



Human and Environmental Risk Assessment  
on ingredients of Household Cleaning Products

# LAS

## Linear Alkylbenzene Sulphonate

(CAS No. 68411-30-3)

**Revised HERA Report**

**April 2013**

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- 7.1 Substance team
- 7.2 HERA environmental task force
- 7.3 HERA human health task force

## 2. *Executive Summary*

Linear alkylbenzene sulphonate (LAS) is an anionic surfactant. It was introduced in 1964 as the readily biodegradable replacement for highly branched alkylbenzene sulphonates (ABS). LAS is a mixture of closely related isomers and homologues, each containing an aromatic ring sulphonated at the *para* position and attached to a linear alkyl chain.

The European consumption of LAS in detergents applications covered by HERA was about 350 kt in 2005. This represents more than 80% of the total European consumption of LAS, which was estimated to be about 430 kt in the year 2005. LAS is one of the major anionic surfactants used on the market. Important application products are household detergents, such as laundry powders, laundry liquids, dishwashing products and all-purpose cleaners. The minor other final uses of LAS, namely in the field of textile and fibres, chemicals, and agriculture, are outside HERA's scope.

### **Environmental assessment**

- The present environmental risk assessment of LAS is based on the HERA methodology document, which in its turn is based on the EU Technical Guidance Document (TGD, 2003). It makes use of the EUSES programme following the HERA detergent scenario (EUSES, 2008). LAS concentrations (PEC values) measured or modelled in the various environmental compartments were compared with extrapolations of the many available eco-toxicity data leading to PNEC values protective of each compartment.
- In raw sewage, the LAS concentration was in the range of 1-15 mg/l. When the sewage was properly treated in activated sludge STPs (Sewage Treatment Plant). LAS was highly removed leading to an effluent concentration in the 0.008-0.27 mg/l range.
- LAS concentration was further decreased by dilution in the receiving waters where it could be found in the <0.002-0.047 mg/l concentration range. LAS degrades rapidly aerobically (half-life in rivers about 3 hours), whereas it does not degrade under anaerobic conditions, except under particular conditions.
- Typical LAS concentrations in aerobic sludge are <0.5 g/kg<sub>dw sludge</sub> (dry weight). In STP anaerobic sludge, the calculated median LAS concentration was 5.6 g/kg<sub>dw sludge</sub> (dry weight) (15.1 g/kg<sub>dw sludge</sub> at 95th percentile). During sludge transportation to the farmland, sludge storage, and application on agricultural soil, aerobic conditions are restored and rapid degradation of LAS resumes.
- In sludge-amended soils, LAS had a maximum half-life of one week (primary biodegradation) and monitored concentrations were around 1 mg/kg<sub>dw soil</sub> (maximum 1.4 mg/kg<sub>dw soil</sub>) at harvesting time. No accumulation in soil and no bioaccumulation in plants could be detected experimentally.
- In freshwater sediments, measured LAS concentrations typically ranged from <1 mg/kg<sub>dw sed.</sub> to a maximum value of 5.3 mg/kg<sub>dw sed.</sub>.

- Ecotoxicity data are abundant and well documented. The aquatic PNEC value (0.27 mg/l) was calculated from: i) a statistical extrapolation including a set of high quality single species chronic data and ii) the no-observed effect concentration of a stream community experimentally exposed to LAS.
- The terrestrial PNEC value (35 mg/kg<sub>dw soil</sub>) was calculated from: i) the equilibrium partitioning method, ii) statistical extrapolation of a set of high quality chronic data on plants and soil fauna, iii) an expert judgement on the toxicity of several microbial processes and functions, and 4) field toxicity studies.
- The sludge PNEC value (49 g/kg<sub>dw sludge</sub>) was back-calculated from the soil PNEC on the basis of the EU TGD scenario (TGD, 2003).
- The sediment PNEC value (23.8 mg/kg<sub>dw sed.</sub>) was calculated from i) the lowest available chronic effect value and an application factor, and ii) the equilibrium partitioning method, the PNEC was normalized for organic carbon content.
- The STP PNEC (5.5 mg/l) was calculated from acute and chronic microbial inhibition data and the use of the relevant application factor (TGD, 2003).
- The risk characterisation as expressed by the PEC/PNEC ratio was below 1 for all environmental compartments. It was concluded that the ecotoxicological parameters of LAS have been adequately and sufficiently characterized and that the ecological risk of LAS is judged to be low.

### **Human health assessment**

- The presence of LAS in many commonly used household detergents gives rise to a variety of possible consumer contact scenarios including direct and indirect skin contact, inhalation, and oral ingestion derived either from residues deposited on dishes, from accidental product ingestion, or indirectly from drinking water.
- The consumer aggregate exposure from direct and indirect skin contact as well as from inhalation and from oral route in drinking water and dishware results in an estimated total body burden of 34.6 mg/kg bw/day. This body burden is substantially higher than the body burden of 0.4 µg/kg bw/day reported in the previous version of this HERA document. The higher estimated body burden is a result of using information from the RIVM report Cleaning Products Fact Sheet (RIVM,2006) to assess the risk to consumers in addition to the AISE overview concerning habits and practices on uses of detergents and surface cleaners in Western Europe (THPCPWE,2002). Furthermore, some additional use scenarios have been identified.
- The toxicological data show that LAS was not genotoxic *in vitro* or *in vivo*, did not induce tumours in rodents after two years daily dosing, and failed to induce either reproductive toxicity or developmental or teratogenic effects. The critical adverse effect identified after repeated long term high dosing of LAS to animals was a change in renal biochemical parameters. A systemic NOAEL of 68 mg/kg bw/day was established.
- Comparison of the aggregate consumer exposure to LAS with the systemic NOAEL results in an estimated Margin of Exposure (MOE) of 1.97. The estimated Margin of Exposure is based on conservative estimations of both exposure and NOAEL (which is a systemic NOAEL given the existence of oral toxicokinetic data). This MOE is substantially less than the MOE of 17000

reported in the previous version of of this HERA document. The lower MOE is a direct result of the higher estimated body burden (see above).

- Neat LAS is an irritant to skin and eyes. The irritation potential of aqueous solutions of LAS depends on concentration. Local effects of hand wash solutions containing LAS do not cause concern given that LAS is not a contact sensitizer and that the concentrations of LAS in such solutions are well below 1% and therefore not expected to be irritating to eye or skin. Laundry pre-treatment tasks, which may translate into brief hand skin contact with higher concentrations of LAS, may occasionally result in mild irritation easily avoided by prompt rinsing of the hands in water. Potential irritation of the respiratory tract is not a concern given the very low levels of airborne LAS generated as a consequence of cleaning sprays aerosols or laundry powder detergent dust.
- In view of the extensive database on toxic effects, the low exposure values calculated and the resulting Margin of Exposure described above, it can be concluded that use of LAS in household laundry and cleaning products raises no safety concerns for the consumers.

### 3. *Substance Characterisation*

Linear alkylbenzene sulphonate (LAS) is an anionic surfactant. It was introduced in 1964 as the readily biodegradable replacement for highly branched alkylbenzene sulphonates (ABS). LAS is a mixture of closely related isomers and homologues, each containing an aromatic ring sulphonated at the *para* position and attached to a linear alkyl chain.

#### 3.1 CAS No. and grouping information

LAS, used on the European market and covered in this focused risk assessment, is represented by the list in Table 1.

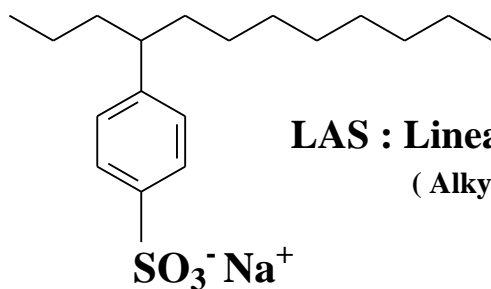
Table 1: CAS and EINECS numbers of LAS in the European market

CAS No.	EINECS No.	NAME
68411-30-3	270-115-0	Benzenesulphonic acid, C <sub>10-13</sub> alkyl derivs., sodium salts
1322-98-1	215-347-5	Sodium decylbenzenesulphonate
25155-30-0	246-680-4	Benzenedodecylsulfonic acid, sodium salt
90194-45-9	290-656-6	Benzenesulphonic acid, mono-C <sub>10-13</sub> alkyl derivs., sodium salt
85117-50-6	285-600-2	Benzenesulphonic acid, mono-C <sub>10-14</sub> alkyl derivs., sodium salt

The present assessment focuses on LAS levels in consumer products used on the European market and found in the various environmental compartments. LAS represented by the CAS No. 68411-30-3 and EINECS No. 270-115-0 is by far the most used on the European market (>98%).

#### 3.2 Chemical Structure and Composition

LAS on the European market is a specific and rather constant mixture of closely related isomers and homologues generated in the manufacture of the raw material Linear Alkyl Benzene (LAB), the LAS precursor, each containing an aromatic ring sulphonated at the “para” position and attached to a linear alkyl chain at any position except the terminal carbons (Schönkaes, 1998; Cavalli et al., 1999b; Valtorta et al., 2000), as shown in the figure below:



## LAS : Linear Alkyl Benzene Sulfonate

( Alkyl Chain : C<sub>10</sub> - C<sub>13</sub> )



The linear alkyl chain has typically 10 to 13 carbon units, approximately in the following mole ratio C<sub>10</sub>:C<sub>11</sub>:C<sub>12</sub>:C<sub>13</sub>=13:30:33:24, an average carbon number near 11.6 and a content of the most hydrophobic 2-phenyl isomers in the 18-29% range (Feijtel et al., 1995b; Feijtel et al., 1999; Cavalli et al., 1999b; Valtorta et al., 2000). This commercial LAS consists of more than 20 individual components. The ratio of the various homologues and isomers, representing different alkyl chain lengths and aromatic ring positions along the linear alkyl chains, is relatively constant across the various household applications. This LAS constant ratio is unique and does not apply to the other major surfactants. Therefore, the present assessment adopted a category approach, i.e., considered the fate and effects of the LAS mixture as described above rather than of each isomer and homologue separately. However, fingerprints in the different environmental compartments are reported.

The linearity of the alkyl chain is between 93% and 98% depending on the different manufacturing processes of LAB, the LAS precursor (Cavalli et al., 1999b). The mono-methyl substituted alkylbenzene sulphonate (iso-LAS) (Nielsen et al., 1997) represent on average 2 to 7% of the raw material. The kind of substitutions of iso-LAS was shown not to limit their biodegradation, which under realistic environmental conditions was comparable to the one of LAS (Nielsen et al., 1997; Dunphy et al., 2000). Non-linear components such as DiAlkylTetralin Sulphonates (DATS) can be present at levels of 3-10% in the LAS derived from AlCl<sub>3</sub> catalysed LAB process (see par. 3.3). This process, however, was less than 5% in 2005 (ECOSOL, 2005).

The data presented in Table 2 are fully described in IUCLID, 1994 and SIDS, 2005 and refer to the commercial C<sub>11.6</sub> LAS or the pure C<sub>12</sub> homologue.

Table 2: Physical chemical data of the commercial C<sub>11.6</sub> LAS (IUCLID, 1994; SIDS, 2005)

LAS	Protocol	Results
Molecular description	Solid organic acid sodium salt	-
Molecular weight (g/M)	(C <sub>11.6</sub> H <sub>24.2</sub> )C <sub>6</sub> H <sub>4</sub> SO <sub>3</sub> Na	342.4
Vapour pressure at 25°C (Pa)	Calculated as C <sub>12</sub>	(3-17) · 10 <sup>-13</sup>
Boiling point (°C)	Calculated as C <sub>12</sub>	637
Melting point (°C)	Calculated as C <sub>12</sub>	277
Octanol-water partition coefficient (log K <sub>ow</sub> )	Calculated as C <sub>11.6</sub>	3.32
Organic carbon-water partition coefficient K <sub>oc</sub> (l/kg)	Calculated as C <sub>11.6</sub>	2500
Critical micelle concentration (g/l)	Experimental	0.65
Water solubility (g/l)	Experimental	250

Sorption coefficient between soil/sediment and water, $K_d$ (l/kg)	Experimental	2-300
Density (kg/l)	Experimental	1.06 (relative) 0.55 (bulk)
pH (5% LAS water solutions)	Experimental	7-9
Henry's constant ( $\text{Pa} \cdot \text{m}^3/\text{mole}$ )	Calculated as $C_{12}$	$6.35 \cdot 10^{-3}$

Molecular weight was calculated according to the structure of the sodium salt of the benzenesulphonic acid with an average  $C_{11.6}$  linear alkyl chain.

Vapour pressure ( $3 \cdot 10^{-13}$  Pa) was estimated for  $C_{12}$ LAS (Lyman, 1985) and calculated ( $17 \cdot 10^{-13}$  Pa) using EPI database by a Syracuse Research Corporation (SRC) software (SIDS, 2005).

Melting and boiling points were calculated using Estimation Program Interface (EPI) database by SRC software (SIDS, 1999).

The octanol-water partition coefficient,  $\log K_{ow}$ , cannot be experimentally measured for surfactants because of their surface-active properties, but only approximately calculated (Roberts, 2000). A  $\log K_{ow}$  of 3.32, for the  $C_{11.6}$ LAS structure was calculated with a method (Leo et al., 1979) modified to take into account the various aromatic ring positions along the linear alkyl chain (Roberts, 1991). This value was used in the aquatic risk assessment carried out in the Netherlands (Feijtel, 1995b). Organic carbon-water partition coefficient ( $K_{oc}$ ) values of 110 and 278 were calculated for  $C_{12}$ benzenesulphonate using regression equations from water solubility and  $\log K_{ow}$  data (Lyman, 1990).

A better indication of this association can, however, be represented by the sludge partition coefficient,  $K_p$  (l/kg), assessed by QSAR analyses (Feijtel et al., 1999; Garcia et al., 2002)). For pure compounds,  $\log K_p$  of 3.0 and 3.5 for  $C_{11}$ LAS and  $C_{12}$ LAS respectively were derived and used in full-scale studies of activated sludge plants (Feijtel, 1995a; Feijtel, 1995b). Laboratory experiments (Temmink et al., 2004) with LAS showed that sorption of the  $C_{12}$ LAS homologue over sludge is a fast and reversible process that can be described by a  $K_p$  value ( $K_p = 3210$  l/kg) in agreement with the above QSAR calculations. Applying the same QSAR for the commercial  $C_{11.6}$ LAS mixture, a  $\log K_p$  value of 3.4 ( $K_p = 2500$  l/kg) can thus be derived and confidently assumed as a measure of the partition of the surfactant between organic matter and water and assimilated to  $K_{oc}$ . An average  $\log K_{oc}$  value of 4.83 was also reported for  $C_{12}$ LAS as a measure of its association with dissolved organic compounds, basically represented by humic acids (Traina et al., 1996).

A critical micelle concentration (CMC) of 0.65 g/l for the commercial  $C_{10-13}$ LAS was reported (Smulders, 2002); the value is in line with that of other anionic surfactants. CMCs were also measured for the different LAS homologues in deionized and hard waters (Garcia et al., 2002).

The reported water solubility and density values were experimentally derived (IUCLID, 1994). pH values in water solutions depend on the free caustic soda content in LAS after neutralisation of the sulphonic acid; in general, 5% water solutions of commercial LAS have pH values in the 7-9 range. Soil/sediment and water sorption coefficients,  $K_d$  (l/kg), were experimentally measured; they ranged from 2 to 300 l/kg, depending on the organic content, and fit the Freundlich equation (Painter, 1992).  $K_d$  sediment values were higher than  $K_d$  soil ones, as a consequence of the higher organic content in sediment than in soil (Marchesi et al., 1991; TGD, 2003).



Using a structure estimation method (Meylan et al., 1991) the Henry's constant for C<sub>12</sub> benzenesulphonate was calculated to be  $6.35 \cdot 10^{-3}$  (Pa · m<sup>3</sup>/mole).

### 3.3 Manufacturing route and production/volume statistics

LAS is produced by sulphonation of LAB with a variety of sulphonating agents. In the past, oleum (fuming sulphuric acid), as well as sulphuric acid were the predominant agents used either in batch reactors or in the so-called "cascade" systems. The sulphonation technology, however, has been considerably improved since the mid 60s and nowadays, although oleum is still used, modern falling film reactors (FFR) (mono-tube or multi-tube) and SO<sub>3</sub> gas are the state of art of the technology in most of the sulphonation facilities in Europe. In these modern plants both the sulphonation of LAB and the sulphation of fatty alcohols are normally practised.

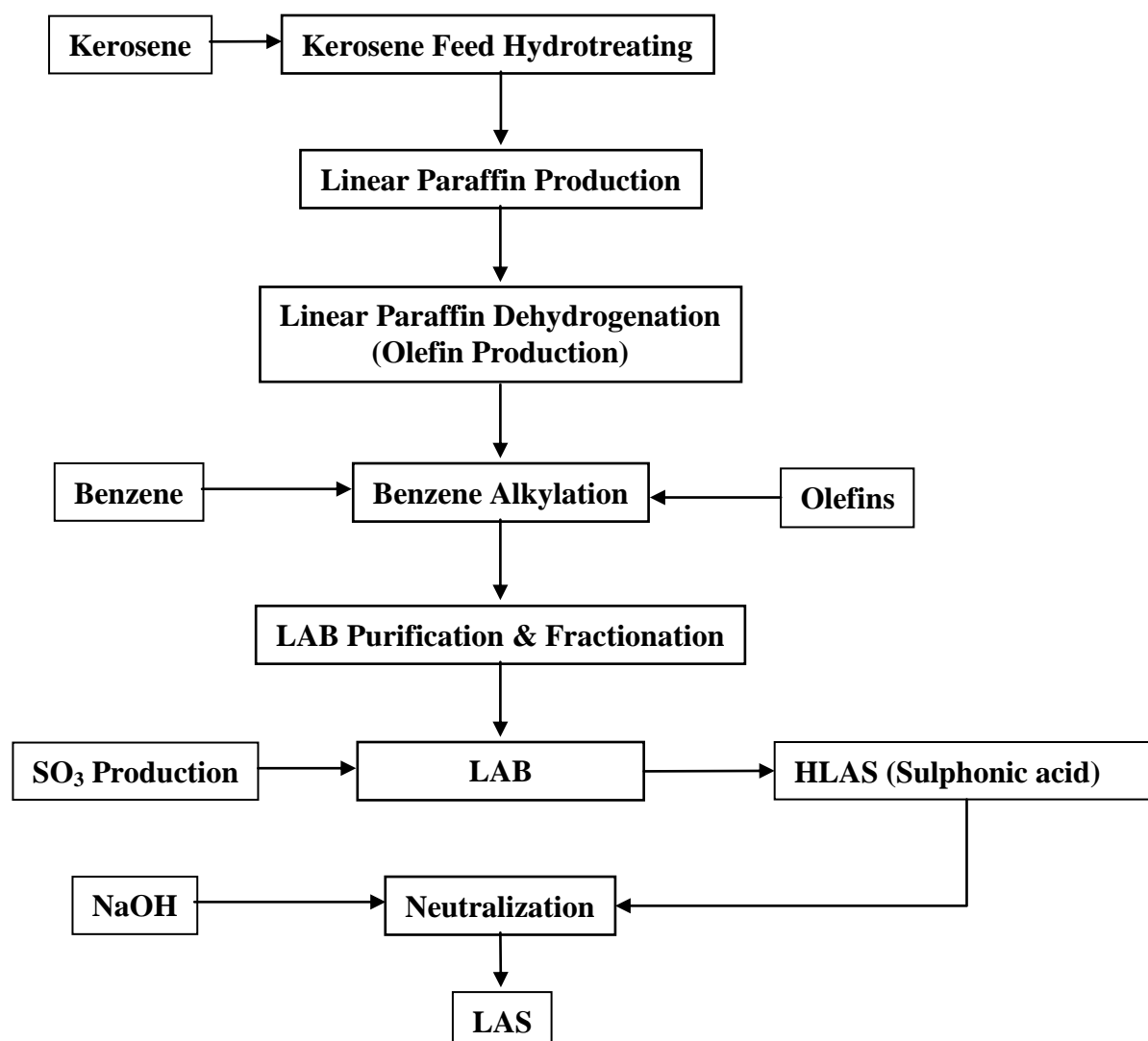
LAB, the precursor of LAS, is manufactured in large scale industrial processes by alkylating benzene with linear mono-olefins or alkyl halides such as chloro-paraffins by using HF or AlCl<sub>3</sub> as the alkylation catalyst (Cavalli et al., 1999b), and recently also over heterogeneous solid super-acids in a fixed-bed reactor (Erickson et al., 1996). LAB production quality, as measured by its bromine and colour indexes as well as by impurities and alkyl chain linearity, has been enhanced over time following significant technological improvements (Marr et al., 2000). Alkylation with AlCl<sub>3</sub> was the first commercial process used in the mid 60s when branched dodecylbenzene (DDB) was replaced by LAB. At the end of the 60s the HF technology was applied for the first time and immediately it became the preferred technology to be installed in the world to produce LAB.

In the mid 90s a new alkylation technology based on heterogeneous catalyst in a fixed-bed reactor, Detal®, appeared on the market (Berna et al., 1994) and was rapidly adopted, as testified by several new units recently installed with this technology. The new technology offers considerable advantages over the old ones, namely: process simplification, elimination of acids handling and disposal (HF, HCl) as well as an overall production yield improvement and improved LAB quality. Production of commercial LAS involves a series of processes as shown schematically in the below scheme.

Total LAB world production capacity in the year 2005 is estimated to be more than 3 million tons, with a split by technology as follows: 75 % HF, 5% AlCl<sub>3</sub>, and 20 % fixed-bed. In Europe, in the year 2005, the estimated installed LAB capacity was around 600 kt/y with a corresponding demand of 325 kt/y (ECOSOL, 2005; CESIO, 2005).

The result of sulphonating LAB is the formation of alkylbenzene sulphonic acid, which has the consistency of a liquid with a high active content, >97% by titration with hyamine (ISO 2271; EN 14480), containing about 1% of unsulphonated matter and 1-2% of H<sub>2</sub>SO<sub>4</sub> (IUCLID, 1994; Schönkaes, 1998). It represents commercially the most important supply form. The acid is then neutralised with a base to give the final LAS surfactant salt. Sodium neutralised LAS is by far the predominant grade. As salt, it can also be supplied in various forms and active contents, for example as paste (50-75%) and powder (80-90%) (Schönkaes, 1998).

## Processing Steps in LAB-LAS Production



### 3.4. Consumption scenario in Europe

The most recent and realistic market survey was completed by the Ecosol companies (ECOSOL, 2005), which estimated a total consumption tonnage of about 430 kt for the year 2005, with a breakdown by household applications of about 350 k, corresponding to more than 80% of the total according to an independent survey of AISE companies.

Table 3: Tonnage consumption estimates of LAS in Europe in 2005

Survey	Total kt	Household Kt
ECOSOL	430	350 (>80% vs. total)

The present focused risk assessment models the use of the highest realistic LAS figure available for the household products, namely 350 kt/y. In addition, the reported monitoring data, related to total

tonnage consumption and degradation in the environment, have been used in the final higher tier risk assessment.

### **3.5 Use application summary**

Most of LAS European consumption is in household detergency (>80%). Important application products are laundry powders, laundry liquids, dishwashing products and all purpose cleaners. The remainder of the LAS (<20%) is used in Industrial and Institutional (I&I) cleaners, textile processing as wetting, dispersing and cleaning agents, industrial processes as emulsifiers, polymerisation and in the formulation of crop protection agents.

## **4. *Environmental risk assessment***

The extensive body of research studies on the environmental properties of LAS present in the literature is reported below.

### **4.1 Environmental exposure assessment**

#### **4.1.1 Biotic and abiotic degradability**

##### **Aerobic biodegradation in aqueous medium**

LAS primary biodegradation is the transformation induced by microorganisms with formation of sulpho phenyl carboxylates (SPCs) as biodegradation intermediates (Swisher, 1987). This biodegradation stage corresponds to the disappearance of the parent molecule and to the loss of interfacial activity and toxicity towards organisms present in the environment (Kimerle et al., 1977; Kimerle, 1989). The change of the interfacial activity of the surfactant during biodegradation has much more importance on the aquatic toxicity than the biodegradation as measured, for example, by the biological oxygen demand (BOD); that was shown by a recent detailed study on the relation between interfacial activity and aquatic toxicity during primary LAS biodegradation (Oya et al., 2010).

Biodegradation proceeds further with i) the cleavage of the aromatic ring and the complete conversion of LAS and SPCs into inorganic substances ( $H_2O$ ,  $CO_2$ ,  $Na_2SO_4$ ) and ii) the incorporation of its constituents into the biomass of micro-organisms (ultimate biodegradation) (Karsa et al., 1995).

One of the first evidences that the alkyl and ring portions of LAS can extensively biodegrade and convert to  $CO_2$  in the environment was shown in a STP simulating laboratory equipment using a  $^{14}C$  ring-labelled commercial product and some pure unlabelled homologues (Nielsen and Huddleston, 1981). The primary biodegradation of LAS, measured by MBAS (Methylene Blue Active Substance) or by specific analytical methods such as HPLC (High Performance Liquid Chromatography), in any OECD tests (OECD, 1993), is >99% (EU Commission, 1997). The ultimate biodegradation measured by DOC (Dissolved Organic Carbon) is in a range going from 80% to >95% for CAS (Continuous Activated Sludge) simulation tests (OECD 303 A), and in the 95-98% range for inherent tests (OECD 302) (EU Commission, 1997).

CAS simulation tests (OECD 303 A) were run for the commercial LAS product in the 9-25°C temperature range (Prats et al., 2003). The acclimation lag phase was significantly different at the various temperatures, being longer at lower temperatures. The percent LAS removal measured by MBAS and HPLC, however, was always similar and high (>95%) in all cases, indicating that the

microorganism community can also reach a proper acclimation and that kinetics are also adequate at low temperatures (Prats et al., 2006; Leòn et al., 2006). These results are in agreement with some stream mesocosm studies which concluded that the mineralization of surfactants under realistic environmental conditions, where various algal species are acclimated following natural temperature fluctuations, was at least maintained and often increased during significant seasonal decreases in temperature (Lee et al., 1997).

The commercial LAS product is readily biodegradable (EU Commission, 1997). The 10-day window is not deemed necessary for assessing ready ultimate biodegradability of surfactants in detergents (CSTEE, 1999). However, in the literature LAS is reported to pass the 10-day window rule as shown by: i) a comparative CO<sub>2</sub> evolution study (Ruffo et al., 1999; Anon, 2002), ii) OECD 301 F tests following the biodegradation by O<sub>2</sub>-consumption and specific C<sub>12</sub>LAS analysis (Temmink et al., 2004) and iii) recent tests run according to the GLP principles, namely, CO<sub>2</sub> evolution test following OECD 301B (LAUSa, 2005), DOC die-away test following OECD 301A (LAUSb, 2005) and mineralization under ISO 14593/1999 test in compliance with the Detergent Regulation 648/2004 (Lòpez et al., 2005). The formation of persistent biodegradation intermediates can be excluded as demonstrated by high tier tests (Gerike et al., 1986; Moreno et al., 1991; Cavalli et al., 1996b). Biodegradation intermediates, i.e. the sulpho phenyl carboxylates (SPCs), are not persistent and their toxicities are several orders of magnitude lower than that of the parent molecule (Kimerle et al., 1977).

Considering the absence of persistent metabolites and the relatively low toxicity of the transient degradation products, the rate of primary biodegradation, rather than that of the ultimate biodegradation is the relevant parameter for risk assessment purposes. Specific analytical methodologies based on High Performance Liquid Chromatography (HPLC), Gas Chromatography/Mass Spectrometry (GC/MS) and Liquid Chromatography/Mass Spectrometry (LC/MS) have been developed for LAS, which provided kinetic data relevant for exposure assessments (Matthijs et al., 1987; Trey et al., 1996; Di Corcia et al., 1999). Relevant kinetics of LAS biodegradation were obtained in a die-away laboratory test applying innovative testing procedures to radio-labelled materials, measuring <sup>14</sup>CO<sub>2</sub> evolution by Liquid Scintillation Counting (LSC) and following the biodegradation by Radio Thin-layer Chromatography (RAD-TLC) (Federle et al., 1997). In these studies, using river water as test medium, the primary biodegradation rate was approximately  $k = 0.06 \text{ h}^{-1}$  ( $t_{0.5} = \text{ca.} 12 \text{ h}$ ) (Itrich et al., 1995) and about 10-15 times lower than that found using activated sludge as test medium (Federle et al., 1997).

Field studies (further described in Section 4.1.3), carried out in some rivers under realistic environmental conditions specifically to measure in-stream removal kinetics of LAS, showed  $t_{0.5}$  in the 1-3 h range indicating that kinetics are faster than those displayed in laboratory studies (Takada et al., 1992; Schröder, 1995; Fox et al., 2000). This is due to the more favourable biodegradation conditions in the real environment vs. those reproduced in laboratory.

Considering the above available field data, **a protective primary biodegradation half-life of 3 hours in aqueous medium was considered in the present risk assessment.**

### **Biodegradation under anaerobic conditions**

#### *Results with Standard Tests that Model Anaerobic Sludge Digesters*

In the existing laboratory screening and simulation tests (ECETOC, 1994; OECD TG 307, 2002; OECD TG 308, 2002; OECD TG 311, 2006; ISO 11734: 1995; ISO 13641-1,-2: 2003), which are extensively reviewed in literature (ERASM, 2007; Berna et al., 2007; Berna et al., 2008), ultimate biodegradation was measured by determining the final gas production (CO<sub>2</sub> and CH<sub>4</sub>) after about two months of incubation. In these studies LAS did not show any significant biodegradation (Steber et al., 1989; Steber, 1991; Federle et al., 1992;; Gejlsbjerg et al., 2004; Garcia et al., 2005).

