Scientific Committee on Health, Environmental and Emerging Risks

SCHEER

Opinion on the potential for anaerobic biodegradability in marine and freshwater of Linear Alkylbenzene Sulphonates (LAS)

Scientific Committees on Consumer Safety on Health, Environmental and Emerging Risks

The SCHEER adopted this document at its plenary meeting on 18 June 2020

ABSTRACT

The SCHEER was asked to evaluate whether Linear Alkylbenzene Sulphonates (LAS) show potential for anaerobic biodegradation in both, marine and freshwater environments. This request was made in light of some recent data and, particularly, in light of a University of Cadiz study that describes the results of four anaerobic biodegradation experiments performed with LAS in different environmental conditions. When fulfilling this request, the SCHEER was asked to take into consideration additional reports and references from the open literature published in the period 2009 – 2019 to support or refute the conclusions of the University of Cadiz study.

It is the opinion of the SCHEER that the experiments of the University of Cadiz were performed in agreement with OECD Test Guideline 308 and that the results of these experiments may be considered reliable albeit with some restrictions in view of a number of weaknesses. In particular there are improvements needed in both the statistical analysis performed and the reporting of the results.

Based on the results provided in the study of the University of Cadiz and related literature it is the opinion of the SCHEER that there is moderate evidence that:

- in marine waters anaerobic degradation of LAS may occur only under particular conditions (e.g.: sandy sediment and low organic carbon content);
- the potential for anaerobic degradation of LAS is negligible in freshwater;
- the conditions in which some anaerobic degradation has been observed (sandy sediments with low organic carbon contents) may be atypical for sites impacted by waste water, where muddy and organic sediments may be encountered more frequently.

The SCHEER is of the opinion that the absence of degradation may lead to accumulation of LAS in anaerobic sediments and thus may present a relevant environmental concern.

Keywords: anionic surfactants, persistence, sorption, biodegradation, marine, freshwater, sediment, wastewater, high production volume chemical

Opinion to be cited as:

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https://ec.europa.eu/transparency/regexpert/index.cfm

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1. SUMMARY

In two previous Opinions (2005 and 2008), the SCHER expressed some concerns about the potential for biodegradation of Linear Alkylbenzene Sulphonates (LAS) under anaerobic conditions. In light of some recent data and, particularly, of a study by the University of Cadiz (Spain) entitled "Anaerobic degradation of a commercial mixture of linear alkylbenzene sulfonates (LAS) in freshwater and marine sediments (OECD TG 308, 2002)", the Commission asked the SCHEER to reconsider the issue of the anaerobic biodegradation of LAS. In particular, the SCHEER was asked to evaluate if LAS shows potential for anaerobic biodegradation in both marine and freshwater environments, following the OECD 308 protocol.

The SCHEER reviewed the study of the University of Cadiz that describes the results of four anaerobic biodegradation experiments performed in different environmental conditions (marine and freshwater, different sediment texture and organic carbon content). Moreover, the SCHEER examined additional reports and references from the open literature on the topic of LAS degradation published in the period 2009 – 2019 to support or refute the conclusions of the University of Cadiz study.

The results of the University of Cadiz study presented evidence of anaerobic degradation in only one experiment (marine, sandy sediments with low organic C content) out of the four performed.

It is the opinion of the SCHEER that the experiments of the University of Cadiz were performed in agreement with OECD Test Guideline 308 and that the results of these experiments may be considered reliable with some restrictions. Indeed, SCHEER also identified a number of weaknesses in the study that have been highlighted in this Opinion.

The recent literature examined, however, seems to substantiate the validity of the study by providing additional evidence that supports the study's main conclusions.

Therefore, it is the opinion of the SCHEER that there is moderate evidence that:

- the potential for anaerobic degradation of LAS is negligible in freshwaters;
- in marine waters, anaerobic degradation of LAS may occur only under particular conditions (e.g.: sandy sediment and low organic carbon content);
- the conditions in which some anaerobic degradation has been observed may be atypical for sites impacted by wastewater, where muddy and organic sediments may be encountered more frequently.

Considering that LAS are compounds that are produced in very high volumes and that they are continuously released in sites impacted by wastewater, it is the opinion of the SCHEER that negligible anaerobic degradation in freshwater and degradation only under certain conditions in marine waters may lead to accumulation of LAS and thus may present an issue of relevant environmental concern.

