REVIEW OF THE ENVIRONMENTAL SAFETY OF LAS

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The European Centre of Studies on Linear Alkylbenzene and derivatives (ECOSOL) is a Sector Group of the Conseil Europeen des Federations de l'Industrie Chimique (CEFIC). It has representatives from:

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Procter & Gamble
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effect of other components of raw sewage cannot be ignored and LAS is degraded faster in the environment than for example ammonia which at elevated levels is toxic to aquatic life. The higher homologues of LAS are more toxic to aquatic life than the lower ones but are removed more rapidly. The degradation products are much less toxic than LAS.

The effect of LAS on organisms in estuaries and coastal waters is not yet fully established but most species are unlikely to be affected at the concentrations observed in marine waters. There is, however, conflicting evidence as to the effect on the red tide algae and the larvae of oysters which might be adversely affected at concentrations found close to sewage outfalls.

Tests with plants grown on sludge-amended soils have indicated that the levels of LAS resulting from sewage sludge application (13 to 47 mg/kg) are unlikely to have an effect as the "no effects" concentration was in excess of 100 mg/kg.

At the current level of use LAS is unlikely to pose a hazard to the environment. This conclusion is based on the results of the literature review and the observation that twenty-five years of continuous use of LAS has not revealed any deleterious effects in the environment.

during sewage treatment. No deleterious effects on sewage treatment have been observed at concentrations below 20 mg/l.

In rivers, concentrations are usually less than 0.05 mg/l, although in some countries levels as high as 1 to 2 mg LAS/l have been reported where untreated wastewater is discharged. In estuaries and coastal waters the concentrations are very low (c. 0.008 mg LAS/l) but near outfalls the concentrations can be as high as 0.03 mg LAS/l.

The evidence shows that under aerobic conditions LAS is completely degraded (mineralised) in the environment. In a few laboratory tests incomplete degradation has been obtained but this can be attributed to factors such as concentration and biochemical activity of inoculum, deficiencies and inbalance in media or lack of biomass in synthetic sewage.

The removal of LAS in efficiently operated sewage treatment works is high (95-99%). The proportion of LAS associated with sewage sludge is approximately 30%. As LAS is not biodegraded under strict anaerobic conditions it is not removed during anaerobic digestion. However, any LAS present in sludge-amended soils degrades at relatively high rates with half lifes ranging from 3 to 35 days.

As concentrations of LAS in the freshwater environment are usually low the LAS present is unlikely to cause any effect on the ecosystem. Where untreated effluent is discharged, environmental concentrations could reach levels sufficiently high to cause detrimental environmental effects. In this situation the

CONTENTS

Page

Page

1. INTRODUCTION

The Water Research Centre (WRc) has been contracted by ECOSOL and the SDIA to assess the environmental impact of linear alkyl benzene sulphonate (LAS) based on existing information.

LAS continues to be the main surfactant used in formulated detergent products in the USA, Japan and Western Europe. The world demand of LAB, the raw material for LAS, is 990 000 tons/year. Converting this figure to LAS by multiplying by 349/247 = 1.4, results in a worldwide LAS consumption of approximately 1.4 million tons/year. The total LAB production in Western Europe is 460 000 tons/year (CEFIC 1987, private communication) which converts to 644 000 tons/year of LAS.

The actual quantity of LAS used in detergents in Western Europe in 1984 was 466 000 tons and it is claimed that the amount used for other applications is negligible (SRI 1985).

The total consumption of laundry detergents <u>per capita</u> in Western Europe is approximately 9.54 kg/year (range 5.6 to 12.8 kg/year) but this includes all types and not just LAS-based detergents. The average consumption of LAS can be estimated from the concentration of LAS in the raw sewage and the sewage flow (see Section 3) and consumption figures of approximately 2.5 g/cap.day have been quoted.

Assuming a population of 320 x 10^6 in Western Europe, this is equivalent to a total yearly tonnage of 292 000, or about 35% less than the estimate of 466 000 tonnes (above). The reasons for this discrepancy are unknown, but biodegradation in the sewers would be a contributing factor.

For this study the reports published by Little (Little 1977, Goyer et al 1981) were used as a starting point. Detailed searches of the literature were carried out and the information obtained was supplemented by references supplied by various companies. In addition, some companies contributed unpublished data together with results of current research. For those papers which were only available in foreign languages, particularly the large number of Japanese papers, only the information available in the English abstract was used for the preparation of the report.

Since the Little report, a few papers have discussed the environmental safety of LAS (eg Holysh et al 1985, Gilbert and Kleiser, in press, de Henau et al 1986b) or of detergent chemicals generally (eg de Henau et al 1986a, Gerike 1987, Woltering et al 1987). These papers were taken into consideration in the preparation of this report.

The present report discusses the various analytical techniques available for the analysis of LAS, the homologues and their isomers and their degradation products (Section 2) and presents the concentrations found for LAS and LAB and the degradation products in the various environmental compartments (Section 3). This is followed by a detailed examination of the fate of LAS in the environment (Section 4) and its likely effect on the ecosystem (Section 5). The final section provides an assessment of the environmental acceptability of LAS (Section 6).

2. ANALYSIS

Various analytical methods are available for studies of LAS and their degradation products.

These include colorimetric methods, gas chromatography and high performance liquid chromatography.

2.1 Colorimetric methods

Colorimetric methods for the determination of anionic surfactants are all based on the formation of an ion association complex with a cationic dyestuff. The complex formed is solvent-extractable whereas the dyestuff is not and thus the complex can be separated from unreacted dye prior to colour measurement.

2.1.1 Methylene-blue active substances - method

Methylene blue has been generally adopted as the reagent dye. The standard method of the International Organization of Standardization (ISO), of the British Standards Institution (BSI) and of other standards organisations is based on Abbott's (1962) modification of the Longwell and Maniece (1955) method. An aliquot of a methylene blue solution is added to the solution containing the surfactant under alkaline conditions which is subsequently extracted with chloroform. Colour standards and a calibration graph are prepared from standard surfactant solutions. In most countries standardised commercial surfactants are used as the anionic surfactant standard (eg sodium lauryl sulphate and sodium dodecylbenzene sulphonate). Manoxol OT (sodium di-octylsulphosuccinate) is used in the UK because it can be obtained in a high state of purity. Results are expressed in terms of the surfactant standard employed. This is important because of the differences in molecular weight of the various standards (eg Manoxol OT = 444, sodium lauryl sulphate = 288, sodium dodecylbenzene sulphonate = 348).

Any substance, anionic surfactant or not, which forms an extractable complex with methylene blue responds to the analysis and the method is therefore not specific to any particular anionic surfactant. Results are therefore expressed as concentrations of Methylene Blue Active Substance (MBAS), eg mg MBAS/l as Manoxol OT.

The limit of detection for the standard method carried out manually is given as 0.05 mg MBAS/l as Manoxol OT for a 100 ml sample, and the range of application is 0.1 to 5.0 mg/l for 100 ml samples (ISO 1984). The method has also been established for air-segmented, continuous-flow autoanalyser use, and here the limit of detection is given as 0.2 mg MBAS/l as Manoxol OT with a range of application of up to 20 mg MBAS/l (DoE/NWC 1982).

Various attempts have been made to improve the specificity of the method by separating LAS from interfering substances, particularly for the analysis of river waters and sea water, using adsorbents such as Amberlite XAD-2. The MBAS method is applied to the eluate containing the surfactant. Hydrolysis of the sample prior to the addition of methylene blue has been used to destroy interferants such as alkyl sulphates and alkyl ethoxysulphates (Saito et al 1981; Saito and Hagiwara 1982; Osburn 1986). Saito and Hagiwara (1982) stressed that about 90 minutes was required for quantitative adsorption by XAD-2 resin if carried out in shake flasks. However, it has to be emphasised that the use of XAD-2 resin and prior hydrolysis does not provide an analysis specific to LAS. Yasuda (1980, 1981), however, found that if the ion exchange resin Amberlite IRA CG 400 was used for the separation, ABS was selectively eluted before LAS which permitted the separate determination of the two surfactant types.

The MBAS method has been applied to the analysis of fresh waters, sea water, and muds (DoE/NWC 1982). Methanol is used to extract LAS and other anionic surfactants from solids. The efficiency of the extraction is reported to be improved by the use of ultrasound (Uchiyama and Takamura 1980).

2.1.2 Other colorimetric methods

In recent years other cationic dyestuffs have been suggested as substitutes for methylene blue.

1-(4-nitrobenzyl)-4-(4-diethylaminophenylazo) pyridinium bromide: Higuchi et al (1980); said to give a lower detection limit.

1-(N-methyl pyridinium-4-ylazo)-4-[4-(diethyl-amino)phenylazo] naphthalene iodide: Higuchi et al (1981, 1982); undergoes a colour change on formation of an ion pair.

bis[2-(5-chloro-2-pyridylazo)-5-diethylamino phenolato] cobalt(III): Taguchi et al (1981); more efficient at extracting lower molecular weight homologues of LAS because of its larger molecular size than methylene blue.

Crystal Violet: Fu and Qi (1985); more sensitive than methylene blue.

1-benzyl-4-(4-diethylaminoazo)pyridinium bromide: Kobayashi and Numata (1984); Huang et al (1985); faster and simpler to use.

2.2 Gas Chromatography (GC)

LASs themselves are not sufficiently volatile to permit direct GC analysis and it is necessary to produce a volatile derivative while preserving the original homologue and isomer pattern (see Section 2.2.2).

In theory the GC analysis of LAS should permit the separation and measurement of each component homologue and its isomers. In practice the degree of resolution that is achieved depends on various factors including the stationary phase, the efficiency of the column and operating conditions such as column temperature and carrier gas flow rate.

Chromatographic limits of detection are expressed in terms of the mass of substance detected. A figure of 50 µg of total LAS has been quoted (Waters and Garrigan 1983) as being preferable for desulphonation. Limits should be applied to individual homologues or separable isomers. Limits of detection in concentration terms are dependent on the volume of sample taken and subjected to the analytical procedure. The aforementioned authors took volumes of up to 8 litres of river water, which give limits of detection of much less than 10 µg/l for total LAS, and sub-µg/l for individual isomers.

2.2.1 Isolation of LAS

Both adsorption and solvent extraction techniques have been employed for the isolation of LAS. For adsorption, ion exchange resins have been reported to be superior to activated carbon because it is difficult to desorb LAS completely from carbon even with large volumes of eluent (Hughes et al 1969).

Solvent extraction of the LAS-methylene blue complex has been used to separate LAS from environmental samples (Waters and Garrigan 1983). The technique has also been applied to extraction of LAS from wet sludges using chloroform/methylene blue extractant (McEvoy and Giger 1985, 1986). These workers carried out a further separation using thin layer chromatography (TLC), the blue band being extracted with methanol. TLC has also been used to separate linear alkyl benzenes (LAB) from environmental samples and from detergent preparations where they are present as impurities

(Eganhouse et al 1983a). The method could presumably be adopted for the isolation of LABs following desulphonation of LAS. TLC was used by Matsutani et al (1979) to separate LAS methyl esters prior to further derivatisation. Derivatisation products have also been separated by silica gel column chromatography (Matsutani et al 1980, Ogino and Nagao 1979).

Other clean-up procedures involve the use of cation exchange chromatography, hydrolysis and solvent extraction to remove hydrolysable and polar and non-polar materials which are potential interferants in GC (Waters and Garrigan 1983). Tetralones and indanones which interfere in GC, have been reported to be formed on desulphonation of biodegradation products of LAS. They can be eliminated by ether extraction (Osburn 1986).

2.2.2 Formation of volatile derivatives

The formation of volatile derivatives (detectable by GC) which retain the homologue and isomer pattern of the parent LAS is carried out by reaction of the sulphonate functional group either to remove it (desulphonation) or to form a derivative. Desulphonation is carried out with strong acid. Waters and Garrigan (1983) described a phosphoric acid desulphonation apparatus designed to minimise the loss of the volatile alkylbenzene product. Using ^{14}C-labelled 2-phenyl C 11 LAS they recovered more than 95% of the radioactivity on desulphonation for 90 minutes at 200-210°C. They quoted Setzkorn and Carel (1963) as reporting an increased yield at the slightly higher temperature of 210-220°C but could not confirm this themselves. Osburn (1986) carried out desulphonation at 215°C for 3 hours. After 1.5 hours not all the LAB product was distilled nor collected in the trap, particularly the C 13 and C 14 homologues which made up the bulk of the LABs collected in the subsequent time period (1.5 to 3 hours). Hughes et al (1969) in common with many other authors were able to demonstrate that the alkylbenzene produced by LAS desulphonation retained the homologue pattern of the original LAS.

A number of derivatisation techniques have been reported. Sulphonyl chlorides are produced by a variety of reagents. McEvoy and Giger (1985, 1986) used phosphorus pentachloride. Thionyl chloride can also be used with the advantage that the by-products are gaseous.

The sulphonyl chlorides can themselves be further reacted to form sulphonic acid esters. Hon-Nami and Hanya (1978) reported the use of thionyl chloride en route to methyl ester formation. Later (1980a,b) they revised their original method by using PCl_5. However, McEvoy and Giger found no advantage in using the methyl ester method over using sulphonyl chloride for derivatisation.

Other derivatisation techniques have been reported by Japanese authors but only abstracts with limited amounts of information are available in English.

Chlorinated derivative: Ogino and Nagao (1979)

Conversion of LAS to the corresponding fluoride using PCl_5 and KF: Tsukioka and Murakami (1983b).

Conversion of methyl esters of LAS to the corresponding thiols: Matsutani et al (1979).

Conversion of LAS to the corresponding alkyl phenol by fusion with alkali: Matsutani et al (1980).

2.2.3 Gas chromatography columns

In GC analysis the basic choice is between packed and capillary columns. A collaborative exercise involving six Czechoslovakian laboratories investigated operating conditions for the determination of LABs derived from LAS. It was concluded that capillary columns were better than packed columns and that a fairly wide range of operating conditions could be tolerated. Impurities in the LABs such as 1,4-dialkyltetralins and 1,3-dialkylindanes caused minor problems (Zeman 1982, 1983).

Capillary column GC is nowadays almost invariably synonymous with high resolution GC and is the technique of choice of present day investigators for LAS-derived substances.

2.2.4 Resolution

Complete resolution of all isomers does not appear to have been achieved even with the high efficiency capillary columns. Even the complex procedure of Eganhouse et al (1983a) involving an initial TLC separation permitted the complete resolution of only three pairs of LAB isomers. Waters and Garrigan (1983) employed temperature programming which gave a better resolution in a shorter time (less than 40 minutes per analysis) than indicated by previous workers; even so the concentrations of some pairs of isomers had to be expressed jointly. The overall recovery for their complete procedure for a C 12 LAS internal standard was 91%.

Mass spectrometers, when used as detectors (GCMS), provide a powerful means of identification and this technique has been used by authors such as Hon-Nami and Hanya (1978, 1980) and McEvoy and Giger (1986).

2.3 High Performance Liquid Chromatography (HPLC)

Like GC, HPLC should in theory be capable of resolving the homologues and isomers of an LAS. Modern techniques are so versatile that the analytical possibilities range from obtaining a single chromatographic peak representing all the LAS present, through single peaks representing all the isomers of each homologue to a near complete resolution of all homologues and their isomers. The degree of resolution of isomers that can be obtained with HPLC is not as great as with high efficiency GC columns. HPLC has the advantage over GC that no derivatisation is necessary to produce volatile substances. It is superior to TLC in being less time-consuming and of much greater efficiency.

As with GC, detection limits for HPLC are expressed in terms of mass injected onto the column, preferably in terms of each homologue. Detection limits of 0.05 µg of each LAS homologue have been reported (eg Matthijs and De Henau 1987). Detection limits in terms of concentration of LAS can be derived and depend on factors including the volume of sample taken for the determination. Matthijs and De Henau (1987) reported values of 10 µg/l for total LAS in aqueous samples and 100 µg/kg in solid samples, when using 100 ml river water and 1 g dried sewage sludge, respectively. Apart from these figures, detection limits of between 0.1 µg/l for each homologue to 40 µg/l for total LAS have been reported.

Interference in HPLC is due to the same causes as interference in GC and clean-up may be necessary; the same techniques have been used as with GC, including isolation as the methylene blue (MB) complex, and separation by resin adsorption or ion exchange. In the case of sludges, sediments and other solids LAS can be extracted with methanol or, to improve selectivity, with methanol and methylene blue.

Takano et al (1975, 1976) used a silica gel column with detection at 225 nm to chromatograph ABS as the MB complexes, the MB remaining on the column, with a detection limit of 0.05 mg/l. Tanaka et al (1976) obtained all the isomers of dodecylbenzene -sulphonate in one peak using high speed LC. The column was Zorbax SIL and the mobile phase was acetic acid-hexane-isopropanol; pre-treatment of environmental samples was carried out on an Amberlite XAD-2 resin column.

In a general paper on reverse phase ion-pair HPLC, Gloor and Johnson (1977) reported that ammonium and trimethylammonium (TMA) chlorides yielded similar high resolutions of LAS isomers and homologues, whereas tributylammonium chloride (TBA) showed a lower degree of resolution because its larger size dominated the retention of the ion pair. They favoured TMA since it yielded higher "capacity factors" with no loss of resolution. Their column was 25 cm x 2.2 mm Micropak MC - a monomolecular layer of C_{18} silane bonded to the surface of 10 µm silica. The relative homologue composition of a standard LAS was found to be almost identical by the HPLC and the GC methods. As opposed to this Jandera and Churacek (1980) reported a method for aromatic sulphonic acids and other strongly polar compounds without the addition of an ion-pairing counter ion. Ion-pairing had been introduced to overcome poor efficiency and low chromatographic selectivity on ion-exchange columns, especially the very strong retention on sym-divinylbenzene ion-exchange resins. Also, without ion-pairing, peaks were very distorted or split with irreproducible shapes. The authors overcame these effects by the addition of a strong electrolyte and used 0.4M Na_2SO_4 as one solvent and 60:40 $MeOH:H_2O$ as the other. The LiChrosorb SI 100 (10 μm) column material was reacted with n-octadecyl-trichlorosilane (C18) and UV-detection was used. Some 20 peaks were obtained with commercial household detergent but were not identified.

Another group (Nakae and Kunihiro 1978) first used a porous styrene/divinylbenzene copolymer as packing with 0.5M perchloric acid as the mobile phase. This system gave fairly good resolution. Later they (Nakae et al 1980) found that of a number of salts examined, 0.1M sodium perchlorate in 8:2 methanol:water gave the best separation of homologues and of the 2-phenyl isomers from the other phenyl isomers. This mobile phase optimised the operation of the UV fluorescence detector, used in the stop-flow mode. No pre-treatment of river water samples was necessary, except for the addition of formaldehyde (if the samples were to be preserved before analysis), since the LAS concentrations were >100 μg/l; but pre-treatment is necessary at the low μg/l values found in the majority of river waters. By using a porous spherical octadecylsilanized silica gel (LiChrosorb RP-18) of average diameter 5 μm and changing the mobile phase to aqueous acetonitrile containing 0.1M sodium perchlorate (Nakae et al 1981) they achieved excellent separation of the phenyl isomers. An important refinement was the addition of a high concentration of sodium dodecylsulphate to reduce the loss of LAS by adsorption. Other inorganic salts - sodium chloride, sodium nitrate, ammonium chloride - in place of perchlorate gave similar separations, but perchlorate gave no absorption in the UV range. Nakamura et al (1981) and Nakamura and Morikawa (1982) also reported enhanced separation by the use of 0.4M sodium chloride. Nakae et al (1981) found that the "capacity factors" decreased with increasing temperature and their results suggested that controlled temperature at 40°C was necessary. The authors suggested that the method would be useful in detecting intermediates and later applied the method (Yoshimura and Nakae 1982a) to identify 3-(4-sulphophenyl)butyrate in biodegradation tests.

