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Toxicity of Linear Alkyl Benzenes (LABs) to the Aquatic Crustacean *Daphnia magna* through Waterborne and Food Chain Exposures

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Linear Alkyl Benzene (LAB) is the parent material for manufacturing the anionic surfactant Linear Alkylbenzene Sulfonate (LAS) which is used in commercial detergent formulations. During the sulfonation reaction a small percentage, currently less than 1% (Verge et al. 1999), of LAB remains unsulfonated and is found as impurity in the LAS. Considering the use of LAS as a detergent and its large production volume, emissions of LAB to aquatic environment must be expected. In fact, LAB concentrations in the range of 1 – 2200 μg/L in water receiving the effluents of municipal wastewater treatment plants have been reported (Gledhill et al. 1991; Takada and Ishiwatari 1991).

Commercial LAB is a mixture of homologues with linear chains containing between 10 (phenyl C_{10}) and 14 (phenyl C_{14}) carbon atoms. In addition, each LAB homologue is a complex mixture of isomers, depending on the phenyl ring position in the alkyl side chain. The water solubility has been studied only for the commercial complex mixtures, while differences among the different homologues and isomers should be expected. Moreover, LAB toxicity to aquatic organisms is expected to be related to non-polar narcosis, and therefore associated to the total body burden, while the only available information on the toxicity of LAB to aquatic organisms focuses on waterborne exposures.

The aim of this work is to compare the acute waterborne toxicity to *Daphnia magna* of phenyl-C_4, C_6, C_8, C_{10}, C_{12}, C_{14}, C_{16}, C_{18} and a commercial-grade mixture (LAB CAS 67774-74-7) versus the short-term toxicity for the same organism using contaminated food (LAB bound to algae) as source for the exposure (Djomo et al. 1996; Taylor et al. 1998; Carbonell et al. 2000).

MATERIALS AND METHODS

Linear Alkyl Benzenes, phenyl- C_4, C_6, C_8, C_{10}, C_{12}, C_{14}, C_{16}, C_{18} and a commercial-grade mixture (LAB CAS 67774-74-7), were supplied by PETRESA (Avda. Partenón, 28042-Madrid, Spain). This commercial mixture contains phenyl-C_{10} to phenyl-C_{13}, with less than 1 % of LAB C_{14} (average molecular weight: 238, alkyl side chain length: 11.5). The composition of LAB CAS 67774-74-7 (Verge et al. 1999), and a standard chromatogram are shown in Figure 1, respectively. *Chlorella vulgaris* var. *viridis* was supplied by Carolina Biological Supply Company (Burlington, NC USA) and grown in Bold´s Basal Medium (BBM) (Bischoff and Bold 1963), according to EU Commission Directive L133/89.

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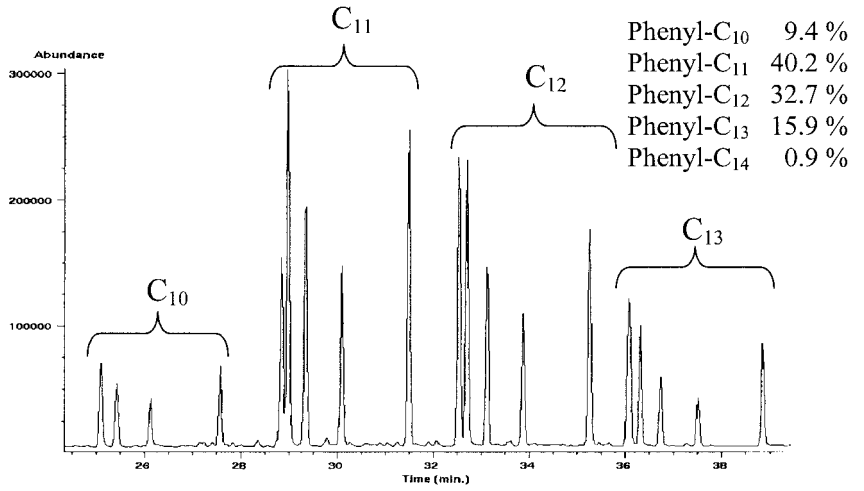

Figure 1. Composition and a standard GC/MS chromatogram of LAB CAS 67774-74-7.