2. MANDATE FROM THE EU COMMISSION SERVICES

2.1. Background

To decide whether further legislative action would be justified concerning the anaerobic biodegradation of surfactants (as indicated in Article 16(2) of the Detergents Regulation), the Commission forwarded to SCHEER, as annex to this mandate, the study of the University of Cadiz on the "Anaerobic Biodegradation of LAS" for evaluation and requested an Opinion on certain issues related to the anaerobic biodegradability of these Linear Alkylbenzene Sulphonates (LAS).

In the SCHER Opinion (SCHER, 2005; adopted in November 2005) some concerns were expressed:

(a) about the terrestrial toxicity of LAS in combination with worst-case environmental conditions;
(b) about the relevance of single tests for evaluating anaerobic biodegradation compared to a combination of different testing conditions.

In its second Opinion (adopted in November 2008), SCHER concluded as follows:

I. Despite the fact that most of the biodegradation studies show that LAS is poorly biodegradable under the anaerobic conditions of the laboratory test methods or in anaerobic digesters of sewage sludge, some findings suggest that partial anaerobic biodegradation of LAS is at least feasible and the environmental data seem to indicate that LAS has at least a potential for degradation under anaerobic conditions.

II. However, further investigation is needed to confirm these results.

2.2. Terms of Reference

In its Opinion of 2005, the results of which were confirmed by a second Opinion on the topic in 2008, the SCHEER called for confirmatory data of the potential of LAS for degradation under anaerobic conditions.

DG Internal Market, Industry and Entrepreneurship and SMEs (GROW) therefore invites SCHEER, in the light of the results of the submitted study and of the latest scientific evidence, to evaluate the following statement:

Linear Alkylbenzene Sulphonates (LAS) show potential for anaerobic biodegradation in both marine and freshwater environments, following the OECD 308 protocol as proposed by SCHER previously.

3. OPINION

The SCHEER is of the opinion that the experiments performed to establish the biodegradation of LAS under specific anaerobic conditions were appropriate, having been performed closely following the OECD Test Guideline 308, and that the results of these experiments may be considered reliable with some restrictions.

Nevertheless, the SCHEER is of the opinion that the quality of the results and the study by the University of Cadiz: "*Anaerobic degradation of a commercial mixture of linear alkylbenzene sulfonates (LAS) in freshwater and marine sediments (OECD TG 308, 2002)*" could have been improved. Some weaknesses are described in detail in the sections below.

On the basis of the SCHEER evaluation of the Cadiz' study results and related literature and also in agreement with the main conclusions of the University of Cadiz study, the SCHEER concludes that there is moderate weight of evidence that:

- in marine waters, the anaerobic biodegradation of LAS has been observed only under particular conditions (i.e., sandy sediments with low organic C content) while in other conditions (muddy and organic C rich sediments), evidence for biodegradation is not statistically significant;
- in freshwaters, the anaerobic biodegradation of LAS is negligible in all the environmental conditions tested;
- the conditions of muddy and organic rich sediments may be frequently encountered in areas impacted by wastewater;
- in any case, the observed degradation led to the production of intermediate organic compounds (SPC: sulfophenyl carboxylates) and not to mineralisation. SPCs are the main degradation products of LAS and although these SPCs may generally be less hazardous than their parent compounds, they may still pose a potential risk for the environment.

Considering that LAS are very high production volume compounds and considering that their emissions are continuous in areas subject to wastewater impact, the SCHEER is of the opinion that there is moderate weight of evidence that negligible degradation in freshwaters and degradation under only certain conditions in marine waters may lead to accumulation of LAS in anaerobic sediments and thus may present a problem of relevant environmental concern.

4. MINORITY OPINIONS

None

5. ASSESSMENT

5.1. Introduction

Linear Alkylbenzene Sulphonates (LAS) are the most important group of surfactants used in detergents and cleaning product formulations, with a global production of more than 3 million metric tons per year (Duarte *et al.*, 2015; HERA, 2013; Knepper and Berna, 2003) and a consumption rate of more than 400,000 ton/y in Western Europe alone (Knepper and Berna, 2003). In function of the type of use, as household and industrial detergents, they are usually present in urban and industrial wastewater. Following their use, surfactants will typically enter wastewater-treatment plants (WWTP), with an average LAS concentration of 1-18 mg/L in influents, where removal has been shown to be highly efficient (95–99% average removal, Hampel *et al.*, 2012; Petrovic and Barcelo, 2003; McAvoy *et al.*, 1998). Despite being efficiently removed in WWTPs, WWTP effluents may still contain LAS levels up to 1.5 mg/L and as a result, LAS are continuously emitted and present in freshwater and marine coastal areas impacted by wastewater (Gonzales-Mazo *et al.*, 1998, Petrovic and Barcelo, 2003).

