C. Verge*, J. Bravo, A. Moreno, and J. L. Berna, San Roque (Cádiz), Spain

Acute toxicity of linear alkylbenzene (L.A.B.) to Daphnia Magna

Linear Alkylbenzene, the raw material for the production of linear alkylbenzene sulfonate (LAS), can enter the environment as a component of the unsulfonated material present in commercial detergent slurries. Although the product is readily biodegradable and does not accumulate, information on the acute toxicity of this product is not available at least for the representative material used in the European detergent market (CAS N° 67774-74-7).

We have carried out toxicity tests (acute) with Daphnia magna using the above-mentioned LAB following OECD-202 guidelines for the acute immobilisation test under two versions; with solvent (acetone) and without.

No toxic effects have been observed within the solubility limits of LAB in water.

Lineares Alkylbenzen, das Ausgangsmaterial zur Herstellung von linearem Alkylbenzensulfonat (LAS) kann als Bestandteil des nicht sulfonierten Anteils in handelsüblichen Waschmittelschlämmen in die Umwelt gelangen. Es ist zwar bekannt daß es leicht abbaubar ist und sich nicht anreichert, es liegen aber keine Zahlen zur akuten Toxizität dieses Produkts vor, zumindest nicht für das auf dem europäischen Waschmittelmarkt gängige Material (CAS N° 67774-74-7). Wir haben daher Tests zur (akuten) Toxizität mit Daphnia Magna durchgeführt. Dabei wurde nach den OECD-202 Richtlinien für den akuten Immobilisationstest nach zwei Versionen vorgegangen: mit Lösungsmittel (Aceton) und ohne. Es wurden keine toxischen Einflüsse innnerhalb der Löslichkeitsgrenzen von LAB in Wasser festgestellt.

1 Introduction

In 1997, the estimated world LAB production was approximately 2 million tons. The majority of this amount (>98%) is converted into the corresponding LAS (Linear Alkylbenzene Sulfonate), which is used as anionic surfactant in commercial detergent formulations.

LAS contains less than 1% of free LAB in the unsulfonated matter (Free Oil-F.O.) as a consequence of incomplete sulfonation reaction. Some studies [1, 2, 3, 5] have shown the presence of LAB in the environment in concentration ranges from a few µg/L in waters up to a few mg/L in rivers and marine sediments.

In order to determine the aquatic toxicity of an LAB that is commonly used in commercial detergent formulations in Europe a study was carried out following the OECD-202 guidelines. A previous assessment of the solubility of LAB in water was also carried out. This communication reports the results obtained.

* Author to whom correspondence should be addressed

2 Materials and methods

2.1 LAB

A commercial LAB produced in an HF alkylation process was used. The homologue distribution is indicated in Table 1.

2.2 Tests

Two types of tests were carried out:

LAB dissolved in acetone
LAB without solvent

In the first case, a standard stock solution of 100 mg/L of LAB in acetone was prepared. Concentrations of 0, 0.05, 0.1, 0.2, 0.4, 0.8, 1.0, 1.2, and 1.4 mg/L were prepared using the above stock solution and the corresponding amount of dilution water (see this paper, par. 2.5, "Acute toxicity").

In the second case, a saturated solution of LAB in water was used (see the solubility part). This saturated solution is used as such and diluted from 100% to 75%, 50%, 25%, and 12.5% with the a.m. dilution water.

2.3 Solubility

The solubility of LAB in deionized water (DIW) was determined adding 4 mL of LAB to 3,200 mL of DIW and stirring slowly for 96 hours [3] (see fig. 1).