The final measured concentration was 15.8 x 10^6 cells/mL. *Daphnia magna* (less than 24 hours old), cultured in our laboratory according to OECD 202 guidelines for testing chemicals, were used in toxicity tests.

The quantitative water accommodated fraction of each LAB was determined as follows: 100 mL samples were taken from the bottom of a conical flask containing 300 mL MilliQ water and 0.3 mL LAB which had been slowly magnetically stirred for 96 h at 20° C (Gledhill et al. 1991; Verge et al. 1999). The whole sample was extracted by a SPE (OASIS HLB 3cc cartridge, Waters Corporation, Milford, MA U.S.A.), and submitted to GC/MS (Zeng et al. 1998; Alonso et al. 2001). LABs concentrations were determined by comparison with alkylbenzene standard solutions, taking the sum of the peak-heights of the cluster as a whole.

To compare the chromatographic peak profile of LAB CAS 67774-74-7 recovered from an aqueous solution with a n-hexane standard solution, 100 mL of MilliQ water spiked with 0.1 mL acetonitrile LAB CAS 67774-74-7 solution were extracted twice with 50 mL of n-hexane, dried, concentrated, evaporated in a rotarory under reduced pressure to near 1 mL and analysed by GC/MS.
Three hundred mL of MilliQ water suspension of *C. vulgaris* (10.2 x 10^6 cells/mL) were stirred for 96 hr (20 °C) with 0.3 mL of LABs. Then, the algae were centrifuged (1000 g, 5 min) and the pellet was washed three times with MilliQ water.
Finally, the pellet was suspended with 300 mL of MilliQ water. Similar experimental conditions have been used by other authors (Muñoz et al. 1996; Tang et al. 1998). For the analytical measurement of the level of LAB in the

contaminated *C. vulgaris*, 70 mL aliquots of algae suspension were centrifuged (1000 g, 5 min) and the pellet was suspended in 5 mL of acetonitrile and sonicated for 10 min. LAB concentrations in acetonitrile solution were determined by GC/MS.

The standard OECD 202 guideline was modified to produce an extended acute toxicity assay for waterborne exposures under semi-static conditions. For each LAB, duplicate groups of ten *Daphnia magna* juveniles (less than 24 hr old), were exposed in 100 mL of reconstituted water containing different LAB concentrations (100 – 12.5 µg/l) using acetone as solvent (< 100 mg/l). Duplicate control and solvent control vessels were also included. Test conditions were: temperature 20 ± 1°C, light-dark cycle of 12 hr and renewal of test medium every 2 d. Animals were starved from day 1 to 5 and then were fed with uncontaminated *C. vulgaris* on day 6 and 8. The number of daphnids alive, dead and affected (partially immobilised, with its swimming capacity decreased) were checked daily. LC_{50} and EC_{50} values were estimated by the log-probit method.

A *D. magna* toxicity test using LAB-contaminated *Chlorella* was performed. This algae was selected because it is easy to culture in the laboratory and is commonly used to feed laboratory cultures of *D. magna* (Bradley et al. 1993, Lambert 1987). For each LAB, duplicate groups of ten *D. magna* juveniles (less than 24 h old) were transferred to 100 mL of reconstituted water and fed with the contaminated *C. vulgaris* (0.125 mL/*Daphnia* on day 1 and 3, and 0.250 mL/*Daphnia* on day 5 and 7 of a 15.8 x 10^6 cells/mL water suspension). Test conditions were: temperature 20 ± 1°C, light-dark cycle of 12 hours and medium test renewal every 2 days. The number of daphnids alive, dead and affected (partially immobilised) were observed daily.