In spite of the high production and emission volumes, the environmental risk of LAS is generally considered not extremely high. For example, the OECD-SIDS Report on LAS (2005) states:

"The chemicals in the LAS category possess properties indicating a hazard for the environment (fish, invertebrates and algae). However, they are of low priority for further work due to ready and/or rapid biodegradation and limited potential for bioaccumulation."

Indeed, the biodegradation of LAS in aerobic conditions is well documented in the literature. However, as is also highlighted in the 2008 SCHER Opinion, there is not enough evidence for the biodegradation in anaerobic conditions and further investigation is needed to better evaluate this issue (SCHER, 2008).

It must be considered that in freshwater and marine coastal areas impacted by wastewater, anaerobic conditions of sediments are not uncommon. Therefore, assessing anaerobic biodegradability of LAS is an issue of high environmental relevance.

5.2. Methodology

For this Opinion, the SCHEER reviewed the study by the Department of Physical Chemistry of the University of Cadiz (Spain) entitled "Anaerobic degradation of a commercial mixture of linear alkylbenzene sulfonates (LAS) in freshwater and marine sediments (OECD TG 308, 2002)" (Lara-Martin and Corada-Fernández, 2016) and the scientific papers from the Cadiz University group that the study is based on.

Additional reports and references from the open literature on the topic of LAS degradation were also examined in order to evaluate experimental evidence in support of or in contrast with the results of the study by the University of Cadiz.

To that end, literature searches were carried out for '**analysis**', **'fate', 'anaerobic degradation' and 'sorption' of 'LAS' in 'marine' and 'freshwater' environments** in the period 2009 – 2019 and – where considered relevant - in preceding years. Abstracts were used for primary screening and papers and reports were accepted or rejected for more detailed reading depending on their relevance to the anaerobic biodegradation of LAS. About 60 papers were identified as relevant.

The weight of evidence approach (SCHEER, 2018) has been followed including 1) clarification of the scientific statement to be assessed from the mandate and problem formulation, 2) identification and collection of the evidence focused on the University of Cadiz study, but also the subsequent sharply-focused literature review using a tightly defined set of keywords, 3) assessment of the Cadiz study and other literature, based on the evaluation of the relevance and reliability of the data, with any limitations and gaps identified and finally 4) the conclusions and their consistency with other relevant literature identified in 2).

5.3. Available information

5.3.1. The study of the University of Cadiz

5.3.1.1. a. Are the methods used for sampling and analysing the sediments suitable and properly applied? b. Are the analytical results for LAS and metabolites (SPCs) methodologically correct and reliable?

Samples from sediments were collected by Van Veen grabs or by sediment cores using PVC tubes. Sampling and storage procedures were done according to commonly used protocols in marine research. Water samples were collected in glass bottles. No details about the cleaning of the sample bottles, Van Veen grabs or PVC tubes were provided in the study.

In the Cadiz studies, sand and clay+silt content was determined by sieving the sediments through 63 micron sieves, which is a well-established method for characterising (marine) sediments.

The organic carbon content of the sediments was determined by a modified version of the titration method developed by Gaudette *et al.* (1974). In later years, alternative methods for determining the organic C content have become available. The most commonly applied method is the catalytic combustion method. Avramidisa *et al.* (2015) have compared the combustion and titration methods and concluded that these have a very good correlation in a wide range of total organic carbon contents.

In the Cadiz studies, analytes were extracted from sediment samples using pressurized liquid extraction (methanol/dichloromethane ratio = 7:3). Cleaning and pre-concentration of these extracts (and water samples) were carried out by solid phase extraction by means of C18 cartridges and eluted with methanol. The extraction efficiency was between 78 and 96% for aqueous samples, and between 89 and 105% for sediment samples. After evaporation of extracts to 1 mL, 20 µL of sample were analysed by liquid chromatography - time-of-flight - mass spectrometry (LC-ToF-MS) in full-scan negative mode in order to identify and quantify LAS homologues as well as anaerobic degradation metabolites.