Linder and Allen (1982) followed Gloor and Johnson (1977) in using an ion-pair mobile phase but used tetrabutylamine phosphate rejected by Gloor and Johnson; the mobile phase also contained tetrahydrofuran. They used as detector UV fluorescence rather than UV, since the latter gave both negative and positive peaks which interfered with peaks from intermediates of LAS biodegradation. The resolution of the system was chosen to produce single peaks for each LAS homologue and was also suitable for detecting aromatic intermediates having at least two C atoms in the alkyl chain. It was assumed for purposes of calculating concentrations that the intermediates gave a response equivalent to that of the parent structure. Samples were stabilised with formaldehyde as was done by Nakae et al (1980, 1981) and stored at 4°C, if necessary, but otherwise needed no treatment. The method was applied to biodegradation test systems, river water, sewage and sewage effluents.

Saito et al (1982) applied normal phase HPLC after concentrating LAS on a weak base anion-exchange resin and eluting. The mobile phase was 0.2% ammonia in ethanol and the detection limit was about 0.02 mg/l (with a UV detector) using a 1 litre sample.

Tsukioka and Murakami (1983a) first converted LAS to the chlorides or methyl esters before applying HPLC using one of three column materials, including μ Bondapak C18. The mobile phase was 85 to 90% methanol and detection was at 250 and 225 nm, respectively, for the chloride and ester. Recovery was ≥90% and the detection limit 0.05 μg. Kudoh and Tsuji (1984) also claimed to improve HPLC separations by first forming derivatives. Kwan and Lin (1984) were able to differentiate between ABS and LAS by RP-HPLC, with a mobile phase of 0.1M sodium acetate in 80% methanol-water, and UV detection.

Another 13 papers from Japan and China were only available in inadequate abstract form and few useful details could be deduced. Two authors (Ohkuma 1981; Yoshikawa et al 1984) used ethanol as eluent, while two others (Yoshimura et al 1984c; Tan and Zhang 1986) employed sodium perchlorate and Du et al (1985) used 0.1% methanolic tetrabutylamine chloride.

Finally, two recent papers have described sensitive and fairly rapid methods for marine samples (Kikuchi et al 1986) and for a wide range of waters and solid samples (Matthijs and De Henau 1987).

Kikuchi et al (1986) reviewed the whole range of methods for LAS analysis in marine samples but rejected them, variously, on grounds of specificity, sensitivity and time required. They concluded that HPLC methods so far described are either not suitable for marine samples, including biota, and/or need too many steps and are too time-consuming. The authors put forward a method which they claim is "simple, rapid, quantitative, sensitive and specific". It has few steps and so lessens the risk of contamination and loss. Although the chromatographic details are those described by Nakae et al (1980, 1981), the mobile phase being 0.1M sodium perchlorate in 45% aqueous acetonitrile, the refinement is in the extraction of LAS from the samples. After preservation (10 ml formaldehyde/l at -1 °C) if necessary, LAS was extracted from filtered aqueous samples with Bond Elut C 18 RP mini-columns. Sediments and fish (first homogenised) were thrice extracted with methanol. Extracts diluted with water were passed through mini-columns. After elution with methanol the eluates were concentrated by nitrogen "blowdown" and the concentrates were chromatographed. The limit of detection for each LAS component was about 0.1 µg/l in water samples and 0.03 µg/g (dry basis) for sediments; recovery was 80% and 87% respectively for water and sediment samples.

De Henau et al (1986b) and Matthijs and De Henau (1987) also considered desulphonation followed by GC or GC/MS to be time-consuming and complex. They, too, adopted the chromatographic method of Nakae et al (1981) but used linear gradient elution with 0.15M sodium perchlorate in 49 to 63% aqueous acetonitrile in 25 min and UV rather than UV fluorescence detection. The use of gradient elution, rather than the isocratic system employed by Nakae et al (1981), led to better separations of LAS homologues. Sludges and soils were first refluxed with methanol and interfering non-ionic matter was removed by passing the extract through an anion-exchange resin (SAX) column, a step not carried out by Kikuchi et al (1986). LAS was eluted from the column with acidified methanol and the eluate was diluted with water and neutralised. The diluted eluate was further purified by passage through a small-scale preparative C8 RP silica column. After rinsing the column with aqueous methanol (again omitted by Kikuchi et al), LAS was eluted with 100% methanol. The final eluate was transferred to a vial, taken down to dryness on a steambath and stored until analysed. Prior to analysis, the solid was taken up in acetonitrile in water (70:30). Unfiltered aqueous samples (sewage 5 ml, effluent 10 ml, river water 100 ml) were passed through the C8 column and then treated as above. Sludges required about 1 g dry solids and sediments about 10 g dry solids; the preparation steps required about one hour per sample, considerably less than for GC-based procedures, although the resolution was not as good as with GC. The detection limit was about 0.05 µg for each LAS homologue and the relative standard deviation was 6%. Recovery of LAS spiked in water, sediment and sludge samples was 94, 87 and 84% respectively.

The methods used by the EAWAG group in Switzerland are about to be published (Marcomini and Giger 1987).

Castles and Ward (in press) have deliberately reduced the resolving power of HPLC columns by substituting the C18 column by a C1 SAS column in order to get each homologue eluted in a compact, single peak. The mobile phase was 0.15M sodium perchlorate in aqueous tetrahydrofuran. Solids were extracted with methanol, and sewage and river water were first isolated on a C2 resin then eluted with methanol, followed by further clean-up with an SAX resin.

2.4 Spectroscopy

Spectroscopic methods have generally been used to identify and characterise unknown compounds rather than to determine them quantitatively.

Infra-red spectroscopy has been used (Hashimoto et al 1973; Yagami et al 1984; Hu et al 1985) to identify and quantify LAS and also to distinguish between linear and branched types. Hellmann (1982) followed the degradation of LAS over 30 d by using IR measurements. However, this method has not been widely applied but plays a back-up, confirmatory role. Other methods are faster and more convenient and there is some doubt (Swisher 1981b; Schaumberg et al 1981) about the number of absorption peaks necessary for full identification.

While UV absorption is used to detect LAS isomers and homologues in many HPLC separations, it is also used to quantify LAS as a class (eg Yagami et al 1984; Saito and Hagiwara 1982; Mizuno et al 1984). For example, Uchiyama (1977) extracted MBAS from aqueous samples with methylene blue and 1,2-dichloroethane. The extract was treated with acid and the liberated LAS was extracted into water; the absorbance at 222 nm was determined. UV methods generally are applicable to biodegradation studies, providing that interferants, eg nitrate, are not present (eg Painter and Durrant 1976; Mizuno et al 1984).

In the view of Swisher (1987), direct application of nuclear magnetic resonance (NMR) spectroscopy to biodegradation and environmental problems is still some way off. Various authors have used NMR to quantify LAS in mixtures, sometimes via their methyl esters (Matsutani et al 1979) and usually after separation by one- or two- dimensional TLC (Yagami et al 1984). The method has also been used to determine the ratio of linear to branched ABS and ABS to "alkane sulphonates" (Matsutani et al 1980; Choi and Kwon 1980). Wainai et al (1983) assigned the chemical shifts in ^{13}C-NMR spectra for ABS containing normal C 10 to C 14 alkyl groups and having the single sulphophenyl group attached to C 1 to C 6 of the chain. These shifts were then used in the quantitative analysis of LAS; the results agreed fairly well with those from GC.

More recently, Thurman et al (1987) have extracted ABS from groundwater at two sites at different distances from sewage infiltration ponds and quantified them by titration. By applying ^{13}C-NMR spectroscopy to the dried extracts they suggested that the water from the nearer well (500 m) contained LAS while the pond at 3000 m contained branched chain ABS.

2.5 Other methods

Wang et al (1975a,b) have improved on their earlier indirect (or back) two-phase titration method for anionic surfactants. In the newer method, which is simpler and less time-consuming than the direct titration and methylene blue methods, the sample is treated with a solution of Azure A to form a complex with the surfactant. Chloroform is added and the mixture is adjusted to pH 3, whereupon the chloroform layer becomes blue. The mixture is then titrated with a quaternary ammonium salt which displaces Azure A and reacts with the surfactant. Since Azure A is not soluble in chloroform, the organic solvent becomes colourless, denoting the end point. However, the method is not specific to LAS. The two-phase titration method has been reviewed, covering 173 references, by Vavrouch and Kuban (1984).

Two indirect non-specific determinations of LAS were described by Matsueda and Morimoto (1980) and Matsueda (1981). In one, the ion-pairs formed between LAS and thiourea-Cu(I) complex are extracted with methyl iso-butyl ketone and Cu is determined by AA spectrophotometry, the concentration range being 0.001 to 20 mg LAS/l. In the second method, sodium is determined by flame photometry after a similar methyl iso-butyl ketone extraction, the limit of detection of LAS being 0.05 mg/l. Although the method is simple and rapid compared with the methylene blue method, the presence of cationic and amphoteric surfactants causes interferences. In another method the amount of ferroin reacting with an anionic surfactant is measured (Abe 1984).

Polarographic methods, such as depression of maxima and voltammetry, have been developed for surfactants (Smyth and Healy 1984; Cosovic et al 1985) but they are not specific and effective separation is necessary for environmental samples. Hart et al (1979) used an indirect polarographic method based on nitration of the benzene ring to determine LAS. It was reliable at 0.5 mg/l and above and was more selective than methylene blue.

Another technique is potentiometric titration using ion-selective electrodes (Dmitriev et al 1981; Vytras et al 1981; Hoke et al 1979) or liquid membrane ion-selective electrodes (Hu et al 1983, 1984). In the latter case ion-pairs were prepared between LAS and either dodecyltriheptyl ammonium iodide or tri-octylmethyl ammonium chloride.

Llenado and Neubecker (1983) and Swisher (1987) have reviewed the progress in ionization techniques in GCMS which unlike electron impact mass spectrometry would allow the direct analysis of strongly polar compounds. This would avoid the need for converting LAS into volatile derivatives, and would allow analysis with a minimum of pre-treatment. They cite papers by Weber et al (1982) and Daehling et al (1982) who incorporated into the field desorption (FD) MS a second stage of MS using collisionally activated dissociation. Although Swisher (1987) thought that Weber et al (1982) seemed to conclude that LAS comprises only the 2-substituted isomers (with minimal amounts of the more internal isomers), nevertheless Swisher suggested that FDMS may well become the method of choice after HPLC has had its hey-day. (This is a contentious view, since these MS techniques are extremely sophisticated and expensive, requiring the total dedication of an experienced mass spectroscopist and may be impractical for routine analysis of LAS.) Other papers using advanced MS techniques, eg fast atom bombardment (FAB), are those by Lyon et al (1984) and Schneider and Levsen (1986).

Ward et al (1987) applied FAB/MS techniques to environmental samples, after evaporation and simple extraction, at concentrations down to 1 µg/l. The method was five times faster, overall, than HPLC methods and the authors anticipated that development of accurate FAB/MS methods would be considerably faster that HPLC procedures.

3. CONCENTRATION OF LAS IN THE ENVIRONMENT

With the more widespread use of specific methods of analysis for LAS, including the homologues and their isomers, more reliable values have been obtained for the concentration of LAS in environmental samples - raw sewage, sewage effluent and sludges, river water and sediments, sea water and sediments and sewage sludge amended soils. However, for some environmental compartments, for instance sludge amended soil, there are still only few data available.

3.1 Sewage and sewage effluent

The data on the LAS concentrations found in sewage (raw or after primary settlement) and sewage effluent in the different countries are given in Tables 3.1 and 3.2.

De Henau et al (1986b) and Rapaport et al (1987) have summarised data on the concentration of LAS in raw sewage in North America. The average for 78 composite samples from 17 plants in the USA was 3.7 ± 1.1 mg LAS/l, while for 9 composites from 3 plants in Canada the mean was 2.0 ± 0.6 mg/l. These values compare with Rapaport's estimates for US raw sewage, based on sales of LAS, water usage

Table 3.1 Concentration of LAS in sewage[1]

Country	No of samples or period	No of treatment works	Concentration of LAS (mg/l)	Reference
USA	-	3	3.8	De Henau et al 1986b
	78	17	3.7 (1.1)	Rapaport et al 1987
	3	1	2.97	Sedlak and Booman 1986a,b
	3	1	3.53*	
	-	-	3.8-6.5	Osburn 1986
Canada	9	3	2.0 (0.6)	Rapaport et al 1987
Switzerland	-	8	2.4 (0.9)	Giger et al 1987
W Germany	-	10	4.0 (0.54-12.4)	Matthijs and De Henau 1987
	-	8	4.8 (1-10)	De Henau et al 1986b
	1 year	1	11.9*	Wagner 1978
UK	1974-1979	Luton (24 h comp)	9.4* (8.6-11.0)	Painter and King 1979a
	"	Stevenage (6 am-12 noon)	26.5* (20-31.3)	Painter and King 1979b
	3	Stevenage (24 h comp)	15.4* (13-17)	Stiff 1987 (pers comm)
Israel		4	9.6-11.0*	Zoller 1985
Japan	-	-	up to 22*	Ogino and Nagao 1979
	-	-	19.2*	Nara et al 1983
China	-	-	5.4-15.7*	Hu et al 1983, 1985

* MBAS
Values in brackets are standard deviations or ranges
[1] The distinction between raw and settled sewage (ie after primary settlement) is rarely reported

Table 3.2 Concentration of LAS in biologically treated sewage (activated sludge, unless indicated otherwise)

Country	No of samples or period	No of treatment works	Concentration of LAS (mg/l)	Reference
USA	-	3	0.06	De Henau *et al* 1986b
	-	4	0.6 (0.3)+	Rapaport *et al* 1987
	-	12	0.05 (0.04)	
	-	1	0.51+ (0.12)	Holysh *et al* 1986
	-	1	0.2-0.4*	Thurman *et al* 1986
	3	1	0.02	Sedlak and Booman 1986a,b
	3	1	0.05	
		1	0.14-0.60+	Osburn 1986
Canada	-	3	0.09 (0.05)	Rapaport *et al* 1987
Switzerland	-	8	0.09 (0.12)	Giger *et al* 1987
W Germany	-	8	0.07	De Henau *et al* 1986b
	-	10	0.07 (0.05-0.11)	Matthjis and De Henau 1987
	1 year	1	0.59* (0.16)	Wagner 1978
UK	-	3	0.1-0.3ø	Waters and Garrigan 1983

+ trickling filter (3) and 1 rotating biological contactor
ø unknown plant
* MBAS
Values in brackets are standard deviations or ranges

(560 l/cap.day), and population, of 4.6 mg/l equivalent to approximately 2.6 g LAS per head per day. In 12 of the US plants treatment with activated sludge reduced the LAS concentration to a mean concentration in the effluent of 0.05 ± 0.04 mg/l. The mean concentration of LAS was 0.09 ± 0.05 mg/l in the effluent of the three Canadian activated sludge plants. However, effluents from four US trickling filter plants contained as much as 0.6 ± 0.3 mg LAS/l. Sewage after receiving only primary sedimentation contained 2.2 ± 0.4 mg LAS/l (USA) and 1.7 to 2.3 mg/l (Canada).

Further examples of the higher effluent concentrations of LAS from trickling filters are given by Holysh et al (1986) who reported 0.51 ± 0.12 mg/l at Rapid Creek, SD, USA, and Thurman et al, (1986) who found 0.2 to 0.4 mg MBAS/l at Otis Air Base, USA.

Sedlak and Booman (1986a,b) studied the removal of LAS in an activated sludge plant at Enid, Ok, USA. The plant was divided into two streams, which were operated at 3.2 (conventional rate) and 0.8 day (high rate) sludge retention time, respectively. The primary treated sewage applied contained 2.97 mg LAS/l and 3.53 mg MBAS/l (means of three 24 h composites) which was reduced to 0.02 and 0.05 mg LAS/l respectively in the conventional and high rate streams, indicating the small effect of loading on LAS concentration in the final effluent. Osburn (1986) reported LAS concentrations in US raw sewage in the range 3.8 to 6.5 mg LAS/l with similar IL-MBAS concentrations. The trickling filter effluents contained 0.14 to 0.60 mg LAS/l but higher values of 0.39 to 1.02 mg/l for IL MBAS indicated that LAS contributed 35 to 60% to the total MBAS in the final effluent.

Similar LAS concentrations of 2.4 ± 0.9 mg LAS/l were reported by Giger <u>et al</u> (1987) for raw sewage from eight Swiss treatment works; corresponding activated sludge plant effluent concentrations were 0.09 ± 0.12 mg/l. German raw sewage had rather higher LAS concentrations, ranging from 0.54 to 12.4, mean 4.0 mg/l, for 10 grab samples (Matthijs and De Henau 1987) while De Henau <u>et al</u> (1986b) reported 1 to 10, mean 4.8 mg LAS/l, for eight plants. In both cases the corresponding effluent concentrations, all from activated sludge plants, were 0.07 mg LAS/l with a range 0.05 to 0.11 mg/l. At the Stuttgart plant, Wagner (1978) found the yearly mean MBAS value in the raw sewage to be 11.9 mg MBAS/l, equivalent to approximately 2.2 g MBAS per head per day (based on a water consumption of 185 l/cap.day), which is very close to the US value estimated above. About 94% MBAS was removed by the activated sludge plant, the average effluent concentration being 0.59 ± 0.16 mg MBAS/l.

In the UK determinations for anionic surfactants have been made only with the Abbott (1962) modification which reduces only some of the interferences of the MBAS method. However, the work of Osburn (1986) and others, shows that MBAS values for raw sewage are usually very similar to those for LAS obtained by GC. The concentration of MBAS usually varies with the size of the treatment works, with smaller works receiving largely domestic sewage having higher concentrations (ie, not diluted by industrial or other effluents). For example at Luton, which receives mixed domestic and industrial wastewaters and is relatively large the average concentration for settled sewage (primary tank effluent) was 9.4 mg MBAS/l (8.6 to 11.0) for the period 1974 to 1979 (Painter and King, 1979a,b). Stevenage sewage, from a relatively small purely domestic area, contained about twice as much LAS with a nine-year (1971-79) mean concentration for settled sewage of 26.5 mg MBAS/l (20.0 to 31.3): these samples were collected between 6 am and noon. Three 24-h composite Stevenage settled sewage samples taken in 1986 (Stiff 1987, pers comm) had concentrations of 13, 16.3 and 17, average 15.4 mg MBAS/l, which represents an average usage per head of 3.2 g/d (based on a water consumption of 208 l/cap.day).

In order to compare values from different countries the standards used for the MBAS measurement must be taken into account. Since for instance Manoxol OT is used in the UK and Marlon A (C 12-LAS) in Germany, the UK MBAS concentration must be multiplied by the ratio of the molecular weight (MW) of Marlon A (C 12-LAS) and the MW of Manoxol OT (= 348/444 = 0.784) in order to be comparable with the German data. Thus the average usage in the UK based on the German standard is 2.5 g/d (3.2 * 0.784 g/d) which is about the same as the values reported for Germany and the USA of 2.2 and 2.6 g/d respectively.

In a survey of over 100 works servicing about 16 million people Swanwick et al (1969) found a mean concentration for raw sewage of 12.5 mg MBAS/l and for settled sewage of 9.7 mg MBAS/l.

The Stevenage and Luton sewages when treated by activated sludge or trickling filters produced effluents containing 0.2 to 0.8 mg MBAS/l. Waters and Garrigan (1983) examined effluents from three treatment plants, of unspecified type; the range of MBAS concentrations was 0.5 to 1.0 mg/l of which 32 to 44% was LAS. Based on usage, water consumption, population and removal, Gilbert and Pettigrew (1984) estimated for the UK an average concentration of LAS of 10 mg/l for raw sewage, 7 mg/l for settled sewage and 0.4 mg/l for final effluent which agrees well with the reported data.

In Israel, MBAS concentrations in raw sewage from five sources varied between 9.6 and 11.0 mg MBAS/l (average 10.3 mg/l) and, where secondary treatment was applied, the concentration in the final effluent was between 0.3 and 1.3 mg/l representing 87 to 97% removal (Zoller 1985).