RESULTS AND DISCUSSION

The calculated aqueous solubility (ng/mL, mean ± std. dev.) at 21-23 °C for phenyl-C_{10}, C_{12}, C_{18}, and for the commercial mixture, were 40.4 (n=2), 48.6 (n=2), 9.62 ± 3.10 (n=5) and 37.2 ± 11.7 (n=3), respectively. The observed value for the commercial LAB mixture is in agreement with those reported in the literature for the same (Verge et al. 1999) or similar (Gledhill et al. 1991) commercial LAB mixtures. Even more, similar solubility values were also obtained for the homologous mixtures, although the total number of individual chemicals (homologues and isomers) was much lower in this case and depends on the length of the carbon chain.
These results clearly indicate that the "apparent dissolution" of one isomer clearly influence the apparent solubility of all the other isomers and homologues. Therefore, the final water concentration of, for example, 2-phenyl-decane, will be different when testing the solubility of 2-phenyl-decane alone, the phenyl-C_{10} homologue containing the other isomers, or the commercial mixture containing also several homologues of different chain length.

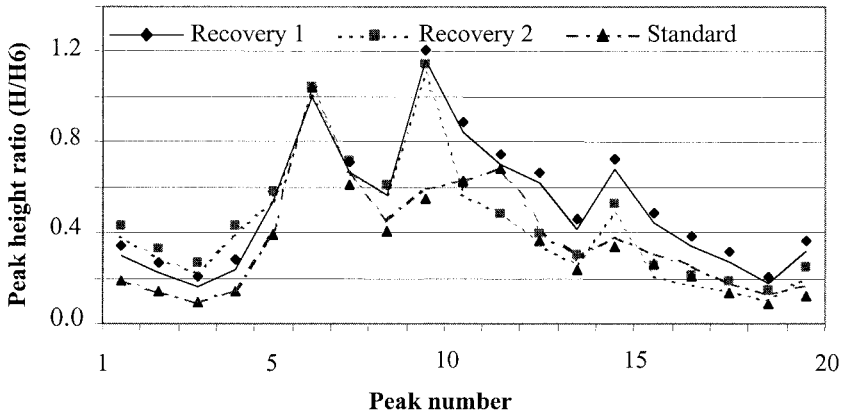

Figure 2. Comparison of chromatographic peak profiles. Recovery 1: from water saturated with CAS 67774-74-7. Recovery 2: from water with CAS 67774-74-7 added by acetonitrile. Standard: n-hexane solution.

In addition, differences in relative abundance of chromatographic peaks were found when comparing the chromatogram of a n-hexane standard solution and the chromatogram of the CAS 67774-74-7 recovered from water. This difference can be appreciated representing peak height ratio (taking peak 6 as base) versus number of peak (Figure 2).

The first significant issue observed in these toxicity tests was the narcotic effect of the LABs on the exposed organisms resulting in reduced mobility of the daphnids. This finding, although expected, is of high relevance for the *D. magna* toxicity test, where the lack of mobility of the organisms is considered as a substitute for lethality. If the observation is not conducted properly, looking carefully at each organism, daphnids with reduced mobility can be accounted as immobile daphnids, while our findings show that these organisms can either die or recover from this condition. In fact, if this aspect is not taken into account, the percentage of immobility can decrease with time, an aspect that is obviously contradictory for a toxicity end point that is expected to be related to mortality. To avoid this problem, we have distinguished two different end points, percentage of affected organisms, which includes those showing reduced mobility, and percentage of immobile organisms, which represents percent lethality.

Table 1. Calculated EC_{50} (µg/l) values of *D. magna* in waterborne LABs experiments

Time (h)	Phenyl C_4	Phenyl C_8	Phenyl C_{10}	Phenyl C_{12}	Phenyl C_{14}	Phenyl C_{16}	Phenyl C_{18}
48	> 100	> 100	> 100	> 100	> 100	> 100	> 100
96	> 100	> 100	82.9	61.9	72.6	> 100	60.6
120	> 100	> 100	35.3	27.8	45.7	40.5	26.7
144	> 100	> 100	24.8	27.8	36.6	29.2	17.4

EC_{50} values, including both lack of and reduced mobility, were calculated from 48 to 144 hr, using a computer basic program (Table 1). All 48-96 hr EC_{50} were higher than the water solubility limit, while the 120-144 hr EC_{50} were at or below the estimated water solubility limit.