Another approach has been recently proposed to assess the anaerobic biodegradation of substances as they relate to sewage treatment. This approach is based on OECD guideline (OECD TG 314, 2008). This standard describes an analytical procedure made by a set of five separate but complementary simulation tests, which assess the primary and ultimate biodegradation of chemicals in the sewer wastewater, in the secondary treatment of the activated sludge system, in the anaerobic sludge digester, in the treated effluent and surface water mixing zone, and in the untreated wastewater directly discharged to surface water. The third test (test C) evaluates biodegradation during anaerobic sludge digestion, in particular aims to demonstrate whether chemicals have the potential for anaerobic biodegradation or not. LAS has been tested with this method: results confirm the absence of anaerobic biodegradation (Procter & Gamble, 2008).

**Conclusion:** LAS does not pass standard tests for anaerobic biodegradation. These tests model anaerobic sludge digesters. The lack of LAS biodegradation in these tests is consistent with the lack of LAS biodegradation noted in anaerobic sludge digesters. Nonetheless, some studies suggest that LAS can biodegrade under anaerobic conditions, but low bioavailability prevents any substantial biodegradation in wastewater treatment plant reactors (Angelidaki et al., 2000a; Mogensen et al., 2003). LAS anaerobic biodegradation has been demonstrated under laboratory and field conditions using other test methods (see below).

#### *Risk Perspective*

The preferred method for disposal of sewage sludge is use as a soil fertilizer. The following information is relevant when considering the fate of LAS in sludge-amended soil:

- 1) Biodegradation under strict anaerobic conditions was shown to have little direct ecological relevance (Heinze et al., 1994; ERASM 2007) and are not formally considered in the EUSES modelling program (see 4.1.4).
- 2) In oxygen-limited conditions, which occur in the real world, LAS biodegradation can initiate and then continue in anaerobic conditions (Larson et al., 1993; Leon et al., 2001).
- 3) Field testing takes precedence over simulation test data. There is a very significant amount of field monitoring data available for LAS in agricultural soils (Jensen et al., 2007; Schowanek et al., 2007)

In addition, the opinion of the Scientific Committee on Health and Environment Risks (SCHER), a committee of experts who serve an advisory role within the European Commission (EC), on the environmental risk posed by detergent surfactants that are poorly biodegradable under anaerobic conditions, such as LAS, is as follows:..."A poor biodegradability under anaerobic conditions is not expected to produce substantial modifications in the risk for freshwater ecosystems as the surfactant removal in the STPs seems to be regulated by its aerobic biodegradability" (SCHER, 2005). This statement was again confirmed by SCHER in its opinion of 2008: "The LAS-HERA report of 2004 contained no recent publications which affected the conclusion of SCHER in its opinion of 2005. Similarly recent publication, later than 2004 (Garcia et al., 2005; Garcia et al. 2006a and b; references cited in LAS-HERA report of 2007), did not give grounds for any change of that opinion" (SCHER, 2008).

**As a consequence, the requirement of ultimate biodegradability under anaerobic conditions cannot be considered an effective measure for environmental protection.**

A specific risk assessment in anaerobic environments would include effects on anaerobic bacteria in anaerobic digesters. It has been shown that LAS at concentrations up to 30 g/kg<sub>dw</sub> sludge does not affect the microbial processes in these digesters (Berna et al., 1989). The LAS effect on the anaerobic sludge digestion process was investigated showing that toxicity on the anaerobic microorganisms depended on the concentration of the bioavailable LAS homologues in the liquid phase of the STP anaerobic digesters; an EC<sub>50</sub> of 14 mg/l was calculated (Garcia et al., 2006b). Poor

primary LAS degradation in anaerobic discontinuous systems was confirmed showing also that the inhibition extent of the biogas production was significantly related to the sludge used as inoculum (García et al., 2006a).

#### *Results with Other Test Methods, Other Anaerobic Digesters and Tests that Model Other Environmental Compartments*

Consideration of the LAS structure suggests that it should be anaerobically biodegradable. First, the LAS structure consists of a sulfonate group attached to the aromatic ring. Certain bacteria are capable of biodegrading such compounds and using them as a sole sulphate source. This has been demonstrated for LAS (Denger et al., 1999).

In addition, LAS has a long alkyl chain (C<sub>10</sub>-C<sub>13</sub>). Long alkyl chains are known to be anaerobically biodegradable by sulphate-reducing, denitrifying and methanogenic bacterial communities (review in Wentzel et al., 2007). LAS anaerobic biodegradation has been reported in the following studies:

- 1) In a modified standard test for anaerobic biodegradation, loss of parent LAS is observed after several months of incubation (Prats et al., 2000a).
- 2) In continuous stirred tank (CST) reactors, 14-25% biodegradation is observed (Angelidaki et al., 2000b; Haggensen et al., 2002)
- 3) In upflow anaerobic sludge blanket (UASB) reactors, 5-44% biodegradation is observed (Sanz et al., 1999; Mogensen et al., 2003).

The most complete set of experiments demonstrating LAS anaerobic biodegradation is on sulphate-reducing marine sediments (Lara-Martin et al., 2007; Lara-Martin et al., 2008; Lara-Martin et al., 2010). Laboratory experiments, performed on anoxic marine sediments spiked with 10-50 ppm of LAS, showed that degradation is feasible, reaching a value of 79% in 165 days, with a half-life time of ca. 90 days. The anaerobic process was also observed in the field with several marine sediment samplings at anoxic depths in the sedimentary column. LAS concentrations in pore waters decreased sharply and the biodegradation intermediates (SPC) reached the maxima. These observations provide the first real evidence of partial degradation of LAS under anaerobic conditions (Lara-Martin et al., 2007; Lara-Martin et al., 2008). A more recent paper claimed to provide for the first time an anaerobic biodegradation pathway for LAS (Lara-Martin et al., 2010).

#### **Biodegradation in soil**

Several measurements of LAS in sludge-amended soil from both laboratory and field studies have been carried out and are reviewed in the literature (De Wolf et al., 1998; Jensen, 1999; Cavalli et al., 1999a). These investigations were performed, after application of sludge containing LAS to soil usually at rates higher than that recommended in agriculture, maximum 5 t DS (Dry Solids)/ha/y (TGD, 2003). For example, the annual sludge spreading averaged 6 t/ha in the UK (Holt et al., 1989; Waters et al., 1989), 32 t/ha in Spain (Berna et al., 1989; Prats et al., 1993), 13.5 t/ha in Switzerland (Marcomini et al., 1988) and 6 t/ha in Germany (Matthijs et al., 1987). In all these studies the calculated LAS removal corresponded to half lives in the range of  $t_{0.5} = 3-33$  days.

The most reliable results in the laboratory were obtained by investigating mixtures of sludge and LAS-spiked soils using <sup>14</sup>C materials, measuring ultimate biodegradation. LAS mineralization rates corresponding to  $t_{0.5} = 13-26$  days (Figge and Schöberl, 1989) and  $t_{0.5} = 7.0-8.5$  days (Gejlsbjerg et al., 2001) were estimated. Mineralization with  $t_{0.5} = 2.1-2.6$  days was obtained after a lag time of 1.9-2.5 days at 10 mg/kg<sub>dw</sub> LAS concentration in soil, which is the highest expected environmental concentration of the surfactant in an agricultural land (Gejlsbjerg et al., 2003).

Laboratory sludge-soil mixtures with <sup>14</sup>C-labelled LAS at concentrations in the µg/kg<sub>dw soil</sub> range, corresponding to predicted steady concentrations (at least after a waiting period of 30 days from sludge application) of the surfactant in sludge-amended soil, were also investigated (Gejlsbjerg et

al., 2004). After relative long lag times (ca. 2 weeks), LAS was mineralized rapidly and extensively showing two phase kinetics: a first rapid mineralization ( $t_{0.5} = \text{ca. } 2 \text{ days}$ ) followed by a slow mineralization phase ( $t_{0.5} = 7.9 \text{ days}$ ), the latter likely governed by sorption and desorption processes in the soil. Even subsurface soils, sampled below a septic system drain field and investigated in laboratory sorption and biodegradation studies using groundwater and radiolabeled materials, showed to have the potential to mineralize LAS (ultimate  $t_{0.5}$  from 0.32 to 8.7 d) (Doi et al., 2002). Other LAS leaching properties in soils and groundwater were investigated to develop a mathematical model for septic systems to predict the fate and transport of consumer product ingredients (McAvoy et al., 2002).

However, most laboratory studies and all field monitoring studies in sludge-amended soil measure the disappearance of LAS, estimating, thus, the primary biodegradation.

In the laboratory tests it was shown that for soil spiked with aqueous LAS and LAS-spiked sewage sludge, the disappearance (primary biodegradation) of the surfactant was more than 73% after 2 weeks (Elsgaard et al., 2001b). A soil mesocosm study showed that the primary degradation of LAS was rapid with  $t_{0.5}$  of 1-4 days (Elsgaard et al., 2003). A field study, at sludge application rates close to those recommended in agriculture (equal or below  $5 t_{\text{dw}}/\text{ha}/\text{y}$ ), estimated  $t_{0.5}$  values in the range of 3-7 days (Küchler et al., 1997).

Accurate data for degradation of LAS in sludge-amended soil under realistic field conditions were reported by Mortensen et al., 2001. Its degradation in soil increased by the presence of crop plants with soil concentrations decreasing from  $27 \text{ mg}/\text{kg}_{\text{dw}}$  to  $0.7\text{-}1.4 \text{ mg}/\text{kg}_{\text{dw soil}}$  at harvesting time after 30 days ( $t_{0.5} < 4\text{d}$ ).

Considering the above available field data, **a conservative protective primary biodegradation half-life of 7 days in agricultural soils was considered in the present risk assessment.**

### Hydrolysis and photolysis degradation

Reactions of hydrolysis (Cross, 1977) and photolysis (Matsuura et al., 1970; Venhuls et al., 2005) of LAS are described in literature (Table 4) in conditions not relevant to the environment. The corresponding results are, thus, not considered in the present assessment.

The set of data on LAS biodegradation properties relevant to this risk assessment are summarized in Table 4.

Table 4: Biodegradation properties

LAS	Protocol	Results	References
Screening, confirmatory	OECD 301 D OECD 303 A	>99 (% primary biod.)*	EU Commission, 1997
Ready test	OECD 301 A, B, D, E, F ISO 1493/1999	Readily biodegradable >70 (% DOC removal) >60 (% CO <sub>2</sub> evolution) >60 (% O <sub>2</sub> uptake)	EU Commission, 1997 Ruffo et al., 1999 Temmink et al., 2004 LAUS, 2005a-b López et al., 2005
Inherent test	OECD 302 A, B	95-98 (% DOC removal)	EU Commission, 1997
Simulation test	OECD 303 A	80->95 (% DOC removal)	EU Commission, 1997

Biodegradation rate in activated sludge	Die-away	$t_{0.5} = 0.6-0.7$ h (prim. biod.) $t_{0.5} = 1.3-1.4$ h (ultim. biod.)	Federle et al., 1997
Biodegradation rate in river water	Die-away Die-away River monitoring	$t_{0.5} = 12$ h (prim. biod.) $t_{0.5} = 18$ h (ultim. biod.) $t_{0.5} = 1-3$ h (prim. biod.)	Itrich et al., 1995 Itrich et al., 1995 Fox et al., 2000
Anaerobic biodegradation	ECETOC  Research study	ca.0 (% ultim. biod.)  5-44 (% prim. biod. in UASB reactors )	AISE/CESIO, 1994  Mogensen et al., 2003
Biodegradation rate in soil	Field study  Laboratory study	$t_{0.5} = 1-7$ d (prim. biod.)  $t_{0.5} = 2-26$ d (ultim. biod.)	Küchler et al., 1997 Elsgaard et al., 2003  Figge et al., 1989 Gejlsbjerg et al., 2001, 2003, 2004
Hydrolysis	Research study	Decomposition: 60-70% in presence of inorganic acids at 150-200°C	Cross, 1977
Photolysis	Research study	Degradation: 80-95% under mercury lamp (200-450 nm)	Matsuura et al., 1970 Venhuls et al., 2005

(\*) measured by MBAS and by additional HPLC analysis

## 4.1.2 Removal

### Sewers

LAS removal rates in sewers, due to a combination of biodegradation, adsorption and precipitation, were measured during field studies in different countries up to a degree of 68% (Moreno et al., 1990; Matthijs et al., 1999). Laboratory studies have demonstrated that the concentration of all surfactants can be significantly reduced in sewers, depending on the length of the sewer, travel time and the degree of microbial activity present in the sewer (Matthijs et al., 1995).

### Laboratory CAS systems

Accurate confirmatory CAS data, using MBAS and specific analytical methods (such as HPLC) or  $^{14}\text{C}$  measurements to determine the LAS removal rate, are available (Schöberl et al., 1988; Cavalli et al., 1996a; Leon et al., 2006). In these tests the removal rate of the parent surfactant was always >99%.



## Sewage Treatment Plants

LAS removal in Activated Sludge Sewage Treatment Plants, (as-STPs), has been documented in several studies and found to be mostly in the 98-99.9% range (Berna et al., 1989; Painter et al., 1989; Waters et al., 1995; Cavalli et al., 1993; Matthijs et al., 1999). This elimination efficiency can be further increased when membrane biological reactors (MBR) will become economically available (Terzic et al., 2005). The LAS removal in as-STPs, measured in five European countries, averaged 99.2% (6 records in the range 98.5-99.9%) (Waters et al., 1995) and 99.4% (4 records in the range 98.9-99.9%) (Holt et al., 2003).

Total LAS removal in Trickling Filter Sewage Treatment Plants (tf-STPs), are lower and more variable and were found in the 89.1-99.1% range (24 records) in Europe with an average value of 95.9% (Holt et al., 2003). These values are higher than those reported for tf-STPs in USA where average removals of 83% (Trehy et al., 1996) and 77% (McAvoy et al., 1993) were recorded.

The following proportions are based on as-STP mass balance studies: 80-90% degraded, 10-20% adsorbed onto sludge and about 1% released to surface waters (Berna et al., 1989; Painter et al., 1989; Cavalli et al., 1993; Di Corcia et al., 1994).

**For EUSES modelling assessment, Predicted Exposure Concentrations (PECs) were calculated assuming 79% degradation, 20% to sludge and 1% release to water (see 4.1.6).**

The dataset of removal rates relevant to this risk assessment are summarised in Table 5.

Table 5: Removal data

LAS	Results	References
Removal in CAS test (%)	>99	Schöberl et al., 1988 Cavalli et al., 1996
Total STP removal (%)	as-STP: 98-99.9 (range) as-STP: 99.2 (arithmetic mean)	Matthijs et al., 1999 Waters et al., 1995
as-STP: degraded (%)	80-90	Berna et al., 1989 Painter et al., 1989 Cavalli et al., 1993 Di Corcia et al., 1994
as-STP: released to water (%)	ca. 1	
as-STP: adsorption into sludge in (%)	10-20	

### 4.1.3 Monitoring studies

Several monitoring studies on LAS in the different environmental compartments are available in Europe. Here below monitoring data for surface waters, ground waters, sludge, soils and sediments are summarized.

#### Surface waters

The present aquatic risk assessment refers specifically to the European monitoring project carried out in five different countries (UK, Germany, Netherlands, Spain, Italy), using a common and agreed protocol in the context of the Dutch risk assessment of surfactants (Feijtel et al., 1995b). The results of this multi-years EU monitoring project were consistent with previous monitoring studies (Berna et al., 1989; Painter et al., 1989; Cavalli et al., 1993) and with other recent monitoring programmes in Europe (Holt et al., 2003). The results illustrate well the actual European LAS content in the as-STP effluents and sludge as well as in the corresponding receiving rivers (Schöberl et al., 1994; Di Corcia et al., 1994; Sánchez Leal et al., 1994; Feijtel et al., 1995a; Holt et al., 1995; Waters et al., 1995; Matthijs et al., 1999).

In the EU monitoring study project LAS levels in raw sewage ranged from 1 to 15 mg/l (Feijtel et al., 1995b; Matthijs et al., 1999). In the same EU project LAS effluent concentrations under normal as-STP operating conditions were altogether in the 8-220 µg/l range with an arithmetic mean of 42.8 µg/l (46 records), considering all the available results.

In the receiving waters downstream the above as-STP effluents, just after the mixing zone, the LAS concentration was in the <2-47 µg/l range with an arithmetic mean of 14.2 µg/l (23 records) (Feijtel et al., 1995b; Matthijs et al., 1999). The highest LAS concentration (47 µg/l) would decrease to <2 µg/l in one day, considering a conservative in-stream biodegradation half-life of 3 hours (see par. 4.1.1).

LAS environmental fingerprints in effluent and surface waters differ from the composition of the commercial material. The relative ratio of the various homologues detected in the aquatic environmental samples is as follows: C<sub>10</sub>:C<sub>11</sub>:C<sub>12</sub>:C<sub>13</sub> = 45:30:23:2 with an average carbon number of 10.8 (Prats et al., 1993; Cavalli et al., 1993; Di Corcia et al., 1994; Tabor et al., 1996). That is a consequence of two processes: i) biodegradation in the water phase which is faster for the higher homologues and ii) adsorption into sediments and suspended solids which is more pronounced for higher homologues.

In another comprehensive European monitoring programme, carried out in the context of the GREAT-ER project (Geography-Referenced Exposure Assessment Tool for European Rivers), thousands of effluent samples from different STPs and samples of river waters were measured in UK for their LAS content over a 2-year period (Holt et al., 2003). All effluents from as-STPs were in the 7-273 µg/l range; those with an additional tertiary treatment were found below 50 µg/l.

In US monitoring studies LAS concentrations in river waters below STP mixing zones were also generally found below 50 µg/l (McAvoy et al., 1993; Trehy et al., 1996; Tabor et al., 1996).

A US study conducted to assess a weight of evidence (WoE) risk of alkyl sulfates (AS), alkyl ethoxy sulfates (AES) and LAS was based on accurate monitoring of STP streams located in 3 different sites (Sanderson et al., 2006). The total LAS concentrations were in the range 2.75-3.96 mg/l in influents, 1.3-2.9 µg/l in effluents and 0.26-3.8 µg/l in the receiving river waters.

A study to evaluate the validity of as-STP fate models was carried out, monitoring the C<sub>12</sub>LAS concentrations under controlled and well-established conditions in a pilot-scale municipal as-STP. C<sub>12</sub>LAS concentrations were 2-12 mg/l in influents, 5-10 µg/l in effluents and 37-69 mg/kg<sub>dw</sub> in the waste aerobic sludge. The removal of the LAS homologue (>99%) was totally ascribed to biodegradation (Temmink et al., 2004).

The tf-STP effluents, on the contrary, have usually higher and more variable LAS concentrations because these plants are not so efficient as the (as)-STPs. BOD<sub>5</sub> removals are in the 85-95% range for tf-STPs (Holt et al., 2000), whereas they are always >95% for as-STPs. tf-STP effluent LAS concentrations, in flow proportional composite samples, were in the 40-430 µg/l range with an average value of 240 µg/l in Europe (Holt et al., 2000; Holt et al., 2003) and up to 1.5 mg/l in the US (Rapaport et al., 1990; McAvoy et al., 1998).

In river waters receiving effluents either from tf-STPs (Fox et al., 2000) or from undersized as-STPs (Gandolfi et al., 2000), LAS was shown to be removed rapidly. Downstream the mixing zones of tf-STP, the LAS concentrations were 0.42-0.77 mg/l and decreased to 72 and 33 µg/l at 4.8 and 3.3 km respectively from the tf-STP outfall (Fox et al., 2000). From an undersized as-STP, LAS concentrations in 24-h composite samples were on average 120 µg/l at the mixing zones and 27 µg/l

at 26 km (Gandolfi et al., 2000). These results indicate that in-stream removal is an efficient process and were used to validate a dynamic quality model to assess the fate of xenobiotics in the river water compartment and benthic sediment (Deksissa et al., 2004).

Other types of discharges, including direct discharges, exist in Europe. Downstream these discharges, higher concentrations of BOD, NH<sub>3</sub>, LAS and other contaminants can be monitored. According to some studies (McAvoy et al., 2003; Dyer et al., 2003), the relative in-stream removal of LAS is higher than the removal of BOD and therefore the impact of untreated discharges on the receiving ecosystem is not caused by LAS but rather by low dissolved O<sub>2</sub> and high unionised ammonia.

As recommended by the TGD (TGD, 2003), only monitoring data of river waters receiving effluents from as-STPs, as well as the highest concentrations found in the European monitoring studies, were considered relevant to the present risk assessment.

**Conclusion: PEC effluent (PEC<sub>STP</sub>) = 0.27 mg/l; PEC river waters = 0.047 mg/l.**

### **Ground waters**

No LAS monitoring data in ground waters are available for Europe. In samples collected in the USA, LAS concentrations were below the detection limit in several monitored wells drilled in an area near a pond system exposed to high concentrations of detergent chemicals for more than 25 years (Larson, 1989). LAS concentrations in ground waters, 500 m downstream a sewage infiltration, were below the analytical detection limit (<10 µg/l). In one well, using an improved analytical methodology, a maximum LAS concentration of 3 µg/l was recorded (Field, 1992).

### **Sludge**

Measured LAS concentrations in sewage sludge have been reviewed (De Wolf et al., 1998; Jensen et al. 1999; Cavalli et al. 1999; Fraunhofer, 2003; Leschber, 2004; Jensen and Jepsen, 2005; Schowanek et al., 2007). Typical LAS concentrations in aerobic sludge are <0.5 g/kg<sub>dw sludge</sub>, higher LAS concentrations are noted in anaerobic sludge (<1 g/kg<sub>dw sludge</sub> up to 30 g/kg<sub>dw sludge</sub>). The highest LAS concentrations in anaerobic sludge (ca. 30 g/kg<sub>dw sludge</sub>) were found in one specific Spanish region in the presence of a very high water hardness (>500 mg/l as CaCO<sub>3</sub>) (Berna et al., 1989). Water hardness data collected by AISE companies are available for Europe and indicate that on average 13% of the European population use water with hardness <70 mg/l, 33% with medium hardness (70-212 mg/l) and 53% with hardness >212 mg/l (Jensen et al., 2006). This high LAS value in Spanish sludge is clearly an outlier.

Although these reports cover LAS concentrations in sludge for a number of wastewater treatment plants in different European countries, they do not represent the situation in one specific country. A comprehensive survey of LAS measurements in aerobic and anaerobic sludge was reported (Jensen and Jepsen, 2005) from the ongoing monitoring program of pollutants in sludge in Denmark. LAS concentrations are annually measured and reported to the Danish EPA for approximately 1,400 waste water treatment plants in Denmark. This survey allowed to derive the Danish LAS distribution in sludge: a mean concentration of 0.24 g/kg<sub>dw sludge</sub> (0.5 to 1.5 g/kg<sub>dw sludge</sub>; 5<sup>th</sup> to 95<sup>th</sup> percentile) (Jensen et al., 2006).

At the European level, approximate sludge distributions were also calculated based on literature data over the time period 1988-2006 (Schowanek et al., 2007). The result of the distribution of the anaerobic sludges (ca. 155 records) was a mean of 5.56 g/kg<sub>dw sludge</sub> (0.49 to 15.07 g/kg<sub>dw sludge</sub>; 5<sup>th</sup> to 95<sup>th</sup> percentile), where the highest point in the data set was the already mentioned Spanish value of ca. 30 g/kg<sub>dw sludge</sub>, a clear outlier.

The LAS homologue distribution in sludge is approximately in the mole ratio  $C_{10}:C_{11}:C_{12}:C_{13} = 7:24:39:30$  with an average carbon number of 11.9, as a consequence of a preferential adsorption of higher homologues (Berna et al., 1989; Cavalli et al., 1993; Di Corcia et al., 1994).

It is worth taking into account possible differences of LAS concentration in wet sludge, freshly produced at STP, from that in dry sludge, aged and dried before its use in agriculture (several months after). It was found that the LAS concentration in the bulk of dry sludge could drop by 74% compared to that of wet sludge (Carlsen et al., 2002). Removal of LAS from sludge can also effectively be performed by composting systems. This methodology for handling sludge in general was extensively discussed in a workshop in Denmark (SPT/EPA, 1999) and was recognised as a useful method to reduce the level of some xenobiotics. Several composting studies have demonstrated that LAS can be removed (>98%) with half-life of 7-9 days (Petersen, 1999; Prats et al., 2000b; Sanz et al., 2006).

**Conclusion: PEC in anaerobic sludge = 5.56 g/kg<sub>dw sludge</sub> (mean 50<sup>th</sup> percentile) and 15.07 g/kg<sub>dw sludge</sub> (95<sup>th</sup> percentile).**

### Soil

Results from several monitoring studies of LAS concentrations in soil are available for various soil types, sludge application rates, and averaging times. For example, concentrations of up to 3.0 mg LAS/kg<sub>dw</sub> were measured in sludge-amended soil at a sludge application rate of 6 t DS/ha/y for extended periods in the UK and Germany (Matthijs et al., 1987; Holt et al., 1989). LAS concentrations in sludge-amended soils were reviewed concluding that they were generally below 20 mg/kg soil, depending on the application rate or sampling time after sludge application (Solbè, 1999). At sludge application rates less than 5 t/ha/y, 30 days after its application, LAS concentrations in soil are expected to be in the low mg/kg range. With sludge application rates higher than those used in the normal agricultural practice (6-10 t/ha/y), LAS concentration in an experimental field of soil-pots with rapes dropped from an initial measured value of 27 mg/kg<sub>dw soil</sub> to 0.7-1.4 mg/kg<sub>dw soil</sub> in soil at harvest time after 30 days (Mortensen et al., 2001).

A series of soils having a known history of sludge amendment and selected to be typical for Denmark were monitored (Carlsen et al., 2002). In regions where the sludge application was carried out according to the prevailing agricultural rules, the concentration of LAS in all soils was found to be <1 mg/kg<sub>dw soil</sub>, well below the soil quality criterion for LAS of 5 mg/kg<sub>dw soil</sub> proposed in Denmark (Jensen et al., 1995). The LAS concentration that can be found in soil at any time after sludge applications, in any case, is always too low to contribute significantly to the mobilization of hydrophobic organic compounds in sludge-amended soil (Haigh, 1996).

**Conclusion: PEC in soil = 1.4 mg/kg<sub>dw soil</sub>.**

### Sediments

Available measured LAS data in fresh water sediments were reviewed (Cavalli et al., 2000). Typical LAS values in sediments below sewage outfalls were found in the 0.5-5.3 mg/kg<sub>dw sed.</sub> range with an arithmetic mean of 2.9 mg/kg<sub>dw sed.</sub> (12 records).

Homologue distributions were also measured for some river sediment samples and the corresponding fingerprint was found similar to that of sludge and soils (Cavalli et al., 2000).

**Conclusion: PEC in sediment = 5.3 mg/kg<sub>dw sed.</sub>**

The set of monitoring data relevant to this risk assessment are summarised in Table 6. The effluent and river data refer to representative EU monitoring studies and to samples collected downstream of (as)-STPs. Most of the data were used in the aquatic risk assessment carried out in the Netherlands (Feijtel et al., 1995b). Sludge and soil data refer to studies developed in the context of the terrestrial risk assessment in Europe (Jensen et al., 2007; Schowanek et al., 2007).

Table 6: Monitoring data

LAS	Results	References
Effluent ( $\mu\text{g/l}$ )	as-STP: 8-220 (range) as-STP: 2-273 (range) as-STP: 42.8 (arithmetic mean) as-STP: 1.3-2.9	Feijtel et al., 1995b Holt et al., 2003 Matthijs et al., 1999 Sanderson et al., 2006
River water ( $\mu\text{g/l}$ )	down as-STP: <2-47 (range) down as-STP: 14.2 (arithmetic mean) down as-STP: 0.3-3.8	Feijtel et al., 1995b Matthijs et al., 1999 Sanderson et al., 2006
Ground water ( $\mu\text{g/l}$ )	0-3	Field et al., 1992
Anaerobic sludge ( $\text{g}/\text{kg}_{\text{dw}} \text{ sludge}$ )	5.56 (median 50 <sup>th</sup> percentile) 0.49-15.07 (5 <sup>th</sup> to 95 <sup>th</sup> percentile)	Schowaneck et al., 2007
River sediment ( $\text{mg}/\text{kg}_{\text{dw}} \text{ sed.}$ )	<1-5.3 (typical range) 2.9 (arithmetic mean)	Cavalli et al., 2000
Soil ( $\text{mg}/\text{kg}_{\text{dw}} \text{ soil}$ )	0.7-1.4, measured at harvest time (30 d) <1, typical agricultural value	Mortensen et al., 2001 Carlsen et al., 2002

#### 4.1.4 Exposure modelling: scenario description

The HERA environmental risk assessment of LAS is based on the Technical Guidance Document for new and existing substances (TGD, 2003). At screening level it makes use of the EUSES programme (EUSES, 2008) to calculate the local and regional exposure to LAS. The total estimated LAS tonnage of 330 kt/y was assumed to follow the down-the-drain pathway to the environment.

The production and formulation releases at local level were not considered because they fall outside the scope of HERA. For the calculation, the HERA exposure scenario was adopted; this scenario assigns 7% of the EU tonnage to the standard EU region, instead of the TGD default 10%, and a factor of 1.5, instead of the TGD default factor of 4, to increase the emissions at local level. These changes introduced by HERA more realistically represent the regional emissions and the local input of substances used in household detergents, as experimentally demonstrated (Fox, 2001). More details and justification of this modification can be found in chapter 2.6 of the HERA methodology document ([www.heraproject.com](http://www.heraproject.com)).

Table 7: HERA exposure scenario

LAS	HERA scenario
Total yearly LAS use in household (HERA scope), kt	350
LAS continental usage going to standard EU region, %	7
Increase factor for local usage	1.5

#### 4.1.5 Substance data used for the exposure calculations

The essential input data used for exposure calculations following the TGD and EUSES are derived from Table 2, 3, 4, and 5, and are summarized in Table 8.