The quality of the analytical methods used was assured with the help of a range of analytical standards. One standard consisted of a mixture of LAS with the following homologue distribution: C10 (10.9%), C11 (35.3%), C12 (30.4%), C13 (21.2%), and C14 (1.1%). An additional pure C16 LAS standard was added to the samples to determine matrix suppression during LC-ToF-MS analysis. For the main degradation products, SPC pure homologue standards ranging from 4ΦC4SPC to 11ΦC11SPC were used. Limits of detection of the method were 0.5 µg/L per homologue in aqueous samples and 10 ng/g dw per homologue in sediments. The analytical methodology has been published in two papers accepted by reputed peer-reviewed scientific journals (Lara-Martín *et al.* 2006, 2010). These papers provide more details on the quality assurance, including optimisation of the recovery of the extraction procedure, repeatability of the method applied, mass spectrometric details, and the influence of ion suppression in sediment, which was determined as a reduction of less than 5% of the signal intensity for the anionics.

The SCHEER concludes that the methods used for sampling and determination of LAS are up-to-date and correct.

5.3.1.2. Are the degradation experiments suitable and performed according to OECD 308?

Several documents from the Cadiz group describing the degradability of LAS under anaerobic conditions were available to the SCHEER. These include Corada-Fernández *et al.* (2018), Corada-Fernández *et al.* (2017) and Lara-Martin and Corada-Fernández (2016). All documents describe the same research and highlight different aspects of the investigations in different journals. Therefore, the SCHEER analysed the document reported in Lara-Martin and Corada-Fernández (2016) more closely as it provided the most details on the experiments performed. The degradation experiments will be dealt with in this section (see below) and the sorption experiments in the next section (5.3.1.3).

The data presented by Corada-Fernández *et al.* (2018) are the same data as those reported in Lara-Martin and Corada-Fernández (2016) and dealt with the degradation and the sorption experiments. In the paper by Corada-Fernández, *et al.* (2018), some problematic situations were highlighted in the area of emission of LAS in untreated sewage water. As long as the system where the discharge was taking place stayed under aerobic circumstances, LAS degradation occurred at an estimated DT50 of c. 73 d. This estimation is based on a degradation figure of 79% degradation after 165 days given in Lara-Martín *et al.*, 2005. That same study, Lara Martin *et al.* (2005), also showed that the concentration of LAS in the sediment was low (maximum about 2µg g^{-1} below about 7 cm depth in the sediment).

Considering the paper by Lara-Martin and Corada-Fernández (2016) in more detail, the SCHEER is of the opinion that the degradation experiments were in general performed according to OECD Guideline 308 protocol (OECD, 2002). However, some details about the maintenance of the anaerobic circumstances were lacking. No details were given on the cleaning of the materials used. In addition, some misunderstandings of the principles of Guideline 308 were noted by the SCHEER:

- It is mentioned in the Cadiz study that the Guidelines "require sampling in pristine areas". This is not correct. What is required is that the sediments used ". . . should not be used if they have been contaminated with the test substance or its structural analogues within the previous 4 years" (OECD 308). The authors state further that sampling took place within the boundaries of a national park (pristine area) as a consequence of their understanding of the Guideline's requirements, but according to the SCHEER this was not necessary. It is the opinion of the SCHEER that, while sites contaminated with the test substance must be excluded due to adaptation of the microbial community, pristine sites may be not fully representative of receptor sites of wastewater emissions.
- In their reports, the authors placed emphasis on the fraction "sand", whilst details of the silt and clay content of the sediments are considered of more relevance in the Guideline. In the soil classification of the USDA, the amount of sand is not relevant.

However, using the data that were available in the report and taking into account the variability of the data in the degradation experiment (see section 5.3.1.4), the SCHEER was able to calculate a DT50-value for the four different experiments: DT50(exp.1) = 64d, DT50(exp.2) = 257d, DT50(exp.3) = 165d and DT50(exp.4) = 58d (the regression analyses of experiments 2, 3 and 4 were not statistically significant (p-value> 0.05). However, the SCHEER calculated an overall DT50 using the combined experiments, which resulted in a statistically significant ($p<0.05$) regression with an average DT50 of 136 d.

The SCHEER searched for more information on the topic of anaerobic degradation of LAS in marine water and freshwater in the scientific literature.