For Japan, Ogino and Nagao (1979) reported up to 22 mg MBAS/l in raw sewage but, surprisingly, could find no LAS by a "simple GC method". Nara et al (1983) found that the average sewage production was 120 l/person-day and the MBAS consumption 2.31 g/person-day. From these data the concentration in raw sewage can be estimated at 19.2 mg MBAS/l. The MBAS consumption in Japan of 2.31 g/person-day compares well with the USA, German and UK figures of 2.6, 2.2 and 2.5 g/person-day respectively (based on same surfactant standard).

Using a liquid-membrane ion-selective electrode method, Hu et al (1983, 1985) reported concentrations of 5.4 to 15.7 mg anionic surfactant/l in raw sewage in China.

Linear alkyl benzenes (LAB) were determined in 11 samples of Los Angeles settled sewage by two methods, Ag NO_3 TLC/GC and GC/MS; the concentrations were 150 ± 69 and 142 ± 62 µg/l respectively (Eganhouse et al 1983a).

Takada and Ishiwatari (1987) reported an average of 1970 ng LAB/l on suspended particles in three settled sewages, while the average for five sewage effluents was 61 ng LAB/l. The corresponding concentrations of LAS were 780 µg/l and 0.46 µg/l respectively.

3.2 River water

It is difficult to interpret much of the data in the literature on concentrations of LAS in river waters because of the information provided. Often the degree of pollution, if any, is not stated and the type and extent of treatment of the discharges into the river are not described. For example, in some countries or parts of countries, sewage is discharged to rivers without treatment or only after partial treatment. In many parts of Japan only the waste water from the toilets is treated whereas the waste water containing the detergent is discharged untreated to rivers. Also, the objectives of some authors are often to study rivers where they expect to find LAS at levels sufficiently high to be determined by current methods. Sometimes the intention is to study the fate of LAS downstream of a known sewage/effluent discharge in order to establish the kinetics of its degradation under environmental conditions.

Thus, the values for concentrations of LAS in river waters reported in the literature are not derived from random samples; they are not representative of the real world. Reported concentrations are heavily biased towards samples from waters affected by effluents and they are therefore not representative of the concentrations of LAS in river waters generally.

In the late 1960's the 'hard' ABS was replaced with the 'soft' ABS which was monitored in Japan by following changes in the ratio of LAS to total ABS in river water using infra-red spectrometry (Miura et al 1968; Ihara et al 1970; Oba et al 1975). The proportion of LAS rose in the years 1967 to 1970 from 20 to 70% and reached 90% by 1973.

Rapaport et al (1987) has produced average concentrations from the large number of values obtained since 1973 in the USA (from the Procter and Gamble monitoring programme) and similar calculations have been carried out for the data available from other countries, Table 3.3. However, since biodegradation of LAS takes place in rivers the concentration depends on the position of the sampling site relative to any discharge and the dilution available (Waters and Garrigan 1983). An attempt has therefore been made to separate the values into two groups: "unpolluted" - some distance downstream of any discharge, and "polluted" - just downstream of a discharge. This separation has inevitably involved the use of judgement since many papers do not include details of the locations of the sampling sites.

Even though methods of analysis employed were not specific in all cases for LAS the concentrations in "unpolluted" surface waters are in most cases much below 0.1 mg/l and often in the region of 0.03 to 0.04 mg/l; the higher concentrations are likely to be associated with treated sewage effluent discharges. However, at sites close to untreated sewage discharges the LAS concentration can exceed 2 mg/l, Table 3.3.

All values reported from Japan were obtained by LAS-specific analytical methods, GC or HPLC, and in some cases MBAS was also determined. The values ranged from traces to 2.5 mg LAS/l and again the higher values were associated with sites close to untreated sewage discharges (Tsukioka and Murakami 1983b) or small streams flowing through "highly developed" areas (Kobuke 1985), Table 3.4. The results also show that the lower the LAS concentration in the river, the lower the proportion of LAS in terms of total MBAS. Uchiyama (1979) followed the fate of LAS along a "small flowing" lake receiving a discharge upstream. Over a distance of 2 km, the LAS concentration fell from 0.6 mg/l to less than the detection limit

Table 3.3 Concentration of LAS (mg/l) in rivers for unpolluted and polluted sites

Country (Ref)	No of studies	Unpolluted sites	Polluted sites	Method
Korea (Bae et al 1982)	1	-	>2.2	not stated
Spain (Moreno Danvila 1987)	1 river	<0.1, 0.19	-	MBAS
	3 sites	<0.1		
Italy (Mancini et al 1984)	1	0.03 (max 0.10)	-	MBAS
(Ruffo et al 1983)	1	0.12	2.0	not stated
Yugoslavia (Dujmov 1984)	1	0.03	-	not stated
Germany, (Fischer 1980)	4 rivers	0.075 ± 0.002	0.2-0.5	MBAS
	many sites	0.03-0.04		IL-MBAS
UK (Waters and Garrigan 1983)	1 (35 samples)	0.039	-	GC
	1 (2 rivers)		0.2-0.5	MBAS
	Above discharge	0.012 (0.008-0.019)		GC
	just below "		0.08 (0.01-0.17)	GC
	5-16 miles below "	0.04 (0.008-0.095)		GC
USA	many	-	-	
	just below discharge		0.099 ± 0.085	GC, HPLC
	< 5 miles below "	0.063 ± 0.03		
	> 5 miles " "	0.041 ± 0.03		
(Rapaport et al 1987)				
Belgium (Institut D'Hygiene et D'Epidemiologie 1985)	189 sites, rivers and canals	0.005-0.2	0.2-6.9	MBAS

Table 3.4 Concentrations of LAS and MBAS in Japanese Rivers*

	LAS (mg/l)	MBAS (mg/l)	LAS/MBAS (%)	Reference
Rivers in Kawasaki		0.092	10	Yoshikawa et al 1984
"		0.529	40	"
Tama River	Trace-0.38	Trace-0.82	50	Yoshimura et al 1984a
Rivers in Fukuoka City	1.6	-	60-70	Ohkuma 1981
Tama River	0.108-0.511	-	37-83	Hon-Nami and Hanya 1980a
Ta River (mean of 5 samples)	0.097	-	-	Nakae et al 1980
Rivers in Niigata Prefecture	0.18 (max)	0.02-2.63	-	Motoyama and Mukai 1981
Hiroshima Prefecture	0.018 (mean)	-	-	Okamoto and Shirane 1982
2 rivers in Ikeda area	0.06, 0.12	-	-	Saito and Hagiwara 1982
Tsurami river	0.484 (mean)	-	-	Yoshikawa et al 1985a
22 rivers	0.018-0.59	-	-	Tsukioka and Murakami 1983b
Rivers in Kobe	0.004-2.5	-	-	Kobuke 1985

* Little or no treatment of waste waters containing detergents before discharge

(unstated) as determined by a UV absorption method. The concentration of MBAS was reduced from 1.46 mg/l to 0.29 mg/l over the same distance.

In the only Korean study (Bae et al 1982), the concentration of anionic surfactants (method not given) exceeded 2 mg/l in the Han river at Seoul and increased downstream. The concentration of LAS-degrading bacteria was 10^2 to 10^3 cells/ml, and the species identified were Pseudomonas, Aeromonas and Enterobacter.

In Europe, data are available from six countries. In the Guadulquivir river in Spain, the MBAS concentration at two sites was <0.1 mg/l and further downstream it was 0.19 mg/l (Moreno Danvila 1987). In aqueduct waters in Florence, Italy, the average concentration was 0.03 mg MBAS/l, with a maximum concentration in surface waters of 0.1 mg MBAS/l (Mancini et al 1984). In a second study (Ruffo et al 1983) 86% of river samples contained 0 to 0.25 mg MBAS/l, with 2% exceeding 2 mg MBAS/l. In the latter study it was found that the concentrations were much lower than in 1973.

In a one year sampling programme, in 1978-79, of various sites on the Jadro river in Yugoslavia, Dujmov (1984) found that the concentration of anionic surfactants (method unstated) increased towards the mouth of the river. Three sections of the river were distinguishable, with concentrations along the river of 0.020 to 0.045 mg/l.

More extensive studies were made in Germany; Fischer (1980) reported on four years' data for many locations on the rivers Neckar, Main, Ruhr and Rhine. The concentration of MBAS fell during the period 1976 to 1979 by 40% in the Neckar, 33% in the Main, 64% in the Ruhr and 40% in the Rhine. The 1979 concentrations (mg MBAS/l) for the four rivers were:

Neckar	0.06 (0.02 - 0.12)
Main	0.08 (0.03 - 0.48)
Ruhr	0.06 (0.02 - 0.11)
Rhine	0.10 (0.03 - 0.21)

Using a more specific analytical method involving an amine-extraction step, Fischer showed that the ABS (= LAS) content of MBAS ranged from 43 to 70% in the Neckar, 54% in the Main, 50% in the Ruhr to 15 to 35% in the Rhine. Thus, the LAS concentrations in these four rivers in 1979 ranged from 0.03 to 0.04 mg LAS/l. Klopp (1987) has reported that the concentration of MBAS in the lower Ruhr river decreased over the period 1979 to 1985, falling from 0.19 mg/l (winter) and 0.09 mg/l (summer) in 1979 to 0.09 and 0.05 mg/l respectively in 1985. During the same period the non-ionic surfactant concentration had remained constant and both types had similar concentrations in 1985. In the UK, Waters and Garrigan (1983) have continued their survey (Waters 1976) of British rivers, using a GC method for LAS determination. The average value for 35 samples was 0.15 mg MBAS/l of which only 26% (0.039 mg/l) was present as LAS. When the samples were classified according to their location the following results were obtained:

	mg MBAS/l	% LAS
above discharge	0.04	30
just below discharge	0.26	31
5 - 16 km below discharge	0.16	24

These values represent the results from three experiments each on four rivers.

Foaming has recently become a problem in two rivers in England and an investigation into its cause, thought to be due to APEs (alkyl phenolethoxylates), is in progress. As part of the study the MBAS content has been determined; in one river the value was 0.1 to 0.5 mg/l, in the other 0.2 to 0.6 mg/l, with no indication of increasing concentrations since 1981 before foaming became a problem.

A single sample of Rhine river water taken in The Netherlands contained 0.015 mg LAS/l, equivalent to only 18% of the MBAS found (Waters and Garrigan 1983).

For Belgium MBAS data for 189 river and canal sites were reported in the 1985 Belgian Surface Water Quality Report (Institut D' Hygiene et D' Epidemiologie 1985). The values ranged from 0.005 to 6.1 mg/l with a mean concentration of 0.2 mg MBAS/l. The very high values were associated with the discharge of untreated sewage.

Most of the US data have been summarised by Rapaport <u>et al</u> (1987). The values in Table 3.3 are averages for about a dozen rivers but they are biased towards higher concentrations, since many of the data were collected in a low dilution stream (Rapid Creek) below a trickling filter plant discharge in Rapid City. Trickling filter effluents tend to have higher concentrations of MBAS and LAS than those from activated sludge plants (see Section 4.3), and the dilution in Rapid Creek was only about four-fold. The concentration of LAS below the outfalls of eight activated sludge plants ranged from 0.01 to 0.05 mg/l, while corresponding values for 14 German and three Canadian activated sludge plants were in the range 0.01 to 0.09 mg/l.

Osburn (1986) reported a concentration of LAS (by desulphonation-GC) as high as 0.27 mg/l, just below the outfall in Rapid Creek, falling to about half of this 5 miles downstream and to below the detection limit after 55 miles. By determining MBAS using his "interference limited" method, he reported the proportion of LAS (of total MBAS) to fall from 67% at the outfall to 10% at 55 miles.

Rapaport et al (1987) have developed a model using the results of the Procter and Gamble monitoring programme for predicting the variability of river LAS concentration below the mixing zones for sewage outfalls as a function of treatment plant flow in the US. Assumptions made were the concentration of LAS in sewage (3.7 mg/l) and the removal rates by activated sludge (98%), trickling filters (80%) and primary treatment (27%). The predicted concentrations agreed well with those observed.

In groundwater 500 m downfield of sewage infiltration ponds at an air base (USA), the concentration of MBAS was 0.4 mg/l, of which 0.3 mg/l was LAS. At 3000 m downfield from the ponds, LAS could not be detected, but MBAS had risen to 2.5 mg/l of which 2.3 mg/l was branched-chain ABS, indicating that the water was "older" than 1965 when ABS was last used (Thurman et al 1987).

Suspended particles from five river sites in Tokyo contained the equivalent of 12.6 μg LAS/l and 249 ng LAB/l (Takada and Ishiwatari 1987).

It may be concluded that, when values for LAS in samples of river water which are affected by gross pollution are eliminated, the most likely range of concentration of LAS in river water is 0 to 0.04 mg/l. For MBAS the corresponding range is 0.005 to 0.2 mg/l, but these values contain material known not to be anionic surfactant. Even where higher levels occur, the evidence is that the downstream concentrations of LAS soon fall to levels approaching those representative of "unpolluted" sites.

3.3 River sediment

A summary of the LAS concentrations found in river sediments from three countries are given in Table 3.5. Most of the values are between 1 and 10 mg LAS/kg dry solids, whereas the higher values, 100 to 200 mg LAS/kg, were found in the vicinity of outfalls of raw sewage or sewage effluent. High values were also reported from Japan; however, in order to establish the reasons for these high values it would be necessary to obtain translations of the relevant papers, although it is already known that domestic wastewater containing surfactant is rarely treated before discharge in Japan.

In two studies MBAS concentrations of river sediments were also determined; Yoshimura et al (1984a,c) found that LAS constituted on average about 30% of total MBAS, while Uchiyama (1979) reported that the proportion of LAS fell from about 25% near the outfall to about 17% two km downstream. Osburn (1986), in the Rapid Creek study, found a different pattern; near the outfall as much as 85% of MBAS was LAS which had fallen to 38% and 27% at 4 and 7 miles respectively downstream of the discharge.
In urban riverine environments in Tokyo, the average concentration of LAS in river sediments collected from 26 sites was 107 mg/kg (range 1 to 567). In sediments from nine Japanese rivers the concentration was on average 107 mg LAS/kg (Takada and Ishiwatari 1987). The same sediments contained 0.01 to 15 mg LAB/kg, average 3.60 mg/kg and the average LAS/LAB ratio was 31.

Table 3.5 Concentration of LAS in river sediments

Country	Concentration (mg/kg dry matter).	Details	Reference
Japan			
	<1-260	51 samples, 23 regions Fukuoka City	Anon 1978
	3.5-86.3	Tama river, few samples	Hon-Nami 1980b
	>260	Nagano region	Tsukioka et al 1980
	0.5-37 median 0.5	20 rivers, Nagano	Katsuno et al 1983
	1.2*	18 rivers, Hiroshima	Okamoto and Shirane 1982
	2.8-10.7 (6.2-31.6)+	Tama river, 19 samples	Yoshimura et al 1984a
	31	Tsurami river, Kawasaki	Yoshikawa et al 1985b
	17-96 (107-378)+	Polluted lake	Uchiyama 1979
	107 (1-567)	9 rivers, Tokyo	Takada and Ishiwatari 1987
Germany			
	1.5-174 1.5-25 1.5-10	14 sites 13 sites 10 sites	De Henau et al 1986b
USA			
	1-275	Rapid Creek	Games 1983
	100-322	Rapid Creek	Holysh et al 1986
	2-5 1-4	) downstream)	Osburn 1986
	174 ± 104 11.2 ± 9.5 5.3 ± 47	Just below outfall <5 miles downstream >5 miles downstream	Rapaport et al 1987

* calculated from 60 µg/l, assuming 95% water
\+ MBAS values

3.4 Estuarine and coastal waters and sediments

Few data have been published on the LAS content of estuarine and coastal waters and sediments. The range of concentrations in seven samples of Tokyo Bay water was <0.003 to 0.014 mg LAS/l; the corresponding concentrations of MBAS were 0.03 to 0.07 mg/l, giving a contribution of LAS to total MBAS of 7 to 20%, average 12% (Hon-Nami and Hanya 1980a). In two other Japanese bays, Katsuura and Moriura, the concentration ranged from 0.003 to 0.016 mg LAS/l (Arimoto et al 1980), and 0.008 mg LAS/l was measured in coastal waters near Hiroshima (Okamoto and Shirane 1982). Kikuchi et al (1986) found concentrations of 0.0008 to 0.03 mg LAS/l in Tokyo Bay water, the highest concentrations being detected in the coastal areas. In Sweden, coastal waters were said (Swedmark and Granmo 1981) to contain usually about 0.01 mg LAS/l, though no experimental evidence was cited.

In the polluted gulf of Fos-sur-Mer near the mouth of the Rhone river, France, surface water contained 0.130 to 0.266 mg MBAS/l and at a 7 m depth the concentration was 0.090 to 0.538 mg MBAS/l (Siron and Giusti 1985). At a nearby unpolluted site - Port-Cros bay - concentrations of 0.010 to 0.090 mg MBAS/l were reported. Mochalov et al (1984) reported that "anionic surfactants" were present in waters of the Baltic Sea at 0 to 0.093 mg/l. Near to the mouth of the Vistula river concentrations were 0.040 to 0.102 mg/l at 3 m depth and 0.015 to 0.135 mg/l in deep waters. In South America, 0.05 to 4.5 mg MBAS (as Manoxol OT)/l, average 0.7 mg/l, was present in samples of bay water near a large discharge of untreated sewage in the Rio Grande (Brazil), (Kantin et al 1981). However, the surfactant here is probably tetrapropylene benzene sulphonate, since at that time the surfactants used in Brazil were not biodegradable.

The range of concentrations of LAS in four Sagami (Japan) river estuarine sediments was 5 to 17 mg LAS/kg dry matter (Utsunomiya et al 1980), and in sediments taken further out to sea, LAS could not be detected. Similarly, sediments in Tokyo Bay contained <0.2 to 69 mg LAS/kg, with the concentration decreasing the greater the distance from the estuary (Kikuchi et al 1986). Coastal sediments in Hiroshima were reported to have 170 μg LAS/l, equivalent to 3.4 mg LAS/kg dry matter assuming a solids content of 5% (Okamoto and Shirane 1982).

Sediments from Klaipeda Bay and near the mouths of the Oder and Vistula rivers in the USSR contained <1 mg "anionic surfactants"/kg dry weight and those in the Gulf of Riga, the central Baltic and Arkonak hollow contained higher concentrations at 1.8 to 3.8 mg "anionic surfactants"/kg (Mochalov et al 1984).

Eganhouse et al (1983b) investigated sediments in the vicinity of a Los Angeles sewage marine outfall and found 1342 mg linear alkyl benzenes (LAB)/kg dry matter for the effluent particulates close to the outfall falling to 21.4 and 1.4 mg/kg at 5 and 20 km distance respectively (the values refer to the top 2 cm of the sediment).

Eganhouse (1986) also reported the presence of the non-naturally occurring LABs in sediment cores some 6 km downstream from the Los Angeles County sewage outfall. At the top 1 cm layer, 20 mg/kg was present, rising to 33 mg/kg at 4 cm, but below 17 cm none was found. The corresponding concentrations of hydrocarbons from the branched-chain benzene sulphonates peaked at 14 cm (12 mg/kg) and were found as far down as 23 cm. The author concluded that these profiles reflect the earlier use of branched chain ABS and the change over to LAS.

3.5 Sewage sludge

The concentration of LAS in sewage sludge is of importance because of the potential effect of LAS on plants and animals when sludge is applied to agricultural land. Only a few papers report data on the LAS content of sewage sludge, which are summarised in Table 3.6. The concentration of LAS in sewage sludge depends on the type of sludge being considered (primary, secondary, digested sludge) but also on the LAS concentration in the sewage.