The biodegradation rate in STP is the default value as assumed by TGD for readily biodegradable substances. It should be noted that this rate is not used in the assessment, as the Simple Treat output is overridden by experimental removal data.  $K_{ow}$  is also not considered in the calculations, which are rather based on  $K_{oc}$ .

The biodegradation rates in water and soil are experimentally measured values as reported in Table 4, whereas the biodegradation rates in aerated sediments and in bulk sediments are the default values as suggested in TGD (TGD, 2003).

The (as)-STP data, as measured by mass balance results and reported in Table 5, are the most protective ones for all environmental compartments. For the fraction to sludge, the extreme high value of the range, namely 0.20, was employed (see 4.1.2).

Table 8: Data for exposure calculations

General name	Linear Alkylbenzene Sulphonate (LAS)	References
Description	(C <sub>11.6</sub> H <sub>24.2</sub> )C <sub>6</sub> H <sub>4</sub> SO <sub>3</sub> Na	-
CAS No.	68411-30-3	-
EINECS No.	270-115-0	-
Average molecular weight (g/mole)	342.4	-
Melting point (°C)	277	SIDS, 2005
Boiling point (°C)	637	SIDS, 2005
Vapour pressure at 25 C° (Pa)	$3 \cdot 10^{-13}$	Lyman, 1985
Water solubility (g/l)	250	IUCLID, 1994
Henry's constant (Pa·m <sup>3</sup> /mole)	$6.35 \cdot 10^{-3}$	Meylan et al., 1991
Octanol-water partition coefficient, log $K_{ow}$	3.32	Feijtel et al., 1995b
Organic carbon-water partition coefficient, $K_{oc}$ (l/kg)	2500	Feijtel et al., 1999
Biodegradation rate in STP	$k = 1 \text{ h}^{-1}$ ( $t_{0.5} = 0.693 \text{ h}$ )	EU Commission, 1997
Biodegradation rate in river water (primary)	$k = 0.23 \text{ h}^{-1}$ ( $t_{0.5} = 3 \text{ h}$ )	Fox et al., 2000
Biodegradation rate in soil (primary)	$k = 0.1 \text{ d}^{-1}$ ( $t_{0.5} = 7 \text{ d}$ )	Küchler et al., 1997
Biodegradation rate in oxic sediments	$k = 0.1 \text{ d}^{-1}$ ( $t_{0.5} = 7 \text{ d}$ )	TGD, 2003
Biodegradation rate in bulk sediments	$k = 0.01 \text{ d}^{-1}$ ( $t_{0.5} = 70 \text{ d}$ )	TGD, 2003
STP removal (%)	99	Waters et al., 1995 Berna et al., 1989 Painter et al., 1989 Cavalli et al., 1993 Di Corcia et al., 1994
Fraction to air by STP	0	
Fraction to water by STP	0.01	
Fraction to sludge by STP	0.20	
Fraction degraded in STP	0.79	

#### 4.1.6 PEC calculations

Column A of Table 9 reports values calculated by EUSES v2.1 (EUSES, 2008) on the basis of data in Table 7 and 8, according to the HERA scenario, considering the tonnage used in household applications (350 kt/y). In-sewer removal (50%) was not taken into account in this calculation.

Column B of Table 9 was not obtained by modelling but by using monitoring data. The values given are the high concentrations of the (as)-STP related monitoring findings in each environmental

compartment, as presented in Table 6. The concentrations listed in column B can, thus, be considered the worst-case PEC of a realistic exposure scenario, excluding, as already said in 4.1.3, data related to (tf)-STPs and other discharges where LAS concentrations are only a marker of poor organic matter removal (McAvoy et al., 2003; Dyer et al., 2003). Data in the aquatic compartment are based on the monitoring results of the European project (Matthijs et al., 1999) and supported by the high tier modelling exercise of the GREAT-ER project (Fox et al., 2000; Holt et al., 2003).

The results of scenario A (modelling) and B (monitoring) are within a factor of 2 for all the environmental compartments except for soil. LAS, however, biodegrades during sludge storage, transport and the waiting period (several months) before its application to soil (Carlsen et al., 2002). A conservative degradation rate of 50% for the pre-application period would lead to a calculated soil concentration of 2.8 mg/kg<sub>dw soil</sub>, closer to the highest measured ones (1.4 mg/kg<sub>dw soil</sub>).

Table 9: Calculated environmental LAS concentrations

	A Modelling of household LAS usages	B LAS monitoring data
Local conc., influent, mg/l	23.7	<b>15</b>
Local conc., effluent, (PEC in STP), mg/l	0.237	<b>0.27</b>
Local conc., sludge, g/kg <sub>dw sludge</sub>	12.1	<b>5.56</b> (50 <sup>th</sup> percentile) <b>15.07</b> (95 <sup>th</sup> percentile)
Local PEC in water, mg/l	0.027	<b>0.047</b>
Local PEC in soil (30 d), mg/kg <sub>dw soil</sub>	10.9	<b>1.4</b>
Local PEC in sediment, mg/kg <sub>dw sed.</sub>	1.51	<b>5.3</b>
Regional PEC in water, mg/l	0.004	-

The monitoring data presented in column B were used in the risk assessment.

#### 4.1.7 Bioaccumulation potential

The purpose of the estimation of bioconcentration is to assess whether there is any potential for the chemical to accumulate in organisms to a high degree and hence, for further transfer up the food chain.

In the absence of measured data, the bioconcentration potential for fish, based on the lipid solubility characteristics of chemicals can be estimated based on QSARs (Quantitative Structure Activity Relationships). Due to the relationship between the bioconcentration of a chemical and its lipophilicity it is possible to predict the BCF for a particular organic compound from its octanol/water partition coefficient ( $K_{ow}$ ). However, bioconcentration predictions based on  $K_{ow}$  are restricted to chemicals with a log  $K_{ow}$  <3 and >7. Such predictions are not applicable to surfactants because of their surface active properties. It must be also born in mind that bioconcentration is not a solely hydrophobicity/diffusion-driven process, and as such organismal (ADME) processes, i.e. Absorption, Distribution, Metabolism, Excretion, should as well be considered. Chemicals with a high molecular weight (MW >700) and certain molecular sizes (length, cross sectional diameters) are not likely to cross the biological membranes and therefore their bioconcentration in fish will be limited. Similarly, chemicals which can be metabolized (biotransformed) by an organism will not bioconcentrate to the extent that would be expected if diffusion was the only process involved. Reliable alternative methods already exist and are being further developed to estimate in vitro the absorption and biotransformation potential of chemicals in fish. These methods will finally limit the cost of in vivo bioconcentration tests on thousands of chemicals.

Early experimental studies on bioconcentration of LAS were not appropriate because of the analytical methods based on radio-analysis, which consistently overestimated the parent concentration present in the aquatic organism and consequently the true bioconcentration (reviewed by Tolls et al., 1994).

An in depth research project on bioconcentration of surfactants was completed and concluded that LAS is not bioaccumulative, likely due to biotransformation (metabolic) processes taking place in the fish, and therefore doesn't transfer through the aquatic food chain (Tolls, 1998).

LAS was studied employing a flow-through test system, in line with the OECD guidelines, using *Pimephales promelas* as test fish. Single homologue and isomer representatives of the commercial LAS were synthesised and then tested, determining their uptake and elimination rates in fish. Specific HPLC analysis in the water phase and in the fish body showed that LAS reaches a steady state concentration in the fish body in about 3 days. Biotransformation contributes to more than 40% of the elimination as shown for the C<sub>12</sub>-2-LAS homologue (Tolls et al., 2000). BCF data for the tested LAS standards ranged between 2 l/kg (6-phenyl C<sub>10</sub>LAS) to 990 l/kg (2-phenyl C<sub>13</sub>LAS), allowing calculating the potential BCF of any LAS mixture (Tolls et al., 1997). BCFs were also calculated for the commercial LAS (C<sub>11.6</sub> alkyl chain length) and a representative sample found in river water (C<sub>10.8</sub> alkyl chain length, see 4.1.3). The respective BCFs were 87 l/kg and 22 l/kg, indicating that the bioconcentration potential of LAS is low and is decreased by environmental processes such as biodegradation and absorption (Tolls, 1998).

This has been confirmed recently by Dyer et al. (2008) and ERASM reports ([www.erasm.org/study.html](http://www.erasm.org/study.html)) evaluating the feasibility of *in vitro* assays with surfactants, including C<sub>12</sub>LAS as prediction tools for their biotransformation and, hence, bioconcentration potential. All fish liver *in vitro* systems investigated are capable of transforming rapidly C<sub>12</sub>LAS. The immortalised hepatocytes are less effective as immortalised cells and tend to lose much of their specific activity. It can be concluded that biotransformation (metabolic) processes in the fish are contributing to the lower than predicted bioconcentration potential of LAS in fish.

*Pimephales promelas* and three invertebrates species were caged in streams during a C<sub>12</sub>LAS model ecosystem experimental study (Versteeg et al., 2003). Total C<sub>12</sub>LAS BCFs for the investigated species ranged from 9 to 116 l/kg. In general, bioconcentration was affected by isomer position, exposure concentration, and species. BCF values tended to decrease as isomer position moved from external (e.g., 2-phenyl) to internal (e.g., 5,6-phenyl). BCFs also decreased as exposure concentration increased. BCFs for *Lumbriculus variegatus* exposed to freshwater sediments spiked with the C<sub>12</sub>-2-LAS homologue were measured and found in the range 0.5-4.7 l/kg depending on the sediment organic content (Mäenpää and Kukkonen, 2006).

**Bioconcentration potential estimation: i) ca. 87 l/kg for commercial LAS mixture (C<sub>11.6</sub> alkyl chain length); ii) ca. 22 l/kg for LAS in river water (C<sub>10.8</sub> alkyl chain length).**

## 4.2 Environmental effects assessment

### 4.2.1 Ecotoxicity

The toxicity database of the present LAS risk assessment basically refers to that used in the risk assessments carried out for the aquatic compartment in the Netherlands (AISE/CESIO, 1995; Van de Plassche et al., 1999a) and to that used in a revisited risk assessment for the terrestrial environment (Jensen et al., 2007).

Robust summaries and validity ratings based on Klimisch scores have been validated for all studies during the compilation of this risk assessment and are available ([www.lasinfo.org](http://www.lasinfo.org)).



#### 4.2.1.1 Aquatic ecotoxicity

The toxicity database for LAS (Kimerle, 1989; SDA, 1991; Painter, 1992; IPCS, 1996) is very rich and well documented. A comprehensive review of environmental information for the aquatic compartment that includes all data of the above mentioned literature is the BKH report (BKH, 1993). This report collects 749 records of toxicity data for LAS, specifically collated for an aquatic environmental risk assessment in the Netherlands (AISE/CESIO, 1995; Feijtel et al., 1995b; Van de Plassche et al., 1999a). The database covers several taxonomic groups; intra- and inter-species variability is large, particularly in case of algae. The reason is due to the fact that data refer to different individual compounds and mixtures of LAS and also to differences in test design as well as to the large range of species sensitivity.

In the aquatic environment, different homologues and isomers are present. Each of these components has a different degree of ecotoxicity, with the shorter chain lengths being less toxic than the longer ones. This trend is illustrated in Table 10, where geometric means of experimental aquatic toxicities of LAS homologues as extracted from the BKH review (BKH, 1993: list 12) are compared for two organisms, an invertebrate (*Daphnia magna*) and a fish (*Pimephales promelas*).

Table 10: Average measured aquatic toxicity (mg/l) of LAS homologues (BKH, 1993)

Alkyl chain	Invertebrate ( <i>Daphnia magna</i> )		Fish ( <i>Pimephales promelas</i> )	
	EC <sub>50</sub>	NOEC	LC <sub>50</sub>	NOEC
C <sub>10</sub>	16.7 (7)	9.8 (2)	39.6 (4)	14 (1)
C <sub>11</sub>	9.2 (17)	-	19.8 (4)	6.4 (3)
C <sub>12</sub>	4.8 (37)	0.58 (7)	3.2 (9)	0.67 (3)
C <sub>13</sub>	2.35 (20)	0.57 (1)	1.04 (10)	0.1 (1)
C <sub>14</sub>	1.5 (13)	0.1 (2)	0.5 (3)	0.05 (1)

No. of records in parenthesis

The average chain length of the environmental fingerprint in water of LAS is C<sub>10.8</sub> (see 4.1.3). However, the actual ecotoxicity of the environmental fingerprint is probably not the same as the ecotoxicity associated with this average structure, because toxicity is not linearly related with chain length. Instead, ecotoxicity increases exponentially with the carbon chain length (see Table 10). Because of that, the contribution to the overall ecotoxicity of the longer (more toxic) homologues is probably more than proportional to their percentage in the fingerprint. Hence, the average structure is expected to be more ecotoxic than the real fingerprint. To take this into account, a toxicity-weighted average structure was calculated as shown in Table 11. To avoid influences of experimental variability, calculated toxicity values, instead of those reported in Table 10, were used for this exercise, obtained by means of QSAR calculations (Könemann, 1981). This resulted in a toxicity weighted average corresponding to a structure of LAS C<sub>11.6</sub>, instead of the original LAS fingerprint average C<sub>10.8</sub>.

Table 11: Toxicity-weighted average structure, LAS C<sub>11.6</sub>

Chain length CL	Homologue % in fingerprint	Calculated LC <sub>50</sub> (mg/l)	Weight % · 1/LC <sub>50</sub>	Weight · CL
10	45	12.48	3.6	36
11	30	4.89	6.1	67.1

12	23	1.91	12.0	144.0
13	2	0.75	2.7	35.1
SUM ⇒			24.4	282.2
Toxicity weighted average structure = SUM (weight · CL) / SUM (weight) ⇒				11.6

The ecotoxicity associated with the C<sub>11.6</sub> alkyl chain is, thus, expected to be representative of the overall LAS aquatic fingerprint. Below, all reported aquatic ecotoxicity data are related to, or normalised (Könemann, 1981), to this weighted average structure.

### Aquatic acute ecotoxicity

Acute toxicity data, selected from the BKH report (BKH, 1993) for the commercial LAS (average carbon numbers near C<sub>11.6</sub>) are summarized in Table 12. *Daphnia magna* and *Pimephales promelas* and *Lepomis macrochirus* were chosen as representative organisms of the toxicity of invertebrates and fish. Data for algae refer to various species. The toxicity values are the geometric means of several records as indicated in parenthesis. However, they were not used directly in the risk assessment, as higher tier data are available.

Table 12: Aquatic acute test results for commercial LAS

Taxon	IC <sub>50</sub> ; EC <sub>50</sub> ; LC <sub>50</sub> (mg/l) Geometric mean
Algae, IC <sub>50</sub>	9.1 (n = 12, SD = ±3.9)
Invertebrate ( <i>D. magna</i> ), EC <sub>50</sub>	4.1 (n = 17, SD = ±2.0)
Fish ( <i>L. macrochirus</i> ), LC <sub>50</sub>	4.1 (n = 12, SD = ±1.7)
Fish ( <i>P. promelas</i> ), LC <sub>50</sub>	3.2 (n = 4, SD = ±1.6)

No. of records in parenthesis with Standard Deviations (SD)

### Aquatic chronic ecotoxicity

Chronic toxicity data from the BKH report are summarised in Table 13 (BKH, 1993). These long term toxicity data are geometric mean NOEC values obtained over fifteen freshwater species and normalised to the average structure of LASC<sub>11.6</sub> (Van de Plassche et al., 1999a).

Test durations for algae were 72 to 120 hours, whereas exposure periods of NOECs for crustacean and fish were at least 21 days. The lowest NOEC is that for the fish *Tilapia mossambica* (0.25 mg/l). All known literature data were incorporated and the use of a geometric mean allows deriving sound NOECs, as used in the Dutch risk assessment (Feijtel et al., 1995b). A validity rating of 1 to 2 (Klimisch et al., 1997) can be assigned to all these toxicity data points.

Table 13: Aquatic chronic NOEC data for commercial LAS (BKH, 1993; Van de Plassche et al., 1999a)

Species	End point	NOEC (mg/l) Geometric mean	Range (mg/l)
<i>Chlamydomonas reinhardtii</i> , alga	growth	12 (1)	-
<i>Chlorella kessleri</i> , alga	growth	3.5 (1)	-
<i>Microcystis</i> sp., alga	population density	0.80 (4)	0.05-6.1
<i>Plectonema boryanum</i> , alga	growth	15 (1)	-
<i>Desmodesmus subspicatus</i> , alga	growth	7.7 (4)	0.8-105
<i>Selenastrum</i> sp., alga	population density	3.8 (9)	0.58-17
<i>Ceriodaphnia</i> sp., crustacean	reproduction	3.2 (1)	-
<i>Daphnia magna</i> , crustacean	mobility	1.4 (12)	0.3-6.6
<i>Chironomus riparius</i> , insectum	emergence	2.8 (1)	-

<i>Paratanytarsus parthenogenica</i> , insectum	growth	3.4 (1)	-
<i>Danio rerio</i> , fish	mortality	2.3 (1)	-
<i>Pimephales promelas</i> , fish	mortality and others	0.87 (14)	0.5-4.8
<i>Poecilia reticulata</i> , fish	reproduction	3.2 (1)	-
<i>Oncorhynchus mykiss</i> , fish	-	0.34 (7)	0.23-0.89
<i>Tilapia mossambica</i> , fish	reproduction	0.25 (1)	-

No. of records in parenthesis

Since the outcome of the BKH report in 1993, several new chronic studies have become available. These studies all have Klimish validity ratings of 1 or 2 and NOEC values within the range of values reported in Table 13. The additional studies are summarised below.

Chronic (32 days) toxicity tests of C<sub>12</sub>LAS to single species (one fish and three new invertebrates), caged in model ecosystem streams, were also obtained (Versteeg et al., 2003). The chronic values, associated to body burden concentrations were: 1 mg/l for the fish *Pimephales promelas*, 0.27, 0.95, and >2.9 mg/l for the invertebrates *Corbicula fluminea*, *Hyalella azteca* and *Elimia* sp. respectively.

Two aquatic plant (other than algae) studies were conducted. In the first study (Maki, 1981), the chronic toxicity of C<sub>11.6</sub> LAS to the aquatic macrophyte (*Elodea canadensis*) was determined in a 28 day model ecosystem test. The nominal test concentrations were 0.5, 1.0, 2.0, and 4.0 mg/l and were confirmed by analytical measurements. Growth inhibition was not observed even at highest tested concentration (4 mg/l). Growth throughout the exposure period approximately doubled the initial biomass of the vegetative shoots used at the start of the exposure. Hence, the NOEC was found to be  $\geq 4$  mg/l. The data are for C<sub>11.6</sub>LAS and no normalization is required.

In the second study (Bishop and Perry, 1981; Bishop, 1980; Van de Plassche et al, 1999a), the duckweed, *Lemna minor*, was exposed to C<sub>11.8</sub>LAS. Endpoints included frond count, dry weight, growth rate and root length after a 7 day exposure period in a flow through study. The measured test concentrations were 0, 2.1, 3.8, 8, 17 and 34 mg/l. The resultant EC<sub>10</sub> value, based on frond number, was 0.21 mg/l. The EC<sub>50</sub> value, also based on frond number, was 2.30 mg/l C<sub>11.8</sub> LAS. Normalizing the EC<sub>10</sub> of 0.21 mg/l to C<sub>11.6</sub> LAS results in a final value of 0.30 mg/l.

In a more recent study (Unilever, 2010), fertilized eggs of rainbow trout (*Oncorhynchus mykiss*, formerly *Salmo gairdneri*) were exposed to mean measured concentrations of 0.03, 0.23, 0.35, 0.63, 0.95 and 1.9 mg/l, for 72 days. The responses recorded included the survival of eggs, time to eyed egg stage, time to hatch, survival and final weight of sac-fry (eleutheroembryos), and time and extent of swim-up (external feeding). The lowest NOEC value found was 0.23 mg/l based on survival of eggs exposed from eyed stage, survival of eggs exposed from fertilization, survival of sac fry, and overall survival from fertilization to swim-up. The data are for C<sub>11.6</sub> LAS and no normalization is required.

Furthermore, a chronic toxicity test (Maki, 1981) with juvenile bluegills (*Lepomis macrochirus*) was conducted on C<sub>12</sub> LAS. Fish growth was determined after 28 days exposure in a flow-through model ecosystem to measured concentrations of 0, 0.5, 1.0, 2.0, and 4.0 mg/l. Results showed that the growth of juvenile bluegills was not affected at 0.5 and 1.0 mg LAS/l, but was reduced at 2.0 and 4.0 mg/l. At the end of the exposure period, fish at 1.0 mg/l LAS had a biomass of 44 g/m<sup>2</sup> compared to 10.5 g/m<sup>2</sup> for the 2.0 mg/l concentration. Based on these effects on growth rate, the NOEC was 1.0 mg/l.

### Model ecosystem studies

A variety of model ecosystem and mesocosm studies have been conducted on LAS. Many of these studies have been evaluated and summarized in two papers (Van de Plassche et al., 1999a; Belanger et al., 2002). NOEC values for standing (lentic) and flowing (lotic) water model ecosystems varied from 0.12 to 3.5 mg/l. The lowest NOEC value ( $\geq 0.12$  mg/l) was observed in an artificial stream study (Tattersfield et al., 1995, 1996).

In a specific stretch of the studied mesocosm (riffle zone) and after a prolonged exposure (56 days), some data appeared to show an exceptional sensitivity of the *Gammarus pulex* (NOEC = 0.03 mg/l), clearly an outlier in the sensitivity distribution. An ERASM study (ERASM, 2000) has tentatively tried to confirm this sensitivity in a 107 days single species laboratory exposure; the NOEC was significantly higher (0.1 mg/l), but the control mortality was particularly high (22-40%), which indicates that the study was not valid for risk assessment purposes (Klimish reliability score: 3).

The fate and effects of a C<sub>12</sub>LAS homologue has been studied in an experimental stream facility (ESF) (Belanger et al., 2002). The C<sub>12</sub>LAS test substance had a high content (35.7%) of its most hydrophobic and toxic 2-phenyl isomer. The 56-day ESF study included a representative community encompassing over 250 taxa. A NOEC of 0.27 mg/l, equivalent to 0.37 mg/l, if normalised to the commercial C<sub>11.6</sub>LAS structure by QSAR calculations (Könemann, 1981), was found. A critical literature review of all mesocosm studies available for LAS (13 studies), including the Tattersfield et. al. studies, was conducted and concluded that a NOEC value of 0.27 mg/l was a reliable and robust value protecting aquatic ecosystems (Belanger et al., 2002). A validity rating of 1 can be applied to this toxicity value (Klimish et al., 1997). This value approximates the LTE (Long-Term Effect) of 0.30 mg/l for LAS present in the DID list (Detergent Ingredient Database) of the European eco-labelling of laundry detergents (EU Commission, 1999).

Table 14: Results of model ecosystem studies for commercial LAS (Van de Plassche et al., 1999a; Belanger et al., 2002)

	Lowest NOEC range (mg/l)
Mesocosm studies	0.12-0.50 (13)

No. of studies in parenthesis.

#### 4.2.1.2 Terrestrial ecotoxicity

A large number of LAS toxicity data, both in laboratory and field, are available for the terrestrial environmental risk assessment. Data refer to the effects of LAS on soil organisms, namely toxicity to soil plants, soil fauna, soil micro-organisms and microbial soil processes (Klopper-Sams et al., 1996; Jensen, 1999; Jensen et al., 2001; Holmstrup et al., 2001a; Elsgaard et al., 2001a).

Using new standard protocols, updated results were obtained to extend the existing toxicity data and to contribute to an improved terrestrial risk assessment (Krogh et al., 2007; Jensen et al., 2007). All available data were obtained with the commercial LAS (average alkyl chain length of C<sub>11.6</sub>). The soil samples were collected in agricultural field. The soil was coarse with a total C content of about 1.5%, representative of cultivated area in Europe. Considering that the toxicities are mainly driven by the LAS pore water concentration, the same toxicity weighted average as that in water was used for the terrestrial and the sediment effects assessments (see par. 4.2.1.1).

The ecotoxicity of surfactants in the terrestrial environment were recently reviewed: eight groups of the most often used surfactants, representing the three largest classes (anionic, non-ionic and cationic), were selected and studied. Soil toxicity data in general are limited. Only for one group, represented by LAS, a full dataset of toxicity is available. The conclusion reported was: "The risk characterizations estimated for LAS are usually significantly lower than 1, what allows for the

conclusion that the ecological risk of this surfactant in the terrestrial environment is relatively low” (Liwarska-Bizukojc, 2009).

The range of the acute and chronic test results on LAS are summarised in Table 15 and Table 16 respectively. A first terrestrial risk assessment, using data available at the time, was presented and discussed at an international workshop (SPT/EPA, 1999) and at a world surfactant Congress (Lokke et al., 2000; Solbè et al., 2000). The figures presented in Table 15 are indicative of acute effects. They were not directly used in the present risk assessment, as higher tier data are available. The figures in Table 16 are a summary of chronic effects, refer to updated results and are used for a revisited terrestrial risk assessment, as described below (Jensen et al., 2007).

Table 15: Terrestrial acute test results for commercial LAS.

Taxon	Range (mg/kg <sub>dry soil</sub> )
Plants, EC <sub>50</sub>	167 – 316
Soil fauna, EC <sub>50</sub>	41 - >1000
Micro-organisms, EC <sub>50</sub>	17 - >1000

Table 16: Terrestrial chronic test results for commercial LAS (Jensen et al., 2007)

Taxon	Range (mg/kg <sub>dry soil</sub> )
Plants, NOEC or EC <sub>10</sub>	52 - 200 (12)
Soil fauna, NOEC or EC <sub>10</sub>	27 - 320 (9)
Micro-organisms, EC <sub>10</sub>	<8 - >793 (10)

No. of records in parenthesis.

### Terrestrial chronic ecotoxicity

Twenty one laboratory chronic data points for plants and soil fauna are available (Jensen et al., 2007). The values and the most sensitive endpoints for each species are indicated in Table 17. Following multi-peer reviews, a validity rating of 1 (Klimisch, 1997) can be assigned to all these chronic toxicity data.

The twelve data for plants were separated for crop and non-crop species, considering that only the former ones would be exposed to LAS via sludge application. The toxicity data were critically analysed reconsidering and consulting the original works. Toxicity results were calculated using graphical estimations and extrapolations with improved software and methodologies (Jensen et al., 2007).

The nine data for soil fauna were separated according to three classes: Oligochaetes, Insects and Arachnids. These toxicity data are basically the ones reported in the previous terrestrial risk assessment (Jensen et al., 2001) with the exception of the updated results for *Aporrectodea caliginosa*, *Enchytraeus sp.* and *Folsomia candida* (Krogh et al., 2007). The dataset was combined to develop a final HC<sub>5,50</sub> of LAS in soil (see par. 4.2.2.2).

As a measure of chronic toxicity, when possible, EC<sub>10</sub> (equivalent to a no-observed effect concentration) were preferred to NOEC (no-observed effect concentration). A full discussion on the relevance of EC<sub>x</sub> in risk assessments has been reported (Bruce and Versteeg, 1992).

The mixture toxicity of LAS with a PAH, pyrene, towards the micro-arthropod *Folsomia sp.* was tested (Holmstrup et al., 1996). No synergistic effects were observed and pyrene bioavailability was not enhanced by LAS in the experiment conditions. According to the authors, LAS is not likely to affect the solubility of PAH in soil at levels below its critical micelle concentration and LAS concentration in soil pore waters are orders of magnitude lower.

Table 17: Plants and soil fauna. Terrestrial chronic toxicity data for commercial LAS (Krogh et al., 2007; Jensen et al., 2007)

Species	Most sensitive end point	Value (mg/kg <sub>dw soil</sub> )	
		EC <sub>10</sub>	Extrapolated NOEC
Plants, non crop species:			
<i>Malvia pusilla</i>	growth	110	-
<i>Solanum nigrum</i>	growth	120	-
<i>Chenopodium album</i>	growth	120	-
<i>Amaranthus retroflexus</i>	growth	110	-
<i>Nigella arvensis</i>	growth	-	52
<i>Galinsoga parviflora</i>	growth	55	-
Plants, crop species			
<i>Brassica rapa</i>	growth	86	-
<i>Avena sativa</i>	growth	80	-
<i>Sinapis alba</i>	growth	200	-
<i>Sorghum bicolor</i>	growth	68	-
<i>Helianthus annuus</i>	growth	116	-
<i>Phaseolus aureus</i>	growth	126	-
Invertebrates: class oligocheates			
<i>Eisenia foetida</i>	growth	277	-
<i>Aporrectodea caliginosa</i>	reproduction	46	-
<i>Enchytraeus sp.</i>	reproduction	27	-
Invertebrates: class insects			
<i>Folsomia fimetaria</i>	reproduction	108	-
<i>Folsomia candida</i>	reproduction	205	-
<i>Isotoma viridis</i>	growth	41	-
<i>Hypogastrura assimilis</i>	reproduction	100	-
Invertebrates: class arachnids			
<i>Hypoaspis aculeifer</i>	reproduction	82	-
<i>Platynocheilus peltifer</i>	reproduction	-	320

Ten chronic soil microbial data points (Table 18) are also available (Jensen et al., 2001; Elsgaard et al., 2001a).

Table 18: Microbial parameters. Effect of commercial LAS on micro-organisms and microbial processes in soil (Jensen et al., 2001; Elsgaard et al., 2001a)

Endpoint	Incubation (d)	EC <sub>10</sub> (mg/kg <sub>dw soil</sub> )
Ethylene degradation	0.5	9
Ammonium oxidation	7	<8
Dehydrogenase activity	7	22

$\beta$ -Glucosidase activity	7	47
Iron reduction	7	<8
Cellulolytic bacteria	7	11
Cellulolytic fungi	7	<8
Cellulolytic actinomycetes	7	8
Basal soil respiration	1-9	>793
PLFA content	11	>488

Effects of both chemical- and bio-surfactants on soil biochemical processes are extensively reported by review papers in literature. Many beneficial applications in microbial, environmental and agricultural biotechnology, oil processing, enzyme technology and other bioprocessing operations are described (Cameotra et al., 2004; Van Hamme et al., 2006; Muller et al., 2007; Singh et al., 2007).