- García *et al.* (2009) investigated four types of commonly used sulphonate-based surfactants (amongst others, alkyl sulphonates) for their aerobic and anaerobic biodegradability. No degradation of the alkyl sulfonates was determined.
- The research of Merrettig-Bruns & Jelen (2009) focussed on the anaerobic degradation of several types of detergents. The LAS detergents they investigated did not show any degradation.
- Traverso-Soto *et al.* (2016) reported experimental K_d values for AEO, nonionics and PEG which are 2 to 3 orders of magnitude lower than the K_ds determined by Corada-Fernández *et al.* for LAS (2016) (see 5.3.1.3). Although a relationship between K_d, bioavailability and degradation is not available, based on this information, it can be expected that LAS will degrade to a lesser extent than AEOs and PEGs.
- Corada-Fernández *et al.* (2013) describe the presence and degradation of the most commonly used surfactants, including anionics (LAS and alkyl ethoxysulfates, AES) in sediments and pore water from several aquatic environments (southwest Spain). Different vertical distributions were observed according to the respective sources, uses, production volumes and physicochemical properties of each surfactant. Sulfophenyl carboxylates (SPCs), which are LAS degradation products, were identified at anoxic depths at some sampling stations. The authors concluded that their presence was related to in situ anaerobic degradation of LAS in marine sediments.
- García-Luque *et al.* (2009) use a dynamic simulation model to assess the biodegradability of LAS and their biodegradation intermediates (SPCs) using environmentally representative LAS concentrations in estuaries and assuming a continuous emission of LAS into the system. They concluded that the disappearance of SPCs indicated that LAS biodegradation was complete along the estuary. According to the SCHEER, this is more likely to be due to dilution and transport than degradation.
- In general, microbial degradation of specific pollutants can increase when the microbial consortia exposed to them have been able to adapt to the presence of the pertinent micropollutants (Poursat *et al.*, 2019). There are no reports for LAS in the scientific literature mentioning adaptation and increased biodegradation.

Based on this information, the SCHEER concluded that there was moderate evidence that LAS could be degradable under anaerobic conditions, albeit with a DT50 of about 64 d, under the most favourable conditions of sandy sediments with low organic carbon contents. An overall, average DT50 for anaerobic degradation of 136 d was calculated by the SCHEER.

5.3.1.3. Are the sorption experiments performed properly, according to OECD 106?

The authors of the Cadiz study mentioned that they performed the adsorption tests according to OECD Test Guideline 106 (OECD, 2000) but several details of the standard test requirements were not reported, possibly representing deviations, e.g. whether or not sufficient information on the test substance was available, whether or not $CaCl_2$ was used for the stock solution, no adsorption isotherm was determined, and only a final K_d-value was given. The number of soils used did conform with the Guideline.

Sediment-water partition coefficients (K_d in L g^{-1}) for LAS homologues in relation to the organic carbon content (in %) and clay + silt content (in %) were determined. The values reported are for experiment 1: K_d = 25 – 30 L g^{-1}, for experiment 2: K_d = 254 –

4304 L g^{-1}, for experiment 3: K_d = 353 – 426 L g^{-1}, and for experiment 4: K_d = 43 – 717 L g^{-1}. The SCHEER noted that the relevant graph (Fig. 8) in the Cadiz study provides log K_d values along the y-axis, whereas these should be K_d values.

The variation in the data was caused by the different homologues tested but also due to the different characteristics of sediments. Normally, $dm^3\ kg^{-1}$ is used as the unit for the substance's sorption capacity in sorption experiments. So, the values in the standard units were 25E3 – 30E3, 254E3 – 4304E3, 353E3 – 426E3 and 43E3 – 717E3 $dm^3\ kg^{-1}$. Based on the kind of substance, the SCHEER would indeed expect values in the high range, as is the case here.

The SCHEER also searched for additional information on sorption of LAS in the scientific literature but was not able to find any.

The SCHEER concluded, although no statistical evaluation of the resulting K_d values was provided, that the experiments were performed substantially following the OECD Test Guideline 106, and that the resulting K_d values could be considered sufficiently reliable.

5.3.1.4. Are the results of the tests statistically reliable and properly presented?

Based on the results of the degradation and sorption experiments, a number of statistical analyses were performed by the Cadiz researchers that are commented on below. In general, however, some detail was lacking and there were instances where alternative statistical analysis would have been more appropriate.

Degradation experiments

There were 4 experiments, 2 each with marine and freshwater sediments, 2 levels of sand content and 2 organic carbon contents. This could be described as a factorial experiment, with sand content, organic carbon and sediment type being factors, although it is not a complete factorial experiment since not all combinations were explored.

24 glass reactors, acclimatised for 1 week, were spiked with 10mg/L LAS and then a time-course experiment (160 days) was performed. At each testing, 2 reactors were analysed, over the time course of 10, 20 30, 40, 60, 90, 120 and 160 days. LAS and degradation products (SPCs) were measured in water and LAS was also determined in the sediment phase.

A separate set of reactors acted as control and were analysed at the beginning and end of the experiment. TOC, pH, redox and oxygen were also measured as covariates. Mass balances were calculated at the end of the experiments.