Sedlak and Booman (1986a,b) studied, over a period of several months, two parallel activated sludge plants at Enid, Oklahoma, USA, one operated with a conventional sludge retention time of 3.2 days and the second stream at a high rate with 0.8 days sludge retention. The primary sludge, that which settles in the primary sedimentation tanks prior to biological treatment, had a concentration of 5.34 to 6.31 g LAS/kg dry sludge, whereas the secondary (activated) sludge from the conventionally operated plant contained only 0.41 g/kg and that from the high rate plant 0.86 g/kg dry sludge. The primary sludge after anaerobic digestion contained 6.66 g/kg compared with 4.25 g/kg after aerobic digestion. Drying of the digested sludges on drying beds significantly reduced the LAS content to approximately 0.15 g/kg. It is of interest to note that the values obtained for LAS by Osburn (1986) were similar to those measured as IL-MBAS indicating that most of the MBAS adsorbed on the sludge was LAS.

The data reported by Sedlack and Booman (1986a,b) for the digested sludges are similar to the results presented by Rapaport <u>et al</u> (1987) for the anaerobic sludges from 5 USA plants (49 grab samples) which had an average LAS concentration of 4.66 ± 1.54 g LAS/kg dry solids. Similar results

Table 3.6 Concentration of LAS in sewage sludge

Type of Sludge	Concentration (g LAS/kg dry matter)	Source	Reference
Primary	5.34, 6.31	2 samples, 1 US works	Sedlak and Booman 1986a,
	73-97*	USA	Osburn 1986
	9.3 (6-14)+	Several UK works	Swanwick et al 1969
Anaerobically digested			
	4.66 ± 1.54	49 grab, 5 USA works	Rapaport et al 1987
	5.2, 6.9	2 USA samples	McEvoy and Giger 1986
	6.66	1 USA works	Sedlak and Booman 1986a.
	63-107*	USA	Osburn 1986
	10-27+	Several UK works	Swanwick et al 1969
	5.9 (3.1-11.9)	7 Swiss works	Giger et al 1987
	6.2 (1.6-11.8)	8 German works	De Henau et al 1986b
	4.9 (1.3-9.9)	10 German works	Matthijs and De Henau 1987
	4.2 ± 1.2	24 Austrian works	Giger et al 1987
Aerobically digested			
	4.25	1 USA works	Sedlak and Booman 1986a,b
	2.1 ± 1.8	5 Austrian works	Giger et al 1987
	2.9	1 Swiss works	McEvoy and Giger 1986
Secondary (activated sludge)			
	0.41 - 0.86	2 sludges, 1 USA works	Sedlak and Booman 1986a,b
	0.09	1 Japanese sludge	Yoshimura 1984
Drying beds (digested sludge)			
	0.15-0.16	2 sludges, 1 USA works	Sedlak and Booman 1986a.b

* expressed as mg/kg wet sludge
\+ MBAS

were obtained for digested sludges from other US (McEvoy and Giger 1986) and European plants (De Henau et al 1986b, Giger et al 1987, Matthijs and De Henau 1987) (Table 3.6) and these values compare well with the predicted value of 5 g LAS/kg (Namkung 1987). The UK data obtained nearly 20 years ago (Swanwick et al 1969) are consistent with the US and other European results when the higher concentration of MBAS in UK sewage is taken into account. For a range of treatment works in hard water areas of England the MBAS concentration of primary sludge varied between 7.7 and 13.0 g/kg dry sludge (mean 9.3 g/kg) and that of digested sludge between 10.9 and 27 g/kg. The average concentration of 9.3 g/kg is in reasonable agreement with the concentration of 8 g/kg predicted by Gilbert and Pettigrew (1984) based on water and LAS usage and on assumptions about treatment plant operation. No recent values for UK sludges have been published.

Litz et al (1987) quote values (for LAS in sludge in Germany) reported by Hellmann (1978) as high as 160 g MBAS/kg, which is 10-15 fold higher than other reported values including those analysed with the methylene-blue method.

Insufficient data are available to conclude whether anaerobic digestion changes the concentration of LAS in sludge. Osburn (1986) reported LAS concentrations, based on wet weight of sludge, of 73 to 97 mg/kg in primary sludge and 63 to 107 mg/kg in anaerobically digested sludge. The corresponding interference limited MBAS values were similar for the primary sludge (92-113 mg/kg) but were about 20% higher for the anaerobically digested sludge (82-125 mg/kg) indicating that the concentration of LAS is slightly changed in anaerobic digestion. In another study, Schaumberg et al (1982) found 5 to 10% of the fulvic acid fraction of anaerobically digested sludge to be LAS and had evidence to suggest that about 20% of the LAS was present as "altered material". The proportion of fulvic acid in the sludge was not given.

There is also some evidence (Sedlak and Booman 1986a,b) that the LAS concentration is reduced by aerobic digestion although Giger et al (1987) concluded that the difference in the case of the Austrian sludges investigated was not statistically significant.

A point on analysis was raised by Ozasa and Tobino (1984) who reported a loss of 9.6% LAS in river sediments stored for 140 days at -30 °C, with a greater relative loss of the lower molecular weight homologues. Many authors analysed their samples very soon after collection or added formaldehyde but McEvoy and Giger (1986) stored their sludge samples for several months at 4 °C and do not indicate whether a preserving agent was added.

3.6 Soils

At a site near Rapid City, USA, a field was treated from 1972 with anaerobically digested sludge at a rate of 21 metric tons/ha-yr which is about twice the usual rate. Analysis very shortly after sludge application, in 1979, showed that little LAS penetrated the soil below 6 inches (<3 mg LAS/kg) and the concentration of LAS in the top 3 inches was 28 (13 to 47) mg/kg (Rapaport et al 1987).

Gilbert and Pettigrew (1984) and De Henau et al (1986b) both calculated the concentration of LAS in sludge-amended soils in Europe, but made different assumptions concerning the rate of application of sludge and the depth of tillage; each assumed no degradation. Nevertheless, the values 16 mg LAS/kg (Gilbert and Pettigrew 1984) and 7 mg LAS/kg

(De Henau et al 1986b) were of the same order of magnitude. However, the actual concentrations measured in Germany were 0.9, 1.1 and 1.3 mg LAS/kg and in the UK 2.2 mg LAS/kg (De Henau et al 1986b), suggesting that degradation takes place. Giger et al (1987) found the concentration of LAS in soil treated with sludge containing 5.5 g LAS/kg to be 45 mg/kg soon after application and 5 mg/kg 104 days later.

In a detailed study in the UK (Holt et al, to be submitted), it was found that the concentrations of LAS in soils taken from 42 fields which had not been treated with sewage sludge for at least six months were <3 mg/kg. Ninety-five percent of the soils contained less than 2 mg/kg and 83% contained less than 1 mg/kg. The range of concentrations in soils taken in mid-1987 from nine fields which received sludge during 1987 was <0.2 to 20 mg LAS/kg (median 2.1 mg/kg). Immediately after application the concentrations in seven soils from 5 fields ranged from 2.5 to 40.3 mg LAS/kg (median 25 mg/kg) which fell to "control" values within 21 to 122 days (see Section 4.2.2).

3.7 Tap water

Waters (1976) used a modified 2-methyl-heptylamine/methylene blue method to determine LAS in tap waters and compared the values obtained with the normal MBAS method. (All values are given as Marlon A.) Dutch tap water, derived from a river containing 0.026 mg LAS/l, contained 0.003 mg LAS/l; the proportions contributed by LAS to total MBAS were 32% and 27% respectively. River water, containing tertiary sewage effluent, taken from a river system in the UK, was found to contain 0.052 mg LAS/l representing 30% of total MBAS. The derived tap water contained 0.007 mg LAS/l, which represented 33% of total MBAS.

Only two other studies reporting LAS concentrations in drinking water were found. Osaka tapwater contained 11.5 to 71.0 µg/l as LAS, determined by GC after clean-up, in an unstated number of samples (Imaida et al 1979), while well-water in Italy contained 0 to 8.4 µg/l (Mancini et al 1984).

In a fourth study, in Japan covering the year 1977, it was reported that 42 "water" samples in 20 regions contained "linear alkane sulphonate (LAS)" and "ABS" below the limit of detection of 0.001 ppm and 0.01 ppm respectively (Anon 1978). The same study reported "waters" in Fukuoka City having 0.63 to 2.9 ppm LAS indicating that these samples were probably not drinking water.

3.8 Average chain-length of LAS in environmental samples

The concentrations of homologues of LAS and their isomers have been reported by a number of groups of workers. Usually the results have been expressed as a percentage of the total LAS present but it is more convenient to express the results as "average chain length". Where the results were given as "percentage of total LAS" they were, for this report, recalculated to "average chain length". (The question of the isomers is dealt with in Section 4.1). Table 3.7 contains a summary of the results. The C 10 to C 13 homologues were found in all samples and sometimes the C 14 homologues were present. In two Finnish sludges (McEvoy and Giger 1986) the C 15 homologue was detected. However, the C 11 and C 12 were the dominant homologues, as is indicated by the average chain length being in the 11 to 12 region.

In their extensive study, Rapaport et al (1987) were able to show a significant statistical difference (at the 0.01 level) between the raw sewage (12.0) and the activated sludge effluent (11.8) values. Similarly, the average chain length for sludges (12.5) was shown to be significantly higher (at the 0.001 level) than the value for raw sewage (12.0). It should be noted, however, that in some individual plants no difference was observed between influent and effluent chain length distributions.

The data in Table 3.7 also indicate that the chain length is higher in river sediments (11.8 to 13) than in river water (10.9 to 11.2); the data of Rapaport <u>et al</u> (1987) show that the difference is significant at the 0.001 level.

The differences between the chain length distributions have been ascribed to the differences in the degree to which the homologues are adsorbed on to solid particles and also to differences in their rate of biodegradation (see other Sections).

4. FATE

4.1 Biodegradation

When interpreting the results obtained in tests for biodegradability the following considerations should be borne in mind.

Biodegradation in the aquatic and terrestrial environments is essentially carried out by ubiquitous and versatile micro-organisms, normally bacteria; test conditions are therefore based on the culturing of these organisms. During aerobic bacterial growth and activity, the organic test chemical is metabolised by different pathways, resulting in the synthesis of new microbial cells (assimilation) and the production of carbon dioxide which releases energy for the synthesis (mineralisation). Some of the hydrogen in the substrate is assimilated into the new cells and the excess is converted to water, while sulphur (eg in LAS), is released as sulphite and oxidised to

Table 3.7 Average chain lengths of LAS in various samples

Type of sample	Source	Average chain length	Reference
Standard LAS	USA	11.3	Osburn 1986
	UK	11.8	Hughes et al 1969
	Switzerland	11.6	McEvoy and Giger 1986
	Japan	11.5	Nakae et al 1980
		11.4	Hon-Nami and Hanya 1980a
		11.4	Yoshimura et al 1984a
Sewage	USA	11.6	Osburn 1986
		12 ± 0.3 (n = 102)	Rapaport et al 1987
	Canada	12.4 ± 0.2 (n = 9)	Rapaport et al 1987
	Germany	11.5 ± 0.2 (n = 10)	Rapaport et al 1987
	UK	11.43	Hughes et al 1969
Sewage effluent	USA		
	trickling filter	12.0 ± 0.1 (n = 41)	Rapaport et al 1987
	act sludge	11.8 ± 0.1 (n = 40)	Rapaport et al 1987
	Canada		
	act sludge	11.8 ± 0.1 (n = 9)	Rapaport et al 1987
	Germany		
	act sludge	11.7 ± 0.2 (n = 10)	Rapaport et al 1987
	UK filter	112	Hughes et al 1969
River water	USA		
	just below outfall	12 ± 0.2 (n = 22)	Rapaport et al 1987
	<5 miles downstream	11.8 ± 0.2 (n = 14)	Rapaport et al 1987
	>5 miles downstream	11.9 ± 0.2 (n = 18)	Rapaport et al 1987
	Japan		
	Ta	11.2	Nakae et al 1980
	Tama	10.9	Nakae et al 1980
	Ta	11.2	Yoshimura et al 1984a
	Tama	10.9	Yoshimura et al 1984a
	(in Kobe)	11.2	Kobuke 1985
River sediments	USA	~ 13 (n = 33)	Rapaport et al 1987
	Japan	11.8 - 12.1	Hon-Nami and Hanya 1980a
		11.9 - 12.2	Yoshimura et al 1984a
Sludge	Anaerobic:		
	USA	12.3 - 12.5	Osburn 1986
		12.5 ± 0.2 (n = 49)	Rapaport et al 1987
	Switzerland	11.9 (11.79 - 11.96) (n = 7)	McEvoy and Giger 1986
	Aerobic:		
	Switzerland	11.9	McEvoy and Giger 1986

sulphate, perhaps by non-enzymic steps. Oxygen in the test chemical is insufficient for these oxidations so that additional molecular oxygen is required for complete oxidation and mineralisation. Nitrogen is normally released as ammonium which in turn is converted to nitrate by specialised bacteria.

The overall reaction may be represented by

$$\underset{\text{test chemical}}{\text{CHONS}} + O_2 + \text{cells} \rightarrow CO_2 + H_2O + NH_4^+ + SO_4^= + \text{new cells}$$

The organic carbon in the test chemical is thus utilised partly for cell synthesis and partly for CO_2 production, and theoretically an organic substance can be completely removed. However, the kinetic rate of oxidation decreases with decreasing concentration of substance, especially at low concentrations, so that over the period that can be realistically allowed for a biodegradation test, 100% removal of the substance will not be achieved. This is reflected in the OECD Test Guidelines (OECD 1981) whereby a substance which is removed by 80% or more in a biodegradation test is deemed to be readily biodegradable. The decision was based on inevitable differences between conditions in the environment and in the test vessels and experience of the behaviour in biodegradability tests of chemicals which are known to be well degraded in the environment.

There is a third possible short-term fate of substrate carbon in the tests; the complex set of metabolic reactions may result in intermediate metabolites which may accumulate in the test medium. This could happen if, for example, the inoculum does not contain all the required species at the required numbers, so that further time is needed for the population of bacteria capable of degrading the intermediates to grow to a sufficient size. This is, again, reflected in the OECD Guidelines (OECD 1981); substances are considered to be readily biodegradable in the environment if only 70% or more of the organic carbon present is removed in the period of the test. This decision is also based on experience with simple, readily degradable chemicals.

The proportion of the theoretical oxygen demand and production of carbon dioxide - the other two parameters often used to assess biodegradation - resulting from the complete mineralisation of the test substance according to the equation

$$C_aH_bO_c + (a + \frac{b}{4} - \frac{c}{2})\ O_2 = a\ CO_2 + \frac{b}{2}\ H_2O$$

cannot be 100%, since some of the carbon is converted to new cells. The actual division between cell synthesis (in which carbon is in a reduced form) and carbon dioxide (and energy) production varies according to the conditions of growth. From experience with common chemicals, the OECD (1981) concluded that results of at least 60% of theoretical oxygen demand or of theoretical carbon dioxide production indicates that the chemical should be classified as readily biodegradable and as being readily removed in the environment. Recent experience in an EEC ring-test of the respirometric method has shown that uptakes of oxygen as low as 40% of theoretical occurred with readily degradable chemicals such as aniline and benzoic acid, while the simultaneously determined removal of organic carbon was as high as 80-90%.

A factor which can reduce the percentage removed in standard tests is the possibility that the test substance may be inhibitory to bacteria at the unrealistically high concentration of test substance, and the high ratio of substance to micro-organisms employed in the standard tests.

4.1.1 Laboratory tests of biodegradability

No essentially new laboratory test methods using synthetic media have been developed since the publication of the test protocol devised by the OECD Chemicals Group (OECD 1981). Briefly, this protocol is a three-tiered system consisting of tests for ready biodegradability, inherent (not degraded in "ready" tests) biodegradability and for removal in simulations of wastewater treatment. The first tier tests consist of determining the removal of the test substance in a simple mineral medium containing the substance as the sole carbon source and a low concentration of micro-organisms, by measuring either the concentration of the test substance, DOC, the production of carbon dioxide or the uptake of oxygen. This test replaces the river die-away method, which, however, is still used. The second tier tests (for inherent biodegradability) expose the chemical to a much higher concentration of micro-organisms and other substrates (allowing co-metabolism) and usually for a longer period. The third tier methods simulate treatment with activated sludge or in percolating filters (using a rotating tube) and are more stringent than the inherent tests.

However, some refinements have been made; for example, Boatman et al (1986) introduced an automatic head-space GC method to determine CO_2 from vials containing acclimatised activated sludge, a synthetic medium and LAS. Birch (1982) adapted the Porous Pot activated sludge method so that sludge could be wasted automatically throughout the whole period of the test. This allows accurate control of the sludge retention time (SRT), so that the influence of SRT and other factors, such as temperature on LAS removal may be readily studied.

There has recently been an emphasis on the use of conditions which more closely represent those in the environment ~~especially~~ relating to the concentration of LAS (normally requiring the use of ^{14}C-labelled LAS), the presence of other substrates and the use of indigenous microbial populations.

Results of biodegradability tests on LAS may conveniently be divided into four groups:

(a) using natural waters with relatively high concentrations of LAS (Table 4.1);

(b) using synthetic media with relatively low (10^2 - 10^3 bacteria/ml) concentrations of inoculum (Table 4.2);

(c) using synthetic media with higher (10^4 - 10^6 bacteria/ml) concentrations of inoculum (Table 4.3); and

(d) simulation or inherent test methods (10^8 - 10^9 bacteria/ml) (Table 4.4).

Tables 4.1 - 4.4 give examples of results from the four types of test methods. Tables 4.5 and 4.6 contain text book data abstracted from Swisher (1987), for which the original papers were not consulted and the information provided is therefore less detailed than that given in Tables 4.1 to 4.4.

It is well known that a number of factors contribute to the degree and rate at which a compound is biodegraded in these tests; for example, concentration and activity of bacteria, concentration of test substance, temperature, pH value, concentration of dissolved oxygen, composition of medium: the results in the Tables illustrate the effects of some of these factors.

4.1.1.1 Biodegradability tests using natural waters

Table 4.1 provides a summary of the results of biodegradability test using natural waters.

Södergren (1966) used nine types of river water to test the biodegradability of primary C 12 LAS. In four waters all the MBAS (2 mg/l) was removed fairly rapidly (5 d), while in three waters as long as 13 d were required. Two of these latter waters were shown to contain little (2.5 µg P/l) or no

Table 4.1. Biodegradation of LAS in die-away tests - natural waters

Compound	Concentration (mg/l)	Removal (%) MBAS or other		Reference
1- ø C12 LAS	2	100 in 5d* 90 in 13d*		Södergren (1966)
LAS 3- ø C11 LAS 3- ø C13 LAS	30-40	98 in 5d (25 °C)+ 95 in 10d (10 °C)		Halvorson and Ishaque (1969)
LAS (EPA)	20	95 in 14d (15 °C) 97 in 7d (35 °C)		Hollis *et al* (1976)
LAS LAS	10	90, 95, 75 in 10d 50 in 16d (seawater)		Moreno Danvila (1987)
LAS	5 10	100 in 7d 100 in 9d		Maurer *et al* (1971)
LAS	5, 10	-	70 ThOD 73 CO_2	Larson and Perry (1981)
LAS	-	>90 in 14d		Petresa (1987)
LAS LAS	5 20	95 in 14d 95 in 30d 85-90 DOC in 36 d		de Oude (1977)

ø para-sulphophenyl
* various river waters
\+ "resting cell suspension" containing 2 x 10^8 cells/ml.

phosphate, whereas the other seven had 20 to 307 µg P/l. Södergren (1966) attributed the low rate of biodegradation to lack of phosphorus, although the varying potential of the bacterial populations present could also have played a part. Halvorson and Ishaque (1969), using a concentrated suspension of cells from a sewage lagoon, found higher rates of removal of MBAS at 25 °C for the 3-phenyl isomers of C 11 and C 13 than at 10 °C (5 d compared with 10 d for 95% removal). No degradation occurred at 2 °C within the 12 d - period of the test. Similarly, Hollis et al (1976) reported faster degradation at 35 °C than at 25 °C or 15 °C, while at 45 °C acclimatisation was difficult. Apparently acclimatisation to LAS was a more important factor in increasing the rate than acclimatisation to temperature. Larson and Perry (1981) showed that even at LAS concentrations of 5 and 10 mg/l, the natural population of river water was able to degrade LAS to carbon dioxide (CO_2), equivalent to 73% of the theoretical amount and to 70% of the theoretical oxygen demand (ThOD). This showed that extensive mineralisation must have occurred, since the OECD Chemicals Group (OECD 1981) concluded that, if more than 60% CO_2 production or 60% ThOD was obtained, the chemical could be considered to be readily and completely biodegraded in the environment. Finally, de Oude (1977) reported high DOC removals in river water (85 to 90% in 36 d) at initial concentrations of 20 mg LAS/l. This high value is comparable with those, in Table 4.5, of 100% in 58 d (Sekiguchi et al 1975a) and 85% in 10 d (Yoshimura et al 1984b). The rate of removal of DOC in seawater was about half that in river water.