To our knowledge, published information on the acute toxicity of LAB to *D. magna* is limited to the data reported by Glendhill et al. (1991) and Verge et al. (1999). The first study reports values between 9 and 80 μg/L, with levels below the solubility limits for two out of the three commercial LAB mixtures studied. The second report is in agreement with our findings, indicating 48hr EC_{50} values far above the solubility limit for the same commercial mixture tested in our study. Both studies used the standard exposure time of 48 hr.

Our test design included a prolonged exposure, up to 7 days, keeping the standard end point for acute toxicity. The results show that the absence of toxicity can be related to the limited exposure period. If the exposure time is expanded to 5 days, the EC_{50} values for waterborne exposures reach the solubility level. This observation is clearly consistent with the assumption of non-polar narcosis as mode of action and toxicity related to the total body burden of LAB. Due to the low water solubility, prolonged waterborne exposures are required to reach the lethal body burden, as has been demonstrated for other poorly soluble hydrocarbons.

The analytical measurements confirmed the binding of LAB to the algae in the algae contamination exercise. LAB concentrations in contaminated *C. vulgaris* used to address the toxicity of LAB to *D. magna* exposed *via* contaminated food ranged from 2.5 to 8.5 μg/mL. Figure 3, shows the percentage of *D. magna* alive, dead and affected (partially immobilised) versus exposure time. As can be observed, the time in which the effect (affected or dead) appears is related to the length of the side chain. For phenyl-C_{10}, toxic effects were observed after 3 d exposure (partiality immobilised), while for phenyl-C_{12}, the effects were already observed in the first day; and phenyl-C_{14} exposure resulted in 100% mortality in less than 24 hr. The toxicity of the commercial mixture was between phenyl-C_{10} and phenyl-C_{12}, in agreement with its side chain average of 11.5

These results are also in agreement with the assumption of non-polar narcosis and toxicity related to total body burden. Lipophilicity is expected to be directly related to the toxicity, and as shown in our study, the longest side chain should have the highest toxicity. For waterborne exposures, lipophilicity plays opposite roles in terms of exposure (water solubility) and toxicity, while for food chain exposures, both exposure and toxicity are directly related to the length of the side chain.
The results obtained in this paper confirm that the toxicity of LAB can be related to non-polar narcosis, and therefore to the total body burden. Due to their low solubility and high binding potential on algae, the exposure through the food chain could become more relevant than direct waterborne exposures.

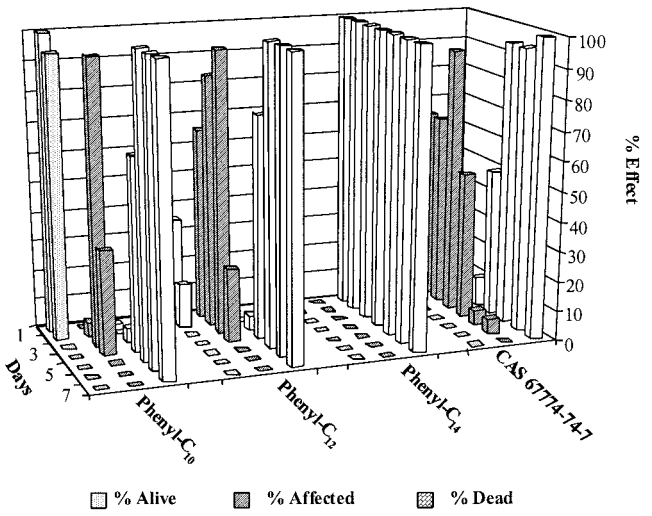

Figure 3. *D. magna* toxicity test. Phenyl-C_{10}, C_{12}, C_{14}, and CAS 67774-74-7 exposure through food chain.