Sorption experiments

In agreement with OECD 106 guidelines, 4 different experiments were performed, using the same sediments as used in the degradation experiments. There were 20 tubes in each experiment and LAS were spiked at different concentrations, and the K_d calculated.

The design principles of using a control and replication were both followed. The statistical analysis of the experimental results and the presentation of the findings could, however, be improved, as detailed below.

Results and analysis

Degradation experiments

The presentation and analysis of the degradation experiments could be improved, e.g. it is not clear in Fig. 10 whether the LAS concentrations in water and sediment for the 4 sediment types are mean values. Given that there is a subsequent comment about the variation in duplicate reactors, it is good practice to include information concerning the variation around the mean values. Also in plotting, it would have been helpful for comparison purposes if the same scales were used in y-axis. The authors also occasionally discuss "significant differences" but it is not clear if they strictly mean statistical significance. Analysis of the profiles shows a decrease over the time course, but again there is no mention of how these % decreases are evaluated as being significant or otherwise.

Finally, the mass balance results are shown in Fig. 16 and a Mann-Whitney test was performed between the reactors at the start and end of the experiment, using a one-tailed test, which showed that a statistically significant change had taken place by the end of the experiment. There is no justification of why this specific test was used.

Finally, the LAS degradation was estimated using linear regression. With the exception of experiment 1, the R^2 values are all very low with the implication that estimation of the half-life will only be valid in experiment 1. The authors should indeed provide evidence of the statistical significance of the regression line. Where the regression line is not statistically significant, there is insufficient evidence to reject the hypothesis of no biodegradation.

Sorption experiments

The results were summarised as an average K_d coefficient, for 4 LAS levels and the several sediment types. A visual exploration of the results of the K_d C10-as a function of C_{10}-C_{13} LAS levels shows an increase but there is no evaluation of the statistical significance of any trend, and there is no discussion concerning the standard errors on the K_d values. This could be improved.

The authors of the study tested for significant differences between experiments 1, 3 and 4 (but did not explain why experiment 2 was not included in this analysis) using a one way ANOVA, but this ignores the organic carbon and texture differences, so the SCHEER is of the opinion that this could have been analysed better (using 2-way ANOVA at least). Fig. 9 also suggests that the experiment should be analysed this way.

Quantification of significance through providing p-values or confidence intervals for the parameters of interest is lacking throughout the study.

The SCHEER concluded that the reporting of the statistical analysis of the degradation as well as the sorption experiments was insufficient.

5.3.1.5. Are the conclusions of the study supported by the experimental results?

The conclusions of the study highlight three main results:

1. 'Transformation of LAS into SPCs via fumarate addition is the main route for the anaerobic degradation of these surfactants'.

This conclusion is supported by the presence of SPCs measured in water in all experiments with the highest concentrations in Experiment 1 and the lowest in Experiment 2, and invariably increasing in time. SPCs were not measured in sediments. Considering the lower hydrophobicity of SPCs in comparison with LAS, it can be expected that SPCs would be mainly present in the water phase, i.e., to a higher extent than LAS. However, concentrations in sediments could be not negligible, and in particular, the longer-chain SPCs (C_9-C_{11}) have been shown to be present in sediments (González-Mazo *et a*l, 1997) and measuring them would make the mass balance of Fig. 16 more complete. The SCHEER speculated that a possible explanation for not reporting SPC amounts in sediments in the mass balance is that, due to their low hydrophobicity, their concentrations could be negligible, even below the limit of detection. However, there is no mention of it in the study.

Considering the environmental risk, the SCHEER agrees with the conclusion that the risk would be lowered due to the lower toxicity of SPCs in comparison to LAS. However, the reference quoted (Argese *et al.*, 1994) is probably not the best one to support this hypothesis. Indeed, this paper reports a very specific endpoint (submitochondrial particle response) that is weakly relevant in ecological terms. A better reference would be the paper of Hampel and Blasco (2002), testing acute toxicity of SPCs on marine fish embryos.

2. 'Anaerobic degradation of LAS in anoxic marine sediments is feasible but strongly dependent on sediment properties'.