4.1.1.2 Biodegradability test synthetic media - low concentration of inocula

Table 4.2 shows that the addition of even small amounts of inoculum to synthetic mineral media usually removed >90% MBAS within 10 d, the limit set by the OECD test (OECD 1971) for the detergents

omp	cen on (mg/l)	Re (% MBAS	DOC	Other	
LAS	5 35	100 in 7d* (to 20 mg/l in 7d)	- -	- -	Swisher (1966c)
JNQ sulphonate	15	90 - 97 in 10d	39 - 63 in 19d+	-	Painter (1978)
JNQ sulphonate	20	75 in 28d	47 in 28d 71 in 39d	-	Brown (1976) "
Marlon A	20	99 in 28d 93 in 7d	75 in 28d 83 in 39d	- -	"
LAS	5	100 in 21d	85 in 19d (COD)	50 in 39d ThOD	Sengul and Muezzinoglu (1980)
LAS	(2-5)	91 in 5d 96 in 10d	-	66 in 30d (ThOD)	Hrsak <u>et al</u> (1981)
C 12 LAS	30	95 in 5d	-	83 in 14d (UV) 40 in 14d (CO_2)	Kravetz <u>et al</u> (1982)
C 13 LAS	30	95 in 28d	50 in 28d	45 in 28d (CO_2)	
LAS	53	-	85 in 28d 40 in 28d*	-	Canton and Slooff (1982)
LAS	16	-	‡(80 in 28d (76 in 28d	-	Gerike and Richterich (1983)
LAS	As in EEC tests	95 in 28d	73 in 28d	65 in 28d (ThOD)	Gerike (1987)
LAS	32	99 in 4d	-	24 in 20d (CO_2)	Boatman <u>et al</u> (1986)
LAS	As in EEC tests	-	75 in 28d	75 in 28d (CO_2)	Gilbert and Kleiser (in Press)

* No inoculum added, medium not sterilised
‡ 2 different carbon analysers
\+ Very little increase by day 55

to be classified as 'soft'. Indeed, even in the absence of an added inoculum 100% MBAS was removed in 7 d at an initial LAS concentration of 5 mg/l (Swisher 1966c). Canton and Slooff (1982) also reported 40% DOC removal in 28 d in unsterilised flasks without inoculation; in both cases it may be concluded that the degradation was carried out by micro-organisms present in the atmosphere. This is a common occurrence especially in some laboratories where sewage or wastewaters are routinely handled. In the presence of mercuric chloride no degradation is observed. The highest rate of removal (99% in 4 days) (Boatman et al 1986) was probably due to the pre-acclimatisation of the inoculum and the concentration of bacteria being much higher than in other experiments reported in Table 4.2.

The removal of organic carbon reported in Table 4.2 ranged from 39 to 85% with an average of about 65%, which is close to the theoretical 66.7% required for complete oxidation of the chain of a C 12 LAS, leaving the benzene ring intact. In a few cases, the percentage removal was much higher than 66%, leaving no doubt that the ring had also been broken in those cases; Canton and Slooff (1982) found 85% DOC removal and Gerike and Richterich (1983) reported 76 and 80%, which were the means, determined by two different types of organic carbon analyser, of six replicate vessels each measured at six occasions. The 75% DOC removal found by Brown (1976) using Marlon A which rose to 83% after 39 d, and the 75% DOC removal and 75% theoretical CO_2 production obtained by Gilbert and Kleiser (in press) also both indicate ring rupture. Although only 47% DOC was found after 28 d for JNQ sulphonate (Brown 1976), the value had risen to 71% after 39 d.

On the other hand, Painter (1978) reported only 39 to 63% DOC removal after 28 d on several occasions using the modified OECD method and very little increase from day 19 to day 55, indicating a long delay in degradation of intermediates, as reported by Leidner et al (1976). Various sulphophenylalkanoic acids were identified during the incubation, also confirming reports by Leidner et al (1976) and some were still present at day 55 when the experiment ended.

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Comparing the results given in Tables 4.2 and 4.3 indicates that MBAS removal was more rapid with higher concentrations of inoculum. However, the difference is relatively small since in a controlled experiment increasing the inoculum concentration, using an acclimatised activated sludge, from 0.7 to 20 mg sludge/l reduced the time to achieve 90% MBAS removal only from 4 to 3 days (Painter and Durrant 1976). The same was true for a sludge grown on a sewage prepared in the laboratory from human excreta, starch, tea leaves, etc; however, the effect of the sludge concentration on the removal of the benzene ring, as measured by UV absorption, was greater in both studies.

Of the five DOC and COD assessments given in Table 4.3 two suggest that breaking of the ring must have occurred, namely those quoting 80 to 81% removal. However, Painter and Durrant (1976) using 20 mg/l acclimatised sludge reported 90% removal of UV absorbance in 5 d, while 0.7 mg/l removed only 50%. Urano and Saito (1985) recorded 98% of ThOD from 3 mg 1-sulphophenyl-C 12-LAS/l, although only 36% ThOD from 10 mg/l in 14 d. Pitter and Fuka (1979a) reported about equal rates of removal of DOC and UV absorbance, indicating roughly equal rates of removal of ring and alkyl chain, but the values were only 53 to 70% in 12 d in spite of using an inoculum concentration of 100 mg acclimatised sludge/l. Larson (1979), in 30 d

Table 4.3 Biodegradation tests - synthetic medium and higher concentration of inoculum

Compound	LAS concentration mg/l	Inoculum	Inoculum concentration mg/l	Removal (%) MBAS	Removal (%) DOC	Removal (%) Other	Reference
2 - Ø C 12 - LAS	40	AS	10	100 in 6d	80 in 21d (COD)	-	Cordon et al (1968)
JNX sulphonate	15	AS	30		-	-	Painter and Durrant (1976)
		ex dom sew		90 in 3d	-	-	"
		ex HE* sew		90 in 7d	-	-	"
		ex HE sew and LAS		90 in 7d	-	-	"
JNX sulphonate	10	Acclim AS	0.7	90 in 4d	-	50 in 5d (UV)	
		Acclim AS	20	90 in 3d	-	90 in 5d (UV)	
		HE AS	0.3	85 in 5d	-	20 in 5d (UV)	
		HE AS	30	90 in 3d	-	60 in 5d (UV)	
LAS	40	Acclim AS	10	90 in 3-10d	56 in 14d	-	Maurer et al (1974)
1 Ø C 12 - LAS (1978)		AS 90		100 in 3d	-	-	Kubodera et al
LAS	25-65	Acclim AS	100	94-97 in 12d	53 to 70 in 12d	53 to 68 in 12d (UV)	Pitter and Fuka (1979a)
LAS I[0] LAS II[0]	10-20	Acclim + AS	30 -	-	81 in 30d 55 in 30d	73 in 30d (CO_2) 48 in (CO_2)	Larson (1979)
LAS	20	"	-	-		75 in 27d (CO_2)	Larson and Wentler (1982)
1 Ø - C 12 - LAS	3 10	AS	30	-	-	98 in 14d (ThOD) 36 in 14d "	Urano & Saito (1985)
C 12 - LAS (^{14}C)	50 μg/l	De-acclim	(unstated)	-		2 in 5d (CO_2)	Freitag et al (1985)
LAS	150	Enrichment from sewage		6 expts:>90 in 7d 2 expts: 52 in 7d		- -	Gard-Terech and Palla (1986)

* sludge from human excreta, starch etc no LAS
\+ homogenised, yielding ~ 2 x 10^5 CFU/ml in final medium
o LAS II had higher proportion of C_{13} + C_{14} alkyl chain lengths and phenyl group was nearer to terminal position than in LAS I

AS activated sludge
Ø para-sulphophenyl

experiments, reported high removals of DOC (81%) and high CO_2 production (73%) for LAS I, but lower values (55 and 48%, respectively) for LAS II which had a higher proportion of C 13 and C 14 alkyl chain lengths and in which the phenyl group was nearer to the terminal position: this is contrary to most other findings (see Section 4.1.5). He attributed this result to the toxic effects of higher chainlength homologues in low biomass systems. In SCAS tests (see Table 4.4) the two products were removed to about the same extent.

Other values for % DOC removals in inoculated media (nature of inocula not stated, Table 4.5) show that with longer periods of incubation the degree of removal is generally higher, although there were some relatively low values after long periods, notably 55% after 56 d (Leidner et al 1976).

Qualitative properties of the inoculum were investigated by Gard-Terech and Palla (1986), who used enriched cultures derived from sewage on eight occasions to inoculate the medium in the AFNOR T 73260 biodegradability test containing as much as 150 mg LAS/l (modified Petrelab 550). More than 90% MBAS was removed in 7 d in six of the tests but only about 50% in the other two (Table 4.3). The authors showed that five of the six successful inocula were very similar containing a community of several bacteria genera; the sixth was different. The populations of the two remaining inocula were different from the other six. The only obvious difference between the more and less successful groups was that the latter were both derived from a mixed industrial-domestic sewage, while four of the six successful inocula came from purely domestic sewage. Further, although prepared in the same way, the less successful population had less ATP (0.1 to 0.3 x 10^{-3} mg/mg protein) than the successful ones (about 2 x 10^{-3} mg/mg protein).

Table 4.4. Biodegradation tests - simulation and inherent tests (temperature, 18-20 °C)

Compound	Concentration (mg/l)	Test	Nominal Retention time of medium (h)	Removal (%) MBAS	DOC	UV	Reference
C 12 - LAS	50	Sim	6	-	-	79	
		SCAS	24	-	-	92	Swisher (1967a)
3Ø - C 12 LAS	50	Sim	6	-	-	90	
		SCAS	24	-	-	93	
6Ø - C12 LAS	50	Sim	6	-	-	85	
		SCAS	24	-	-	85-94	
Marlon A	10-20	Sim	6	92	33	-	Brown (1976)
			3	95	41	-	
			3	87	41	-	
			3	-	92 → 57	-	
JNQ sulphonate	20	Sim	3	97 ± 2*	80 ± 8*	-	Painter (1979)*
	20	Sim	3	37†	-	-	Painter and King (1978)
				92, 94^{oa}	-	-	
				63^{a}, 70^{oa}	-	-	
				97^{b}, 92^{ob}+	-	-	
JNQ sulphonate	23-45	Sim	6	91-97	79-88	-	Brown *et al* (1981)
JNQ sulphonate	25	Sim	3 (single units)	95, 89‡	89, 78‡	83, 69‡	Brown *et al* (1982)
			3 (coupled units)	94, 93‡	76, 79‡	75, 76‡ (COD)	"
LAS (low in 2Ø)		Sim	-	96	85	90	Moreno Danvila (1987)
LASI		SCAS	24	-	92	-	Larson (1979)
LASII					93	-	
LAS	21	Sim	10	89-98	-	-	Hrsak *et al* (1981)
	46			36, 51, 85^{c}	-	-	
LAS	20			96	-	8	
(sole C source)	50			64	-	10	
LAS	13	Sim	3	90-95	73±6	-	Gerike and Jasiak (1985)
		(metabolite)	3	-	94.9±1.2	-	

Table 4.4. continued

Compound	Concentration (mg/l)	Test	Retention time (h)	Removal (%) MBAS	DOC	UV	Reference
LAS		Sim	3(?)	100	-	80-90	Petresa (1987)
LAS		Sim					
				(Sludge RT) 3d 6d 9d	-	-	Gilbert and Kleiser (in Press)
		15^oC	3	95 95 97	-	-	
		9^oC		95 97 96	-	-	
		SCAS	24	-	88-93	-	"
		Z/W	28d	-	95	-	"

* average of 10 months operation
\+ OECD synthetic sewage deficient in phosphorous
o 9.5 ^oC
ǂ daily wastage of 1/6 sludge volume
a limed sewage, removes phosphate
b a + phosphate
c 3 different inocula
ø para-sulphophenyl
Sim = simulation test (activated sludge)
SCAS = Semi-continuous activated sludge
Z/W = Zahn/Wellens test

Table 4.5 Percentage DOC removal of various LAS products in die-away tests

Data taken from Swisher (1987)

Compound	Method	Time (d)	% DOC removal	Reference
LAS	Sea-water River water	58 58	57 100	Sekiguchi et al (1975a)
C 10-C 13 LAS	Riverwater	10	85	Yoshimura et al (1984b)
Marlon A	Syn medium	23	16-33	Wuhrmann and Mechsner (1974)
C 10-C 13	"	28	75	Gledhill (1975)
C 11-C 14			68	
C 12-C 14			59	
Marlon A	"	56	55	Leidner et al (1976)
C 10-C 14	"	10	70	Okumura et al (1976)
Technical	"	30	90	Lotzsch et al (1979)
LAS	"	8	25	Miura et al (1979)
Marlon A	"	42	85	Ruffo and Arpino (1979)
Commercial	"	12	38-63	de Fulvio et al (1980)
LAS	"	20	72	Narkis and Schneider-Rotel (1980)
LAS	"	19	64-70	Gerike (1984)
C 10-C 13	"	13	68 (80, UV)	Pecenik et al (1984)
LAS	"	21	71	

4.1.1.4

Biodegradability test - simulation and inherent tests

Results of simulation tests are given in Tables 4.4 and 4.6. The results show again that primary biodegradation of LAS occurs readily as most values given for MBAS removal are >90%. Results in Table 4.4 citing removals of less than 80% can be explained as follows:

Table 4.6. Percentage DOC removal from various LAS products in simulation tests

Data taken from Swisher (1987)

Compound	Method	Time	% DOC removal	Reference
LAS	River simulation	43h	55	Oba et al 1976
LAS	Trickling filter	-	79 ± 9 82 ± 2	Gerike et al (1980)
LAS	SCAS	19d	98	Gerike and Fischer (1981)
High 2ØLAS	Continuous activated	6h (RT)	80 (81, UV)	Swisher (1981c)
Low 2ØLAS	sludge	6h (RT)	85 (83, UV)	Swisher (1981c)
High MWT LAS		"	82 (79, UV)	Swisher (1981c)
LAS	"	3h (RT)	73 ± 6*	Berth et al (1984)

* This may be the same value given by Gerike and Jasiak in Table 4.4
ø para-sulphophenyl

Phosphate deficiency was responsible for the low MBAS removal of only 37% obtained by Painter and King (1978) with the OECD 'synthetic sewage'. (The OECD recipe (OECD 1971) contained no added phosphates.) When a different 'synthetic sewage' containing sufficient phosphate was used, MBAS removal increased to over 90%. Similarly when lime was added to the sewage to remove phosphate the MBAS removal was reduced to 63%, but was increased again to 97% by the addition of 2.5 mg phosphate - P/l.

The inoculum used can also have an influence on MBAS removal. Hrsak et al (1981) observed that only 36 and 51% of the initial concentration of 46 mg LAS/l were removed when the test units were inoculated with River Sava water and a forest soil respectively. The removal increased to 85% when a wastewater was used.

When LAS is dosed to activated sludge units as sole carbon source the initial LAS concentration can also have an influence on its removal. When 20 mg LAS/l was dosed 96% MBAS was removed compared to only 64% when the initial dose was 50 mg LAS/l. The corresponding values for the removal of UV absorbance were 8% and 10%, respectively (Hrsak et al 1981). Swisher (1982) suggested that these low values were caused by abnormal operation such as the low concentration of activated sludge solids in Hrsak's units, as well as insufficient time - apparently only 5 d was allowed at each 'steady' state. Another contributory reason could have been the use of the original OECD 'synthetic sewage' (OECD 1971) which was phosphorus deficient. (The EEC Directives on surfactants require incorporation of phosphorus in the synthetic sewage only since 1982.)

Temperatures between around 20 °C and 9 °C had little effect on MBAS removal, at least under the conditions used by Painter and King (1978) - 3 h sewage retention, 2500 mg solids/l - and by Gilbert and Kleiser (in press), who used Birch's (1982) modified porous pot method (3 h sewage retention, sludge retention 3, 6 or 9 d). In the latter experiment, it is of interest to note that ammonia was less well degraded than LAS. The results clearly show that LAS will be efficiently removed with short SRTs even in winter, while ammonia will not.

There is clear evidence that LAS can be completely mineralised to a high degree, since DOC removal in the most potent OECD methods for inherent biodegradability, SCAS and Zahn-Wellens, has been reported as 92-93% (Larson 1979), 98% (Gerike and Fischer 1981), 82-93% DOC (Gilbert and Kleiser in press) - all by SCAS - and 95% by Zahn-Wellens

(Gilbert and Kleiser in press). UV absorbance in the SCAS test was reduced by 92, 93 and 85 to 94% for three forms of LAS C 12 mixed, 3-phenyl C 12 and 6-phenyl C 12, respectively (Swisher 1967a). Also, the more recently developed powerful method, known as the 'metabolite' test (Gerike and Jasiak 1985), removed 94.9 ± 1.2% DOC.

However, these methods are more effective than sewage treatment and the removal of DOC in the simulation methods for sewage treatment can be expected to be, and is, somewhat lower at 73 to 89%. The exceptions are the data of Brown (1976) and Hrsak et al (1981) who reported low DOC and UV absorbance removal respectively. It is likely that the synthetic sewage used in these tests was deficient in phosphorus, since for instance Brown (1976) reported only 84% DOC removal of added glucose. It should be noted that Swisher (1967a, and elsewhere) used "sewage" containing phosphorus, rather than the OECD recipe.

The period of operation of the Husmann or porous pot activated sludge units was also found to be important. Painter (1979) operated his units for 10 months after an initial 'running-in' period of 3 weeks; MBAS removal had reached a plateau after 3 weeks. The removal of DOC took more than twice as long to reach steady state. During the first few months, desulphonation and GC analysis of effluents revealed eight peaks which were identified as sulphophenylalkanoic acids with 3 to 8 carbon atoms in the chain, or their ring-closed derivatives indanones and tetralones formed during chemical desulphonation of the test samples. It was estimated that the total concentration of these intermediates was very small (representing only micrograms/l) and after a few more months they were no longer detected. Moreno Danvila (1987) also observed the slower attainment of maximum DOC removal with MBAS removal reaching a plateau after only 10 d, while DOC removal was still slowly increasing at the end of the test (42 d). Brown et al (1981) recorded 79 to 88% DOC removal in Husmann units treating concentrations of JNQ sulphonate as high as 45 mg/l; this could have been due to increasing the sewage retention time from 3 h to 6 h, which is more comparable with the time used in practice.

Brown et al (1982) explored the effect of operating units differently. Sludge was wasted from the units, in the OECD manner, three times per week to maintain a sludge concentration of 2500 mg/l and also by wasting one sixth of the volume of sludge in the aeration tank every day to achieve a more constant sludge retention time of 6 d. The units were operated in the conventional 'single' mode and in the 'coupled' mode, described by Fischer et al (1975). Wasting sludge daily, compared with thrice weekly, slightly lowered the % MBAS and DOC removal in the units operated in the single but not in the coupled mode (Table 4.4). Coupled units operated in the OECD manner removed MBAS by the same proportion as single units, whereas DOC removal was slightly lower at 76% compared with 89% in single units.