Some key soil physico-chemical and bio-chemical parameters show to be temporarily affected by sludge amendment of soil (Dunbabin et al., 2006). As to LAS, for example:

- the presence of LAS in agricultural soil stimulated the uptake of N, P and K with a surfactant dose of 15-30 g/m<sup>2</sup>; Ca and Mg were reduced (Moreno-Caselles et al., 2006); the average LAS doses in agriculture, however, with anaerobic sludge are much lower (2.8 g/m<sup>2</sup>) (Schowanek et al., 2007);
- laboratory studies on the growth of isolated soil bacteria cultures in presence of 50  $\mu$ g/ml LAS concentration indicate that application of sewage sludge (also wastewater or pesticides formulations) containing LAS to an agricultural soil could be considered a potential risk for selected aerobic heterotrophic soil microbiota and their microbial activities (Sanchez-Peinado et al., 2008).

As LAS degrades rapidly and the sludge integrates in the soil, such effects disappear rapidly. In addition, it is difficult to distinguish whether any observed effect is due to the sludge organic matter itself, LAS (ca. 10%, the lowest sludge organic fraction) or other components (e.g. metals) and to understand whether the disturbance is adverse and permanent. In any case, field studies have never provided evidence of adverse and permanent impact of LAS in sludge on these parameters.

Specific effects of surfactants, present in municipal wastewaters, considering in particular the main soil regulatory factors, haven't been much considered (Muller et al., 2006). Regulatory requirements relevant to "pristine/natural" soil should not be used for agricultural soil that receives sewage sludge. Again, as already said before, it is also impossible to separate effects related to the organic carbon of sewage sludge solids itself, and perhaps to other persistent contaminants, from effects of biodegradable surfactants.

On the contrary, no significant effects to the microbial community were observed after prolonged exposure to heterogeneous LAS distributions in agricultural soil following sludge amendment. For example:

- no effects were observed in the soil even at LAS concentrations >31 g/kg<sub>dw sludge</sub> (Brandt et al., 2003);
- LAS at the concentration levels of 22 and 174 mg/kg<sub>dw soil</sub> in sandy agricultural soil (worst-case scenario in terms of high bioavailability and toxicity in the soil environment) was rapidly degraded (>93% in 4 weeks) and had little or no significant influence of the functional diversity of aerobic heterotrophic bacterial community (Winther et al., 2003);
- effects of LAS (at concentrations of 10 or 50 mg/l for periods of time up to 21 days) on the bacterial community of a microcosm system consisted of agricultural soil columns were evaluated, applying a molecular-based community-level analysis. The structures of

three bacteria communities (*Alphaproteo-*, *Actino-* and *Acido-bacteria*) were analysed. The conclusions were that the alphaproteobacterial population identified in the work was enriched in the LAS polluted soil, suggesting its relevant role and ability to biotransform and degrade LAS. LAS had no remarkable effects on the other two community bacteria, even when present at concentrations widely exceeding those reached in soil immediately after sludge application (Sánchez-Peinado et al., 2010).

Micro-organisms and overall soil processes were thus considered protected by the PNEC derived from the relative higher sensitivity of plants and invertebrates (Brandt et al., 2003; Petersen et al., 2003) and therefore not considered in the risk assessment.

Field observations are also available (Jensen, 1999; Jensen et al., 2001; Brandt et al., 2003) and are summarized in Table 19. The application of LAS-containing sludge generally stimulated the microbial activity and, hence, the abundance of soil fauna and growth of plants. Paddy growth was stimulated when LAS was <80 mg/kg<sub>dw soil</sub> (Liang-Qing et al., 2005). It was found that application of LAS-containing sludge on soil did not produce any short- and long-term adverse effects on microbial functions and processes or the abundance and diversity of soil invertebrates.

Table 19: Field studies for commercial LAS (Jensen et al. 2001; Figge and Schöberl, 1989)

Taxon	Range (mg/kg <sub>dry soil</sub> )
Soil ecosystem, NOEC	>15
Biomass, NOEC	>16, >27

A laboratory agricultural ecosystems study used a “plant metabolism box” to measure the growth of grass, beans, radishes and potatoes for a period up to 106 days after application of sludge spiked with radiolabelled LAS material (Figge and Schöberl, 1989; Figge and Bieber, 1999). At LAS soil concentrations of 16 and 27 mg/kg<sub>dw soil</sub>, no significant uptake and accumulation by plants and no adverse effects on the biomass were observed. Jensen et al. (2001) concluded that soil LAS concentrations of 5 to 15 mg/kg<sub>dw soil</sub> did not cause any harm to the soil ecosystem. Selected microbial populations in sandy soils (low organic matter content) surrounding sludge bands spiked with high levels of LAS were also studied (Brandt et al., 2003). In this study the observed disturbance of the soil microbial community lasted only two months and was confined to soil close to sludge, confirming that LAS doesn’t pose any significant threat to the function of the microbial community in sludge-amended soils.

#### 4.2.1.3 Sediment ecotoxicity

The organic carbon content of the sediment may influence the bioavailability and therefore the toxicity of the test substance. Therefore, for comparison of sediment tests, the organic carbon content of the test sediment should be within a certain range. The organic carbon content of a standard sediment is set to 5 % (TGD, 2003). It is recommended that the organic carbon content of the test sediments is between these two values. As some of the available data are tested with sediments that have an organic carbon content that fall outside the ranges, all results are converted to a standard sediment, which is defined as a sediment with an organic matter content of 5%.

Toxicity information is available for sediments and is summarized in Table 20. A NOEC of 319 mg/kg<sub>dw sed.</sub> (Klimish score of 1) was observed for the larvae of a benthic organism, *Chironomus riparius* (Pittinger, 1989; Kimerle, 1989). The organic carbon content of the tested sediment was 4.2%. The organic carbon normalized NOEC is 380 mg/kg<sub>dw sed.</sub> New toxicity experiments for the same organism, looking at larval growth and mortality, were performed using two different sediments spiked with both radiolabelled and unlabelled C<sub>12</sub>-2-LAS homologue (Mäenpää and Kukkonen, 2006). After 10-days exposure, NOECs were 362 mg/kg<sub>dw sed.</sub> and 537 mg/kg<sub>dw sed.</sub>



(Klimish score of 1). The organic carbon content of the sediments were 1.06% and 1.57%, respectively. The organic carbon normalized NOECs are 1,710 mg/kg<sub>dw sed.</sub> for both sediments. For one sediment the NOEC as body residue (measure of internal exposure) was 30 mg/kg larval wet weight.

A tubificid species, *Branchiura sowerbyi*, a benthic filter organism, was exposed for a long period (220 days) to a sediment with LAS concentrations varying from 26 to 7 mg/kg<sub>dw sed.</sub> (Klimish score of 1, absence of any observed effect) over the exposure period and no effects were observed in any of the test concentrations (Casellato et al., 1992). While the absence of reported toxicity is reassuring, it appears that the range of exposure concentrations was too low to derive a toxicity data directly useful in risk assessment. However, the results of this test do not invalidate the PNEC calculation. Two freshwater mollusc species, *Unio elongatulus* and *Anodonta cygnea*, were exposed to sediments with LAS concentration >200 mg/kg<sub>dw sed.</sub> (Klimish score of 2, due to lack on description of the experimental details) without noticing any adverse effects (Bressan et al., 1989).

Chronic studies were conducted with *Lumbriculus variegatus* and *Caenorhabditis elegans* (Comber et al., 2006). As to the first species, a 28 days NOEC of 81 mg/kg<sub>dw sed.</sub> was derived for survival, reproduction and growth, using sediment spiked with radio-labelled material, the organic carbon content of the sediment was 1.7%. The organic carbon normalized NOEC is 238 mg/kg<sub>dw sed.</sub>. For the second species, a 3 day NOEC of 100 mg/kg<sub>dw sed.</sub> was obtained for egg production, the organic carbon normalized NOEC is 294 mg/kg<sub>dw sed.</sub>. Both experiments are well described (Klimish score of 1).

LAS sorbed to sediments was assessed for its level and potential perturbations on benthos; comparative sediment contamination analyses came to the conclusion that LAS risk for both aquatic and sediment compartment is low (Sanderson et al., 2006).

Table 20: Sediment chronic test results for commercial LAS

Species	Most sensitive end point	NOEC (mg/kg <sub>dw sed.</sub> )	Organic carbon normalized NOEC (mg/kg <sub>dw sed.</sub> )	Organic carbon content (%)	References
<i>Chironomus riparius</i>	reproduction, survival	319	380	4.2	Pittinger, 1989 Kimerle, 1989
		362, 537	1,710	1.06, 1.57	Mäenpää and Kukkonen, 2006
<i>Unio elongatulus</i> <i>Anodonta cygnea</i>	survival survival	>200 >200	- -	- -	Bressan et al., 1989
<i>Lumbriculus variegatus</i>	survival, reproduction, growth	81	238	1.7	Comber et al., 2006
<i>Caenorhabditis elegans</i>	egg production	100	294	1.7	Comber et al., 2006

It is also worth mentioning LAS safety in the coastal marine environment.

LAS is highly biodegradable, not only under aerobic conditions in sea water (Leon et al, 2004), but also under anaerobic conditions in marine sediments (Lara-Martin et al., 2007; Lara-Martin et al.,

2008). Monitoring studies have shown that LAS is only present in coastal sediments close to points of municipal and industrial discharges (Petrovic et al., 2002).

Laboratory experiments, performed on anoxic marine sediments spiked with 10-50 ppm of LAS, showed that degradation is feasible reaching a value of 79% in 165 days, with a half-life time of ca. 90 days. The anaerobic process was also observed in the field with several marine sediment samplings: at anoxic depths in the sedimentary column, LAS concentrations in pore waters decreased sharply and the biodegradation intermediates (SPC) reached the maxima. These observations were claimed as the first real evidence of a partial degradation of LAS under anaerobic conditions (Lara-Martin et al., 2007; Lara-Martin et al., 2008). An anaerobic biodegradation pathway for LAS has recently been described (Lara-Martin et al., 2010).

Sortition and desorption experiments with two marine sediments were carried out using C<sub>12</sub>-2-LAS molecule to study its toxicity on a marine mud shrimp, *Corophium volutator*, in water-only exposure as well as in spiked sediments (Rico-Rico A et al., 2009). Pore water LC<sub>50</sub> values were calculated in the range 100-700 µg/l. These values are considerably higher than pore water concentrations for LAS (maximum 15 µg/l) found in marine sediments of coastal areas close to wastewater discharges (Lara-Martin et al., 2006).

The mud snail *Hydrobia ulvae* was exposed to marine LAS-spiked sediments: LC<sub>50</sub> toxicity values were comprised between 203 mg/kg (48 h) and 94 mg/kg (9 d) (Hampel et al., 2009). The results confirm that *H. ulvae* is an appropriate candidate organism for routine marine sediment toxicity testing with surfactants.

#### **4.2.1.4 Ecotoxicity to sewage microorganisms**

The 3-h EC<sub>50</sub> of LAS for microorganisms present in the aerobic activated sludge was experimentally measured at 550 mg/l (Verge et al., 1993; Verge et al., 1996). Assuming an average content of suspended matter in the activated sludge of 3 g/l, the EC<sub>50</sub> value corresponds to about 18% LAS in sludge on dry basis (i.e., 183 g LAS/kg<sub>dw</sub> sludge).

A consortium of two bacteria (*Pantoea agglomerans* and *Serratia odorifera*) was isolated from a STP sludge. They complement each other in the ability to degrade LAS. Optimizing their culture growth conditions, complete laboratory mineralization of 200 mg/l LAS was obtained within 48-72 h (Khleifat et al., 2006).

Laboratory toxicities of commercial surfactants were carried out using a specific type of microorganism isolated from a STP activated sludge (the phosphate-accumulating bacterium: *Acinetobacter junii*). The anionic surfactants were the most toxic, with LAS having a 50% growth inhibition of 0.15-1.8 mg/l (Ivankovic et al., 2009).

A NOEC value of 35 mg/l, normalised to the C<sub>11.6</sub>LAS structure, was found for *Pseudomonas putida* after a growth inhibition test (Feijtel et al., 1995b).

The microbial population present in the STP activated sludge digesters was not found to be inhibited even by a high and atypical concentration (30 g/kg<sub>dw</sub> sludge) of LAS in sludge (Berna et al., 1989).

#### **4.2.1.5 Reassurance on absence of estrogenic effects**

LAS was also investigated to check whether it could be an endocrine disruptor, using an estrogens-inducible yeast screen (Routledge et al., 1996; Navas et al., 1999) and the vitellogenin assay with

cultured trout hepatocytes (Navas et al., 1999). LAS as well as its biodegradation intermediates, Sulpho Phenyl Carboxylates (SPC), did not display any estrogenic effects.

## 4.2.2 PNEC calculations

### 4.2.2.1 Aquatic PNEC

In a previous environmental risk assessment of LAS for the aquatic compartment (Van de Plassche et al., 1999a), NOECs for fifteen freshwater species were considered (Table 13), a dataset that justified the application of a statistical extrapolation method (Aldenberg & Slob, 1993). They were normalised to the average structure C<sub>11.6</sub> LAS by the use of QSARs. A geometric mean NOEC for each species was calculated. HC<sub>5,50</sub>, the median value of the 5<sup>th</sup> percentile of the log-normal distribution including all available NOEC values, was derived and was 0.32 mg/l. This value is in good agreement with the lowest available freshwater NOEC, found for the fish *Tilapia mossambica* (0.25 mg/l).

Various mesocosm studies (Tattersfield et al., 1995; Tattersfield et al., 1996; Belanger et al., 2002) indicate that the lower limits of mesocosm studies can be considered between 0.12 to 0.5 mg/l. Following a critical review of all the mesocosm studies, however, it was also concluded that a NOEC = 0.27 mg/l for a C<sub>12</sub>LAS homologue, corresponding to 0.37 mg/l when normalised to the C<sub>11.6</sub> LAS structure, is the most reliable, robust and defensible mesocosms value, to which an application factor of 1 has to be applied (Belanger et al., 2002). The reasons for this are many, but include:

- presence of a large number of sensitive flora and fauna, accompanied by a high degree of overall biodiversity (a total of 149 alga species and 6 phylogenetic divisions; 117 benthic invertebrates including insects, molluscs, crustaceans, and aquatic worms; 77 macroinvertebrate taxa collected in drift; 110 adult insect species);
- 16 weeks of colonization and exposure, longer than single species chronic toxicity tests represented in the database;
- use of a large array of endpoints, including many that reveal subtle and indirect effects; endpoints combine relevant environmental aspects of fate (biodegradation, chemical metabolism, sorption, and exposure verification) with effects (invertebrate, autotrophic and heterotrophic periphyton);
- the experimental stream facility (ESF) has a long history of biological and chemical data that has been used to interpret and re-interpret past studies (Belanger et al., 1994, 1995, 2000); two pairs of studies have been conducted to assess repeatability and findings have been consistent in different years (Belanger, 1992; Belanger et al., 2000 and unpublished data);
- ESF streams have relatively low levels of variability and are sampled intensively (i.e., at relatively high levels of replication) (Lowe et al., 1996; Belanger et al., 2000);
- ESF stream population and community structure has been compared to local and regional flora and fauna to ensure that the ESF communities are representative of sensitive ecosystems (Belanger et al., 1995; Dyer and Belanger, 1999); ecological investigations of nutrient dynamics of ESF streams support their being representative of headwater streams at the relevant discharge levels (Peterson et al., 2001).

It seems reasonable and in agreement with the results on single species to assign a PNEC value of 0.27 mg/l to the PNEC of LAS in the water compartment.

**Conclusion: PNEC in water = 0.27 mg/l.**

### 4.2.2.2 Terrestrial PNEC

In a typical disposal scenario, LAS enters soil predominantly via addition of (anaerobic) sewage sludge to agricultural land.

Modelling approach: The terrestrial PNEC of LAS can be calculated by using the TGD equilibrium partitioning method (EqP - TGD, 2003, Part II: eq. 72, page 117). On the basis of a local PNEC in water of 0.27 mg/l and assuming a value of 2500 l/kg as partition coefficient between organic matter and water (see 3.2), a value of 11.9 mg/kgdw soil can be obtained. No additional safety factor is required for LAS because the substance has a log Kow <5. This value is in the same order of magnitude as the values derived below based on the all available experimental toxicity results for soil organisms.

Analysis of soil experimental data: In a previous environmental risk assessment carried out for LAS in the soil compartment (Jensen et al., 2001), the estimation of PNEC, performed for soil fauna and plants using a data set of twenty three records and applying a statistical extrapolation method (Wagner et al., 1991), was 4.6 mg/kgdw soil. This PNEC was calculated as the HC5,50, the median value of the 5th percentile of the log-normal distribution, and includes the microbial processes and functions that have been examined (Jensen et al., 2001).

Comparison with the EqP approach and with available more recent information suggest that this value can be considered as rather low/conservative. Following an extensive review and update of the plant and invertebrate ecotoxicological data, and a further interpretation of the relevance of the microbial endpoints for the functioning of the soil ecosystem, the terrestrial risk assessment of LAS has been revisited (Jensen et al., 2007). The new PNEC, using a data set of twenty one toxicity values (as reported in Table 17), was derived at 35 mg/kgdw soil.

The opinion of SCHER (2008) however disagrees with the argument that soil microbial functions (and with particular reference to iron reduction) are adequately covered by the proposed PNEC of 35 mg/kgdw soil, and considers that an evaluation of the relevance of LAS effects on microbial activity is essential for a proper PNECsoil derivation. Thus, SCHER considers that the information provided is not sufficient for justifying the newly proposed PNEC value of 35 mg/kg. In this respect, HERA experts remark that at present there is no consistent and universally accepted framework of how microbial species, and in particular single biochemical endpoints, should be included in a soil or sediment risk assessment for a given chemical. The EU TGD (2003) provides only very basic guidance in this respect, emphasizing the function of “primary producers” (plants), “consumers” (soil fauna) and “decomposers” (mainly microbes). Given the enormous diversity and metabolic/genetic flexibility of microbial communities, and the variability and diversity of potentially measurable microbial endpoints in soil, a careful interpretation is required. Each result should be evaluated for its true environmental relevance with respect to the size of the effect, duration, essential soil function impairment, etc., and not necessarily the lowest observed number should therefore be retained as a NOEC.

The salt speciation of LAS and the soil type were included in the evaluation and did not significantly modify the toxicity of LAS to soil organisms (Holmstrup et al., 2001b; Jensen et al., 2001). Dosage of LAS via sewage sludge, instead, generally reduced the effects for microbial parameters, showing also recovery potentials for most parameters as a result of prolonged incubation (Elsgaard et al., 2001b). Disturbance of soil microbial community were confined to soil close to sludge and disappeared after two months (Brandt et al., 2003). In addition, field observations (Table 19) after experimental sludge amendment at high application rates concluded that LAS, at an average soil concentration of > 15 mg/kgdw soil, does not seem to be detrimental to the soil ecosystem in the long term (Jensen et al., 2001). The HERA experts therefore judge that the impact of LAS on the soil community has been adequately assessed, in particular if one

combines the laboratory data with the holistic weight of evidence provided by available controlled field studies at high LAS levels. These show no impact on ‘ecosystem service’ parameters such as soil fertility and crop yield (see studies reported in Schowanek et al. 2007, where a probabilistic pan-European risk assessment for LAS in soil is also presented). With respect to the protection of the agro-ecosystem, reference is also made to discussion on setting protection levels on the basis of ‘ecosystem services’ in the EU Commission document (2012) “Addressing the new challenges for Risk Assessment” ([http://ec.europa.eu/health/scientific\\_committees/consultations/public\\_consultations/scenihr\\_consultation\\_16\\_en.htm](http://ec.europa.eu/health/scientific_committees/consultations/public_consultations/scenihr_consultation_16_en.htm))

**Conclusion: PNEC in soil = 35 mg/kg<sub>dw soil</sub>.**

#### 4.2.2.3 Sludge PNEC

A sludge PNEC, also called sludge quality standard (SQS), of LAS can be back-calculated from the soil PNEC taking into account the TGD (TGD, 2003) scenario for exposure of sewage sludge on agricultural soil and the soil PNEC of 35 mg/kg<sub>dw soil</sub> (see par. 4.2.2.2). A PNEC of 49 g/kg<sub>dw sludge</sub> was calculated (for details of its calculation and interpretation we refer to Schowanek et al., 2007)(\*).

**Conclusion: PNEC in sludge = 49 g/kg<sub>dw sludge</sub>.**

#### 4.2.2.4 Sediment PNEC

As for soil, sediment PNEC of LAS can be calculated using the TGD equilibrium partitioning method (TGD, 2003: Part II, eq. 70, page 113). The resulting PNEC is 14.9 mg/kg<sub>dw sed.</sub>

Good quality chronic data on sediment toxicity for LAS are available for five species representing different living and feeding conditions. An application factor of 10 can be applied to the lowest available NOEC figure normalized for organic carbon, deriving a conservative PNEC for sediment of 23.8 mg/kg<sub>dw sed.</sub>

The available sediment toxicity data, as reported in Table 20, in particular those relative to oligochaetes, well represent the different benthic taxa (Comber et al., 2006) and are recommended by the European TGD (TGD, 2003) in the sediment testing for the risk assessment of chemicals.

**Conclusion: PNEC in sediment = 23.8 mg/kg<sub>dw sed.</sub>**

#### 4.2.2.5 STP PNEC

Although the lowest effect concentration is a NOEC value of 35 mg/l, normalised to the C<sub>11,6</sub>LAS structure, for *Pseudomonas putida* after a growth inhibition test, this value will not be taken into account. Results of the cell multiplication inhibition test with *P. putida* should only be used for calculation of the STP PNEC in cases where no other test results employing mixed inocula are available. As a respiration inhibition test with activated sludge is available, results from this study will be used to derive the STP PNEC (TGD, 2003). Thus the most relevant reported effective concentration for STP organisms is the 3-h EC<sub>50</sub> value of 550 mg/l for activated sludge. This value with an application factor of 100 gives a PNEC of 5.5 mg/l, as recommended by the TGD.

**Conclusion: PNEC in STPs = 5.5 mg/l.**

## 4.3 Environmental risk assessment

PEC and PNEC values with the corresponding PEC/PNEC ratios are summarized in Table 21.

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(\*) A LAS limit value in sludge of 1.3 g/kg<sub>dw sludge</sub> is actually in force in Denmark (Executive Order 823 DK).

Table 21: Risk characterization

LAS	PEC	PNEC	PEC/PNEC
Water, mg/l	0.047	0.27	<b>0.17</b>
Soil (30 d), mg/kg <sub>dw soil</sub>	1.4	35	<b>0.04</b>
Sludge, g/kg <sub>dw sludge</sub>	5.56 (50th percentile) 15.07 (95th percentile)	49	<b>0.11</b> <b>0.31</b>
Sediment, mg/kg <sub>dw sed.</sub>	5.3	23.8	<b>0.22</b>
STP, mg/l	0.27	5.5	<b>0.05</b>

This assessment shows that the use of LAS in HERA applications results in risk characterisation ratios (PEC/PNEC) less than one. To demonstrate this, higher tier exposure and effects data were needed. PEC values were estimated based on monitoring data for each environmental compartment and PNEC values were based on chronic effects data. This conclusion can be generalized to all LAS usages in Europe including the non-HERA minor applications, since exposure has been based on the actual LAS concentrations measured in the various environmental compartments.

## 5. *Human health assessment*

### 5.1 Consumer exposure

#### 5.1.1 Product types

LAS is one of the major anionic surfactants used in laundry and cleaning products. LAS is commonly used in many household detergents, including laundry powders, liquids, and tablets (at a typical concentration range from 3% to 22%), laundry bleach additives (at a typical concentration range from 3% to 11%), hand dishwashing liquids (at a typical concentration range from 2% to 30%), and all-purpose cleaning powders, liquids, sprays, and tablets (at a typical concentration range from 1% to 37%). LAS is also used in some industrial applications, such as in the fields of textile and fibers, chemicals, and agriculture and in cosmetics and glues. These other uses of LAS are minor relative to the laundry and cleaning applications (which represent about 80% of the total use of LAS in the market) and are outside the scope of HERA. They are not evaluated in this assessment.

#### 5.1.2 Consumer Contact Scenarios

Based on the product types, the consumer contact scenarios that were identified and considered in this assessment include: direct and indirect skin contact, inhalation of aerosols from cleaning sprays, and oral ingestion derived either from residues deposited on dishes, from accidental product ingestion, or indirectly from drinking water.

#### 5.1.3 Consumer exposure estimates

There is a consolidated overview concerning habits and practices of use of detergents and surface cleaners in Western Europe which was tabulated and issued by AISE (THPCPWE,2002). This table reports the consumer's use of detergents in g/cup, tasks/week, duration of task and other uses of products. All exposure estimates that follow were calculated using relevant data from this table. Information from the RIVM report Cleaning Products Fact Sheet - To assess the risks for the consumer has also been used (RIVM,2006)

(Editorial note: across this section and throughout the report the term “conservative” is used frequently to refer to the nature of an estimation of exposure. For clarification, the term “conservative” is always meant here as indicating the higher end of likely exposure).

### 5.1.3.1 Direct skin contact from hand washed laundry

During the hand-wash laundry, the diluted laundry liquid comes into direct contact with the skin of hands and forearms.

The following worst case should address this scenario:

- The exposed area is the skin surface area of forearms and hands, which is 1900 cm<sup>2</sup> (RIVM, 2006).
- It is assumed that not the total amount of diluted product is in contact with the skin but only a layer of 0.01 cm around the exposed skin (TGD,2003). The exposure area is 1900 cm<sup>2</sup>, therefore the amount of diluted product is 19 cm<sup>3</sup>, or 19 g (RIVM,2006).
- The concentration of laundry detergent for the hand-wash is 0.1% to 1%. Worst-case, the weight fraction of the diluted detergent is 1% of the used detergent powder/liquid (AISE,2002).
- Taking the above into account, the following local dermal exposure can be calculated:

$$0.01 \text{ (dilution factor)} \times 19 \text{ (g)} / 1900 \text{ (cm}^2\text{)} = 0.10 \text{ mg/cm}^2\text{/day}$$
$$\mathbf{Exp_{dermal,local} = 0.10 \text{ mg/cm}^2\text{/day}}$$

Assuming a frequency of 104 times per year, and a body weight of 65 kg, the resulting dermal systemic dose is:

$$0.01 \text{ (dilution factor)} \times 19 \text{ (g)} / 65 \text{ (kg)} / 365/104 \text{ (frequency)} = 0.83 \text{ mg/kg bw/day}$$
$$\mathbf{Exp_{dermal,sys} = 0.83 \text{ mg/kg bw/day}}$$

### 5.1.3.2 Direct skin contact from laundry tablets

Contact time is so low and area of contact with skin is so small that the amount absorbed percutaneously is considered insignificant.

### 5.1.3.3 Direct skin contact from pre-treatment of clothes

Direct skin contact with LAS is possible when clothing stains are being removed by spot-treatment with a 60 % (600 mg/ml) detergent paste powder (THPCPWE,2002) or neat liquid. As only a fraction of the skin surface area of the hands (840 cm<sup>2</sup>) (TGD,2003) is exposed, it can be assumed that the amount of LAS systemically available via percutaneous absorption, if any, is quite low.

The following worst case should address this scenario:

- Highest concentration of LAS in powder laundry detergents amounts to 22% (internal AISE data). Therefore highest concentration of LAS in hand washing paste (600 mg/ml) is approximately 132 mg/ml. Highest concentration of LAS in liquid laundry detergents amounts to 14% (140 mg/ml) (internal AISE data). Because liquid detergents may be used neat for pre-treatment, the worst case value of 14% will be used in the calculation.

- Contact of hands into solution would expose a maximum of 840 cm<sup>2</sup> (TGD,2003). This value is very conservative because only a fraction of the two hands surface skin will be exposed.
- Assuming a film thickness of 100 µm (0.1 mm or 0.01 cm) (TGD,2003) on the hands and an assumed applied amount of 0.65 g (RIVM,2006), the following local dermal exposure can be calculated:

$$0.14 \text{ (dilution factor)} \times 0.65 \text{ (g)} / 840 \text{ (cm}^2\text{)} = 0.11 \text{ mg/cm}^2\text{/day}$$

$$\mathbf{Exp_{dermal,local} = 0.11 \text{ mg/cm}^2\text{/day}}$$

Assuming a frequency of 128 times per year, and a body weight of 65 kg, the resulting dermal systemic dose is:

$$0.14 \text{ (dilution factor)} \times 0.65 \text{ (g)} / 65 \text{ (kg)} / 365/128 \text{ (frequency)} = 0.49 \text{ mg/kg bw/day}$$

$$\mathbf{Exp_{dermal,sys} = 0.49 \text{ mg/kg bw/day}}$$

#### 5.1.3.4 Direct skin contact and inhalation from hand dishwashing

##### *Dermal*

When doing the dishes, there is dermal exposure to the diluted dishwashing liquid.

The following worst case should address this scenario:

- Highest concentration of LAS in hand dishwashing solution is  $6.54 \cdot 10^{-5}\%$  (RIVM,2006).
- Immersion of hands and forearms into solution would expose about 1900 cm<sup>2</sup> (RIVM,2006).
- Assuming a film thickness of 100 µm (0.1 mm or 0.01 cm) (TGD,2003) on the hands and an assumed applied amount of 15000 g, the following local dermal exposure can be calculated:

$$6.54 \cdot 10^{-7} \text{ (dilution factor)} \times 15000 \text{ (g)} / 1900 \text{ (cm}^2\text{)} = 5.16 \cdot 10^{-3} \text{ mg/cm}^2\text{/day}$$

$$\mathbf{Exp_{dermal,local} = 5.16 \cdot 10^{-3} \text{ mg/cm}^2\text{/day}}$$

Assuming a frequency of 426 times per year, and a body weight of 65 kg, the resulting dermal systemic dose is:

$$6.54 \cdot 10^{-7} \text{ (dilution factor)} \times 15000 \text{ (g)} / 65 \text{ (kg)} / 365/426 \text{ (frequency)} = 0.18 \text{ mg/kg bw/day}$$

$$\mathbf{Exp_{dermal,sys} = 0.18 \text{ mg/kg bw/day}}$$

##### *Inhalation*

When doing the dishes, there is inhalation exposure to the diluted dishwashing liquid.

