be examined so as to take account of all problematic concentrations occurring in indoor air. The analytes were dissolved in methanol and doped at the rate of 1 µl each for charging onto the Tenax® TA in the carrier stream into the glass wool. Methanol evaporates and all of the substances reach the Tenax. The solvent passing through the sorbent without any interaction.

The advantages of the process compared with conventional methods are obvious: less instrument equipment and time needed, easy handling and easy to integrate into laboratory practice. This means that presented method is an alternative to the established procedures, such as the use of test gases.