As is to be expected, high removals of DOC lead to high removals of the ring at 79 to 90% measured as UV absorbance (Tables 4.4 and 4.6), with the exception of the 8 and 10% reported by Hrsak et al (1981) discussed above.

For the following papers only the abstracts were consulted: Mizuno et al (1984) found rapid MBAS removal in a river water die-away test whereas UV absorbance started to decrease only several days after complete removal of MBAS. Abe and Kobayashi (1984, 1985) followed the disappearance of C 12-LAS by HPLC, DOC and 'ferroin active substances' in Tama and Nogawa river waters. Rates of removal of LAS indicated by HPLC and ferroin were in fair agreement and removal of isomers obeyed Swisher's 'distance' principle. The 2- and 3- phenyl isomers were removed more rapidly in Nogawa river water than in water from the river Tama. Organic carbon almost completely disappeared in 20 to 25 d and the rate of LAS removal was increased by stirring suggesting that the concentration of dissolved oxygen was limiting in unstirred vessels. The lag time before LAS removal started at 10 °C was about three times longer than at 20 °C, and at 10 °C some LAS remained even after 50 d. Kikuchi (1985, abstract only) also determined the effect of temperature on the removal of LAS in Tama river water. The fluctuation of LAS and its intermediates over 53 d in water taken from Hiroshima Bay was followed by HPLC (Takimoto et al 1982). Mizuno et al (1984) also assessed the biodegradation of LAS in sea-water.

Takamine et al (1985) found that LAS degraded after 28 d in four OECD screening tests (AFNOR, Sturm, Closed Bottle and Modified OECD) by more than the 'pass' levels of 70% DOC and 60% ThOD or $ThCO_2$; HPLC showed that the 'homologues and isomers' disappeared within 28 d. Janak and Kulek (1983) compared the media used by Pitter, Fischer and the French AFNOR method and found that LAS degraded equally well in all three. The importance of media was studied by Vaicum (1971), who found, for example, that dried meat extract gave much lower removal of MBAS than the paste form. (The question of media composition is discussed in detail by Swisher 1987, pp269-273.)

In a Sturm-type test, Itoh et al (1974, abstract only) used GC to determine CO_2 produced, as did Boatman et al (1986). LAS was degraded by 95% MBAS in 3 d and by 50 to 60% DOC in 7 d in aerated fermenters; degradation to CO_2 was complete but slow (Hrsak and Johanides 1975). Degradation of LAS was reported to be slower in hard than in soft water. Other papers are Setsuda et al (1974), Sakaguchi et al (1975) and Yagi (1980).

Discussion

(a) Biodegradability test using low concentration of inoculum.

The comparatively poor degradation of LAS in some tests, as determined by DOC and by UV absorbance, has its roots in the design of the OECD die-away test for surfactants. The conditions of the test were set to allow the 'soft' standard to lose 90% of its methylene blue activity (related to foaming) within 14 days and would result in no more than 35% MBAS loss from the 'hard' standard in the same time. The inoculum required to achieve this was normally just a few drops to a few ml of good quality sewage effluent per litre which is equivalent to only 0.5 to 2.5 x 10^2 bacteria/ml in the medium. When this test was applied to organic compounds in general and DOC was used as the indicator of degradation, it was found that many compounds did not degrade well within the extended period (28 d) allowed. In other tests adopted by the OECD and by ISO (eg AFNOR, Sturm, MITI and ISO tests), the recommended inoculum results in cell concentrations in the test medium of around 10^5/ml. Many compounds 'failing' the Modified OECD die-away test were degraded to a much greater extent in these tests. Not only are the numbers of cells/ml of importance, but the larger number ensures a greater diversity of metabolic activity not guaranteed, although sometimes present, in the test with a small inoculum. Another contributory factor for some of the low degradability reported was that the original surfactant method stipulated a test concentration of only 5 mg MBAS/l but many workers have greatly exceeded this concentration. The modified OECD test permits 5 to 40 mg C/l.

The above factors combine to give the variable and often low results obtained with screening tests using low concentrations of inocula; the populations are not balanced and long delays occur during which part of the population endeavours to produce the necessary enzymes to carry out the second stage of metabolism - the oxidation of the aromatic alkanoic acids. Also, the toxicity to micro-organisms of the higher concentrations of LAS could be an important factor leading to low degradation results in low-biomass test systems.

(b) Biodegradation - simulation tests

The laboratory simulation tests also tend to give lower LAS removal than occurs in full-scale sewage treatment works. Firstly, it is necessary to use 'synthetic sewage', which has a limited range of organic compounds and has relatively few bacteria. Natural sewage contains a wide range of inorganic and organic compounds which may encourage degradation by co-metabolism, but more importantly, it contains a wide variety of microbial species, with a wide spectrum of metabolic activity, which constantly re-inoculate the activated sludge or biological film. Secondly, of necessity laboratory tests are restricted in duration. The OECD confirmatory test, for example, allows up to 6 weeks for acclimatisation followed by 3 weeks of 'steady' operation. Evidence presented here and elsewhere (eg Rutgers et al 1987) suggests that in continuous culture systems selection of the optimal population can take 60 to 70 times the retention time of the bacteria (sludge) rather than the 6 to 7 (or even lower)-fold recommended for the test. For a sludge retention time of 6 d, the 9 weeks allowed in the OECD test offers only a 10-fold factor; whereas a 60-fold factor would require a test lasting about a year, ie 60 x 6 = 360 d.

Because of the unsatisfactory nature of laboratory tests as at present carried out, there has been a move to systems which much more closely mimic the conditions in the environment, especially in river waters and in sediments; these systems and the results obtained are described in the next section (4.1.2).

4.1.2 Biodegradability tests yielding kinetic data

The main drawback of the currently applied biodegradability tests - the so-called laboratory screening tests - which use either synthetic media, or natural waters, is that the test substance (LAS) has to be present at least at 5 to 10 mg C/l to allow a reasonably accurate determination of the dissolved organic carbon (DOC). These concentrations are much higher than those found in natural waters, even when polluted. Also, the results are expressed arbitrarily as percentage removed, which is unhelpful since the value depends on the initial concentration of LAS and neither can the result be readily applied to the natural environment for calculating LAS profiles. For wastewater application this problem is less important as the concentrations present in sewage are usually in the range 2 to 20 mg MBAS/l. Experience with other chemicals suggests that in many cases the kinetics of their degradation at the mg/l level are different from those at the µg/l level. There are sometimes threshold concentrations below which the chemical is not biodegraded. On the other hand, some chemicals may degrade well at concentrations in the region of µg/l but may be inhibitory at the mg/l level.

Addressing these problems, Larson (1979) and Larson and Payne (1981) proposed the use of 1st order equations to describe the production, during biodegradation, of CO_2 from compounds such as LAS and the uptake of oxygen in respirometers. The equations could take account of any delay or lag before degradation began. One form of the equation he used is

$$y = a\,(1-e^{-k(t-c)})$$

where y = CO_2 evolved in time t,
a = final amount of CO_2 produced,
k = first order constant (d^{-1})
c = lag time (d).

The data generated were fitted to equations, and best estimates of a, k and c were obtained. The author stressed that, although these constants related to ultimate biodegradation, they were overall values arising from a complex series of reactions.

Results for the high (5 to 20 mg/l) concentration range of LAS in synthetic media are given in Table 4.7. The kinetic constant k ranged from only 0.1 to 0.24 d^{-1}. An LAS (II) having a higher proportion of C 13 and C 14 homologues showed a longer lag than LAS(I), but its reaction constant was higher. Neither the source of the

Table 4.7. Kinetic data of LAS degradation in die-away tests at high and low LAS concentrations

Compound	LAS concentration	Inoculum or type of water	Lag period, c (d)	Rate constant k (d^{-1})	Percent CO_2 removed (the 'plateau') a	Reference
		<u>In synthetic media</u>				
LAS I		acclim As	5	0.1	73	Larson (1979)
LAS II	all at 5 to 20 mg/l		7.5 - 10	0.17 - 0.21	67	
LAS		acclim As	3.4	0.24	73	Larson and Perry (1981)
LAS		"	5.5	0.1	78	Larson (1980)
		sewage	2.7	0.13	79	
		sewage	3.0	0.12	79	
		effluent	4.6	0.12	73	
LAS		acclim As	-	-	75	Larson and Wentler (1982)
LAS		"	-	0.17	71	Larson (1983)
		<u>In natural waters or suspensions</u>				
LAS	10 mg/l	river	4.2	0.38	70 (O_2)	Larson and Perry (1981)
		0	3.0	96 (MBAS)		
C12 LAS*	50-500 µg/l	river, below outfall	0	0.5	73†	Larson and Payne (1981)
		and sediment	0	0.95	80	
		above outfall	3.1	0.05	70	
		and sediment	3.2	0.26	72	
"	5 µg/l	river	-	-	82	Larson and Wentler (1982)
"	50 µg/l	river, above outfall	-	0.07	68	Larson (1983)
		below outfall	-	0.52	71	
C13 LAS	5 µg/l	ground water slurry	-	0.63	80	Larson (1984)
"	10-100µg/l	subsurface sediment	-	0.62	76	Ventullo <u>et al</u> (1988a)

* contains uniformly labelled ring $-^{14}C$
† measured as $^{14}CO_2$ except where indicated otherwise
As activated sludge

micro-organisms nor their concentration, in the range 10^3 to 10^6 colony-forming units/ml, seemed to affect the kinetic constant. Lag periods of 3 to 5 d generally occurred before CO_2 was produced. The yield of CO_2 was on average 75% (67 to 79) of the theoretical, indicating a high degree of ultimate biodegradation.

Table 4.7 also shows the results obtained with natural waters or suspensions, in which more balanced populations exist than in inoculated synthetic media and which contain other organic substances which may aid biodegradation. In one experiment 10 mg LAS/l was added to river water; a very high rate constant of 3.0 d^{-1} (for MBAS) was obtained and 96% MBAS was removed. However, no oxygen uptake was observed for the first 4 days but the rate of ultimate biodegradation (measured as O_2 demand) was higher, at 0.38 d^{-1}, than the values obtained for synthetic media (0.1 to 0.2 d^{-1}), and 70% of the theoretical oxygen demand (ThOD) was taken up. In the remaining experiments ^{14}C ring-labelled C 12- and C 13- LAS were added to natural waters at concentrations from 5 to 500 µg/l and the yields of CO_2 given in Table 4.7 relate to ^{14}C-labelled material. The rates generally were higher in river water exposed to sewage effluent than in synthetic media and the presence of sediment (500 mg/l) increased the rate. Even water taken above the sewage effluent outfall showed activity (0.05 to 0.07 d^{-1}) towards LAS, after a lag of 3 d, and this rate was enhanced by the addition of appropriate sediment. The degree of removal of ring-^{14}C was high, average 75% (68 to 82%) of the theoretical, again indicating extensive mineralisation of the benzene ring.

Ventullo and Larson (1988) extended their kinetic studies, using the short-term heterotrophic potential method, to microbial communities grown on plexiglass plates in a river which were then exposed, in bioassay tubes suspended in the river, to 1 to 5 mg LAS/l. Growths scraped from the plates and suspended in the river water were amended with ^{14}C ring-labelled C 13-LAS at a number of concentrations between 3 and 6000 µg/l. Incubation lasted until 10 to 20% of the added label was metabolised (usually 2 to 48 h) when the ^{14}C assimilated and $^{14}CO_2$ production were determined and the metabolised fraction, f, of ^{14}C added was calculated. From the Michaelis Menten type equation,

$$v = \frac{f}{t} A = \frac{V_M \times A}{K_a + A}$$

where v = rate of degradation ($ng/cm^2.h$)

f = fraction metabolised in time t

A = concentration of LAS added (ng/cm^2)

V_M = maximum rate of degradation ($ng/cm^2.h$)

K_a = concentration of LAS at which v = $V_M/2$, (ng/cm^2),

estimates of V_M and K_a were calculated using a computer program. Turnover times were calculated from t/f. For samples taken above and below an outfall, V_M values were 1.5 $ng/cm^2.h$ (0.11 µg/l.h) and 4.3 $ng/cm^2.h$ (0.32 µg/l.h), respectively. After acclimatising <u>in situ</u> to 1 mg LAS/l for 21 d the corresponding rates were 134 $ng/cm^2.h$ and 592 $ng/cm^2.h$, respectively.

Similarly, Ventullo <u>et al</u> (1988b) have shown that exposure of bacteria in Acton Lake to 5 mg LAS/l for 21 d greatly increased their ability to degrade LAS (^{14}C ring-labelled C 13-LAS) - from 2.6 ng/l.h to 586 ng/l.h. The MPN, (most probable numbers) of LAS-degraders increased by about 10-fold. Other papers in preparation also describe 10- to 20-fold increases, after adaptation, in rates of degradation of LAS in sediment/water microcosms (Shimp, in preparation); in field studies a 20-fold increase in degradation rate was observed in epilithic communities with adaptation, but although sediment communities in situ showed rapid degradation there was no increase in rate with adaptation (Shimp, in preparation).

Among work nearing completion (at Procter and Gamble) are studies of the effects of inorganic and organic amendments on adaptation to biodegradation of C 13-LAS and on a model laboratory system for measuring removal of C 13-LAS simulating wastewater treatment.

Larson (1987) also found no threshold concentration of LAS below which biodegradation did not occur; the limits tested were 0.025 ng/l in the aquatic environment, 0.05 ng/l in the epilithon and 0.5 ng/l in sub-surface sediments.

From the results referred to here, and others, Larson concludes that LAS biodegrades extensively in a variety of environmental compartments and the rates exhibit 1st order kinetics at trace (μg/l) concentrations in natural systems. The rates are high relative to the estimated residence time of LAS in the specific compartments and biodegradation is a significant removal mechanism in these compartments.

sts
LAS

^{14}C-labelled LAS homologues and isomers have been used to study biodegradation and adsorption of LAS. In biodegradation tests their use enables measurement of CO_2 evolution from LAS in the presence of other degrading compounds, especially at realistic environmental concentrations. The method also provides information on the fate of both ring and chain moieties of LAS by determining the distribution of ^{14}C between CO_2, biomass and the residues in solution. (Examples of its application for determining the 1st order kinetic constant for CO_2 evolution for a variety of environmental conditions are given in Section 4.1.2.)

Table 4.8 lists the yields of $^{14}CO_2$ from a number of ^{14}C ring-labelled homologues of LAS and, where measured, the proportions of the initial ^{14}C contained in the biomass produced and remaining in solution in culture filtrates from die-away tests.

Gledhill (1975) reported an average of 63% $^{14}CO_2$ production in 28 d for a variety of LAS mixtures using concentrations of 30 mg LAS/l which had an inhibitory effect on the rate of removal of higher homologues. On average, 18% ^{14}C remained in solution. The biomass was not investigated. Steber (1979) used only 5 mg LAS/l for a longer period, 42 d, and found about the same yield of $^{14}CO_2$ but more ^{14}C remaining in the culture medium. At least some of this 26 to 30% ^{14}C was probably associated with the biomass since the medium was not filtered. Using much higher concentrations (30 to 60 mg LAS/l) of a specific C 12 isomer, Lotzsch et al (1979) found somewhat lower $^{14}CO_2$ production (50%) and 30% ^{14}C left in solution after only 12 d incubation. The remaining ^{14}C (21%) was associated with the biomass separated by filtration of the medium.

Larson and his co-workers (Larson and Payne 1981, Larson and Maki 1982, Larson and Wentler 1982 and Larson 1987) used much lower concentrations of LAS in river water and obtained higher yields (75 to 85%) of $^{14}CO_2$ than those found by others using synthetic media and higher concentrations. In all of these cases, <5% of the radiolabel initially added remained in solution at the end of the test.

Table 4.8. Biodegradation of LAS using ^{14}C-labelling as in die-away tests

	Chain Length	Time (d)	^{14}C distribution (%) in CO_2	Biomass	DOC	Reference
Synthetic media						
	12-13	28	63	-	18	Gledhill (1975)
	10-13	42	57-62	-	26-30*	Steber (1979)
	4 phenyl C 12	12	50	21	30	Lotzsch et al (1979)
Estuary water						
	13	58	50	-	-	Larson and De Henau (1988)
River water						
	12	12	75	-	<5	Larson and Payne (1981)
	12	15	82	-	<5	Larson and Payne (1981)
	12	19	82	-	<5	Larson and Maki (1982)
	12	21	85	-	<5	Larson and Ventler (1982)
	10-14	28	80	-	<5	Larson and De Henau (1988)
Surface sediment						
	10-14	14	80	-	-	Larson and De Henau (1988)
Sub-surface sediment						
	13	21	80	-	-	Ventullo et al (1988a)
	13	12	76	-	-	Larson (1984)

* contains C in suspension

Huddleston and his group (Huddleston and Nielsen 1979a,b, Nielsen et al 1980a,b and Nielsen and Huddleston 1981) have carried out ^{14}C balances using the semi-continuous activated sludge (SCAS) test operated at a nominal hydraulic retention time of 1 d but probably higher depending on the mode of operation. The SCAS units, which were $^{1}/_{10}$th of normal size reached equilibrium in only 4 d when filled with activated sludge and receiving 10 mg unlabelled LAS/l. On changing to 10 mg ^{14}C - labelled LAS/l a further 7 d were required to achieve equilibrium with regard to ^{14}C; average results obtained in the following 13 d of "steady-state" operation are given in Table 4.9. About two-thirds of the ^{14}C - whether in the ring or in the alkyl chain - were converted to $^{14}CO_2$; 18 to 28% was associated with the sludge and 8 to 20% remained in solution in the effluent. The authors showed that biodegradation continued in the effluent by subjecting the supernatant of the centrifuged sludge at the end of the experiment to a modified Sturm test, which increased the yield of $^{14}CO_2$ to 93% after 91 d and to 98% after 190 d. The remaining few percent ^{14}C was in the biomass.

Analyses of the SCAS effluent, containing 10% of the original ^{14}C, by HPLC and other techniques showed that 1.5% of the original ^{14}C was associated with the compounds containing the intact ring and 8.5% with broken-ring intermediates.

The conclusion from these SCAS tests is that LAS can be mineralised completely given sufficient time - that is, it is inherently biodegradable. It cannot, however, be concluded that LAS behaves in the same manner in sewage treatment processes. The conditions of the SCAS test are much less stringent than those in sewage treatment in so far that in the SCAS tests the sewage retention time is 24-36 h and, since no sludge is deliberately wasted, the sludge retention time equals the duration of the test. Thus, the retention time of the sludge which determines the nature of the population, especially its specific growth rate and the chances of adaptation, was far greater in the SCAS tests. Also, the subsequent modified Sturm test was excessively long - there are no rivers taking 190 d to reach the sea. As against this criticism, in

Table 4.9. Biodegradation of ^{14}C ring-labelled LAS: sewage treatment simulation

Chain length	Hydraulic retention time (d)	^{14}C distribution (%) in CO_2	Biomass	DOC	Reference
Semi-continuous activated sludge					
11-13	1	62	18	20	Huddleston and Nielsen (1979a,b)
11-13	1	62	28	8-10	Nielsen et al (1980a, 1980b)ǂ
	1+91*	93	6	1	Nielsen et al (1980b)
	1+190*	98	2	0	Nielsen and Huddleston (1981)
Continuous activated sludge					
10-13	3h+	42-61	21-26	14-20	Steber (1979)
13	6h	80	7	11	Shimp (1988)

* 91 or 190 d in a modified Sturm test
\+ ^{14}C-labelled LAS dosed only for 1-5 d; other conditions as OECD confirmatory test
ǂ chain also labelled with ^{14}C.

Huddleston's experiments a synthetic sewage was used which is known to be less favourable for inducing biodegradation than domestic sewage.

Only two reports have been seen in which ^{14}C-labelled LAS was treated in a true simulation of sewage treatment. Steber (1979) used activated sludge units $^1/_{10}$th of the size of the vessels recommended in the OECD confirmatory test, presumably because of the limitation of the amount of labelled material available. The units were filled with activated sludge from a local treatment works and fed with OECD "synthetic sewage" at a hydraulic retention time of 3 h for only two weeks.