After this time, 2 litres of the sample (water equilibrated fraction) are taken from the bottom of the vessel using an aspiration pipette. The solubilized LAB is extracted from the solution 3 times with n-hexane, then all extracts were

Table 1. Homologue distribution of the LAB used in this study %wt

<Ph. C_{10}	0.9
Ph. C_{10}	9.4
Ph. C_{11}	40.2
Ph. C_{12}	32.7
Ph. C_{13}	15.9
Ph. C_{14}	0.9
2 Ph alkanes	17
Tetralines	<0.5
Mol. Weight	239.6

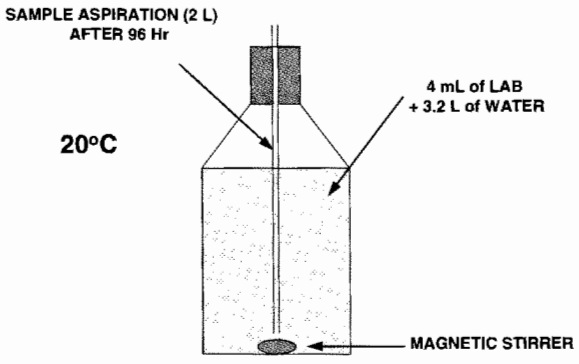

Fig. 1. Experimental arrangement for the solubility tests of LAB

combined in a microreactor and n-C_6 was evaporated until dryness.

2.4 GC-MS Analysis

25 µg of internal standard (2-Phenyl C_8) with a purity >95% are added to the dry residue to obtain a final volume of 1 mL with hexane for subsequent GC-MS analysis.

The GC-MS analysis was carried out using the following operating conditions:

Capillary column: cross linked silica fused, 30 m × length, 0.25 mm ID, 0.25 µm × film thickness.
Phase : SPB 1,
Initial T^u: 100°C, maintained for 6 minutes;
Final T^u: 230°C;
Rate : 3°C/min.
Detector : Mass-SIM Mode (Selected Ion Monitoring)

2.5 Acute toxicity

The OECD-202 guideline has 2 parts: The first one deals with the "48 hours EC-50 acute immobilisation test", the second with the reproduction test. Only the first part has been followed in this work. *Daphnia magna*, not more than 24 hours old, laboratory bred, are used in this test. Four groups of 5 Daphnias were used for each test concentration as well as for the controls.

Dilution water was prepared according to the Afnor T-90.301 method with the following saline content:

$NaHCO_3$.....0.2 g/L
$CaCl_2$ .....0.224 g/L
K_2SO_4.....0.026 g/L

The temperature was kept at 20°C during the test.

The percentage of immobile daphnias after 48 hours of exposure is used to calculate the EC-50% using a computer standardised program (Probit).

3 Results and discussion

3.1 LAB in acetone–water

Toxicity results obtained with acetone in water, and LAB dissolved in acetone are summarised in Tables 2 and 3. As shown in Table 2 the toxicity of acetone in water vs. Daphnia is very low in the range of used concentrations (0–1.4 ppm). In the worst case, only two Daphnias out of 20 were immobilised.

Table 3 shows the toxicity results obtained for LAB solved in acetone–water. The EC-50%, 48 hours, using probability calculations, is 1.1 ppm with the limits 0.96-1.18 ppm. These figures are one to two orders of magnitude higher than those reported by *Gledhill* [3] (9-80 µg/L for EC-50, 96 hours, using another LAB product from the USA).

We understand, however, that the use of a solvent for toxicity tests of insoluble materials is not realistic, as the final result could be either due to the inherent toxicity of the molecule or to physical stress-toxicity due to enhanced adsorption of the test compound. Better understanding of the toxicological properties can be achieved using pure LAB without any solvent present.

Table 2. Toxicity of acetone solutions (no LAB) ("Immobilised Daphnias after 48 hours of exposure")

		Acetone concentration, ppm							
Replica	Control	0.05	0.1	0.2	0.4	0.8	1.0	1.2	1.4
1	0	0	0	0	0	1	0	1	1
2	0	0	0	2	0	0	0	0	0
3	0	0	0	0	0	1	0	1	1
4	0	0	0	0	1	0	1	0	0

Table 3. Toxicity of LAB emulsion in acetone-water ("Immobilised Daphnias after 48 hours of exposure")

		LAB concentrations, ppm							
Replica	Control	0.05	0.1	0.2	0.4	0.8	1.0	1.2	1.4
1	0	0	1	3	1	2	2	3	5
2	0	1	1	0	1	1	2	2	5
3	0	0	1	0	0	1	2	3	5
4	0	1	0	0	0	2	1	3	5