The following worst case should address this scenario:

- Highest concentration of LAS in hand dishwashing solution is  $6.54 \cdot 10^{-5}\%$  (RIVM,2006).



- The exposure duration is the time of being in the kitchen, which is estimated at 60 min. The application duration is set at 16 min. (RIVM,2006)
- The room volume is 15 m<sup>3</sup> (kitchen). The release area, based on the surface area of the sink, is set at 0.15 m<sup>2</sup>. (RIVM,2006)
- Default values are used for ventilation rate (2.5 hr<sup>-1</sup> = 6.9·10<sup>-4</sup> s<sup>-1</sup>), applied amount (15000 g), molecular weight matrix (18 g/mol) and mass transfer rate (2100 m/min) (RIVM,2006).
- Based on the above, the inhalation exposure to LAS is estimated using the ConsExpo v4.1 evaporation model:

$$\mathbf{Exp_{inhalation} = 3.90 \cdot 10^{-20} \text{ mg/m}^3/\text{day}}$$

This amount does not contribute significantly to the total exposure of LAS.

### 5.1.3.5 Indirect skin contact from wearing clothes

Residues of components of laundry detergents may remain on textiles after washing and could come in contact with the skin via transfer from textile to skin. The amount of LAS deposited on fabric remaining after 10 repeats of a typical washing process with typical laundry detergents was experimentally measured to be in the order of 2.5 mg of LAS per g of fabric (Rodriguez et al., 1994). However, this amount of compound deposited on the textile depends on the type of chemical and on the product itself. Therefore, extrapolating to the total amount of detergent residues is not feasible (RIVM,2006).

- It is assumed that clothers are worn every day, for 24 hours; resulting in a frequency of exposure of 365 year<sup>-1</sup>.
- The leachable fraction is the relative amount of chemical which can leach from a product, i.e. the fraction of deposits of the detergent which can leach from textile. This fraction is determined to be 0.0023 (RIVM,2006).
- The average weight of the product that is worn on the body is estimated at 1000 g (RIVM, 2006).
- The exposed area is 17600 cm<sup>2</sup>.
- Assuming a skin contact factor, the part of the product that is in contact with bare skin, of 0.8, the following local dermal exposure can be calculated:

$$1000 \text{ (g)} \times 0.8 \times 0.0023 / 17600 \text{ (cm}^2\text{)} = 0.105 \text{ mg/cm}^2/\text{day}$$

$$\mathbf{Exp_{dermal,local} = 0.11 \text{ mg/cm}^2/\text{day}}$$

Assuming a frequency of 365 times per year, and a body weight of 65 kg, the resulting dermal systemic dose is:

$$1000 \text{ (g)} \times 0.8 \times 0.0023 / 65 \text{ (kg)} = 28.31 \text{ mg/kg bw/day}$$

$$\mathbf{Exp_{dermal,sys} = 28.31 \text{ mg/kg bw/day}}$$

### 5.1.3.6 Inhalation of detergent dust during washing processes

Charging the washing machine with laundry powder may lead to generation of dust particles and may lead to inhalation exposure.

- The powder laundry detergents contain up to 22% LAS.
- The exposure duration includes picking up the package, opening it, filling the machine and closing the package, and is set at 15 s (0.25 min.) (RIVM,2006).
- The room volume is 1 m<sup>3</sup>; room volume is interpreted here as ‘personal volume’, a small area of 1 m<sup>3</sup> around the user.
- Default values are used for ventilation rate (2 hr<sup>-1</sup> = 5.6·10<sup>-4</sup> s<sup>-1</sup>), and applied amount (2.7·10<sup>-4</sup> mg).
- In the worst case assumptions that all of the dust is inhaled during machine loading and that this task is done once daily, the inhalation exposure to LAS is estimated to be:

$$\text{Exp}_{\text{inhalation}} = [(2.7 \cdot 10^{-4} \text{ (mg)} \times 0.22 \times e^{-5.6 \cdot 10^{-4} \text{ (s}^{-1}) \times 15 \text{ (s)}}) / 1 \text{ (m}^3)] \times 1.7 \cdot 10^{-4} \text{ (day)} = \mathbf{1.03 \cdot 10^{-8} \text{ mg/m}^3/\text{day}}$$

This amount does not contribute significantly to the total exposure of LAS. Similarly, lint formation during drying of fabrics in tumble-dryers which vent indoors is considered not to contribute to inhalation exposure of LAS, since washed fabrics do not contain any relevant amount of LAS (see above).

### 5.1.3.7 Inhalation of and skin contact with aerosols from cleaning sprays

LAS is present in some surface cleaning spray products at a typical concentration range of 3% to 6% (internal AISE data).

#### *Inhalation*

When cleaning a surface using a spray cleaner, inhalation exposure to the aerosols from the cleaning spray can occur.

The following worst case should address this scenario:

- Highest concentration of LAS in spray cleaner is 6 % (internal AISE data).
- The exposure duration is the time of being in the room, which is estimated at 60 min. The application duration is set at 0.41 min. (RIVM,2006)
- The room volume is set at 15 m<sup>3</sup>, the room height at 2.5 m (RIVM,2006).
- The mass generation rate is 0.78 g/s, and the weight fraction non-volatile is 0.06.
- Default values are used for ventilation rate (2.5 hr<sup>-1</sup> = 6.9·10<sup>-4</sup> s<sup>-1</sup>), inhalation cut-off diameter (15 μm), density non-volatile (1.8 g/cm<sup>3</sup>) and airborne fraction (0.2) (RIVM,2006).
- Based on the above, the inhalation exposure to LAS is estimated using the ConsExpo v4.1 exposure to spray model:

$$\text{Exp}_{\text{inhalation}} = \mathbf{1.31 \cdot 10^{-5} \text{ mg/m}^3/\text{day}}$$

#### *Dermal*

When cleaning a surface using a spray cleaner, dermal exposure to the aerosols from the cleaning spray can occur.

The following worst case should address this scenario:

- Highest concentration of LAS in spray cleaner is 6 % (internal AISE data).
- Immersion of hands and forearms into solution would expose about 1900 cm<sup>2</sup> (RIVM,2006).
- Assuming a contact rate of 100 mg/min (1.67 mg/s) and a release duration 24.6 s, the following local dermal exposure can be calculated:

$$1.67 \text{ (mg/s)} \times 24.6 \text{ (s)} \times 0.06 / 1900 \text{ (cm}^2\text{)} = 1.29 \cdot 10^{-3} \text{ mg/cm}^2\text{/day}$$
$$\text{Exp}_{\text{dermal,local}} = 1.29 \cdot 10^{-3} \text{ mg/cm}^2\text{/day}$$

Assuming a frequency of 365 times per year, and a body weight of 65 kg, the resulting dermal systemic dose is:

$$1.67 \text{ (mg/s)} \times 24.6 \text{ (s)} \times 0.06 / 65 \text{ (kg)} / 365/365 \text{ (frequency)} = 3.78 \cdot 10^{-2} \text{ mg/kg bw/day}$$
$$\text{Exp}_{\text{dermal,sys}} = 3.78 \cdot 10^{-2} \text{ mg/kg bw/day}$$

#### 5.1.3.8 Oral exposures to LAS

Oral exposures can be assumed to originate from drinking water and food (fruits and vegetables) and from residues over eating utensils and dishes washed in hand dishwashing detergents (machine dishwashing products do not contain LAS).

- It is assumed that every day dinnerware is used for food and drinks, resulting in a frequency of 365 year<sup>-1</sup>.
- The value for amount of water left on dishes is 5.5 · 10<sup>-5</sup> mL/cm<sup>2</sup> and the value for the area of dishes in daily contact with food is 5400 cm<sup>2</sup>. The concentration of the dishwashing water is 1.4 g/L. Using these data, the ingested product amount is 5.5 · 10<sup>-5</sup> mL/cm<sup>2</sup> x 5400 cm<sup>2</sup> x 1.4 mg/mL = 0.42 mg (RIVM,2006).
- Assuming a weight fraction of 0.3 and a body weight of 65 kg, the oral exposure to LAS is estimated to be:

$$0.42 \text{ (mg)} \times 0.3 / 65 \text{ (kg)} = 1.94 \cdot 10^{-3} \text{ mg/kg bw/day}$$
$$\text{Exp}_{\text{oral,sys}} = 1.94 \cdot 10^{-3} \text{ mg/kg bw/day}$$

#### 5.1.3.9 Inhalation and skin contact from laundry pretreatment products: Spray spot removers

##### *Inhalation*

When pretreating laundry with spray spot remover, inhalation exposure to the aerosols from the spray spot remover.

The following worst case should address this scenario:

- Highest concentration of LAS in spray spot remover is 14 % (internal AISE data).

- The exposure duration is the time per task for the use of laundry-pre-treatment, which is estimated at 10 min (AISE data). The spray duration is set at 0.05 min (3 s) (RIVM,2006).
- The room volume is set at 10 m<sup>3</sup>, the room height at 2.5 m (RIVM,2006).
- The mass generation rate is 1.5 g/s, and the weight fraction non-volatile is 0.14.
- Default values are used for ventilation rate (2 hr<sup>-1</sup> = 5.6·10<sup>-4</sup> s<sup>-1</sup>), inhalation cut-off diameter (15 μm), density non-volatile (1.8 g/cm<sup>3</sup>) and airborne fraction (0.2) (RIVM,2006).
- Based on the above, the inhalation exposure to LAS is estimated using the ConsExpo v4.1 exposure to spray model:

$$\mathbf{Exp_{inhalation} = 3.51 \cdot 10^{-6} \text{ mg/m}^3/\text{day}}$$

### *Dermal*

When pretreating laundry with spray spot remover, dermal exposure to the aerosols from the spray spot remover can occur.

The following worst case should address this scenario:

- Highest concentration of LAS in spray spot remover is 14 % (internal AISE data).
- The exposed area would be two hands, 840 cm<sup>2</sup>.
- Assuming a contact rate of 46 mg/min (0.77 mg/s) and a release duration of 3 s, the following local dermal exposure can be calculated:

$$0.77 \text{ (mg/s)} \times 3 \text{ (s)} \times 0.14 / 840 \text{ (cm}^2\text{)} = 3.83 \cdot 10^{-4} \text{ mg/cm}^2/\text{day}$$

$$\mathbf{Exp_{dermal,local} = 3.83 \cdot 10^{-4} \text{ mg/cm}^2/\text{day}}$$

Assuming a frequency of 128 times per year, and a body weight of 65 kg, the resulting dermal systemic dose is:

$$0.77 \text{ (mg/s)} \times 3 \text{ (s)} \times 0.14 / 65 \text{ (kg)} / 365/128 \text{ (frequency)} = 1.74 \cdot 10^{-3} \text{ mg/kg bw/day}$$

$$\mathbf{Exp_{dermal,sys} = 1.74 \cdot 10^{-3} \text{ mg/kg bw/day}}$$

### **5.1.3.10 Skin contact from laundry pretreatment products: Liquid spot removers**

When using a liquid spot remover to remove spots from laundry, dermal exposure to the spot remover can occur.

The following worst case should address this scenario:

- Highest concentration of LAS in spray spot remover is 14 % (internal AISE data).
- The exposed area would be two hands, 840 cm<sup>2</sup>.
- Assuming an applied amount of 650 mg, the following local dermal exposure can be calculated:

$$650 \text{ (mg)} \times 0.14 / 840 \text{ (cm}^2\text{)} = 0.11 \text{ mg/cm}^2/\text{day}$$

$$\text{Exp}_{\text{dermal,local}} = 0.11 \text{ mg/cm}^2/\text{day}$$

Assuming a frequency of 128 times per year, and a body weight of 65 kg, the resulting dermal systemic dose is:

$$650 \text{ (mg)} \times 0.14 / 65 \text{ (kg)} / 365/128 \text{ (frequency)} = 0.49 \text{ mg/kg bw/day}$$
$$\text{Exp}_{\text{dermal,sys}} = 0.49 \text{ mg/kg bw/day}$$

### 5.1.3.11 Inhalation and skin contact from liquid cleaner products: Oven cleaner (spraying)

This scenario describes the cleaning of a cold oven once every fortnight with a trigger spray. The oven has a surface area of 0.9 m<sup>2</sup> (30 cm x 40 cm x 45 cm). After spraying the oven door is closed and the product has to soak.

#### *Inhalation*

The following worst case should address this scenario:

- Highest concentration of LAS in oven cleaner is 10 % (internal AISE data).
- The exposure duration is the time of being in the room, which is estimated at 60 min. The spray duration is set at 0.5 min (30 s) (RIVM,2006).
- The room volume is set at 15 m<sup>3</sup>, the room height at 2.5 m (RIVM,2006).
- The mass generation rate is 0.78 g/s, and the weight 6.9·10<sup>-4</sup> s<sup>-1</sup>, inhalation cut-off diameter (15 µm), density non-volatile (1.8 g/cm<sup>3</sup>) and airborne fraction (0.2) (RIVM,2006).
- Based on the above, the inhalation exposure to LAS is estimated using the ConsExpo v4.1 exposure to spray model:

$$\text{Exp}_{\text{inhalation}} = 1.90 \cdot 10^{-6} \text{ mg/m}^3/\text{day}$$

#### *Dermal*

The following worst case should address this scenario:

- Highest concentration of LAS in spray spot remover is 10 % (internal AISE data).
- The exposed area would be half of two hands, 430 cm<sup>2</sup>.
- Assuming a contact rate of 46 mg/min (0.77 mg/s) and a release duration of 30 s, the following local dermal exposure can be calculated:

$$0.77 \text{ (mg/s)} \times 30 \text{ (s)} \times 0.1 / 430 \text{ (cm}^2\text{)} = 5.35 \cdot 10^{-3} \text{ mg/cm}^2/\text{day}$$
$$\text{Exp}_{\text{dermal,local}} = 5.35 \cdot 10^{-3} \text{ mg/cm}^2/\text{day}$$

Assuming a frequency of 26 times per year, and a body weight of 65 kg, the resulting dermal systemic dose is:

$$0.77 \text{ (mg/s)} \times 30 \text{ (s)} \times 0.1 / 65 \text{ (kg)} / 365/26 \text{ (frequency)} = 2.52 \cdot 10^{-3} \text{ mg/kg bw/day}$$

$$\mathbf{Exp_{dermal,sys} = 2.52 \cdot 10^{-3} \text{ mg/kg bw/day}}$$

### 5.1.3.12 Skin contact from liquid cleaner products: Oven cleaner (cleaning)

After treatment with the cleaner, the oven is wiped clean with a wet cloth or sponge and one has to ringe frequently. It is assumed users will not wear gloves, therefore the dermal exposure has been determined.

The following worst case should address this scenario:

- Highest concentration of LAS in spray spot remover is 10 % (internal AISE data).
- The exposed area would be half of two hands, 430 cm<sup>2</sup>.
- Assuming an applied amount of 200 mg, the following local dermal exposure can be calculated:

$$200 \text{ (mg)} \times 0.1 / 430 \text{ (cm}^2\text{)} = 4.65 \cdot 10^{-2} \text{ mg/cm}^2\text{/day}$$

$$\mathbf{Exp_{dermal,local} = 4.65 \cdot 10^{-2} \text{ mg/cm}^2\text{/day}}$$

Assuming a frequency of 26 times per year, and a body weight of 65 kg, the resulting dermal systemic dose is:

$$200 \text{ (mg)} \times 0.1 / 65 \text{ (kg)} / 365/26 \text{ (frequency)} = 2.19 \cdot 10^{-2} \text{ mg/kg bw/day}$$

$$\mathbf{Exp_{dermal,sys} = 2.19 \cdot 10^{-2} \text{ mg/kg bw/day}}$$

### 5.1.3.13 Inhalation and skin contact from liquid cleaner products: Bathroom cleaners (mixing & loading)

Bathroom cleaning liquids are periodically applied as descaling products. In this scenario the mixing and loading of bathroom cleaning liquid in a bucket of water is described.

#### *Inhalation*

The following worst case should address this scenario:

- Highest concentration of LAS in spray spot remover is 2.2 % (RIVM,2006).
- The exposure duration is the time per task for the use of bathroom cleaners, which is estimated at 0.75 min. The application duration is set at 0.3 min (18 s) (RIVM,2006).
- The room volume is set at 1 m<sup>3</sup>; room volume is interpreted here as 'personal volume', a small area of 1 m<sup>3</sup> around the user (RIVM,2006). The release area is 20 cm<sup>2</sup>.
- The mass transfer rate is 2.04 · 10<sup>3</sup>, and the molecular weight matrix is 26 g/mol.
- Default value is used for ventilation rate (2 hr<sup>-1</sup> = 5.6 · 10<sup>-4</sup> s<sup>-1</sup>), and the amount used is 500 g.
- Based on the above, the inhalation exposure to LAS is estimated using the ConsExpo v4.1 evaporation model:

$$\text{Exp}_{\text{inhalation}} = 2.37 \cdot 10^{-19} \text{ mg/m}^3/\text{day}$$

This amount does not contribute significantly to the total exposure of LAS.

#### *Dermal*

The following worst case should address this scenario:

- Highest concentration of LAS in spray spot remover is 2.2 % (RIVM,2006).
- The exposed area would be one palm, 215 cm<sup>2</sup>.
- Assuming an applied amount of 10 mg, the following local dermal exposure can be calculated:

$$10 \text{ (mg)} \times 0.022 / 215 \text{ (cm}^2\text{)} = 1.02 \cdot 10^{-3} \text{ mg/cm}^2/\text{day}$$
$$\text{Exp}_{\text{dermal,local}} = 1.02 \cdot 10^{-3} \text{ mg/cm}^2/\text{day}$$

Assuming a frequency of 4 times per year, and a body weight of 65 kg, the resulting dermal systemic dose is:

$$10 \text{ (mg)} \times 0.022 / 65 \text{ (kg)} / 365/4 \text{ (frequency)} = 3.71 \cdot 10^{-5} \text{ mg/kg bw/day}$$
$$\text{Exp}_{\text{dermal,sys}} = 3.71 \cdot 10^{-5} \text{ mg/kg bw/day}$$

#### **5.1.3.14 Inhalation and skin contact from liquid cleaner products: Bathroom cleaners (cleaning)**

Bathroom cleaning liquids are periodically applied as descaling products. In this scenario the mixing and loading of bathroom cleaning liquid in a bucket of water is described.

#### *Inhalation*

The following worst case should address this scenario:

- Highest concentration of LAS in spray spot remover is 2.2 % (RIVM,2006).
- The exposure duration is the time per task for the use of bathroom cleaners, which is estimated at 25 min. The application duration is set at 20 min (1200 s) (RIVM,2006).
- The room volume is set at 10 m<sup>3</sup> (RIVM,2006). The release area is 6.4·10<sup>4</sup> cm<sup>2</sup>.
- The mass transfer rate is 2.04·10<sup>3</sup>, and the molecular weight matrix is 18 g/mol.
- Default value is used for ventilation rate (2 hr<sup>-1</sup> = 5.6·10<sup>-4</sup> s<sup>-1</sup>), and the amount used is 260 g.
- Based on the above, the inhalation exposure to LAS is estimated using the ConsExpo v4.1 evaporation model:

$$\text{Exp}_{\text{inhalation}} = 9.26 \cdot 10^{-18} \text{ mg/m}^3/\text{day}$$

This amount does not contribute significantly to the total exposure of LAS.

### *Dermal*

The following worst case should address this scenario:

- Highest concentration of LAS in spray spot remover is 2.2 % (RIVM,2006).
- The exposed area would be the hands and forearms, 1900 cm<sup>2</sup>.
- Assuming an applied amount of 19000 mg, the following local dermal exposure can be calculated:

$$19000 \text{ (mg)} \times 0.022 / 1900 \text{ (cm}^2\text{)} = 0.22 \text{ mg/cm}^2\text{/day}$$
$$\mathbf{Exp_{dermal,local} = 0.22 \text{ mg/cm}^2\text{/day}}$$

Assuming a frequency of 4 times per year, and a body weight of 65 kg, the resulting dermal systemic dose is:

$$19000 \text{ (mg)} \times 0.022 / 65 \text{ (kg)} / 365/4 \text{ (frequency)} = 7.04 \cdot 10^{-2} \text{ mg/kg bw/day}$$
$$\mathbf{Exp_{dermal,sys} = 7.04 \cdot 10^{-2} \text{ mg/kg bw/day}}$$

### **5.1.3.15 Inhalation and skin contact from liquid cleaner products: Floor cleaners (mixing)**

Floor cleaners, which contain soap, are meant for daily or periodically removing all kinds of grease and dirt from different sorts of floors.

#### *Inhalation*

The following worst case should address this scenario:

- Highest concentration of LAS in spray spot remover is 5 % (RIVM,2006).
- The exposure duration is the time per task for the use of floor cleaners, which is estimated at 0.75 min. The application duration is set at 0.3 min (18 s) (RIVM,2006).
- The room volume is set at 1 m<sup>3</sup>; room volume is interpreted here as 'personal volume', a small area of 1 m<sup>3</sup> around the user (RIVM,2006). The release area is 20 cm<sup>2</sup>.
- The mass transfer rate is 2.04 · 10<sup>3</sup>, and the molecular weight matrix is 22 g/mol.
- Default value is used for ventilation rate (0.5 hr<sup>-1</sup> = 1.3 · 10<sup>-4</sup> s<sup>-1</sup>), and the amount used is 500 g.
- Based on the above, the inhalation exposure to LAS is estimated using the ConsExpo v4.1 evaporation model:

$$\mathbf{Exp_{inhalation} = 1.23 \cdot 10^{-17} \text{ mg/m}^3\text{/day}}$$

This amount does not contribute significantly to the total exposure of LAS.

### *Dermal*

The following worst case should address this scenario:

- Highest concentration of LAS in spray spot remover is 5 % (RIVM,2006).
- The exposed area would be one palm, 215 cm<sup>2</sup>.
- Assuming an applied amount of 10 mg, the following local dermal exposure can be calculated:



$$10 \text{ (mg)} \times 0.05 / 215 \text{ (cm}^2\text{)} = 2.33 \cdot 10^{-3} \text{ mg/cm}^2\text{/day}$$

$$\mathbf{Exp_{dermal,local} = 2.33 \cdot 10^{-3} \text{ mg/cm}^2\text{/day}}$$

Assuming a frequency of 104 times per year, and a body weight of 65 kg, the resulting dermal systemic dose is:

$$10 \text{ (mg)} \times 0.05 / 65 \text{ (kg)} / 365/104 \text{ (frequency)} = 2.19 \cdot 10^{-3} \text{ mg/kg bw/day}$$

$$\mathbf{Exp_{dermal,sys} = 2.19 \cdot 10^{-3} \text{ mg/kg bw/day}}$$

### 5.1.3.16 Inhalation and skin contact from liquid cleaner products: Floor cleaners (cleaning)

Floor cleaners, which contain soap, are meant for daily or periodically removing all kinds of grease and dirt from different sorts of floors.

#### *Inhalation*

The following worst case should address this scenario:

- Highest concentration of LAS in spray spot remover is 5 % (RIVM,2006).
- The exposure duration is the time per task for the use of floor cleaners, which is estimated at 240 min. The application duration is set at 30 min (1200 s) (RIVM,2006).
- The room volume is set at 58 m<sup>3</sup> (RIVM,2006). The release area is 2.2·10<sup>5</sup> cm<sup>2</sup>.
- The mass transfer rate is 2.04·10<sup>3</sup>, and the molecular weight matrix is 18 g/mol.
- Default value is used for ventilation rate (0.5 hr<sup>-1</sup> = 1.3·10<sup>-4</sup> s<sup>-1</sup>), and the amount used is 8800 g.
- Based on the above, the inhalation exposure to LAS is estimated using the ConsExpo v4.1 evaporation model:

$$\mathbf{Exp_{inhalation} = 5.43 \cdot 10^{-15} \text{ mg/m}^3\text{/day}}$$

This amount does not contribute significantly to the total exposure of LAS.

#### *Dermal*

The following worst case should address this scenario:

- Highest concentration of LAS in spray spot remover is 5 % (RIVM,2006).
- The exposed area would be the hands and forearms, 1900 cm<sup>2</sup>.
- Assuming an applied amount of 19000 mg, the following local dermal exposure can be calculated:

$$19000 \text{ (mg)} \times 0.05 / 1900 \text{ (cm}^2\text{)} = 0.5 \text{ mg/cm}^2\text{/day}$$

$$\mathbf{Exp_{dermal,local} = 0.5 \text{ mg/cm}^2\text{/day}}$$

Assuming a frequency of 104 times per year, and a body weight of 65 kg, the resulting dermal systemic dose is:

$$19000 \text{ (mg)} \times 0.05 / 65 \text{ (kg)} / 365/104 \text{ (frequency)} = 4.16 \text{ mg/kg bw/day}$$

$$\text{Exp}_{\text{dermal,sys}} = 4.16 \text{ mg/kg bw/day}$$

### 5.1.3.17 Accidental or intentional overexposure

Accidental or intentional overexposure to LAS may occur via household detergent products, which may contain up to 30% of LAS.

No fatal cases or serious injuries arising from accidental ingestion of LAS by humans are known to us. The accidental or intentional overexposure to LAS directly is not considered a likely occurrence for consumers, but it may occur via household detergent products. The German Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV,1999) published recently a report on products involved in poisoning cases. No fatal case of poisoning with detergents was reported in this report. Detergent products were not mentioned as dangerous products with a high incidence of poisoning.

Equally, in the UK, the Department of Trade and Industry (DTI) produces an annual report of the home accident surveillance system (HASS). The data in this report summarizes the information recorded at accident and emergency (A&E) units at a sample of hospitals across the UK. It also includes death statistics produced by the Office for National Statistics for England and Wales. The figures for 1998 show that for the representative sample of hospitals surveyed, there were 33 reported accidents involving detergent washing powder (the national estimate being 644) with none of these resulting in fatalities (DTI,1998). In 1996 and 1997, despite their being 43 and 50 reported cases, respectively, no fatalities were reported either.

Accidental exposure of the eye to LAS will occur in consumers only via splashes or spills with a formulated product. Therefore, the eye irritation potential has to be considered in the context of accidental exposure.

## 5.2 Hazard assessment

### 5.2.1 Summary of the available toxicological data

#### 5.2.1.1 Toxicokinetics

The absorption, distribution, metabolism and elimination of LAS (radio-labelled with <sup>35</sup>S; chain length: C<sub>10-14</sub>) were studied in male Charles River rats (Michael,1968). LAS was administered as an aqueous solution. The compound was readily absorbed from the gastrointestinal tract (80-90% of the dose). Most of the absorbed <sup>35</sup>S was eliminated within 72 hours and 60-65% of the absorbed dose was eliminated in the urine, with sulfophenyl butanoic and sulfophenyl pentanoic acid as metabolites. These metabolites were not reabsorbed from the kidney tubules. 35% of the absorbed <sup>35</sup>S was excreted in the bile and then reabsorbed completely from the gastrointestinal tract.

Although the metabolites in the bile were not identified, it was shown that no unchanged LAS was eliminated via this pathway. The authors suggested that metabolism proceeded via omega oxidation with subsequent beta-oxidation. Retention of radioactivity was not observed in any organ.

LAS is well absorbed via the gastrointestinal tract of pigs treated with 3.3 mmol/animal <sup>35</sup>S-Na-dodecylbenzene sulphonate (Havermann et al.,1959). At 200 hours after oral administration, the radioactivity was relatively high in bristles and bones, while low in liver, kidney and spleen. After 10 weeks only traces of radioactivity were still in the body. 40 hours after the administration, 40% of the dose was excreted into the urine and 60% of the dose via the faeces.

Rhesus monkeys (*Macaca mulatta*) were dosed orally with  $^{14}\text{C}$ -LAS at a dose level of 150 mg/kg (Cresswell et al.,1978). Plasma radioactivity concentrations reached a maximum of 41.2  $\mu\text{g/ml}$  at 4 hr and then declined during the following 6-24 hours with a biological half-life of about 6.5 hrs. After 7 consecutive daily doses of 30 mg/kg, both plasma concentrations of radioactivity and the biological half-life were almost identical to those observed after single administration. Two hours after the last dose, the highest radioactivity was observed in the stomach. Radioactivity was also observed in the intestinal tract, kidneys, liver, lung, pancreas, adrenals and pituitary. At 24 hours, concentrations were highest in the intestinal tract, probably indicating biliary excretion. Since the concentrations in the tissues were in general lower than in plasma, no specific accumulation of LAS occurred. When  $^{14}\text{C}$ -LAS was injected into the skin, most of the radioactivity remained at the site of injection. During the 120 hours after single oral (30 mg/kg) or subcutaneous (1 mg/kg) doses, average rates of excretion were between 63% and 74% in the urine and between 9% and 26% in the faeces. TLC of the urine extracts after oral or subcutaneous doses showed that only trace amounts of unchanged LAS were present. Five metabolites were excreted but they were not identified. Incubations with beta-glucuronidase/sulfatase did not affect the metabolites, indicating that the metabolites were probably not present as the corresponding conjugates.

Rats were dosed orally with  $^{14}\text{C}$ -NaLAS and radioactivity was detected 0.25 hr after administration, reaching a maximum at 2 hrs (Sunakawa et al.,1979). The biologically half-lives were calculated to be 10.9 hrs. The distribution was high in the digestive tract and in the bladder at 4 hours after administration. Concentrations were also high in the liver, kidney, testis, spleen and lung. Sixty-eight hours after administration, the rates of excreted radioactivity were 47% in the urine and 50% in the faeces.