In the first period of 2 weeks, unlabelled LAS was dosed at 10 mg/l and equilibrium was reached with regard to LAS removal in this time. Ring ^{14}C-labelled LAS was then substituted for unlabelled surfactant, again at 10 mg/l, for 5 d when the test was terminated. Although the effluent concentration of ^{14}C reached a plateau after only 1 d, $^{14}CO_2$ production rose during the 5 d period and was still rising at 5 d. The yield of $^{14}CO_2$ was 42 to 61%, the proportion of ^{14}C associated with the sludge was 21 to 26% and the ^{14}C remaining in solution was 14 to 20%. Of the ^{14}C contained in the sludge one third was adsorbed on the sludge and two-thirds, or about 15% of total ^{14}C applied, was assimilated by the biomass.

Analysis of the effluents indicated that original LAS amounted to between 1 and 1.4% of the ^{14}C dosed, other aromatic compounds, mainly phenylalkanoic acids, constituted 9 to 15% while 2 to 4% was attributed to lower molecular weight compounds. About 80% of the last group still contained the sulphonate group and a very small proportion consisted of steam-volatile acids. Similar proportions of the same groups of compounds were found in the few determinations made on filtrates from OECD die-away tests.

Since 15% ^{14}C in the benzene ring had been assimilated by bacteria and 42 to 61% was converted to $^{14}CO_2$, it can be concluded that 57 to 76% of the benzene ring had been biologically degraded. Expressed in another way, 80 to 86% of the ring C had been removed from the sewage during its passage through the treatment unit.

4.1.4 Bacteria degrading

In extrapolating to 'real world' conditions, three factors suggest that the degree of removal would be even higher than those found by Steber. First, the retention time of sewage in practice is usually longer than the 3 h stipulated in the OECD confirmatory test, which also permits 6 h. Secondly, municipal sewage aids the biodegradation of xenobiotics by introducing a wider spectrum of micro-organisms and by encouraging co-metabolism. Lastly, Steber's tests lasted at most 19 d so that the microbial population may not have had time to adjust to the new conditions - eg synthetic sewage, higher growth rate, ^{14}C - and the concentration of sulphophenylalkanoic acids, present in the effluent at about 1 to 1.5 mg/l, may well have decreased with longer operation.

A second study, by Shimp (1988, in press), also shows extensive degradation of LAS under simulated sewage-treatment conditions. He fed 6 litre scale activated sludge units with municipal sewage which already contained LAS but which was also amended with ^{14}C ring-labelled C 13-LAS. The experimental period lasted for 1 week after an acclimation period of 2 weeks, and ten samples of influent, effluent and sludge were analysed. (Carbon dioxide could not be determined since it was impractical at the rates of aeration employed.) The effluent contained 11.3% of the influent ^{14}C indicating a removal of 88.7%. In a mass balance, the unaccounted ^{14}C was 80% of the influent concentration and the bulk of this was thought to be $^{14}CO_2$, since in batch tests with sludge taken from the units 70% of the initial ^{14}C-labelled C 13-LAS was converted to CO_2 in 28 d. The author plans to modify his system to enable evolved $^{14}CO_2$ to be determined.

4.1.4 Bacteria capable of degrading LAS

Many bacterial and a few fungal isolates have been made which degrade/grow on LAS and many standard laboratory pure cultures have also been found to degrade/grow on the surfactant; however, in all cases only primary degradation resulted, ie removal of MBAS but not mineralisation. Swisher (1987) cites about 90 bacterial and a few fungal species, which grow on and are capable of at least primary biodegradation of LAS. For 17 species conflicting results were obtained with some workers reporting LAS degradation whereas others found no effect. The reasons for the discrepancies could have been associated with problems of identification, different culture conditions and the degree of acclimatisation.

Many of the genera reported to degrade LAS are commonly found in the environment, for example Pseudomonas, Aerobacter, and Alcaligenes. Oba (1971) isolated these species together with Micrococcus, Flavobacterium and Paracolobactrum from a cesspool-percolating field, and found that all of them assimilated LAS; Cook et al (1975) added Moraxella, isolated from sewage, to the list.

Harrison et al (1976), found that although a Pseudomonas sp isolated from a biological filter was able to degrade (primary) LAS without a lag, it could not grow on LAS as the sole source of carbon and energy. It was able to degrade LAS without lag even after many days growth on glutamate alone. When grown on glutamate, 100 mg LAS/l caused complete inhibition, while slight inhibition was observed at 10 mg LAS/l. Inhibition of the respiration rate of pre-grown cells, taken from a chemostat, depended on the concentration of the cells; 30 mg LAS/l completely inhibited the respiration of a suspension of 250 mg cells/l, while the inhibitory threshold was around 150 mg LAS/l at cell concentrations of 750 to 1000 mg/l. Using high concentrations of these cells the rate of removal of LAS was found to be 20 to 100 mg MBAS/g cells.h when the initial LAS concentration was between 40 and 100 mg/l. However, the rate of removal was reduced to only 1 to 2 mg MBAS/g cells.h, when the concentration of LAS fell below about 15 mg/l, and when the initial concentration was in this region. No explanation could be found for this unexpected result.

Bacterial isolates usually have much less activity towards LAS than the biological film or activated sludge from which they were isolated. Phillips (1978) isolated from activated sludge Serratia marcescens, Arthrobacter globiformis, Pseudomonas alcaligenes and P. putida. Each individual species gave a primary biodegradation rate less than that obtained with the activated sludge. Even a mixture of all four species isolated degraded LAS more slowly than the sludge. However, when the four species were grown together in 'floc' form, the degradation rate was equal to that for the activated sludge, implying that adsorption on to the flocs plays an important part in the degradation.

On the other hand, Divo and Cardini (1980, 1981) isolated a Pseudomonas sp (called MICRO-C) from a shake-flask culture containing commercial LAS as the sole source of carbon which was able to grow on LAS at a concentration as high as 1000 mg/l with a yield of 38 g cells per 100 g LAS. The maximum concentration of LAS at which the organisms were still capable of degradation was higher for those isomers in which the sulphophenyl group was closer to the end of the alkyl chain. The maximum at which the 2-sulphophenyl isomers of C 10 - C 13 homologues degraded (MBAS disappearance) in 10 d was 5000 mg/l, for the 3-sulphophenyl isomers it was 2000 mg/l, while for the 5- and 6-sulphophenyl isomers tested the maxima were as low as 50 mg/l. The C 10 4-sulphophenyl isomer also had a maximum of 50 mg/l whereas for the only other 4-sulphophenyl isomer tested (C 13) the value was 2000 mg/l.

The effect was apparently not caused by inhibition since in binary mixtures of isomers MICRO-C degraded the susceptible isomer at least at the same rate as when tested separately, even though the second isomer was present at a concentration well above its maximum value. In some pairs, the presence of the more readily degradable isomer assisted the degradation of the less degradable isomer, possibly by speeding up the process of enzyme induction. MICRO-C removed all the MBAS but was unable to degrade any of the isomers beyond the aromatic stage; however, addition of a mixed culture to the MICRO-C culture removed the intermediates.

The complexity of the mixed populations capable of the degradation of LAS is further illustrated by the studies of Hrsak et al (1978, 1982b). They investigated the enrichment of, and changes in, LAS-degrading bacteria in a Husmann-type activated sludge test, using the phosphate-supplemented OECD synthetic sewage. Nine species of bacteria were identified, five of the genera Pseudomonas and two each of Achromobacter and Acinetobacter. In six experiments at rates of 0.025 to 0.1 h^{-1} and using 20 to 50 mg commercial LAS/l, the cultures were dominated by an Acinetobacter sp on four occasions and by Pseudomonas fluorescens and another Pseudomonas sp on one occasion each; in no culture did all nine survive. At the high rate (0.1 h^{-1}) only 64% MBAS was removed which increased to 96% at the lower rates (0.025, 0.05 h^{-1}); no removal of the benzene ring was observed at the high rate and only 13 to 23% at the low rates. In single, pure culture experiments the predominant Acinetobacter sp could not degrade LAS unless the medium was supplemented with yeast extract and nutrient broth. After several sub-cultures on yeast extract - nutrient broth without LAS present, the organism lost its ability to degrade LAS. Because of this, the authors suggested that Acinetobacter sp was stimulated in mixed cultures by amino acids, nucleic acids and autolysates from the other species present. The unidentified predominant Pseudomonas sp could use LAS as the sole carbon and energy source but it could only partly degrade sulphophenylundecanoic acid and sulphophenylheptanoic acid, leaving the benzene ring intact, and could not degrade sulphophenyl valeric or p-sulphobenzoic acids. The third predominant species, P. fluorescens, could not metabolise LAS in pure culture either in mineral medium or in the presence of yeast extract and nutrient broth; the other six species also came into this category.

Yoshimura (1984) isolated bacteria from activated sludge and from rivers and found that some isolates, species of Pseudomonas, Alcaligenes, Necromonas and Moraxella, could degrade the alkyl chain of LAS but not the benzene ring, while one strain - an unidentified Gram-negative rod - could not degrade LAS but could degrade the ring of the intermediates. The first group of organisms degraded LAS and sulphophenylundecanoic acid (but not 3-sulphophenylbutyric acid) forming seven aromatic intermediates. The unidentified rod degraded 3-sulphophenylbutyric acid, opening the ring; a binary mixture of this organism and one of the first group removed 100% LAS, sulphophenyl-undecanoic acid and 3-sulphophenylbutyric acid.

The rate of biodegradation of LAB isomers by pure cultures of a Pseudomonas sp was higher for those isomers in which the phenyl group was closer to the end of the alkyl chain (Bayona et al 1986) - similar to Swisher's "distance principle" for LAS. The authors consider that this shows, contrary to Swisher's view, that it is not the $-SO_3H$ group which determines the selective isomeric degradation; Swisher (1987) proposed that the $-SO_3$ group might be bound to the oxidative enzyme at a certain distance away from the enzyme site at whic[h] oxidation of the alkyl chain was initiated.

Following on earlier reports (Davis and Gloyna, 1967, 1969) of the degradation of "ABS" by some species of algae, Biedlingmaier and Schmidt (1983) found that a strain of Chlorella fusca grew on a medium containing up to 100 mg LAS/l as the sole source of sulphur. With LAS the yield of algal cells was 27% of that produced with sodium sulphate as the sulphur source. The authors suggest four possible routes by which the alga could desulphonate LAS, namely, hydrolysis to yield sulphite plus an alcohol, reductive cleavage to form a hydrocarbon, oxidation by oxygen giving an unstable α-hydroxy derivative yielding sulphite and an aldehyde and, lastly, reduction to the corresponding mercaptan and cleavage of the thiol group. The fate of the rest of the molecule was not considered.

Papers available only in abstract form. Jiang et al (1981) contacted a wastewater with fixed cells of a Pseudomonas sp in polyacrylamide gels (in a column?) which removed up to 60% of the LAS in the wastewater. The cells had a half-life of about 20 d. "Acclimated" bacteria from the Han river degraded Korean-made LAS by 90% (MBAS?) in 6 d, whereas imported LAS took only 2 to 3 d (Kim and Park 1983). An isolate from sewage in the Taeger (Korea) area was identified as being similar to Klebsiella pneumoniae and was capable of growth on 1000 mg "linear alkylate sulphonate"/l but was inhibited by 40% at 20 000 mg/l (Hong et al 1984). Stavaskaya et al (1986), using flow-through continuous cultures, found that different species of Pseudomonas predominated at different LAS concentrations and at different retention times.

One species, P. alcaligenes 'TR', removed 90% of a solution containing 400 mg "sulphonol"/l in packed reactors operated at a rate of 0.04 h^{-1} and temperatures between 16 and 21 °C (Rotmistrov et al 1986). Optimum conditions for this species were pH 7.4 and temperature 35 °C; sodium and potassium concentrations of 10^{-3}M increased biodegradation efficiency more than three-fold, but Ni, Cu, Fe and Zn inhibited growth (at 10^{-3}M?).

Other papers which may contain relevant data are Menerse and Atalay (1982) and Govzdyak et al (1985).

No papers dealing with named species capable of degrading the aromatic intermediates were found.

4.1.5 Effect of LAS structure on biodegradability

There is ample evidence, summarised by Swisher (1987), that the primary biodegradability of homologues of LAS (usually C 10 - C 13, but even C 6 - C 16) in general increases as the chain length increases, and that for isomers, the greater the distance between the sulphonic group and the terminal methyl group on the alkyl chain, the faster the degradation. The latter is known as the "distance principle". These phenomena have been observed in batch experiments (eg Huddleston and Allred 1962, Swisher 1963, Setzkorn et al 1964) and in laboratory scale continuous activated sludge units (eg Bock and Wickbold 1966; Mann and Reid 1971; Wickbold 1974; Divo 1976). However, there are exceptions to these general rules and it seems that the factors determining the relative removal rates are complex and interactive. Some factors are:

- concentration of the LAS homologue or isomer,

- possible inhibitory effects of LAS,

- presence of other homologues or isomers,

- concentration of suspended solids,

- state of acclimatisation of the inoculum,

- presence of LABs if desulphonation - GC is used.

For example, Swisher (1972) reported the isomers of C 12-LAS to degrade (primary biodegradation) in die-away tests, when present as a mixture of all five isomers, in the following order of the position of the sulphophenyl group: 2-, 3-, 4-, 5- and 6-. However, when added separately at 30 mg/l the order changed to 3-, 2-, 4-, 6-, 5- for primary biodegradation and for ring opening it was 3-, 2-, 6-, 5-, 4-. Also in weekly transfer tests, C 14 degraded more slowly than C 11 in the first three transfers, but the rates were comparable in the fourth and subsequent steps (Swisher 1966c).

Gledhill (1975) reported that high molecular weight (longer chain) LAS degraded to CO_2 more slowly than lower molecular weight LAS in shake-flask systems inoculated with sewage or soil. He attributed this to the higher inhibition caused by the higher-chain homologues, added at 30 mg/l, since the effect was reduced or eliminated on incremental addition of LAS during the first week.

The effect of mixtures of homologues and isomers, at least in relation to a pure culture (Pseudomonas), was illustrated by Divo and Cardini (1980) who found that the concentration above which an isomer could not be degraded (primary) was increased, often considerably such as from 50 to 500 mg/l, in the presence of a second isomer having a much higher threshold. This also confirmed that the cause of the non-biodegradation of the first isomer was not inhibition. It was further found that, whereas the pure culture degraded 4-sulphophenyl C 13-LAS (but not γ-sulphophenylheptanoic acid) as well as mixed cultures, on adding different mixed cultures to the pure culture, once the LAS had disappeared, the key intermediate γ-sulphophenylheptanoic acid was particularly slowly mineralised on four out of five occasions. The authors attributed this to a reduced probability of finding organisms which in nature are capable of degrading this alkanoic acid, but suggested that many more tests would be required to test the hypothesis.

A further example of the complexity of the subject is given by Schöberl (1979). He followed the fate in OECD activated sludge units of 5-sulphophenyl C 10-LAS, which was the least (52%) removed isomer when Marlon A alone was treated (Divo 1976; Bock and Schöberl 1977). (Marlon A contains a wide range of homologues and isomers.) When added singly at 20 mg/l, this isomer was removed poorly (21%), but when 10 mg/l was dosed together with 10 mg Marlon A /l, the removal of the isomer was 90% (primary). The author puts forward an enzymic explanation; Marlon A when fed alone cannot induce sufficient build-up of the necessary methyl-oxidising enzymes, since it contains too little (~ 1%) of the isomer. Neither can the isomer alone, in the 3 h retention time, produce sufficient enrichment of the appropriate enzymes but this can be achieved in the mixture of 10 mg/l each of Marlon A and the isomer.

There are reports that environmental samples show a distribution of homologues and isomers little different from that of commercial LAS. Simko et al (1965) found the same for effluents from a full-scale activated sludge plant and Sullivan and Swisher (1969) for Illinois River water. Waters and Garrigan (1983) observed no effect regarding chain length but were able to demonstrate a marked predominance of internal isomers of the C 10 - C 1[illegible] homologues.

The biodegradability of two sorts of impurities in commercial LAS, namely linear alkylbenzenes (LAB) and dialkyltetralin sulphonates (DATS), has been assessed (Procter and Gamble, unpublished). In batch tests using 1 mg ^{14}C ring-labelled C 12-LAB/l with an activated sludge inoculum, 68% of the theoretical $^{14}CO_2$ was produced in 65 d under aerobic conditions, but none under anaerobic conditions. Similarly, in river water supplemented with 10 g/l river sediment 69% $^{14}CO_2$ was collected in 56 d. Homologues of DATS were less degradable; in screening tests using 10 and 20 mg/l over 26 d only 6, 16, 19, 59 and 35% theoretical CO_2 was produced from the C 10-C 14-DATS, respectively. However, in river die-away tests 84% MBAS was removed from 1 to 10 mg C 11.3-DATS/l in 9 d and 98% after 30 d. The half-life for MBAS loss was 5.3 to 5.8 d, compared to 0.5 to 0.7 d for C 11.3-LAS; in SCAS tests 59% DOC was removed from DATS (presumably C 11.3).

Papers for which abstracts only were consulted:
Yoshimura and Nakae (1982b) confirmed the disappearance of LAS homologues and isomers in the order C 13 to C 10 and of the 2-phenyl isomers being faster than that of the internal (4-, 5-, 6-) isomers in both effluents and sludge. In a later paper Yoshimura (1984) found that LAS adsorbed on to activated sludge in hydrophobic sequence and then biodegraded in the same sequence (ie longer chain faster than shorter chain) and again confirmed the "distance principle". After treating 15 mg/l of C 10 - C 13-LAS for 3 months in a continuous-flow activated sludge unit, no LAS was detected either in the effluent or sludge.

Fujiwara et al (1975), working with a model river, also confirmed both rules. Abe and Kobayashi (1984) also observed the "distance principle" for isomers of C 12-LAS in river water and later (Abe and Seno 1985a) confirmed both rules for soil systems. On the other hand, Utsunomiya et al (1986) could find no evidence from analysis of effluent and sludge that LAS with longer chains were more effectively removed than those with shorter chains.

Three recent papers on LABs are relevant. Eganhouse (1986) reported that the LAB isomer distribution showed a marked depletion of the external (ie 2-, 3-) isomers with depth in marine sediments suggesting microbial attack. In pure cultures of Pseudomonas sp, Bayona et al (1986) found degradation to be analogous to that of LAS with both rules applying. Takada and Ishiwatari (1987) determined the ratio of internal to external isomers of LAB and found the ratio to increase from 0.8 in commercial detergents to 1.7 and 1.5 in river water suspensions and sediments, respectively. In sewage aerated for six days the ratio increased about ten-fold (from 0.67 to 7.0). Other experiments showed that partitioning between water and suspended solids did not change the isomeric composition.

The evidence above concerning LAS relates to concentrations in the range 1 to 10 mg/l or higher. More recently, Larson and De Henau (1988) have investigated the removal of LAS homologues and isomers at much lower concentrations (10 to 100 μg/l) using ^{14}C-labelled material in various environmental compartments and measuring the rate of $^{14}CO_2$ production. They have found no clear indication of significant differences between homologues or between isomers.

However, in each case there is certainly no trend one way or the other. For example, in Rapid Creek river water and sediments the 1st order degradation constants, k, for five homologues were:

C Number	10	11	12	13	14	Av
k, water (d^{-1})	0.99	0.71	0.89	0.88	0.59	0.81 ± 0.1[illegible]
k, sediment (d^{-1})	1.26	1.10	1.19	1.18	0.87	1.12 ± 0.1[illegible]

The average k values represent half lives of 20 ± 2 h and 15 ± 2 h for water and sediment, respectively. It should be noted that whereas the values, given earlier, for percentage removal related to MBAS or LAS removal (by HPLC or DS-GC), those obtained by Larson represent ultimate biodegradation, since $^{14}CO_2$ was measured. The yield of $^{14}CO_2$ was ~ 80% of the theoretical and nearly all of the remaining ^{14}C was present as biomass on the membrane after the river water had been filtered.