Table 4. Toxicity of LAB dissolved in water (Immobilised Daphnias at 48 hours exposure)

		Dilution used				
Replica	Control	Saturated	75%	50%	25%	12.5%
1	0	0	1	1	0	0
2	1	1	0	0	1	0
3	0	0	0	0	0	0
4	0	0	1	0	0	0

3.2 LAB in water

The solubility of commercial LAB in DIW following the above described method, is 40 µg/L. In order to perform the toxicity test, a saturated solution of LAB (40 µg/L) was diluted to 75% (30 µg/L), 50% (20 µg/L), 25% (10 µg/L), and 12.5% (5 µg/L) respectively. Table 4 shows the toxicity values for the samples diluted in this way. The results indicate that the toxicity level (EC-50%) of LAB is well above its solubility limit in water.

4 Discussion

Neither the LAB solved in acetone/water nor the LAB solved in water, show toxicity values as those reported in the literature.

Realistic environmental concentrations of LAB in water, are in all cases well below the solubility limit and consequently the toxicity vs. Daphnia above 40 µg/L. Soluble LAB is not toxic to this crustacean.

LAB produced today with modern technologies has a considerably higher purity than the product produced until the mid 80's, which is a consequence of several optimisation steps in the production process in the last two decades. This could explain the differences found in the aquatic toxicity when comparing a product from today and that produced in the US at the time of *Gledhill*'s report [3].

The interpretation of aquatic toxicity data of insoluble substances however, should differentiate between the inherent toxicity of the substance (QSAR) and the physical toxic effects due to the insolubility of the product.

Bibliography

1. *Takada, H.* and *Ishiwatari, R.:* Linear Alkylbenzene in urban riverine environments in Tokyo: Distribution, source, and behavior. Environ. Sci. Technol. *21* (1987) 875–883.
2. *Eganhouse, R. P.:* Long chain Alkylbenzenes: Their analytical chemistry, environmental occurrence and fate. Int. J. Environ. Anal. Chem. *26* (1980) 241–263.
3. *Gledhill, W. E., Saeger, V. W.,* and *Trehy, M. L.:* An aquatic environmental safety assesment of Linear Alkylbenzene. Environ. Tox. and Chem. *10* (1991) 69-178
4. OECD Guidelines for testing of chemicals. (1984).
5. *Murray, A. P.,* and *Gibbs, C. F.:* Linear Alkylbenzenes in sediments of Port Phillip Bay (Australia). Mar. Environ. Res. *23* (1987) 65–76.
6. *Bayona, J. M., Albaigués, J., Solanas, A. M.,* and *Grifol, M.:* Selective aerobic degradation of linear alkylbenzene by pure microbial cultures. Chemosphere *15* (1986) 595–598.

Acknowledgement

We are grateful to E. González for her technical assistance in this study.

The authors of this paper

Mrs. *Coral Verge Lopéz* graduated in Fundamental Chemistry at Malaga University and obtained her degree in 1988. Afterwards she obtained a 6 month scholarship in the Cepsa Group, and joined the Petresa in the same year and worked in the Environmental Laboratory for several years. At present she works in the Research and Customer Service Laboratory and takes part in the 3rd Ph.D. course at the University of Cádiz.

Mr. *José E. Bravo* graduated in Chemistry at Granada University in 1965. In 1969 he joined Petresa where he is Chief Assistant Chemist of the Chemical Department.

Mr. *Alfonso Moreno* graduated in Chemistry at the University of Granada (Spain) in 1965. Since 1969 he has worked for Petresa's petrochemical complex in San Roque (Spain), where he is Head of the Chemical Department.

Mr. *José L. Berna* graduated in Chemistry at the University of Zaragoza (Spain) in 1967 and at Madrid University 1969 in Petroleum Technology. In1971 he joined Petresa as research chemist and is at present Director of Research and Development of this company.

Received: January 28, 1999