This conclusion is supported by the results of experiments 1 and 2. LAS degradation may occur in conditions of sandy sediments with low organic carbon contents (experiment 1). In muddy sediments with higher organic carbon contents, the higher affinity of LAS to the solid particles reduces their availability thereby hampering or preventing the transformation of LAS and the formation of SPCs (experiment 2). In each case, the hypothesis being tested is that there is no biodegradation observed in the experiments, and experiments 2, 3 and 4 are not statistically significant at the 5% level, which means that there is insufficient evidence for the SCHEER to reject the hypothesis that there is no biodegradation in favour of the alternative that there is biodegradation. The statistical power of each experiment (or the probability to detect biodegradation **if it were present**) is also likely to be low due to the small sample size. At the same time, the SCHEER is able to show that experiments 3 and 4 are statistically significant at a p-value of 0.1 (i.e. the chances of observing these results under the hypothesis of no biodegradation is less than 10%).

It may be hypothesised that conditions where both relatively high C and clay contents are present (as in experiment 2) are more frequently encountered in impacted estuaries and coastal areas subject to urban sewage emissions.

3. 'Evidence for anaerobic degradation of LAS in non-polluted freshwater sediments is inconclusive'.

This conclusion is based on the results of experiments 3 and 4. A slight decrease over the time period, while not statistically significant at 5%, may be observed in experiments 3 and 4 and a small increase of SPC levels was also observed in water.
The SCHEER agrees with the hypothesis that this result may be a function of the composition of the bacterial community. However, this confirms that the anaerobic degradation of LAS is not a general issue, it is negligible in the freshwater conditions tested, and may only be possible in specific conditions.

In synthesis, it is the opinion of the SCHEER that the conclusions of the study are supported by the experimental results of the study with a moderate weight of evidence.

5.3.2. Additional information from the literature

5.3.2.1. Is there some evidence in the literature supporting results in contrast with those provided by the study?

In the recent literature examined, there is additional evidence to support the main conclusions of the study (e.g., Merettig-Bruns and Jelen, 2009; Duarte *et al.*, 2015; Wu *et al.*, 2019). However, there is also evidence that degradation of surfactants is slower in marine than in freshwater environments (Capone and Kiene, 1988; González-Mazo *et al.*, 1997; Khleifat, 2006). It was suggested that this comparatively slower degradation might be explained by marine microbial communities being less abundant (Wang *et al.*, 2012) and less active than their freshwater counterparts toward xenobiotic chemicals (Jackson, 2015), which is in contrast with the findings of the Cadiz study.

5.3.2.2. Evidence for extrapolation of this study to the environment (in the real world)?

LAS have been detected in seawater and marine sediments near the outfalls of untreated urban wastewaters and in highly polluted harbours e.g., in Europe in the German Bight of the North Sea (Bester *et al.*, 2001), Baltic Proper and Little Belt (Folke *et al.*, 2003, Hampel *et al.*, 2012), river Tagus estuary (Hampel *et al.*, 2009), Gulf of Cadiz (González-Mazo *et al.*, 1997), and in other Spanish coastal marine sediments (León *et al.*, 2001; Temara *et al.* 2001; DelValls *et al.*, 2002; Petrovic *et al.* 2002).

Apart from these areas, the distribution of LAS has been studied in sediments from a salt marsh and an estuary of the Bay of Cadiz (southwest of Spain) (Lara-Martín, *et al.*, 2005), in the Tagus estuary (Hampel *et al.*, 2009), and in the central lagoon of Venice, Italy (Marcomini *et al.*, 2009), where the spatial distributions appeared to be rather homogeneous over the entire central lagoon (Marcomini *et al.*, 2009). LAS levels were similar to those previously reported in the Bay of Cadiz (Lara-Martín *et al.*, 2005), in the North Sea (Bester *et al.*, 2001) and in Japanese lakes (Inaba and Amano, 1988), but significantly lower than those found in more polluted areas on the Spanish coasts that are subjected to untreated wastewater discharges (González-Mazo *et al.*, 1998; Petrovic *et al.*, 2002; DelValls *et al.*, 2002). In Tokyo Bay sediments, LAS contents decreased offshore and fell below 0·01 µg/g 10 km off the mouths of the rivers. These results indicate the rapid degradation of LAS in the coastal zone (Takada *et al.*, 1992).

Taking as model the Gulf of Cadiz, either for fresh and for marine areas, the degradation of surfactants as LAS is treated differently:

- It could be slower in marine than in freshwater environments, explained by marine microbial communities less active and lower bacterial densities than their freshwater counterparts toward xenobiotic chemicals (Jackson, 2015), in contrast with the present study (Biel-Maeso *et al.*, 2018).
- in oxygen-limited conditions, which occur in the real world, LAS biodegradation can be initiated in anaerobic conditions (Larson *et al.*, 1993; León *et al.*, 2001, 2004, 2006), and can biodegrade under methanogenic conditions (Angelidaki *et al.*, 2000) or in sulphate-limited environments where LAS is the only source of sulfur (Denger and Cook, 1999).