In fact, all the LABs and commercial mixture studied in this work, did not show toxicity at the exposure time recommended in the OECD acute toxicity assay on *D. magna* at the solubility limit. Induced, longer exposure times were required to obtain EC_{50} values at or below the solubility limit. However, algae exposed to LAB saturated water solutions accumulated enough LAB to produce effects on more than 50% of the daphnids feeding on contaminated algae suspensions in a few hours. These results suggest that for these sparingly soluble hydrocarbons, the exposure *via* food can be more relevant than waterborne exposures. Our results also indicate that special care must be considered when testing and evaluating the environmental hazard and risk of poorly soluble hydrocarbons. First, the presence of particulate organic matter, including microorganisms in the test medium and vessels must be carefully considered, because the hydrocarbons could be bound on these particles reaching toxic concentrations, producing inconsistencies among results produced in different assays. Second, the immobility of the organisms should be carefully considered to discriminate between narcotised animals, which can recover from the effect, and immobile organisms.

Regarding the toxicity of hydrocarbon mixtures, the results obtained in this study suggest that the issue is more complex than expected. The interaction among homologues and isomers has been demonstrated and this effect seems to be related to a more complex issue than a simple relation to lipophilicity or Pow. Additional studies are required to clarify the consequences of this interaction on the toxicity of LAB and other hydrocarbon mixtures.

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REFERENCES

Alonso C, Fernández C, García P, Tarazona JV, Carbonell G (2001) SPME/GC-MS for the determination of Linear Alkyl Benzenes (LABs) in environmental samples. In: Gelpi E (ed) Advances in Mass Spectrometry, vol 15. John Wiley & Sons Ltd, p 855

Bischoff B, Bold L (1963) Phycological studies IV. Publ. 1318, University of Texas. USA

Bradley MC, Naylor C, Baird DJ, Barber I, Soares A, Calow P (1993) Reducing variability in *Daphnia* toxicity tests: A case for further standarization. In: Soares A, Calow P (eds). Progress in the Standarization of Aquatic Bioassays. Lewis, Boca Raton, FL, USA, pp 57-70

Carbonell G, Ramos C, Pablos MV, Ortíz JA, Tarazona JV (2000) A system dynamic model for the assessment of different exposure routes in aquatic ecosystems. Sci Total Environ 247: 107-118

Djomo JE, Garrigues P, Narbonne JF (1996) Uptake and depuration of polycyclic aromatic hydrocarbons from sediment by the zebrafish (*Brachidanio rerio*). Environ Contam Toxicol 15: 172-180

Gledhill WE, Saeger VW, Trehy ML (1991) An aquatic environmental safety assessment of linear alkylbenzene. Environ Toxicol Chem 10:169-178

Lambert W (1987) Feeding and nutrition in *Daphnia*. Mem Is Italian Idrobiol 45: 143-192

Muñoz MJ, Ramos C, Tarazona JV (1996) Bioaccumulation and toxicity of hexachloronezene in *Chlorella vulgaris* and *Daphnia magna*. Aquat Toxicol 35: 211-220

Takada H, Ishiwatari R (1991) Linear alkylbenzenes (LABs) in urban riverine and coastal sediments and their usefulness as molecular indicator of domestic wastes. Water Sci Technol 23:437-446

Tang J, Hoagland KD, Siegfried BD (1998) Uptake and bioconcentration of atrazine by selected freshwater algae. Environ Toxicol Chem 17: 1085-1090

Taylor G, Baird DJ, Soares AMVM (1998) Surface binding of contaminants by algae: consequences for lethal toxicity and feeding to *Daphnia magna*. Environ Toxicol Chem 17:412-419

Verge C, Bravo J, Moreno A, Berna JL (1999) Acute toxicity of linear alkylbenzene (L.A.B.) to *Daphnia magna*. Tenside Surf Det 36:127-129

Zeng EY, Cheng D, Khan AR, Vista CL (1998) Validity of using linear alkylbenzenes asmarkers of seawage contamination with interference from tetrapropylene-based alkylbenzenes. Environ Toxicol Chem 17 (3): 394-397