$^{35}\text{S}$ -LAS ( $15 \cdot 10^8$  cpm) was administered topically, once, onto the back skin of rats and guinea pigs (Chikara Debane,1978). Absorption and distribution in major organs and blood were studied. Urine was collected 24 hours after topical application of the test substance. In the guinea pig, the amount of  $^{35}\text{S}$  excreted in the urine was about 0.1% of the total administered dose. Organ distribution in the rat was about 5 times greater than in the guinea pig and "relatively large amounts" of  $^{35}\text{S}$  were noted in the liver and kidneys.

Conclusion: when 0.2-0.5% LAS is topically applied once, it is approximately absorbed by 0.1-0.6%; there was no accumulation in specific organs; the test chemical was quickly excreted in the urine after being metabolised.

Studies (Howes,1975) with isolated human skin preparations as well as in vivo investigations of percutaneous administration of LAS to rats have demonstrated that penetration through skin and subsequent systemic absorption of this surfactant does not occur to any significant extent at 24 to 48 hours.  $^{14}\text{C}$ -LAS was applied on the clipped dorsal skin of the rats, which was washed after 15 min. No radioactivity was detected in urine or faeces.

LAS was not detected in the uterus of pregnant ICR mice administered with a single oral dose of 350 mg/kg bw on day 3 of gestation (Koizumi et al.,1985).

## Conclusions

- LAS is readily absorbed by the gastro-intestinal tract (80-90% of the administered dose)
- The LAS absorption through intact skin is very poor (0.1-0.6% of the administered dose)
- LAS is distributed to most organs, except uterus, and the major part is metabolised in the liver to sulfophenyl carboxyl acids
- LAS metabolites are eliminated primarily via the urine and faeces

- Main urinary LAS metabolites are sulfophenyl butanoic and sulfophenyl pentanoic acids; most of them are normally excreted within 24 hours.
- Accumulation of LAS or of its main metabolites has not been observed after repeated oral administration
- The good absorption of LAS by the gut and its very poor adsorption by the skin is an interesting observation. To explain it, one could speculate that the gut microflora may be adapting with time and causing metabolism of LAS before it is absorbed.

### **5.2.1.2 Acute toxicity**

#### **5.2.1.2.1 Acute oral toxicity**

Six acute toxicity tests are available, five with rats (Hüls-a,1984; Hüls-b,1984; Hüls-c,1984; Ito et al.,1978; Huntingdon,1984; Murmann,1984), and one with mice (Ito et al.,1978).

In a well documented and conducted study with rats (Huntingdon,1984), according to GLP and the OECD 401 method, clinical observations, at doses near the LD<sub>50</sub> values (1980 mg/kg bw), were piloerection, hunched posture, abnormal gait (waddling), lethargy, decreased respiratory rate, ptosis, pallor of the extremities and diarrhoea. Recovery was apparently complete by day 4 for survivors. Deaths occurred within 24 hours after administration. Autopsy of rats that died revealed isolated cases of pallor of the kidneys or spleen. Terminal necropsy findings for survivors were normal.

The oral acute toxicity of the test substance in rats was also examined in another study (Murmann,1984). Groups of 5 male and 5 female rats were exposed orally via gavage to 0, 1075, 1220, 1360, or 1710 mg/kg bw of test substance (all doses reported were adjusted from the original for 86% activity). The animals were then monitored for 14 days for mortality and clinical signs. Body weights were measured on days 7 and 14, and necropsies were performed at the end of the study. No effects on body weight were observed, but all animals showed some signs of toxicity. Symptoms beginning about 30 minutes past application included diarrhea, squatting attitude, breathing difficulties, nose bleeding, ataxia, and lethargy. These symptoms had disappeared in surviving animals by 120 hours. In the animals that died before the end of the study, red mucous was seen in the stomach and intestine. In the surviving animals, hyperemia of the stomach was noted, along with abnormalities of the stomach, liver, spleen, kidneys, and the peritoneum. Mortality was seen at all dose levels, with 4 of 10 animals at the lowest dose level dying. All animals at the highest dose level died. The acute oral LD<sub>50</sub>, when adjusted for active content was 1080 mg/kg bw.

#### **Conclusion**

The acute oral LD<sub>50</sub> for rats was 1080 mg/kg bw. Mortality was seen at all dose levels. In addition, all animals showed some signs of toxicity, with symptoms including diarrhea, squatting attitude, breathing difficulties, nose bleeding, ataxia, and lethargy, though all of these symptoms had disappeared in surviving animals by 120 hours. In the surviving animals, hyperemia of the stomach was noted, along with abnormalities of the stomach, liver, spleen, kidneys, and the peritoneum. According to the CLP-Regulation, the test substance is a Category IV toxicant (H302: Harmful if swallowed).

#### **5.2.1.2.2 Acute inhalation toxicity**

Acute inhalation data are available for LAS (Kinney,1985). Groups of six 8-week old rats underwent nose-only exposures to aerosol atmospheres containing 65, 120, 260 or 310 mg/m<sup>3</sup> particulate LAS (C<sub>12</sub>, 98% activity) for 4 hours, followed by 14 days of observations for clinical signs. During exposures, rats in all groups had clear to red nasal discharge. During the recovery

period, rats exhibited dose dependent weight loss 1 day post exposure followed by normal weight gains. No mortality or adverse clinical signs occurred at concentrations up to 260 mg/m<sup>3</sup>. At 310 mg/m<sup>3</sup>, MMAD (Mass Median Diameter) = 2.5 microns, one rat died during the exposure and two rats died one day post exposure.

It is important to note that this laboratory exposure is not representative of the possible LAS exposure during actual production or use and, therefore, its relevance is limited. In the study, animals were given high exposures to respirable-sized particles, which were generated by special, difficult laboratory procedures. LAS particles of that size do not occur under normal conditions. Spray products containing LAS are designed to produce large particle sizes. These large particles are needed for efficient delivery of the spray to the surface being cleaned. This results in particle sizes that are much larger than the respirable particle sizes used in testing and, therefore, would not be able to reach far into the lungs where effects could occur. Given this lack of relevance to real-world exposure potential, the use of this study for risk assessment purposes is limited.

### **Conclusion**

Given this lack of relevance to real-world exposure potential, the use of this acute inhalation study for risk assessment purposes is limited. Due to the irritant nature of LAS, it is expected that high LAS aerosol concentrations may be irritating to the upper respiratory tract.

#### **5.2.1.2.3 Acute Dermal Toxicity**

The acute dermal toxicity of LAS was investigated in three rabbit studies (Monsanto,1971; Monsanto-a,1972; Monsanto-b,1972). Toxicity effects were found at doses ranging from 251 mg/kg bw to 794 mg/kg bw. Control groups were not used. The number of animals was 1 per dose substance and not from the same sex. Clinical observations were reduced appetite, reduced activity, increased weakness and collapse. Necropsy findings consisted of haemorrhagic lungs, liver discoloration, enlarged gall bladder, and gastro-intestinal inflammation (only observed in the animals that died).

A limit test study was performed on rats, according to the OECD 402 Method and GLP (Huntingdon-a,1986). There were no deaths following a single dermal application of 2000 mg/kg bw of LAS at 47% of active matter. No signs of systemic reaction to treatment. Well-defined or slight erythema and slight oedema were observed at all test sites after removal of the occlusive dressings on day 2. These reactions were unresolved before progressive hardening of the skin that was first detected on day 4. All test sites were entirely covered by scab formation from day 7. Sloughing from the scabbed skin began at various times between day 7 and day 12 and was completed before termination. Low bodyweight gains or loss of bodyweight were recorded for one male and three female rats on day 8. The three female rats also showed low bodyweight gains between day 8 and 15. Terminal necropsy findings were normal in all animals.

The clipped skin on the backs (approximate 10% of the area) of five male and five female rats was exposed to a dose of 2000 mg/kg bw LAS and kept under an occlusive dressing for 24 hours, then observed for another 14 days after the dressing was removed and the skin washed in warm water. The treated areas were examined daily for signs of dermal irritation and assessed according to the standard scoring system for erythema, eschar and oedema. On day 15 all animals were sacrificed and given a macroscopic post-mortem examination of internal organs. No mortality was observed at exposures to 2000 mg/kg of the undiluted test material. There were no signs of systemic reaction. Well defined or slight erythema and slight oedema were observed at all test sites after removal of the occlusive dressings, and these reactions were unresolved before progressive hardening of the skin was first detected on day 4. All test sites were entirely covered by scab formation from day 7.

Sloughing from the scabbed skin began at various times between day 7 and day 12 and was completed before test termination. Therefore, results indicate slight erythema and slight oedema but no acute mortality. The dermal LD<sub>50</sub> is > 2000 mg/kg bw.

### **Conclusion**

The quality of the data of studies by Monsanto,1971; Monsanto-a,1972; Monsanto-b,1972 on rabbits has to be rated as non reliable, mainly due to the fact that the animals were not sufficient number (only 1 per dose) and were not of the same sex. No information was provided about the concentration of the tested substance.

Reliable results come from a well-performed and documented limit test on rats (Huntingdon-a,1986), with a LD<sub>50</sub> >2000 mg/kg bw at the LAS concentration of 47% (LD<sub>50</sub> >1000 mg/kg bw at 100%). In the study by Kynoch,1986 no mortality was seen at exposures to 2000 mg/kg of undiluted LAS and no other signs of systemic reactions were observed. However, well defined or slight erythema and slight oedema were observed at all test sites immediately after removal of the occlusive dressings, and these reactions were unresolved before progressive hardening of the skin was first detected on day 4. All test sites were entirely covered by scab formation from day 7, and complete sloughing from the scabbed skin was completed before test termination. Therefore, results indicate slight erythema and slight oedema but no acute mortality from dermal exposures, with a dermal LD<sub>50</sub> of > 2000 mg/kg. Therefore the substance is not classified for acute dermal toxicity under CLP.

#### **5.2.1.3 Skin Irritation**

Several skin irritation studies on rabbits are available for LAS at the concentration of about 50%. (Huntingdon-b,1986; Hüls,1983; Kaestner-a,1987; Kaestner,1977; BIOLAB-a,1989). Findings of all the studies were consistent, showing similar irritation effects.

The most reliable study (Huntingdon-b,1986) was performed on three animals with a semi-occlusive application, according to the OECD Guideline 404 and GLP. LAS concentration as active matter was 47%. Three rabbits were exposed to 0.5 ml of the test substance dermally for 4 hours on clipped skin under a gauze pad held in place by an adhesive dressing. Examination of the treated skin was made approximately 30 minutes after removal of the patch and daily through 14 days. Grading and scoring of the dermal reactions was performed using the standard numerical scoring system. Irritation was noted in all animals at the first observation (maximum score of 2). Symptoms worsened, and desquamation, necrosis, and hyperkeratinization was noted by day 4. Symptoms resolved in one animal by day 12, but in the other two animals, symptoms were seen through the end of the observation period. The primary dermal irritation index was 2.17.

In other studies (BIOLAB,1988; BIOLAB-b,1989; BIOLAB,1983) LAS was tested at 1%, 2.5% and 5%, according to the modified Draize test. Six rabbits were used with a 24-hour application on intact and abraded skin. An occlusive dressing was applied in all experiments. For LAS at 1% and 2.5% no effects were found. The 5% dilution is considered a moderate irritant according to the Draize criteria.

### **Conclusion**

LAS in aqueous solutions, after a 24 hour application on intact and abraded skin of rabbits under occlusive dressing, did not show any irritation effects at concentrations of 1% and 2.5%, while it was moderately irritating at the concentration of 5% (Draize criteria). At higher concentrations, 47-50%, LAS is irritating, on the base of the available tests on the intact skin of rabbits with a 4 hour application under occlusive or semi-occlusive dressing. Irritation symptoms worsened after

exposure, and desquamation, necrosis, and hyperkeratinization were noted by day 4 in all animals. These resolved in one animal by day 12, but in the other two animals symptoms continued through the end of the observation period. Therefore, LAS is considered a Category 2 skin irritant.

#### **5.2.1.4 Eye irritation**

Four eye irritation studies on rabbits are available for LAS at the concentration of about 50% (Kaestner-b,1987; Hüls-b,1983; Huntingdon-c,1986; BIOLAB-c,1989). Findings of all the studies were consistent and showed significant irritation effects.

The most reliable and documented study (Huntingdon-c,1986), performed according to GLP and OECD Guidelines, was conducted on three rabbits with LAS at 47%. Groups of three rabbits had 0.1 ml of test substance placed in each of their eyes. In one group, the eyes were not rinsed. In the second group, the eyes were rinsed after 4 seconds of exposure. In the third group, the eyes were rinsed after 30 seconds of exposure. Observations were made one hour and 1, 2, 3, 4, 7, 14, and 21 days after exposure. Severe irritation was noted in the animals whose eyes were not rinsed. This irritation was not resolved in one of these animals at the end of 21 days. Irritation was also seen in the animals rinsed after 30 seconds, although the irritation was not as severe, and the effects were fully reversible within 14 days. Mild irritation was seen in the animals rinsed after 4 seconds. These effects were fully reversible within 7 days. Since OECD guideline 405 for eye irritation studies calls for an exposure of at least 24 hrs, the results for unrinsed eyes were used for classification.

Another study (BIOLAB-c,1989), conducted with LAS at 50% on six rabbits, showed significant irritation effects on iris and conjunctivae. These effects were persistent at day 6.

LAS was tested at lower concentrations as well. In two Japanese studies (Iimori et al.,1972; Oba et al.,1968), no abnormalities were found for animals treated with a test solution at 0.01% LAS. Slight and considerable irritation of the conjunctivae at 0.05% LAS, considerable irritation at 0.1% LAS within 2 hours, which disappeared at 24 hours, and marked reactions at 0.5% LAS (severe irritation and oedema, increased secretion, turbidity of the cornea and disappearance of the corneal reflex) for 24 hours. The eye tended to recovery and the effects disappeared completely after 120 hours. Averaged irritation scores are not available and the effects at 24, 48 and 72 hours cannot be quantitatively evaluated.

In two other studies (BIOLAB,1984; BIOLAB,1988), LAS at concentrations of 1% and 5% was tested on six rabbits, according to the OECD guidelines. Findings were that LAS is not irritating to eye at 1%, while it is moderately irritating at 5%. However, it is not classifiable as an irritant according the EU criteria.

#### **Conclusion**

LAS is not irritating to eye at 1%. It is moderately irritating at 5% (not classifiable as an irritant according the EU criteria). It is irritating to eye at concentrations of 47-50%.

Severe irritation was noted in the animals whose eyes were not rinsed and was not resolved by day 21. Milder irritation was observed in animals that had the test substance rinsed from their eye after 4 or 30 seconds, and effects seen in these rinsed animals were reversible within 7 or 14 days. Based on the irreversible irritation observed in the unrinsed animals, LAS is considered a Category 1 eye irritant (H318: Causes serious eye damage).

#### **5.2.1.5 Sensitisation**

##### **Tests on animals**

There are several studies available and the most reliable ones were selected (Hüls,1988; Procter & Gamble,1985; RBM,1985).

In the first study (Hüls,1988), performed with the Guinea Pig Maximisation Test (OECD method), LAS was used at 50%. With applications of LAS solutions at 0.1% as intracutaneous and at 3% as epicutaneous, negative results were obtained for all tested animals.

In the second study (Procter & Gamble,1985), carried out under the Buehler test (OECD method) and GLP, 10 animals (5M/5F) remained untreated and were used as controls to be treated at a first challenge, 10 animals (5M/5F) remained untreated and were used as additional controls to be treated at a second challenge, and 20 animals (10M/10F) were treated with LAS. Induction concentration was 1.0% LAS in water; first and second challenge concentrations were 0.8% LAS in water. Zero of 20 animals responded in the treated group; 0/10 animals responded in the control group.

In the last study, the potential of the test substance to be sensitising to skin was investigated (RBM,1985). Ten male and ten female guinea pigs were given intradermal injections of 25% test solution. Control animals (5 male and 5 female) were given injections of vehicle only. One week later, a second induction was done by dermal exposure to 25% test solution for 24 hrs. Control animals were again exposed to vehicle only. On day 21, the challenge exposure was performed. All animals were exposed to 12.5% test solution dermally. Exposure was for 24 hrs, with observations made at 48 and 72 hrs after the start of exposure. No positive reactions were noted.

### **Tests on human volunteers**

Two Human Repeat Insult Patch Tests are available.

In the first (Procter & Gamble,1997) 95 volunteers were treated with LAS at 0.10% (w/v) on the upper arms, under occlusive patch conditions. Test material was applied for 24 hours, 3 times a week, for 3 weeks during the induction period. After a 14- to 17-day rest, a 24-hour challenge patch was applied on the original and alternate arm sites. There was no evidence of skin sensitisation on the 95 subjects who completed the test.

In the second test 2294 volunteers were exposed to LAS as a raw material and 17,887 exposed to LAS in formulations (Nusair et al.,1988). No evidence of skin sensitisation was found.

An occlusive epicutaneous test was carried out on volunteers in Europe. LAS was applied once at 1%. The test duration was 6 days. The authors concluded that LAS was sufficiently compatible to the skin (Matthies,1989).

### **Conclusion**

No sensitisation potential was found for LAS when tested either on animals or on human volunteers.

#### **5.2.1.6 Repeated Dose Toxicity**

##### **5.2.1.6.1 Oral route**

LAS was administered for 90 days in the diet to groups of 15 male and 15 female rats at doses of 50 and 250 mg/kg bw/day (Oser,1965). Control groups were used. No behavioural abnormalities were noted during the test period. Growth responses were equal in all groups. There were no differences in food intake or in efficiency of food utilisation. The clinical data showed no abnormal variations in any of the dose groups. The relative organ weights and the histopathological evaluation did not show significant differences among the dose groups except for a slight liver weight increase in females of the highest dose group.



The NOAEL is 50 mg/kg bw/day.

LAS was administered for 90 days in the diet to 10 male/females rats per dose groups. Doses were 0.02%, 0.1% and 0.5% (8.8, 44, 220 mg/kg bw/day) (Kay et al.,1965 ). No adverse effects were found upon the following parameters: growth, food efficiency, survival, haematologic values, urinary analytical values, absolute and relative organ weights, gross and histopathological changes.

The NOAEL is 220 mg/kg bw/day, the highest tested dose.

LAS was administered for six months at doses of 0.07%, 0.2%, 0.6%, 1.8% in the diet (40, 115, 340 and 1030 mg/kg bw/day) to 10 rats per each sex (Yoneyama et al.,1972). Control groups were used. The 1.8% group showed diarrhoea, markedly suppressed growth, increased weight of the cecum, and remarkable degeneration of the renal tubes. The 0.6% (340 mg/kg bw/day) group showed slightly suppressed growth, increased weight of the cecum, increased activity of serum ALP, a decrease in serum protein and degeneration of the renal tubes. The 0.2 % (115 mg/kg bw/day) group showed increased weight of the cecum and slight degeneration of the renal tubes. The 0.07% (40 mg/kg bw/day) group showed no adverse effects related to the administration of LAS.

A NOAEL of 40 mg/kg bw/day was estimated.

LAS was administered for 9 months at doses of 0.6% and 1.8% (260 and 780 mg/kg bw/day) in the diet to male/female rats (8 animals per groups). Control groups were included (Yoneyama et al., 1976). In the 1.8% dose group, the body weight gain was suppressed and haematological and serum-biochemical adverse effects were observed in both treatment groups of both sexes. The weight of the cecum of the male rats and the weight of the liver and cecum of the females in the high dose groups were significantly increased. Enzymatic examinations of the liver and kidneys revealed changes in enzyme activities in the 1.8% groups.

A NOAEL of 260 mg/kg bw/day was estimated.

LAS was administered for 2, 4 and 12 weeks, at the a single dose of 1.5% in the diet (750 mg/kg bw/day) to groups of 5 male rats, with control groups (Ikawa et al.,1978). LAS suppressed body weight gain, and the relative liver weight was increased after 2 weeks of LAS administration. Serum biochemical examinations revealed significant increases in ALP and GTP at each observation period and significant decreases in cholesterol and protein in 4 weeks. Enzymatic examinations of the liver revealed decreases in G6Pase and G6PDH and an increase in isocitrate dehydrogenase (IDH) at each observation period. Enzymatic examinations of the renal cortex revealed decreases in G6Pase and 5'-nucleotidase at each observation period, an increase in LDH at 12 weeks, and an increase in IDH at 2 and 4 weeks. Enzymatic examinations in the renal medulla revealed a decrease in Na,K-ATPase, an increase in LDH at 12 weeks, a decrease in IDH at 2 weeks, and an increase in IDH at 12 weeks.

Effects were found at the only dose tested, equivalent to 750 mg/kg bw/day.

LAS was administered to male/female rats for 9 months in drinking water, at doses of 0.07%, 0.2% and 0.6% (85, 145, 430 mg/kg bw/day) (Yoneyama et al.,1976). Control groups were used.

Haematological examination revealed no significant changes in any experimental group and no organ weight changes were observed. Body weight gain was suppressed in the males of the highest dose group and also serum-biochemical and enzymatic parameters of the liver and kidney were affected. A significant decrease in renal Na,K-ATPase was seen in the group given 145 mg/kg bw/day of LAS.

The NOAEL is 85 mg/kg bw/day and the LOAEL is 145 mg/kg bw/day.

LAS was administered by gavage to male/females rats (12 animals per dose group) for one month, at daily doses of 125, 250, 500 mg/kg bw/day (Ito et al.,1978). Control groups were used. Diarrhoea was observed in the 500 mg/kg group and soft stools were observed in the other 2 groups. Body weight gain was suppressed in all the male groups and in the female 500 mg/kg bw/day group.

Haematological examinations revealed no abnormalities. Serum-biochemical examinations revealed several differences among the mid and high dose group compared to the control group. The weight of the spleen and the heart significantly decreased in the male high dose group. In the female high dose group, the weight of the liver increased while the weight of the heart and thymus decreased.

Histological findings of the liver revealed no abnormalities.

The NOAEL was 125 mg/kg bw/day.

LAS was administered to mice for six months in drinking water, at the dose of 100 ppm, corresponding to 20 mg/kg bw/day (Watari et al.,1977). No data about sex and number of animals are available. Control groups were used. The animals were sacrificed at 1, 2, 3, and 6 months. Some animals were observed an additional 2 months without test substance administration. Liver slices were investigated using electron microscopy. Hepatic damage was observed at one and six months. In mice examined after the two-month recovery some hepatic damage was seen, while other cellular effects had reversed, indicating that the liver cells had recovered.

LOAEL = 20 mg/kg bw/day

Groups of 8 or 9 male/females mice were given diets containing LAS at concentrations of 0.6 and 1.8% (corresponding to 500 and 1000 mg/kg bw/day) or drinking water containing LAS at concentrations of 0.07%, 0.2% and 0.6% for 9 months (corresponding to 100, 250, 600 mg/kg bw/day for males and to 100, 250, 900 mg/kg bw/day for females) (Yoneyama et al.,1976). Control groups were used.

**LAS in diet:** in the mice given 500 mg/kg bw/day, body weight gain was not suppressed, but the weight of the liver increased in male and female mice. Enzymatic examinations revealed significant decreases in LDH of the liver and in acid phosphatase of the kidneys in the male mice.

**LAS in drinking water:** body weight was depressed at the highest dose for male and females, increase in liver weight in females, significant decreases in renal Na,K-ATPase.

LOAEL: 500 mg/kg bw/day (in diet)

NOAEL: 250 mg/kg bw/day (in water)

A 28-day study on male/female Rhesus monkeys (*Macaca mulatta*) was conducted (Heywood et al.,1978). LAS was given to four groups of three males and three females at doses of 30, 150, 300 mg/kg bw/day per gavage (po) and simultaneously with 0.1, 0.5, or 1.0 mg/kg bw/day subcutaneously (sc). Control groups were used. At 300 (po) and 1.0 (sc) mg/kg bw/day, the monkeys vomited frequently and usually within 3 hours of administration. An increased frequency of loose or liquid faeces was recorded for animals receiving 150 (po) and 0.5 (sc) mg/kg bw/day. These effects are probably related to the inherent irritative effects of LAS rather than to its systemic toxicity. Fibrosis of the injection sites was found among the entire test group, the incidence and severity being dose related. Ophthalmoscopy, laboratory examination of blood and urine, organ weight analysis and histopathological investigation did not detect any further treatment-related responses.

The demonstrated systemic NOAEL is 150 mg/kg bw/day (po) + 0.5 mg/kg bw/day (sc), since animals vomited at the higher dose level and may not have been truly exposed to LAS.

In a well-documented study of teratogenicity (Nolen et al.,1975) (see 5.2.1.9), a mixture of 55% of tallow alkyl ethoxylate sulphate and 45% of LAS was fed to two generations of rats either continuously to males and females during the 8-week growth period or only to females during the organogenesis period. Control groups were used. Seven groups of 25 male and 25 female rats received dietary levels of the mixture of 0.1%, 0.5% and 1% (50, 250 and 500 mg/kg bw/day). The corresponding doses of LAS were 22.5, 112.5 and 225 mg/kg bw/day. No significant effects were seen in weight gain, organ/body weight ratios, haematology values and histopathology during both the first generation 8-week period and the second-generation period.

The NOAEL for LAS is the highest dose tested of 225 mg/kg bw/day.

In a three-generation study with rats for reproductive toxicity (Palmer et al.,1974) (see 5.2.1.8), findings of the oral administration for 60 days of LAS in a commercial light duty liquid detergent (17% LAS and 7% alkyl ethoxylate sulphate) are available. This study is well documented and complies with guidelines recommended by the US-FDA and GLP. Dietary concentrations of 0, 0.08%, 0.4% and 2% (0, 40, 200 and 1000 mg/kg bw/day) of the formulation were continuously administered throughout three generations for 60 days prior to mating. The corresponding administration of LAS was of 0, 6.8, 34 and 170 mg/kg bw/day. The number of parental animals per group, control groups included, were 11 males and 22 females for the F0 generation and 10 males and 20 females for F1b and F2b. Among parental animals over the three generations there were no signs of adverse effects to treatment. Food consumption and bodyweight changes showed no consistent relationship to dosage. The terminal necropsy revealed no changes attributable to treatment.

The NOAEL for LAS is the highest dose tested of 170 mg/kg bw/day.

#### 5.2.1.6.2 Inhalation

Long-term studies on LAS inhalation are not available. Given the irritant nature of LAS, it is expected that repeated inhalation of LAS might be irritating to the respiratory tract.

#### 5.2.1.6.3 Dermal route

LAS was applied for 15 days to the backs of male rats, at daily doses of 0.5 g of solutions at 20 and 30% (about 286 and 427 mg/kg bw/day) (Sadai et al.,1972). On the 16<sup>th</sup> day of the experiment, the animals were assessed. Body weight gain was suppressed in the 20% group (286 mg/kg bw/day) and the body weight was decreased in the 30% group (427 mg/kg bw/day). An infiltrating, yellowish-reddish brown crust was observed after 2-3 days in the lower dose group, and after 1-2 days in the high dose group. After 4-6 days the crust was abraded and erosion occurred at the abraded site. Histological examinations of the application site revealed severe necrosis of the region from the epidermis cuticle to the upper layer of the dermis, severe infiltration of leukocytes in the necrotic site, and diffuse inflammatory cell infiltration of all layers of the corium. The effects on body weight are to be considered related to the LAS irritation.

The LOAEL for these effects is 286 mg/kg bw/day, the lower dose tested.

The repeated dose toxicity tests are summarised in Table 23.

Table 23: Summary of the repeated dose toxicity tests

Animal	Route	Duration	NOAEL mg/kg bw/day	LOAEL mg/kg bw/day	Doses mg/kg bw/day	Reference
Monkey	Gavage+ subcutaneous injection	28 days	150 (po) + 0.5 (sc)		30,150,300 (po) + 0.1, 0.5, 1.0 (sc)	Heywood et al.,1978
Rat	Gavage	1 month	125	250	125, 250, 500	Ito et al.,1978
Rat	Oral feed	60 days	170		6.8, 34, 170	Palmer et al., 1974
Rat	Oral feed	2 months	225		22.5, 112.5, 225	Nolen et al.,1975
Rat	Oral feed	90 days	50	250	50, 250	Oser et al.,1965
Rat	Oral feed	90 days	750 <sup>(*)</sup>		750	Ikawa et al.,1978
Rat	Oral feed	90 days	220		8.8, 44, 220	Kay et al.,1965
Rat	Oral feed	6 months	40	115	40,115,340, 1030	Yoneyama et al.,1972
Mouse	Drinking	6 months		20 <sup>(**)</sup>	20	Watari et al.,1977

	water					
Rat	Oral feed	9 months	260	780	260, 780	Yoneyama et al.,1976
Rat	Drinking water	9 months	85	145	85, 145, 430	Yoneyama et al.,1976
Mouse	Oral feed	9 months	< 500	500	500, 1000	Yoneyama et al.,1976
Mouse	Drinking water	9 months	100	250	100, 250, 750	Yoneyama et al.,1976
Rat	Dermal	15 days	< 286	286	286, 427	Sadai et al.,1972

(\*) the only dose tested

(\*\*) effects disappeared during the course of the study

## Conclusion

LAS was tested for toxicity in several repeated dose toxicity experiments by the oral and dermal routes in rodents (rats, mice) and non-rodents (monkeys).

In monkeys dosed for 28 days by gavage and subcutaneous injection, the observed effects were diarrhoea at 150 mg/kg bw/day (oral) +0.5 mg/kg bw/day and vomiting at 300 mg/kg bw/day (oral) +1 mg/kg bw/day (subcutaneous), but effects of systemic toxicity were not found (Heywood et al.,1978).

In some studies, with duration from 1 to 3 months, no effects were observed in rats at oral doses (by gavage or in diet) from 125 to 750 mg/kg bw/day, except for a slight liver increase in females administered with 250 mg/kg bw/day for 3 months.