Estuarine and coastal environments are considered the most productive and sensitive ecosystems on Earth; hence, exposures can have significant environmental implications (Terzic *et al.*, 1992).

The modelling of the fate of linear alkylbenzene sulphonate (LAS) has been achieved (EXAMS study by Games, 1982) taking into account that these chemicals were introduced into a stream from a single point-source input of domestic sewage. Steady state concentrations of LAS in the water and sediment predicted using the EXAMS model agreed fairly well with the measured concentrations [EPA 600/9-85/018]. Unlike the fugacity or SLSA model, the EXAMS model could correct the changes in the transformation properties caused by changes in the properties of individual compartments (Games, 1982; Branson and Dickson, 1981).

Trehy *et al.* (1996) reported that LAS is a ubiquitous contaminant on the sediments at the bottom of the Mississippi River and that dissolved LAS is present mainly downstream from the sewage outfalls of major cities. The removal of the higher LAS homologues and external isomers indicates that sorption and biodegradation are the principal processes affecting dissolved LAS. Sorbed LAS appears to degrade slowly (Tabor and Barber, 1996).

The data presented above reveal that the marine environment may act as a sink for surfactants as a result of sorption to particles that settle onto the sediment bed, potentially leading to their burial and accumulation. Rubio *et al.* observed <6% LAS recovery from marine sediments and concluded (by fitting experimental data to a Freundlich model) that sorption of surfactants to marine sediments was irreversible (Rubio *et al.*, 1996).

5.4. Conclusions

In agreement with the main conclusions of the study, it is the opinion of the SCHEER that the potential for anaerobic degradation of LAS is negligible in freshwaters and may occur in marine waters only under particular conditions.

It is also the opinion of the SCHEER that the conditions in which some anaerobic degradation has been observed (sandy sediments and low organic carbon content) may be atypical for sites impacted by wastewater, where muddy and organic sediments may be encountered more frequently.

These opinions are supported by an overall assessment of a moderate weight of evidence: good evidence from a primary line of evidence but evidence from several other lines is missing (important data gaps).

However, it is the opinion of the SCHEER that the Cadiz study presents some limitations and weaknesses. In particular:

- SPC amounts are not reported in the mass balance and no explanations are provided for that.
- The results of the degradation experiments are only reported in figures (e.g., Figs. 10, 14, etc.), not in numeric tables. Therefore, to perform additional statistical assessments or calculations, approximated values derived from the figures had to be used.
- There are improvements needed in both the statistical analysis performed and the reporting of the results.
- The reference quoted to support the low toxicity of SPCs is not appropriate.
- In Figs. 8 and 9, the title of the Y-axis is wrong; it is not "log K_d", it is "K_d", the scale of the axis is logarithmic.

Despite these flaws, it is the opinion of the SCHEER that the methodology was applied correctly, according to OECD 308 Guideline, and that the results obtained may be considered reliable.

Considering that LAS are compounds produced in very high volumes and continuously emitted in sites impacted by wastewater, it is the opinion of the SCHEER that the negligible anaerobic degradation in freshwaters and degradation in marine waters only under certain conditions may be a problem of relevant environmental concern. It must be also considered that estuarine and coastal environments are among the most productive yet sensitive ecosystems on earth; hence, exposures can have significant environmental implications.

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7. GLOSSARY OF TERMS, UNITS

An extended glossary of technical terms can be found in: Knepper TP, Barceló D, de Voogt P, eds. (2003) Analysis and fate of surfactants in the aquatic environment. Wilson & Wilson's Comprehensive Analytical Chemistry, Vol.40. Elsevier, Amsterdam.

8. LIST OF ABBREVIATIONS

AEO	Alcohol ethoxylates
AES	Alcohol ethoxysulphates
C18	Octadecyl-silica stationary phase used in LC and solid phase extraction
EXAMS	Exposure Analysis Modelling System
GLP	Good Laboratory Practices
LAS	Linear alkylbenzene sulphonates
LC-ToF-MS	Liquid chromatography coupled to time-of-flight mass spectrometry
NPE	Nonylphenol ethoxylates
PEG	Polyethylene glycols
SIDS	Screening information data set
SPC	Sulphophenyl carboxylate
USDA	United States Department of Agriculture
WWTP	Wastewater treatment plants