Ultra-structural changes in liver cells were observed at the dose of 20 mg/kg bw/day in one 6-month study in mice which were dosed orally (drinking water), but effects were not seen at higher doses in other studies. These changes seem to be reversible as they disappeared in the course of the study (as did liver effects reported at higher doses in two 24-month carcinogenicity studies in rats (see 5.2.1.7), in which proliferation of hepatic cells and other effects were observed after one and six months and later disappeared). Since these alterations later disappeared, they are considered to represent adaptation to the administration of LAS.

Increased weight of the cecum and slight degeneration of the renal tubes were noted in a 6-month study at the dose of 115 mg/kg bw/day administered by oral feed (Yoneyama et al.,1972). The dose with no adverse effects was 40 mg/kg bw/day.

In a 9-month study in rats, a significant decrease in renal Na,K-ATPase was seen at the oral dose (drinking water) of 145 mg/kg bw/day, while no effects were seen at 85 mg/kg bw/day (Yoneyama et al.,1976).

In two other 9-month studies by the same authors, oral administration of higher doses (250 and 780 mg/kg bw/day), to mice in drinking water and to rats in diet, resulted in suppressed body weight gain, changes in weight of spleen, heart, thymus, cecum, liver, and degeneration of renal tubes. Also haematological, serum-biochemical and enzymatic alterations were seen in liver and kidneys. The NOAELs were 100 and 260 mg/kg bw/day respectively.

Repeated dermal application on rats of 280 mg/kg bw/day of LAS for 15 days, the only dose tested, caused local irritation effects and, most likely as a consequence, suppression of the body weight gain (Sadai,1972).

## **NOAEL**

In view of the available information it is not possible to determine which single study among those summarized above is the most reliable or appropriate for the determination of a NOAEL. Because of that, based on the data from all the studies, a NOAEL of 85 mg/kg bw/day is proposed, which was derived from a 9 month oral study and corresponds to the NOAEL which is closest to the lowest available oral LOAEL of 115 mg/kg bw/day.

### **5.2.1.7 Genetic Toxicity**

#### **5.2.1.7.1 *In vitro***

##### **Bacterial tests**

A reliable and well documented test (Hüls,1993) was conducted according to OECD Guidelines and GLP on TA 98, TA 100, TA 1535, TA 1537, TA 1538 Salmonella typhimurium strains with and without metabolic activation. Concentrations tested were 8-5000 µg/plate and the cytotoxicity concentration was >5000 µg/plate, both with and without metabolic activation. The LAS concentration was 91.3%. Negative and positive controls were used.

Another bacterial mutagenicity study (Ames test) (Schoeberl,1993) using Salmonella typhimurium strains TA 1535, TA 1537, TA 98 and TA 100, as well as TA1538, at test concentrations of 8, 40, 200, 1000 and 5000 µg/plate is also available. All strains tested negative with and without S9 activation.

Two other Ames tests are available. Although conducted on limited Salmonella typhimurium strains compared those recommended by the OECD Guidelines and with no information about cytotoxicity and controls, they showed negative results (Inoue et al.,1980; Sunakawa et al.,1981). Concentrations tested were up to 200 µg/plate in the first assay and up to 500 µg/plate in the second one. The LAS concentration was 20%.

One recombination assay (Inoue-a et al.,1979) is available on *Bacillus subtilis*, with and without metabolic activation. Concentrations tested were up to 50 µg/plate. The LAS concentration was 99.5%. Results were negative.

An *Escherichia coli* reverse mutation assay, with and without metabolic activation, reported negative results (Inoue-a et al.,1979). No data on the concentration tested were given.

##### **Non bacterial tests**

A transformation test with Syrian hamster embryo (SHE) cells without metabolic activation was conducted with negative results (Inoue et al.,1980). Concentrations tested were up to 50 µg/plate. The LAS concentration was 22.2%.

In the second test (Anon.,1995), the potential of LAS to cause mutations in mammalian cells was examined. Chinese Hamster Ovary (CHO) cells were exposed to concentrations of 0, 0.6, 1, 1.8, 3, and 6 µg/ml without S9, and 0, 6, 10, 18, 30, and 60 µg/ml with S9. The cells were then examined for cytogenicity and mutation frequency. Ethyl methane sulfonate and 3-(20-)methylcholanthrene were used as positive control substances. Preliminary tests show the test substance was cytotoxic at concentrations of 50 µg/ml or greater with metabolic activation, and 100 µg/ml or above without metabolic activation. There was no biologically significant increase in mutation frequency in the treated groups. Therefore, results show that LAS was not mutagenic to CHO cells both in the presence and absence of S9.

The third study (Murie and Innes,1997) examined the potential of LAS to cause chromosomal

aberrations in mammalian cells. Chinese hamster ovary cells were exposed to concentrations of 2.5, 5, 10, 15, 20, 26, 33, and 39 µg/ml with S9, and 20, 39, 58, 78, 104, 130, and 156 µg/ml without S9. No biologically significant results were seen in treated cultures in the absence of metabolic activation. In the presence of metabolic activation the results were more equivocal. In the first of three tests, no cytotoxicity, and no increase in chromosome aberrations were observed at doses of 10 or 20 µg/ml and 100% cytotoxicity was observed at 39 µg/ml. In the second test, a steep cytotoxicity curve was observed between 10 and 20 µg/ml with a cell count of 68/90 % at the 10 µg/ml dose and no living cells remaining at the 20 µg/ml dose. An increased in aberration frequency could be observed at the 10 µg/ml dose. No increase in aberration frequency has been observed at lower doses which also did not show any cytotoxicity. To gain clarity on the positive result an additional test was conducted. Here, no cytotoxicity and no increase in chromosomal aberration frequency have been observed at the 10 µg/ml dose. At the 15 µg/ml dose the cell number was reduced to 25 % which is why this dose group cannot be evaluated due to excessive cytotoxicity. These results indicate that LAS is weakly clastogenic at cytotoxic concentrations but negative at concentrations below cytotoxic concentrations in this *in vitro* assay.

## Conclusion

In bacterial test and in a test with mammalian cells the substance did not induce gene mutations. In a chromosomal aberration test LAS was weakly clastogenic at cytotoxic concentrations but negative at concentrations below cytotoxic concentrations.

### 5.2.1.7.2 *In vivo*

A cytogenetic assay (chromosomal aberrations) on male mice was carried out (Inoue et al., 1979). Doses of 200, 400, 800 mg/kg bw/day of LAS were administered by gavage for 1 and 5 days. The maximum dose was half the LD<sub>50</sub>. Bone marrow was examined 6, 24, 48 hours after administration. There was no significant difference in the incidence of chromosomal aberrations between any of the groups given LAS and the negative control group. Mitomycin was used as a positive control and induced severe chromosomal aberrations.

Another cytogenetic assay was performed on male rats and male mouse (Masabuchi et al., 1976). LAS was administered by oral feed for 9 months, at a dose of 0.9% in rats (450 mg/kg bw/day) and in mice (1170 mg/kg bw/day). Chromosomes of the bone marrow cells were examined. There were no significant differences in the incidences of chromosomal aberrations between the experimental and control groups.

A dominant lethal assay is available (Masabuchi et al., 1976). LAS was administered as oral feed for 9 months to 7 male mice, at the dose of 0.6% (300 mg/kg bw/day). One of the male mice was mated with 2 female mice that were not given LAS. The pregnant mice were laparotomized on day 13 of gestation to determine the numbers of luteal bodies, implantations, surviving foetuses, and dead foetuses. There were no significant differences in fertility, the mortality of ova and embryos, the number of surviving foetuses, or the index of dominant lethal induction between the experimental groups and the control group. Details are not available about eventual signs of toxicity and the number of animals is very limited.

In one micronucleus assay on male mice (Kishi et al., 1984), a single intraperitoneal application at the dose of 100 mg/kg bw was administered. There were no differences in the incidences of polychromatic erythrocytes with micronuclei in the bone marrow cells between the treated group and the control group.

An *in vivo* mammalian micronucleus study is available on the structurally related substance LAS Acid (CAS#85536-14-7, Benzenesulfonic acid, C10-13-sec alkyl derivatives). In this study (Fedtke,1991), 40 male and 40 female mice were given a single oral dose by gavage of 1122 mg/kg LAS Acid (read across) and evaluated for chromosome aberrations. Only a single dose has been evaluated which was in the range of the acute oral LD50 value for LAS Acid in rats (LD50 = 1470 mg/kg). Furthermore, slight cytotoxicity has been observed after 48 hours. No statistically significant or biologically relevant increases in the number of polychromatic erythrocytes with micronuclei were observed; therefore the test material is considered negative for cytogenicity.

### **Conclusion**

LAS was tested in cytogenetic assays in rat and mouse, in a dominant lethal assay in rat, and in an micronucleus test in mice. None of these tests indicated any genetic toxicity of the test compound *in vivo*. An additional micronucleus study with mice conducted on the structural analogue LAS Acid further supports that LAS is not clastogenic *in vivo*.

The positive result in the *in vitro* chromosome aberration study using a rodent cell line (CHO cells) derived from cancer tissues that is lacking proper cell cycle control has to be seen in the context of the extensive *in vivo* data. *In vivo* studies do assess genotoxicity under more realistic conditions, including exposure. Therefore, LAS is not considered a genotoxic compound.

#### **5.2.1.8 Carcinogenicity**

Four studies are available.

A test was conducted on male/female rats (Buehler et al.,1971). Doses of LAS (98.1%) of 0.02%, 0.1% and 0.5% (10, 50, 250 mg/kg bw/day) were administered for 2 years in the diet. A control group was used. No information about the method used was given. Fifty animals per dose group and sex were tested. Adverse effects on growth or feed efficiency were not observed during the experiment. Five males and females from each of the groups at 8 and 15 months and all survivors at 24 months were necropsied, haematologic values were determined, and tissues were taken for histologic studies.

These examinations revealed no consistent dietary-induced changes, which could be considered a toxic response. In addition, animals, which showed significant loss of weight, development of tumours or other evidence of abnormalities, were sacrificed and tissues were preserved for study. The incidence of tumours and the common incidental diseases were similar in all dietary groups.

In a second study, Wistar rats were exposed for 2 years at doses of 0.01%, 0.05%, 0.1% LAS (34.55%) in drinking water, corresponding to 20, 100, 200 mg/kg bw/day. (Tiba et al.,1972). Control groups were used. There were no changes due to the administration of LAS in regard to growth, mortality, the weight of major organs, or histopathological findings. There is no description of tumours in the report.

In a third study, male/female Wistar rats were fed LAS at doses of 0.04, 0.16, 0.6% (20, 80 and 300 mg/kg bw/day) for 1, 3, 6, 24, or more months (Fujii. et al.,1977; Yoneyama et al.,1977). A control group was used. Groups of 5 rats of both sexes were fed for 1, 3, 6, and 12 months and groups of 15 rats of both sexes were fed for 24 months or more. During the experiment, the 0.6% group showed slight increases in weights of liver and cecum, and in GPT and ALP in serum. LAS administration had no adverse effects upon the intake of food, body weight gain, general condition, and mortality or mean survival period. On the basis of these results, it was concluded that the diet containing LAS at a concentration of 0.6% (300 mg/kg bw/day) did not have any adverse effects on rats.

Detailed histopathological examinations were made on the rats. At one month, proliferation of hepatic cells in the liver and slight swellings of the renal tubes and narrowing of the tubular lumen in the kidneys were found in the 0.16% and 0.6% groups. Since these findings disappeared later on, they were thought as being adaptation phenomena to the administration of LAS. There were no histopathological lesions attributable to LAS administration in any of the organs in the rats, which were fed for 24 months or more. Various types of tumours were observed in different organs, but findings suggestive of tumorigenicity of LAS were not present.

Male and female rats were exposed up to 26 months to LAS at the dose of 0.1% in drinking water (200 mg/kg bw/day) (Endo et al.,1980). A control group was used. A group of 62 rats of both sexes were treated with LAS and a control group of 37 rats of both sexes were given pure water. Five to 12 of the rats in the experimental group and 3 to 12 rats in the control group at 3, 6, 12, and 18 months, and all surviving rats between 24 and 26 months were sacrificed for pathological, biochemical, and haematological examinations. The administration of LAS had no effect on the intake of water, mortality, body weight gain, or general condition. In pathological examinations, looseness, atrophy, and fatty change of the hepatic cells in the liver were found in the experimental group at 6 months, together with significant increases in GOT, GTP and bilirubin. In haematological examinations no effects due to LAS were observed.

### **Conclusion**

Even though the studies are conducted per-1980 and were not performed and/or evaluated according to GLP and current requirements (number of animals, doses, scope of investigations) the information that they provide is still useful. All the studies were well conducted according to common practice at the time and toxicity was observed at the higher dose tested in some of the studies. All of the studies consistently showed lack of evidence of carcinogenicity in all species tested (rats and mice). There is no reason to believe that LAS has carcinogenic potential.

#### **5.2.1.9 Reproductive toxicity**

A four-generation reproduction study is available on male/female Wistar rats (Endo et al.,1980). Animals were administered 0.1% LAS in drinking water (corresponding to 70 mg/kg bw/day). A control group was used. The administration of LAS had no adverse effects on fertility, parturition, gestation period, or lactation in any of the generations. Five to 10 rats of both control and experimental groups were sacrificed at 12 weeks for pathological examinations. No effects of LAS administration were observed.

A three-generation reproduction study was conducted on male/female rats. LAS was administered in the diet at doses of 0.02, 0.1, 0.5% (14, 70, 350 mg/kg bw/day) (Buehler et al.,1971). A control group was used. Animals were fed for 84 days to the 4 groups of weaning rats, each consisting of 50 animals of both sexes (FO-generation). Twenty females from each dose group were mated with 20 males from the same group. The first litters of each generation (F1a-generation) were sacrificed at 21 days of age. Ten days after the first litter was sacrificed, all females were re-mated with different males from the same group. The F2a-generation was sacrificed at the F1a-generation. From the resulting F1b-generation, 20 males and females of each group were selected at weaning to continue their respective diets for 80 to 85 days until they were mated to produce the F2b-generation. This generation was treated with LAS for a further 8 weeks and mated again. The first litter (F3a) was sacrificed; the F3b-generation was treated until the animals were weaned. General reproduction including fertility gestation, parturition, neonatal viability, lactation, and post-weaning growth was normal for all test groups and did not deviate from the controls in each generation. No gross abnormalities were noted. No definitive adverse effects due to the test material were noted in the haematology and pathology.

NOAEL Parental: 350 mg/kg bw/day

NOAEL F1 Offspring: 350 mg/kg bw/day



NOAEL F2 Offspring: 350 mg/kg bw/day  
The NOAEL is the highest tested dose.

Another three-generation study on rats is available for a commercial light duty liquid detergent, containing 17% LAS and 7% alkyl ethoxylate sulphate (Palmer et al.,1974). This study is well documented and complies with guidelines recommended by the US-FDA and GLP. Dietary concentrations of 0, 0.08%, 0.4% and 2% (0, 40, 200 and 1000 mg/kg bw/day) of the formulation were continuously administered throughout three generations for 60 days prior to mating. The corresponding administration of LAS was of 0, 6.8, 34 and 170 mg/kg bw/day. The number of parental animals per group, control groups included, were 11 males and 22 females for the F0 generation and 10 males and 20 females for F1b and F2b. Among parental animals over the three generations there were no signs of adverse effects of treatment. Food consumption and bodyweight changes showed no consistent relationship to dosage. The terminal necropsy revealed no changes attributable to treatment. The mating performance, the pregnancy rate and the duration of gestation were unaffected. Among litter parameters, statistically significant differences were occasionally recorded, but as these showed non-consistent dosage related trends, they were considered to be unrelated to treatment. The incidences of sporadic deaths and total litter losses were unrelated to dosage. The incidence of malformations was unaffected by treatment. Additional organ weight analysis, histopathology and skeletal staining of representative young from the F3b generation revealed no changes that could be conclusively related to treatment. The NOAEL for LAS is 170 mg/kg bw/day, corresponding to the highest tested dose.

## **Conclusion**

Results of two tests on three generations and one on four generations did not show any adverse effects on reproduction at any of the doses tested. Based on these studies, a NOAEL of 350 mg/kg bw/day, corresponding to the highest tested dose, is assessed.

### **5.2.1.10 Developmental Toxicity and Teratogenicity**

#### **5.2.1.10.1 Oral route**

Female rats and rabbits were administered 0.1% LAS in drinking water, corresponding to 383 mg/kg bw/day for rats and to 3030 mg/kg bw/day for rabbits (Endo et al.,1980). Control groups were used. LAS was given to 40 rats (20 controls) and 22 rabbits (11 controls) from day 6 to 15 (rats) and day 6 to 18 of pregnancy (rabbits). The effect on the dams was a slight inhibition of body weight gain in the rabbits. The litter parameters of both species did not show any significant differences from those of the controls. Delayed ossification was observed in rabbits, but there was no increase in malformations in either the rabbits or the rats.

NOAEL Maternal: 383 mg/kg bw/day (rat)

LOAEL Maternal: 3030 mg/kg bw/day (rabbit)

NOAEL Foetuses: 383 mg/kg bw/day (rat)

LOAEL Foetuses: 3030 mg/kg bw/day (rabbit)

Palmer et al. conducted studies on female rats, mice and rabbits (Palmer-a et al.,1975). They were all conducted according to GLP and standard guidelines and their results are summarised below:

#### **Rat study** (Palmer-a et al.,1975)

Twenty animals per dose group were used. Animals were daily administered at day 6-15 of pregnancy by gavage with LAS at doses of 0.2, 2, 300, 600 mg/kg bw/day and sacrificed at day 20 of gestation. A control group was used. The body weight gain was retarded in the highest dose group from the start of dosing and showed partial recovery toward the end of the dosing period. One animal died in this group, but it could not be conclusively related to treatment. The toxic effects were associated with disturbance of the gastrointestinal tract. Pregnancy rates were comparable at all dosage.

No differences were observed among the dose groups and the control group with respect to: number of litters, viable young, litter weight, foetal weight, embryonic deaths, implantations, corpora lutea, pre- and post-implantation embryonic loss, major malformations, and minor visceral or skeletal anomalies or incidence of pups with extra ribs.

NOAEL Maternal: 300 mg/kg bw/day

NOAEL Foetuses: 600 mg/kg bw/day

**Mice study** (Palmer-a et al.,1975)

Animals were administered 0.2, 2, 300, 600 mg/kg bw/day LAS by gavage on days 6-15 of pregnancy, then sacrificed on day 17 of pregnancy (Palmer-a et al., 1975). A control group was used. Among parent animals, treatment at 300 and 600 mg/kg bw/day was associated with increased mortality (35% and 90% respectively). At 300 mg/kg bw/day weight gain was retarded only during the first four days. No assessment could be done at 600 mg/kg bw/day due to the high mortality rate. Necropsy revealed an almost invariable picture of tympanites, sometimes associated with gastritis. Pregnancy rates were essentially comparable for all groups. At doses with no maternal toxicity, no differences were observed among the dose group and the control group with respect to: number of litters, viable young, litter weight, foetal weight, embryonic deaths, implantations and post-implantation embryonic loss. At these doses the incidences of major malformations and minor abnormalities were not affected. At doses with maternal toxicity there was increased foetal loss and reduced litter size due almost entirely to total litter loss, which was considered to be a secondary effect due to the maternal toxicity. The incidences of major malformations and minor abnormalities were not significantly affected, apart from a higher, but not statistically significant, incidence of skeletal anomalies at 300 mg/kg bw/day (extra ribbed pups). Given the large difference between the maternal no-effects dose of 2 mg/kg bw/day and the LOAEL dose (300 mg/kg bw/day), this study, although well documented and conducted according to GLP, does not allow determination of a reliable maternal NOAEL.

Since no assessment was possible at 600 mg/kg bw/day, due to the high mortality rate of parent animals, the NOAEL for litters is 300 mg/kg bw/day.

**Rabbits study** (Palmer-a et al.,1975)

Animals were administered 0.2, 2, 300, 600 mg/kg bw LAS by gavage at days 6-18 of pregnancy, then sacrificed at day 29 of pregnancy (Palmer-a et al.,1975). A control group was used. At 300 and 600 mg/kg bw/day, parent animals showed anorexia, diarrhoea, weight loss and death; mortality rates were 85 and 100% and necropsy revealed changes in the gastrointestinal tract. At 0.2 and 2 mg/kg bw/day, treatment did not adversely affect bodyweight changes and pregnancy rates of parent animals. The influence of maternal toxicity at higher doses restricted assessment of the effects on litter parameters to animals treated with lower dosages, which showed no adverse effects on litter parameters.

This study, although well documented and conducted according to GLP, does not allow determination of reliable NOAELs, given the large difference between the maternal no-effects doses of 2 mg/kg bw/day and the maternal LOAEL dose (300 mg/kg bw/day) that is also the dose for which effects on litters could not be determined due to the high mortality rate in parent animals.

A test was conducted on female mice. Doses of 40, 400 mg/kg bw/day LAS was administered daily by gavage from day 0 to day 6 of pregnancy or from day 7 to 13 of pregnancy (Takahashi et al.,1975). Thirteen to fourteen mice were used in each dose and control groups. In mice given 400 mg/kg bw/day, the pregnancy rate was 46.2% compared to 92.9% in the controls. There was no increase in malformations. Although no information on maternal toxicity is available, it appears likely that maternal toxicity was present at the high dose group.

NOAEL Maternal: 40 mg/kg bw/day

NOAEL Foetuses: 400 mg/kg bw/day

Female mice were administered doses of 10, 100, 300 mg/kg bw/day LAS daily by gavage at day 6 through day 15 of pregnancy (Shiobara et al.,1976). There were 25 to 33 mice in each dose group and a control group was used. The dams showed inhibition of body weight gains in all groups, especially in the high dose group. In this group, two dams died, and there was one case of premature delivery and death of all foetuses. There were findings such as decreased body weight and delayed ossification among the living foetuses, but there was no increase in malformations.

LOAEL Maternal: 10 mg/kg bw/day

NOAEL Foetuses: 300 mg/kg bw/day

Pregnant female rats were fed doses of 0.1%, 1.0% LAS (16 rats/dose) in the diet (80 and 780 mg/kg bw/day) from day 0 to 20 of gestation (Tiba et al.,1976). Control groups were used, but information about the numbers of animals is not available. At the LAS dose of 780 mg/kg bw/day there were no abnormalities in the body weight gains of the dams, or in the occurrence and maintenance of pregnancy. The values of the litter parameters did not differ from those of the controls and there was no evidence of teratogenicity. The number of offsprings was rather low in the highest dose group, and the weaning rate of 78.3% was lower than the 100% rate observed in the controls. However, there were no abnormalities in body weight gain, organ weights or functions in the offsprings.

NOAEL Maternal: 780 mg/kg bw/day

NOAEL Foetuses: 780 mg/kg bw/day

In a well-documented study, a mixture of 55% of tallow alkyl ethoxylate sulphate and 45% of LAS was fed to rats and rabbits (Nolen et al.,1975).

**Rats.** Seven groups of 25 male and 25 female rats were kept at dietary levels of 0.1%, 0.5% and 1% of the surfactant mixture (50, 250 and 500 mg/kg bw/day). The corresponding doses of LAS were 22.5, 112.5 and 225 mg/kg bw/day. The surfactant mixture was fed to two generations either continuously to males and females during the 8-week growth period or to females during the organogenesis period (days 6-15) of six pregnancies. Control groups were used. No significant effects were seen in weight gain, organ/body weight ratios, haematology values and histopathology during both the first generation 8-week period and the second-generation period. No adverse effects were noted on conception, foetal viability or post-natal survival in either generation of rats. There were no statistical differences among the groups of rat foetuses examined for birth defects. Of 1210 rat foetuses, the overall incidence of abnormal young was 9%.

NOAEL Maternal: 225 mg/kg bw/day (rat)

NOAEL Foetuses: 225 mg/kg bw/day (rat)

**Rabbits.** Pregnant rabbits were given 50, 100, and 300 mg/kg bw/day of the surfactant mixture by intubation on days 2-16 of gestation during a single pregnancy (22.5, 45 and 135 mg/kg bw/day of LAS). No symptoms of maternal toxicity and no adverse effects in foetuses were noted. Of 855 rabbit foetuses, 5.7% were abnormal, but the incidences of defective foetuses in the test groups were not significantly different from those in controls.

NOAEL Maternal: 135 mg/kg bw/day (rabbit)

NOAEL Foetuses: 135 mg/kg bw/day (rabbit)

In the three generation study for reproductive toxicity with rats (Palmer et al.,1974), already mentioned in 5.2.1.8, there were no signs of adverse effects of treatment over the three generations at dietary concentrations of a formulation containing 0, 6.8, 34 and 170 mg/kg bw/day of LAS. Food consumption and bodyweight changes showed no consistent relationship to dosage. The terminal necropsy revealed no changes attributable to treatment. The incidence of malformations was unaffected by treatment. Additional organ weight analysis, hystopatology and skeletal staining of representative young from the F3b generation revealed no changes that could be conclusively related to treatment.

NOAEL both for parental animals and foetuses is 170 mg/kg bw/day of LAS, corresponding to the highest tested dose.

#### **5.2.1.10.2 Dermal route**

Pregnant female rats were exposed daily at days 2 through 15 of gestation to LAS at doses of 0.03%, 0.3%, or 3% on the shaved skin as 0.5 ml aqueous solution (0.6, 6, 60 mg/kg bw/day) (Palmer-b et al.,1975). A control group was used.

Maternal toxicity: at the high dose, local irritation was observed, resulting in a slightly lower body weight gain and hypersensitivity. Teratogenicity: no differences were observed among the dose groups and the control group with respect to: number of litters, viable young, litter weight, foetal weight, embryonic deaths, implantations, corpora lutea, pre- and post implantation embryonic loss. The incidences of major malformations, minor visceral or skeletal anomalies, and skeletal variants were not different between controls and dose groups even at maternal toxic doses.

NOAEL Maternal: 6 mg/kg bw/day

NOAEL Foetuses: 60 mg/kg bw/day

Another dermal study was conducted on female rats (days 0 through 21 of gestation), with daily exposure at 1.0%, 5.0%, and 20% of LAS (20, 100, and 400 mg/kg bw/day). Controls groups were used (Daly et al.,1980).

Maternal toxicity: the dams treated with 400 mg/kg bw/day and 100 mg/kg bw/day showed inhibition of body weight gain and local skin effects that compromised the integrity of the skin and caused overt toxicity, like inhibition of the body weight gain.

Teratogenicity: there were no findings indicative of effects of LAS on the foetal parameters evaluated. There were no indications of teratogenic or embryotoxic effects.

NOAEL Maternal: 20 mg/kg bw/day

NOAEL Foetuses: 400 mg/kg bw/day

Doses of 0.03, 0.3, or 3% LAS (5, 50, and 500 mg/kg bw/day) in aqueous solution were applied daily onto the shaved skin of females mice (days 2 through 13 of gestation) (Palmer-b et al.,1975). The dosage volume was 0.5 ml, which was applied to an area of skin (2 x 3 cm). Controls groups were used. At the high dose, severe local irritation was observed resulting in body weight loss and hypersensitivity, which was also observed at the medium dose. Teratogenicity: at the lowest dose, the dose with no maternal toxicity, no differences were observed among the LAS group and the control group with respect to: number of litters, viable young, litter weight, foetal weight, embryonic deaths, implantations, corpora lutea, pre- and post-implantation embryonic loss. The incidences of major malformations minor visceral or skeletal anomalies, and skeletal variants were not different between controls and the tested group. Maternally toxic dosages were associated with a significantly increased foetal loss and consequent reduction of litter size. This was due almost entirely to total litter losses, as values for the one surviving litter at the highest dose were similar to the control litters. At the medium dose, the moderate degree of maternal toxicity correlated with a moderate effect on litter values in that, whilst the higher incidence of embryonic deaths differed significantly from control values, the consequent reduction in litter size was not statistically significant. With regard to major malformations and minor skeletal or visceral anomalies, the assessment of litters was not possible in the highest dose group due to the low survival. At the low doses, no treatment related increases of the incidences of major malformations or minor skeletal and visceral anomalies were observed.

The maternal NOAEL is 5 mg/kg bw/day.

Given the large difference between the no observed effects dose for litters of 50 mg/kg bw/day and the dose of 500 mg/kg bw/day, for which the assessment of litters was not possible due to the low survival, this study does not allow determination of a reliable foetal NOAEL.

Female mice were daily treated (day 0 through day 13 of pregnancy) with a single LAS dose of 2.2% (110 mg/kg bw/day) (Sato et al.,1972). An area of 4 x 4 cm on the backs of mice was depilated and LAS was applied at a dose of 0.5 ml/mouse/day. No information about control groups. No abnormalities were seen in the dam or foetuses.

NOAEL Maternal: 110 mg/kg bw/day

Female mice were treated daily from day 6 through day 15 of pregnancy at dermal doses of 0.03%, 0.3%, 3% (15, 150, and 1500 mg/kg bw/day) of LAS (Imahori et al.,1976). Control groups were used. The 1500 mg/kg bw/day group showed a clear decrease in the pregnancy rate (67.9%) when compared with a rate of 96.3% in the controls. However, there were no decreases in the litter size, and no changes in the litter parameters with the exception of a slight decrease in foetal body weight. There were no significant increases in the incidence of malformations in the foetuses.

NOAEL Maternal: 150 mg/kg bw/day

NOAEL Foetuses: 1500 mg/kg bw/day

LAS (99.5%) was administered daily via subcutaneous injection to female mice at doses of 0.35, 1% in water (20, 200 mg/kg bw/day) from day 0 to 3 or day 8 to 11 of pregnancy (Takahashi et al.,1975). There were 12-19 mice in each treatment group. Control groups were used. When dams were administered the 200 mg/kg bw/day solution from day 0 to 3 of pregnancy, there was an initial decrease in body weight and necrosis at the injection sites. The number of pregnancies decreased in the mice given the 1% solution compared to the controls (61.5% vs. 93.3%). There were no significant changes with respect to litter parameters, major malformations or minor abnormalities.

NOAEL Maternal: 20 mg/kg bw/day

NOAEL Foetuses: 200 mg/kg bw/day

Female rabbits were exposed (days 1 through 16 of gestation) to aqueous solutions of LAS at 0.03%, 0.3%, or 3% on shaved skin (0.9, 9.0, and 90 mg/kg bw/day) (Palmer-a et al.,1975). Control groups were present. The dosage volume was 10 ml, which was applied to an area of skin (12 x 20 cm) from which the fur was removed. At the highest dose, local irritation was observed in parental animals, resulting in body weight loss and hypersensitivity. The medium dose caused retarded body weight gain and hypersensitivity. At the medium and low dose, no differences were observed among the dose groups and the control group with respect to: number of litters, viable young, litter weight, foetal weight, embryonic deaths, implantations, corpora lutea, pre- and post-implantation embryonic loss. The high dose was associated with a slightly, but not significantly, higher foetal loss and lower litter size. The incidences of major malformations, minor visceral or skeletal anomalies, and skeletal variants were not different between controls and dose groups even at maternal toxic doses.

NOAEL Maternal: 0.9 mg/kg bw/day

NOAEL Foetuses: 90 mg/kg bw/day, corresponding to the highest dose tested

LAS was applied at the concentration of 20% to the dorsal skin of pregnant mice during the pre-implantation period. On day 3 of gestation the embryos were flushed from the uterus (Nomura et al.,1980). Some dead, deformed and growth-retarded embryos were observed in the treated group. Although the authors stated that these effects were not due to maternal toxicity since no maternal organs were affected, this statement is probably not correct in view of the high concentration of LAS and its irritation effects. A secondary effect due to maternal toxicity appears much more likely. This is also corroborated by a study in which LAS were not detected in the uterus of pregnant ICR mice administered a single oral dose of 350 mg/kg bw on day 3 of gestation (Koizumi et al.,1985) (see 5.2.1.10).

The developmental and teratogenicity tests are summarised in Table 24.

Table 24: Summary of the developmental and teratogenicity tests

Animal	Route	Exposure in pregnancy	NOAEL maternal mg/kg bw/day	NOAEL Teratogenicity mg/kg bw/day	Dose mg/kg bw/day	Reference
Rat	Drinking water	Day 6-15	383	383	383	Endo et al.,1980
Rat	Oral feed	Day 0-20	780	780	80, 780	Tiba et al.,1976
Rat	Oral feed	Day 6-15 + 60 days prior mating	225	225	22.5, 112.5, 225	Nolen et al.,1975
Rat	Gavage	Day 6-15	300	600	0.2, 2, 300, 600	Palmer-a et al.,1975
Mouse	Gavage	Day 7-13	40	400	4, 40, 400	Takahashi et al.,1975
Mouse	Gavage	Day 6-15	10	300	10, 100, 300	Shiobara et al.,1976
Mouse	Gavage	Day 6-15	(2)	300	0.2, 2, 300, 600	Palmer-a et al.,1975
Rabbit	Gavage	Day 2-16	135	135	22.5, 45 ,135	Nolen et al.,1975
Rabbit	Drinking water	Day 6-18	3330 (LOAEL)	3330 (LOAEL)	3030	Endo et al.,1980
Rat	Dermal	Day 2-15	6	60	0.6, 6, 60	Palmer-b et al.,1975
Rat	Dermal	Day 0-21	20	400	20, 100, 400	Daly et al.,1980
Mouse	Dermal	Day 0-13	110	110	110	Sato et al.,1972
Mouse	Dermal	Day 6-15	150	1500	15, 150, 1500	Imahori et al.,1976
Rabbit	Dermal	Day 1-16	0.9	90	0.9, 9, 90	Palmer-b et al.,1975
Mouse	SC	Day 0-3 or Day 8-11	20	200	20, 200	Takahashi et al., 1975

## Conclusion

LAS was evaluated for developmental/teratogenic effects on rats, mice and rabbits. Some findings of maternal toxicity were found at low or relatively low doses, administered to rats and mice dermally and by gavage, but they are associated with the irritation effects of LAS, either on the skin or the gastrointestinal tract.

Two studies, one with rabbits administered by gavage (Palmer-a et al.,1975) and one with mice administered dermally (Palmer-b et al.,1975), although well documented and conducted according to standard guidelines, did not allow determination of reliable NOAELs, due to dose ranges that are too large between the doses with no effects (2 and 50 mg/kg bw/day, respectively) and maternal toxic doses (300 and 500 mg/kg bw/day, respectively) which resulted in high mortality rates of dams and litter losses.

In two studies a clear decrease in the pregnancy rate of mice was noted, associated with the toxic doses of 400 mg/kg bw/day administered by gavage (Takahashi et al.,1975) and 1500 mg/kg bw/day administered dermally (Imahori et al.,1976), but no effects on the litters parameters or malformations were found. In other studies no effects were found both in parental animals and litters at oral doses up to 780 mg/kg bw/day and at dermal doses up to 400 mg/kg bw/day for litters. The most reliable are those by Nolen and Palmer.

In a study LAS at 20% in aqueous solution was applied to the dorsal skin of pregnant mice during the pre-implantation period (Nomura et al.,1980). Some dead, deformed and growth-retarded embryos were observed in the treated group. This was interpreted as a secondary effect due to maternal toxicity

at this high LAS concentration and its irritation effects, also corroborated by a study in which LAS was not detected in the uterus of pregnant ICR mice administered a single oral dose of 350 mg/kg bw on day 3 of gestation (Koizumi et al.,1985) (see 5.2.1.10).

To sum up: some effects, such as embryo death or deformities, decrease in pregnancy rate and litter loss were noted in some studies at maternal toxic doses. However, no decreases in the litter size, no changes in the litter parameters, no malformations or significant differences in skeletal defects were observed at oral doses up to 780 mg/kg bw/day and dermal doses up to 1500 mg/kg bw/day.

## **5.2.2 Identification of critical endpoints**

### **5.2.2.1 Overview on hazard identification**

LAS shows an oral LD<sub>50</sub> of 1080 mg/kg bw (this value is adjusted for 86% activity) and a dermal LD<sub>50</sub> of > 2000 (undiluted substance). The usual concentration sold to formulators is 50%. According to CLP-Regulation, the test substance is a Category IV toxicant (H302: Harmful if swallowed).

LAS did not show any irritation effects on rabbit's skin at concentrations from 1% to 2.5%, while it is moderately irritating at a concentration of 5%. In the range from 47% to 50% it is irritating to skin, according to the EU criteria. Irritation symptoms worsened after exposure, and desquamation, necrosis and hyperkeratinization were noted by day 4 in all animals. These resolved in one animal by day 12, but in the other two animals symptoms continued through the end of the observation period. Therefore, LAS is considered a Category 2 skin irritant.

The substance is non-irritating at 1% and moderately irritating at 5% (not classifiable as an irritant, according to the CLP criteria), while it is severely irritating to eye at the concentration of about 50%. Severe irritation was noted in the animals whose eyes were not rinsed and was not resolved by day 21. Milder irritation was observed in animals that had the test substance rinsed from their eye after 4 or 30 seconds, and effects seen in these rinsed animals were reversible within 7 or 14 days. Based on the irreversible irritation observed in the unrinsed animals, LAS is considered a Category 1 eye irritant. Reliable data on acute inhalation are not available, but given the irritant nature of LAS, it is expected that high LAS aerosol concentrations may be irritating to the respiratory tract.

LAS is not a contact sensitiser, on the basis of both animal and human volunteer tests.

LAS was tested for toxicity in several repeated dose toxicity experiments by the oral and dermal routes in rodents (rats, mice) and non-rodents (monkeys).

In monkeys dosed by gavage and subcutaneous injection, the observed effects were diarrhoea at 150 mg/kg bw/day and vomiting at 300 mg/kg bw/day, but effects of systemic toxicity were not found.

Ultra-structural changes in liver cells were observed at the dose of 20 mg/kg bw/day in one 6 months study in mice which were dosed orally (drinking water), but effects were not seen at higher doses in other studies. These changes seem to be reversible as they disappeared in the course of the study (as did liver effects reported at higher doses in two 24-month carcinogenicity studies in rats in which proliferation of hepatic cells and other effects were observed after one and six months and later disappeared). Since these alterations later disappeared, they are considered to represent adaptation to the administration of LAS.

Increased weight of the cecum and slight degeneration of the renal tubes were seen in a 9-month rat study at the dose of 115 mg/kg bw/day administered by oral feed.

In another 9-month study in rats, a significant decrease in renal Na, K-ATPase was seen at the oral dose (drinking water) of 145 mg/kg bw/day, while no effects were seen at 85 mg/kg bw/day. Oral administration (in diet or drinking water) for 9 months of higher doses in other studies with mice and rats (from 250 to 780 mg/kg bw/day) resulted in suppressed body weight gain, changes in weight of spleen, heart, thymus, cecum, liver, and degeneration of renal tubes. Also haematological, serum-biochemical and enzymatic alterations were seen in liver and kidneys.

Repeated dermal application on rats of 280 mg/kg bw/day of LAS for 15 days caused local irritation effects and, as a consequence, suppression of the body weight gain.

In view of the available information it is not possible to determine which single study among those summarized above is the most reliable or appropriate for the determination of a NOAEL. On the basis of data from all the studies a NOAEL of 85 mg/kg bw/day is proposed, which is the closest value to the lowest available LOAEL (115 mg/kg bw/day).

In all *in vitro* and *in vivo* assays there is no indication of genetic toxicity for LAS.

The oral long term studies performed did not indicate any potential for carcinogenicity of LAS and showed no effects or histopathological findings at doses up to 300 mg/kg bw/day.

Results of studies on reproduction fail to show any adverse effects at any of the doses tested. Based on these studies, a NOAEL of 350 mg/kg bw/day, corresponding to the highest tested dose, is estimated.

For the developmental toxicity/teratogenicity, some findings of maternal toxicity were found at low or relatively low doses, administered dermally and by gavage to rats, mice and rabbits, but they are associated with irritation effects of LAS, either on the skin or the gastrointestinal tract. In other oral studies no effects were found in parental animals up to 780 mg/kg bw/day. Some effects, such as embryo death or deformities, decrease in pregnancy rate and litter loss, were noted in some studies at maternal toxic doses, but in general no decreases in the litter size, no changes in the litter parameters, no malformations or significant difference of skeletal defects were observed at oral doses up to 780 mg/kg and dermal doses up to 1500 mg/kg bw/day.

#### **5.2.2.2 Adverse effects related to accidental exposure**

The oral toxicity is greater than 1080 mg/kg bw (adjusted for 86% activity) and the dermal toxicity is greater than 2000 mg/kg bw (undiluted substance) for LAS. LAS is present in detergent formulations at 30% as a maximum.

LAS is severely irritating to the eye at concentrations of about 50%, while is moderately irritating at 5% and non-irritating at 1%. The irritating effects diminished with rinsing after the exposure.

LAS is irritating to skin at a concentration of about 50% after 4 hours of exposure, while it is moderately irritating at a concentration of 5%, and not irritating at 2.5%, after 24 hours exposure.

### **5.2.3 Determination of NOAEL or quantitative evaluation of data**

#### **Repeated dose toxicity**

Many studies are available for the repeated dose oral toxicity. In view of the available information it is not possible to determine which single study is the most reliable or appropriate for the determination of a NOAEL. Because of that, based on data from all the studies, a NOAEL of 85 mg/kg bw/day is proposed, which is the NOAEL value closest to the lowest available LOAEL (115 mg/kg bw/day). This NOAEL is the dose with no effects on renal biochemical parameters that has been observed in a 9-month study of oral toxicity in rats.



## **Carcinogenicity**

The oral long-term studies performed did not indicate any potential for carcinogenicity of LAS and showed no effects or histopathological findings at doses up to 300 mg/kg bw/day.

## **Reproductive toxicity**

Results of studies on reproduction fail to show any adverse effects at any of the doses tested. Based on these studies, an oral NOAEL of 350 mg/kg bw/day, corresponding to the highest tested dose, is assessed.

## **Developmental toxicity and teratogenicity**

Some findings of maternal toxicity were found at low or relatively low doses, administered dermally and by gavage to rats, mice and rabbits, but they are associated with irritation effects of LAS, either on the skin or the gastrointestinal tract.

In other oral studies no effects were found in parental animals up to 780 mg/kg bw/day.

To sum up, some effects, such as embryo death or deformities, decrease in pregnancy rate and litter loss were noted in some studies at maternal toxic doses, but in general no decreases in the litter size, no changes in the litter parameters, no malformations or significant difference in skeletal defects were observed at oral doses up to 780 mg/kg bw/day and at dermal doses up to 1500 mg/kg bw/day.

## **5.3 Risk assessment**

### **5.3.1 Margin of exposure calculation**

The Margin of Exposure (MOE) is the ratio of the No Observed Adverse Effect Level (NOAEL) or an appropriate substitute to the estimated or actual level of human exposure to a substance. A systemic NOAEL for LAS was determined using the 9 months oral NOAEL of 85 mg/kg bw/day in the rat (see 5.2.3) and a bioavailability of 80% (Michael,1968) following gastrointestinal absorption. The resulting value of **68 mg/kg bw/day** was used as the **systemic NOAEL** to calculate the MOE values in the different exposure scenarios detailed below.

Conversion from oral NOAEL to **inhalation NOAEC** results in a NOAEC of **74 mg/m<sup>3</sup>/day**, which was used to calculate the MOE values for inhalation exposure.

#### **Exposure scenario: direct skin contact from hand washed laundry**

For calculation of the MOE, the systemic NOAEL of 68 mg/kg bw/day was divided by the daily systemic dose of 0.83 mg/kg bw/day estimated for the dermal exposure to LAS from hand washed laundry.

$$\text{MOE}_{\text{direct skin}} = \text{systemic oral NOAEL} / \text{estimated systemic dose} = 68/0.83 = \mathbf{82}$$

#### **Exposure scenario: direct skin contact from pre-treatment of clothes**

For calculation of the MOE, the systemic NOAEL of 68 mg/kg bw/day was divided by the daily systemic dose of 0.49 mg/kg bw/day estimated for the dermal exposure to LAS from pre-treatment of clothes.

$$\text{MOE}_{\text{direct skin}} = \text{systemic oral NOAEL} / \text{estimated systemic dose} = 68/0.49 = \mathbf{139}$$

**Exposure scenario: direct skin contact from hand dishwashing**

For calculation of the MOE, the systemic NOAEL of 68 mg/kg bw/day was divided by the daily systemic dose of 0.18 mg/kg bw/day estimated for the dermal exposure to LAS from hand dishwashing.

$$\text{MOE}_{\text{direct skin}} = \text{systemic oral NOAEL} / \text{estimated systemic dose} = 68/0.18 = \mathbf{378}$$

Other possible direct skin contact scenarios, such as short direct contact with laundry powder or laundry tablets result in even lower estimated systemic doses and will give larger MOE. These are not further considered in this risk assessment.

**Exposure scenario: indirect skin contact from wearing clothes**

For calculation of the MOE, the systemic NOAEL of 68 mg/kg bw/day was divided by the daily systemic dose of 28,31 mg/kg bw/day estimated for the dermal exposure to LAS from wearing fabrics washed in laundry detergents.

$$\text{MOE}_{\text{indirect skin}} = \text{systemic oral NOAEL} / \text{estimated systemic dose} = 68/28.31 = \mathbf{2.40}$$

**Exposure scenario: inhalation of and skin contact with aerosols from cleaning sprays**

For calculation of the MOE, the systemic NOAEL of 75 mg/kg bw/day was divided by the daily systemic dose of  $1.31 \cdot 10^{-5}$  mg/kg bw/day estimated for the exposure to LAS from inhalation of aerosols generated with surface cleaning sprays.

$$\text{MOE}_{\text{inhalation aerosols}} = \text{systemic oral NOAEL} / \text{estimated systemic dose} = 74/1.31 \cdot 10^{-5} = \mathbf{5.65 \cdot 10^6}$$

For calculation of the MOE, the systemic NOAEL of 68 mg/kg bw/day was divided by the daily systemic dose of  $3.78 \cdot 10^{-2}$  mg/kg bw/day estimated for the exposure to LAS from skin of aerosols generated with surface cleaning sprays.

$$\text{MOE}_{\text{skin contact}} = \text{systemic oral NOAEL} / \text{estimated systemic dose} = \\ 68/3.78 \cdot 10^{-2} = \mathbf{1.80 \cdot 10^3}$$

**Exposure scenario: inhalation of detergent dust during washing processes; powder detergents**

The dose of LAS from inhalation of detergent dust during the washing process was estimated to amount to  $1.03 \cdot 10^{-8}$  mg/kg bw/day. The MOE that could be calculated from this low exposure is higher than  $10^9$ . Such low exposure does not contribute significantly to the total LAS exposure and will therefore not be considered in the risk assessment.

**Exposure scenario: oral route from residues left on dishware**

For calculation of the MOE, the systemic NOAEL of 68 mg/kg bw/day was divided by the daily systemic dose of  $1.94 \cdot 10^{-3}$  mg/kg bw/day estimated for the oral route from residues left on dishware.

$$\text{MOE}_{\text{oral route}} = \text{systemic oral NOAEL} / \text{estimated systemic dose} = \\ 68/1.94 \cdot 10^{-3} = \mathbf{3.51 \cdot 10^4}$$

**Exposure scenario: inhalation and skin contact from laundry pretreatment products: Spray spot removers**

The dose of LAS from inhalation from laundry pretreatment products (spray spot removers) was estimated to amount to  $3.51 \cdot 10^{-6}$  mg/kg bw/day. The MOE that could be calculated from this low exposure is higher than  $10^7$ . Such low exposure does not contribute significantly to the total LAS exposure and will therefore not be considered in the risk assessment.

For calculation of the MOE, the systemic NOAEL of 68 mg/kg bw/day was divided by the daily systemic dose of  $1.74 \cdot 10^{-3}$  mg/kg bw/day estimated for the exposure to LAS from skin of aerosols generated with surface cleaning sprays.

$$\text{MOE}_{\text{skin contact}} = \text{systemic oral NOAEL} / \text{estimated systemic dose} = \\ 68/1.74 \cdot 10^{-3} = \mathbf{3.91 \cdot 10^4}$$

**Exposure scenario: skin contact from laundry pretreatment products: Liquid spot removers**

For calculation of the MOE, the systemic NOAEL of 68 mg/kg bw/day was divided by the daily systemic dose of 0.49 mg/kg bw/day estimated for the exposure to LAS from skin of aerosols generated with surface cleaning sprays.

$$\text{MOE}_{\text{skin contact}} = \text{systemic oral NOAEL} / \text{estimated systemic dose} = \\ 68/0.49 = \mathbf{139}$$

**Exposure scenario: inhalation and skin contact from liquid cleaners: Oven cleaners (spraying)**

The dose of LAS from inhalation from liquid cleaners (oven cleaners (spraying)) was estimated to amount to  $1.90 \cdot 10^{-6}$  mg/kg bw/day. The MOE that could be calculated from this low exposure is higher than  $10^7$ . Such low exposure does not contribute significantly to the total LAS exposure and will therefore not be considered in the risk assessment.

For calculation of the MOE, the systemic NOAEL of 68 mg/kg bw/day was divided by the daily systemic dose of  $2.52 \cdot 10^{-3}$  mg/kg bw/day estimated for the exposure to LAS from skin of aerosols generated with surface cleaning sprays.

$$\text{MOE}_{\text{skin contact}} = \text{systemic oral NOAEL} / \text{estimated systemic dose} = 68 / 2.52 \cdot 10^{-3} = 2.70 \cdot 10^4$$

**Exposure scenario: skin contact from liquid cleaners: Oven cleaners (cleaning)**

For calculation of the MOE, the systemic NOAEL of 68 mg/kg bw/day was divided by the daily systemic dose of  $2.19 \cdot 10^{-2}$  mg/kg bw/day estimated for the exposure to LAS from skin of aerosols generated with surface cleaning sprays.

$$\text{MOE}_{\text{skin contact}} = \text{systemic oral NOAEL} / \text{estimated systemic dose} = 68 / 2.19 \cdot 10^{-2} = 3.11 \cdot 10^3$$

**Exposure scenario: skin contact from liquid cleaners: Bathroom cleaners (mixing & loading)**

For calculation of the MOE, the systemic NOAEL of 68 mg/kg bw/day was divided by the daily systemic dose of  $3.71 \cdot 10^{-5}$  mg/kg bw/day estimated for the exposure to LAS from skin of aerosols generated with surface cleaning sprays.

$$\text{MOE}_{\text{skin contact}} = \text{systemic oral NOAEL} / \text{estimated systemic dose} = 68 / 3.71 \cdot 10^{-5} = 1.83 \cdot 10^6$$

**Exposure scenario: skin contact from liquid cleaners: Bathroom cleaners (cleaning)**

For calculation of the MOE, the systemic NOAEL of 68 mg/kg bw/day was divided by the daily systemic dose of  $7.04 \cdot 10^{-2}$  mg/kg bw/day estimated for the exposure to LAS from skin of aerosols generated with surface cleaning sprays.

$$\text{MOE}_{\text{skin contact}} = \text{systemic oral NOAEL} / \text{estimated systemic dose} = 68 / 7.04 \cdot 10^{-2} = 9.66 \cdot 10^2$$

### **Exposure scenario: skin contact from liquid cleaners: Floor cleaners (mixing)**

For calculation of the MOE, the systemic NOAEL of 68 mg/kg bw/day was divided by the daily systemic dose of  $2.19 \cdot 10^{-3}$  mg/kg bw/day estimated for the exposure to LAS from skin of aerosols generated with surface cleaning sprays.

$$\text{MOE}_{\text{skin contact}} = \text{systemic oral NOAEL} / \text{estimated systemic dose} = 68 / 2.19 \cdot 10^{-3} = \mathbf{3.11 \cdot 10^4}$$

### **Exposure scenario: skin contact from liquid cleaners: Floor cleaners (cleaning)**

For calculation of the MOE, the systemic NOAEL of 68 mg/kg bw/day was divided by the daily systemic dose of 4.16 mg/kg bw/day estimated for the exposure to LAS from skin of aerosols generated with surface cleaning sprays.

$$\text{MOE}_{\text{skin contact}} = \text{systemic oral NOAEL} / \text{estimated systemic dose} = 68 / 4.16 = \mathbf{16.3}$$

### **Exposure scenario: oral route from accidental ingestion and accidental contact with the eyes**

Occasional ingestion of a few milligrams of LAS as a consequence of accidental ingestion of laundry and cleaning products is not expected to result in any significant adverse health effects to humans given the low toxicity profile of LAS. This view is reinforced by the fact that poison control centers, such as for example those in Germany, have not reported a case of lethal poisoning with detergents containing LAS.

Contact of hand wash solutions containing LAS with the skin is not a cause of concern given that LAS is not a contact sensitiser and that the concentrations of LAS in such solutions are well below 1%. As reported in section 5.2.1.2 of this assessment, aqueous solutions of LAS at concentrations up to 2.5% failed to show any irritation effects on rabbit skin after 24 hours of occlusive application.

Accidental contact of hand wash solutions containing LAS with the eyes is not expected to cause more than a mild irritation on the basis of the experimental data as reported in section 5.2.1.3.

### **Total consumer exposure**

The consumer exposure from direct and indirect skin contact as well as from inhalation and from oral route in drinking water and dishware results in an estimated total body burden of 34.6 mg/kg bw/day. Comparison with the systemic NOAEL of 68 mg/kg bw/day yields an MOE of 1.97.

$$\text{MOE}_{\text{total}} = \text{systemic oral NOAEL} / \text{estimated systemic dose} = 68 / 34.6 = \mathbf{1.97}$$

## **5.3.2 Risk characterization**

### **5.3.2.1 Systemic toxicity**

Scenarios relevant to the consumer exposure to LAS have been identified and assessed using the margin of exposure or equivalent assessments. The Margin of Exposure for the combined estimated systemic dose is 1.97.

The estimated Margin of Exposure is based on conservative estimations of both exposure and NOAEL (which is a systemic NOAEL given the existence of oral toxicokinetic data). The critical adverse effect identified associated to the NOAEL was a change in renal biochemical parameters. Other than that, the toxicological data show that LAS was not genotoxic in vitro or in vivo, did not induce tumors in rodents after two years daily dosing, and failed to induce either reproductive toxicity or developmental or teratogenic effects at the highest doses tested. Based on the above, the presence of LAS in consumer products does not raise any safety concerns associated to systemic toxicity.

### **5.3.2.2 Local effects**

Neat LAS is an irritant to skin and eyes. The irritation potential of aqueous solutions of LAS depends on the concentration.

Contact of hand wash solutions containing LAS with the skin is not a cause of concern given that LAS is not a contact sensitizer and that the concentrations of LAS in such solutions are well below 1%. As reported in section 5.2.1.3 of this assessment, aqueous solutions of LAS at concentrations up to 2.5% failed to show any irritation effects on rabbit skin after 24 hours of occlusive application.

Accidental contact of hand wash solutions containing LAS with the eyes is not expected to cause more than a mild irritation on the basis of the experimental data as reported in section 5.2.1.4.

In the course of laundry pre-treatment, skin contact with concentrated powder paste or neat liquid detergent (in the worst case containing up to 14% LAS) may occur. If it does, contact is confined to a fraction of the skin of the hands (palms or fingers), is of very short duration (typically a few minutes at most) and the initial high LAS concentration is usually diluted out rapidly in the course of the pre-treatment task. Failing to rinse hands in water after contact with the laundry pre-treatment paste or liquid may result in transient skin irritation in the hands, which is expected to be mild in nature and effectively avoided by prompt washing with water.

Potential irritation of the respiratory tract is not a concern given the very low levels of airborne LAS generated as a consequence of cleaning sprays aerosols or laundry powder detergent dust (see sections 5.1.3.6 and 5.1.3.7).

LAS is present in household liquid detergent products at concentrations that range from 1% to 30%. Accidental spillage of neat product into the eye is to be avoided as can be expected to result in likely irritation. Immediate rinsing of the eyes with water for several minutes should follow accidental spillage of neat product. The experience from many years of marketing of household liquid detergent products containing LAS is that accidental eye spillage results at worst in transient irritation, which heals after a few days with no irreversible effects to the eye.

### **5.3.2.3 Acute effects**

Occasional ingestion of a few milligrams of LAS as a consequence of accidental ingestion of laundry and cleaning products is not expected to result in any significant adverse health effects to humans given the low toxicity profile of LAS. This view is reinforced by the fact that poison

control centers, such as for example those in Germany and UK, have not reported any case of lethal poisoning with detergents containing LAS.

### 5.3.3 Summary and conclusions

The presence of LAS in many commonly used household detergents gives rise to a variety of possible consumer contact scenarios including direct and indirect skin contact, inhalation, and oral ingestion derived either from residues deposited on dishes, from accidental product ingestion, or indirectly from drinking water.

The consumer aggregate exposure from direct and indirect skin contact as well as from inhalation and from oral route in drinking water and dishware results in an estimated total body burden of 34.6 mg/kg bw/d.

The toxicological data show that LAS was not genotoxic *in vitro* or *in vivo*, did not induce tumors in rodents after two years daily dosing, and failed to induce either reproductive toxicity or developmental or teratogenic effects at the highest doses tested. The critical adverse effect identified after repeat long term dosing of LAS to animals was a change in renal biochemical parameters. A systemic NOAEL of 68 mg/kg bw/day was established.

Comparison of the aggregate consumer exposure to LAS with the systemic NOAEL results in an estimated Margin of Exposure of 1.97. The estimated Margin of Exposure is based on conservative estimations of both exposure and NOAEL (which is a systemic NOAEL given the existence of oral toxicokinetic data).

Neat LAS is an irritant to skin and eyes. The irritation potential of aqueous solutions of LAS depends on concentration. Local effects of hand wash solutions containing LAS do not cause concern given that LAS is not a contact sensitiser and that the concentrations of LAS in such solutions are well below 1% and therefore not expected to be irritating to eye or skin. Laundry pre-treatment tasks, which may translate into brief hand skin contact with higher concentrations of LAS, may occasionally result in mild irritation easily avoided by prompt rinsing of the hands in water. Potential irritation of the respiratory tract is not a concern given the very low levels of airborne LAS generated as a consequence of cleaning sprays aerosols or laundry powder detergent dust.

In view of the extensive database on toxic effects, the low exposure values calculated and the resulting large Margin of Exposure described above, it can be concluded that use of LAS in household laundry and cleaning products raises no safety concerns for the consumers.

## 6. References

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## **7. Contributors to this report**

### **7.1 Substance team**

- **Manufacturers of LAS**

- ECOSOL (European Center of Studies on LAB-LAS), a CEFIC sector group formed by:
  - PETRESA
  - SASOL Italy
  - WIBARCO

- **Formulators**

- PROCTER & GAMBLE

The substance team is in debt with the members of HERA Human Health and Environmental Task Forces as well as with the Industry coalition for the OECD/ICCA SIDS assessment of LAS for their valuable comments and suggestions during the preparation of the report.

## **7.2 HERA environmental task force**

- AISE
- BASF
- CIBA Speciality Chemicals
- Clariant
- Dow Corning
- Henkel
- Petresa.
- Procter & Gamble, Eurocor.
- Rhodia
- Sasol Germany
- Sasol Italy.
- Shell Chemicals
- Solutia Services International
- Solvay
- Unilever

## **7.3 HERA human health task force**

- BASF
- Bayer
- CIBA
- Clariant
- Colgate-Palmolive
- Degussa-Hüls
- Henkel
- McBride
- Procter & Gamble, Eurocor.
- Shell Chemicals
- Unilever

## **7.4 Industry coalition for the OECD/ICCA SIDS assessment of LAS**

- Colgate
- Crompton
- Dial
- Huntsman

- John Adams Associates
- Kao
- Petresa
- Procter & Gamble
- Sasol It
- Sasol N.A.
- Stepan
- Venoco
- Weinberg Group
- YPF