Homologues were shown to degrade independently of one another; for instance, at the 100 μg/l level in river water C 10-LAS and C 14-LAS, separately and in 1:10 and 10:1 mixtures, gave the same k values and removals of ^{14}C as $^{14}CO_2$ (~ 75%).

Using activated sludge at 1500 to 3000 mg solids/l and 1 mg LAS/l, the half-life of C 10 to C 14 homologues was 1.5 to 2.2 d and 60 to 70% $^{14}CO_2$ was evolved in 21 d (Ward T E 1986). These values were similar to those for ^{14}C-labelled benzoic acid and phenylacetic acid, and again there was little difference between the five homologues.

No explanation for the difference between the results at high and low concentrations is yet available.

Most isomers of all the homologues contain an asymmetric C atom and thus would exist in enantiomorphic forms; no reports have been seen of any effects such forms have on biodegradability.

4.1.6 Metabolic pathways

Several authors (eg Heyman and Molof 1968; Swisher 1972; Willetts 1973; Cain 1976, 1981) have described the known and other possible metabolic pathways by which micro-organisms degrade and mineralise LAS. Swisher (1987) gives an up-to-date account of the subject with supporting evidence but concludes that knowledge of the pathways is still incomplete. The problem is complex partly because LAS does not have a single unique molecular structure (it consists of at least four homologues (C 10 - C 13) each of which is present as four or more isomers depending on the position of the sulphophenyl group on the alkyl chain) but also because of the known versatility of micro-organisms in attacking organic compounds. The proposed pathways are based on knowledge of the metabolic intermediates identified in culture and die-away test filtrates, or in effluents from simulated sewage treatment units, and on comparisons with the known metabolism of similar straight-chain aliphatic hydrocarbons and the benzene ring.

The majority view of the metabolic pathway is based on the proposal made by Heyman and Molof (1968), who suggested that the alkyl chain is first attacked by ω-oxidation of one terminal C atom forming a sulphophenylalkanoic acid via $-CH_2OH$ and $-CHO$. The C atom attacked is the one farthest from the phenyl group. The carboxylic acid formed is then converted by β-oxidation to another sulphophenylmonoalkanoic acid containing two C atoms fewer than the original molecule, producing acetic acid or a derivative in the process. It is thought that α-oxidation also occurs to form a sulphophenylmonoalkanoic acid with only one fewer C atom but this is believed to be a rarer reaction.

Under some laboratory conditions, a delay sometimes occurs between the removal of methylene-blue reactivity and the breakdown of the ring. The intermediate at which this delay begins, which is different for different isomers, is called by Swisher (1987) the 'key' intermediate. The range of bacteria usually present in laboratory test media lacks the ability to degrade the key intermediate and requires acclimatisation before the degradation can proceed further.

The process of β-oxidation continues producing monocarboxylic acids of lower molecular weight; at some stage the other terminal C atom is attacked which leads to the formation of a series of sulphophenyldialkanoic acids. The type of acid formed is determined by the number of C atoms in the original LAS, as well as by the position of the phenyl group in the case of odd-numbered LAS homologues. For example, all C 12-LAS are thought to form sulphophenylsuccinic acid, while some C 11-LAS are oxidised to sulphophenylmalonic acid and others are degraded to sulphophenylglutamic acid. Decarboxylation reactions of the succinic and malonic acids in turn lead to sulphophenylbenzoic and sulphophenylacetic acids respectively.

At this stage the ring is believed to be broken by one of three established pathways, namely normal or meta-breakdown of a catechol derivative or via homogentisic acid, but these pathways have not yet been confirmed.

Confirmation is also not yet available on the timing of desulphonation. Early papers (eg Cain and Farr 1968, Willetts 1973, Seassaro 1973) suggested that hydrolytic or reductive desulphonation occurred with some species of bacteria even before the alkyl chain was oxidised, but the overwhelming evidence indicates that alkyl chain ω- and β-oxidations predominate as the first place of attack. At present there is also no proof whether desulphonation occurs before or after opening of the ring. However, the final product is sulphate produced either by enzymic autoxidation of the released sulphite or by catalytic oxidation by metals such as cobalt. Bock and Schöberl (1977) cited the presence of sulphoadipic acid in an OECD confirmatory test effluent as evidence that at least some micro-organisms could break the ring before desulphonation and suggested that the sulphonic acid group was removed before the citric acid cycle was reached. No rebuttal has been made of Swisher's many justified criticisms of Willett's claims (1973) concerning desulphonation as the initial step. No further reports that sulphite is released early in the degradation have been published and no identifications of phenylalkanoic acids (the putative intermediates) have been reported. It must be concluded that desulphonation as the first step must be considered to be a rare event.

The Heyman and Molof (1968) proposal could well be the principal metabolic pathway but the detection of other compounds, inconsistent with this route, indicates alternative pathways. For example, the presence of aliphatic sulphonates, carboxylated and non-carboxylated compounds (eg Krüger 1964; Steber 1979) suggests ring opening before desulphonation and the detection of sulphocinnamic and other possible sulphophenylalkanoic acids (Kölbel et al 1967, Taylor and Nickless 1979) indicates at least a minor change in the pathway.

4.1.6.1

Intermediates

The question of the existence and nature of intermediates or "dead-end" degradation products is important not only for the verification of the metabolic pathways, but also for the consideration of the effects of LAS on the environment (see Section 5). Confirmation of some of the intermediates mentioned in Section 4.1.6 was given by Kubodera et al (1978), who studied C 12 primary alkylbenzenesulphonate labelled with ^{14}C in the alkyl chain. After chemical desulphonation and GC analysis, tetralones and 1-indanone were identified, indicating the presence of 4-phenylbutyric acid (the main constituent) and 3-phenylpropionic acid. Two other peaks were unidentified but naphthalene was also reported to be present as a major component at the end of the incubation (5 d); the origin of the napthalene was unexplained.

Divo and Cardini (1980) used a pure culture of a Pseudomonas sp designated MICRO-C, to follow the degradation of C 10 - C 13-LAS at the very high concentration of 1 g/l. Intermediates were either isolated in pure form or concentrated as much as possible for examination by IR and NMR. The only intermediates identified were relatively short-chain sulphophenylmonocarboxylic acids and in all cases they accounted for almost all of the organic material present. Some LAS isomers yielded only one intermediate while most of those examined yielded two, the minor one being present at about a quarter of the concentration of the major (Table 4.10). The Pseudomonas species MICRO-C was unable to degrade the intermediates further in the 30 d incubation period but when a mixed culture was added to the centrifuged supernatant the intermediates were degraded rapidly and completely.

Table 4.10. Metabolic intermediates formed in culture broths by the degradation of LAS isomers by MICRO-C (a Pseudomonad)*

LAS	Major intermediate	Minor intermediate
2- Ø - C 10	3- Ø butyric acid	5- Ø hexanoic acid
2- Ø - C 11	4- Ø valeric acid	none
2- Ø - C 12	3- Ø butyric acid	5- Ø hexanoic acid
2- Ø - C 13	4- Ø valeric acid	6- Ø heptanoic acid
3- Ø - C 11	3- Ø valeric acid	5- Ø heptanoic acid
3- Ø - C 12	4- Ø hexanoic acid	none
3- Ø - C 13	3- Ø valeric acid	5- Ø heptanoic acid
4- Ø - C 13	4- Ø heptanoic acid	none

Ø = para-sulphophenyl
* = Divo and Cardini (1980)

In experiments (Divo and Cardini 1980) initially inoculated with a mixed culture (no description given), the kinetics of degradation of the sulphophenylcarboxylic acids were on the whole less reproducible than the kinetics of degradation of the alkyl chain, probably because of differences in the mixed inocula used. This showed itself by the inconsistent appearance in the mixed culture experiments of the different sulphophenylmono-alkanoic acids which had disappeared by the end of incubation, 15 to 20 d. Since no significant quantities of other substances were detected, the authors concluded that the sulphophenylmonoalkanoic acids were the only intermediates that could reach detectable concentrations.

Leidner et al (1980) reported that p-(1-butyl and p-(2-butyl) benzene sulphonates did not degrade in the OECD die-away test (DOC removal) but their carboxy analogues did. Similarly, seven phenylalkanoic acids (phenylacetic, 2- and 3-phenylpropionic, 3- and 4- phenylbutyric, 4- and 5-phenylvaleric) were degraded in the test; 2-phenylbutyric acid, however, was not degraded and

2-phenylpropronic acid degraded only after a longer delay than occurred with the other six. These results are only relative, since the inoculum used - a filtered soil suspension - was unadapted and resulted in a very low density of bacterial cells.

Nielsen et al (1980a,b) and Nielsen and Huddleston (1981), using HPLC-MS, sought benzenoid compounds with fewer than 3 carbon atoms in the side chain such as benzene sulphonic acid and p-sulphobenzoic acid, in SCAS effluents. Although it is not categorically stated, it is understood that none was found.

Linder and Allen (1982) applied HPLC to die-away test media and obtained evidence of at least three intermediates which were not identified. Yoshimura and Nakae (1982 a, b; abstracts only) reported a number of peaks (HPLC), in activated sludge-LAS mixtures, most of which disappeared within 24 h; only 3-sulphophenylbutyrate was identified. Similarly, Takimoto et al (1982, abstract only) using HPLC, followed the fluctuation of LAS and its intermediates in mixtures of LAS, sea water and oyster shell (?) taken from Hiroshima Bay. No details were given.

Yoshimura (1984) found several peaks (HPLC) in batch experiments using LAS-activated sludge mixtures containing concentrations of suspended sludge solids as high as 25 000 mg/l (normal range is 3000 to 5000 mg/l); most of the peaks disappeared within 24 h. Pure isolates from activated sludge formed seven intermediates, including 2- and 3- sulphophenylbutyric acids and a sulphophenylhexanoate from the degradation of C 12-LAS but none of the intermediates disappeared from the cultures. The isolates were able, however, to degrade the longer chain acid sulphophenylundecanoate. The author suggested that his isolates, like that of Divo and Cardini (1980), grew only on the alkyl chain; other species, isolated by the author, were able to degrade the lower sulphophenylalkanoates. Mixtures of the two types of isolates degraded C 12-LAS and sulphophenylundecanoic acid completely.

A method for detecting stable biodegradation metabolites was devised by Gerike and Jasiak (1985, 1986) and applied to LAS. The test is based on the OECD confirmatory activated sludge simulation test operated in the 'coupled' mode. This coupling was achieved by daily centrifuging half of the sludge in each of two sludge units and interchanging the centrifuged sludge between the units, but returning the supernatants back to their original vessels. Also daily, the effluents collected over the previous 24 h were amended with concentrated nutrient for the control vessel and nutrient plus LAS to the second vessel. The amended effluents were dosed back to their respective units during the course of the following 24 h. The test proceeded for >50 days until a steady state was reached. The percentage removal of dissolved organic carbon (DOC) calculated by a series of complex equations from the known amount of LAS-carbon in the influent and the concentration of DOC determined in the two effluents, taking into account the transfer of LAS (and any intermediates) from the test unit to the control unit. The removal of DOC was 94.9 $\pm$ 1.2%, leaving about 5% DOC as unbiodegraded residue. This residue could have been 'stable' intermediates but the authors suggest that the 5% probably comprised 'components and impurities of the original technical material, such as substituted indanes, sulphones, etc'. It might be worthwhile to repeat the test using purer LAS material, although, as the authors themselves state, the method has not been extensively applied and is not particularly appropriate for surfactants.

Eganhouse (1986), in considering the origins of linear alkyl benzenes (LAB) found in the environment, concluded that there was no evidence of either microbial or chemical reductive desulphonation of LAS to produce the corresponding LAB. His view was that LABs, which do not occur naturally, found their way into the environment through the use of detergents as impurities of the LAS, since a small proportion of the LABs remain unsulphonated in the synthesis process.

All the above intermediates have been shown to be biodegraded, the rate of disappearance depending on the structure of the particular intermediate and on the type of culture present. The longer chain sulphophenylalkanoates degraded relatively rapidly and, indeed, were not detected under some conditions. Natural microbial populations such as those present in river water produced faster breakdown than synthetic media inoculated with soil extracts or low concentrations of sewage effluents. In balanced acclimated populations, eg activated sludge or river water, all the species required for complete mineralisation are present in the necessary proportions and temporary delays in the degradation process do not occur. However, in die-away tests containing unacclimated, unbalanced populations, especially with abnormally high concentrations of LAS and perhaps deficient and insufficiently buffered media, delays occur after the formation of the sulphophenylalkanoic acids until sufficient numbers of the required species are reached to mineralise the intermediates.

No new intermediates have been reported; indeed, it seems surprising that given the potential of HPLC analysis, so few studies on the intermediates have been published or attempted. The presence of relatively long chain sulphophenyldialkanoic acids has not been unequivocally established, though

Taylor and Nickless (1979) may have found two. Neither have esters between sulphophenylcarboxylic acids and their pre-cursors, sulphophenyl alcohols, been reported (such esters have been identified in degradation studies of long chain aliphatic hydrocarbons). Similarly, none of the above types of compounds, but without the sulphonic acid group, has been reported, and, apart from the work of Swisher (1987), attempts at such detection have not been published. Techniques with appropriate detection limits are now available for the analysis of such compounds, so that it should be possible to resolve the question of intermediates, and their possible ecotoxicity, and the more important question of completeness of mineralisation.
Most reports of the presence of intermediates refer to laboratory die-away tests and very little work has been carried out with environmental samples, presumably because of the analytical problems. However, these problems have largely been overcome and attention should now be paid to the occurrence of these compounds in environmental samples.

4.2 Removal in soil and subsurface sediment

LAS reaches the soil by two main routes; by discharge of septic tank effluents, and by spreading of sewage sludge in some cases undigested but usually following anaerobic digestion. Irrigation water can also be a source of LAS reaching the soil. Septic tank effluent contains LAS in the range <1 to 10 mg/l. Irrigation water usually contains less than 1 mg LAS/l but it can have higher concentrations if it is abstracted from polluted sources. Sludge is usually applied in the wet form but dry application is also practised. Digestion does not reduce the LAS concentration of the sludge (see Section 4.3) while drying on open beds for several months reduces the LAS concentration to between 5 and 10 % of that of the wet sludge (Sedlak and Booman 1986a,b).

4.2.1

Septic tank effluent

Reneau and Pettry (1975) reviewed earlier work on the behaviour of MBAS in natural soil systems. Removals of up to 97% MBAS were observed after passage of sewage effluents through 1.2 m of pine-forest soil at an application rate of 5 cm wastewater per week over a 3-y period. Even greater removals were found with cropland, but under winter conditions and higher application rates MBAS rapidly penetrated to depths of 5 m after 6 months. Other studies revealed that regions of LAS contamination seemed to coincide with poorly drained soils; if saturation occurred and the concentration of dissolved oxygen decreased, LAS would not be biodegraded.

Reneau (1979) monitored the concentration of LAS (MBAS) in shallow ground waters receiving septic tank effluents. He found that the concentration of MBAS decreased logarithmically along the line of flow, and that the rate of removal was sufficient to ensure that the LAS had virtually disappeared well before the water reached the local stream. At one site the concentrations of MBAS 0.5 m from the drainfield were 0.8 (winter) and 0.5 mg/l (summer) respectively, and at 6 m the concentrations were reduced to 0.1 mg/l. At another site it was calculated that 99% was removed within 8 m. There was no direct evidence in any of the studies that the removal of LAS was due to biological action.

Thurman et al (1986) reported that, at an Air Base in the USA where the treatment plant effluent discharged to an aquifer, LAS (as MBAS) had been removed rapidly in the first 300 to 600 m down gradient of the sand beds. The study also revealed that branched-chain ABS, banned since 1965 in the USA, had not disappeared but had travelled in the aquifer at the same rate as specific major anions and cations and boron, and was well separated from the LAS.

4.2.2 Soil/sludge

Two reports from Japan indicate that LAS, including the benzene ring, was degraded by the indigenous microbial population present in soils. Inoue et al (1978) found as much as 90% removal in 30 d with a half-life, calculated by Ward and Larson (1988), of 10 d. Kawashima and Takeno (1982, abstract only) measured the production of $^{14}CO_2$ from the Ca and Na salts of ^{14}C ring-labelled C 12-LAS in two soils. The pattern of $^{14}CO_2$ evolution was similar for the two salts reaching 35 to 50% of the theoretical value in 60 d. Ward (1987) calculated from the authors' data that the half-life was 12 to 20 d and also that up to 40% of ^{14}C was in the soil residue which was assumed to be biomass and soil organic matter. The abstract states that neither salt seemed to be absorbed by peanut plants up to 30 d after foliar application.

Abe and his co-workers (Abe et al 1982, Abe 1984, Abe and Seno 1985a, 1987) used the soil perfusion method to determine the removal of LAS at concentrations of up to 100 mg/l. Initial LAS concentrations of 50 to 100 mg/l, measured as LAS and DOC, were removed after a few days lag. DOC removal was at a lower rate, but both rates depended on the chemical and physical properties of the soil. Subsequent LAS additions were removed without lag, and no lag was observed when the initial LAS concentration was 20 mg/l. In one abstract (Abe 1984) it is claimed that C 12-LAS exhibited a longer lag than the C 10, C 11 and C 13 homologues, although all four subsequently degraded at about the same rate. On the other hand, in another abstract (Abe and Seno 1985a) the rate is said to be clearly dependent on the length of the alkyl chain and position of the sulphophenyl group, according to the Swisher rules. In a later paper in English, Abe and Seno (1987) also claim that the Swisher rules are obeyed. All the LAS (expressed as ferroin-reactive substances) was removed within 10 d and DOC in 50 d in one soil, while in a second soil a residue of about 10% DOC remained after 60 d. No half-life values were given.

In the UK, Gilbert and Kleiser (in press) have found half-life periods of 8 to 10 d with 60% of $^{14}CO_2$ being evolved in 25 d and 80% being reached after 100 d. Acetate was removed by samples of the same soils at a higher rate; however, only 40% of $^{14}CO_2$ was evolved in 10 d rising to only 60% after 100 d. This was thought to be due to assimilation of ^{14}C by the biomass and subsequent slow release by endogenous metabolism.

Soil taken from grassland in Switzerland which had been fertilised with 5 t sludge/hectare.y for 10 y, was analysed for LAS by HPLC (Giger et al 1987). Samples of soil taken during the 104 days immediately following the last application showed decreasing LAS concentrations from 45 mg/kg to 5 mg/kg and Ward and Larson (1988) have calculated that the rate constant for this decay was 0.16 d^{-1} and the half-life 4.4 d. Giger et al considered that the concentration on day 104 (5 mg/kg) was still a cause for concern and possible toxicity effects to soil populations should be investigated.

In the USA, soils, which had received anaerobically digested sludge for 5 to 10 y, were amended with various ^{14}C ring-labelled LAS homologues and the rate of $^{14}CO_2$ production was measured (Ward and Larson 1988). Degradation was delayed for 1 to 4 d in soil from Rapid City, SD, and the half-life for $^{14}CO_2$ was 18 to 26 d with an average yield of 65%. Initial concentrations of 2.5, 25 and 250 mg/kg each gave a $^{14}CO_2$ yield of about 70%, with half-lives of between 18 and 26 d, and 35 d for the highest concentration. Soil taken in 1984 from Harleysville, Pa, exhibited longer delays of 15 to

20 d but similar half-lives of 18 to 19 d and yields of 69 to 73%. However, in 1986 the soils from Harleysville were much more active, the half-lives being only 1.8 to 2.1 d and the yield about 50%. The activity of the soil towards hexadecane also increased, the half-lives decreasing from 12 d to 5.9 d.

Thus, the range of values for the half-life for $^{14}CO_2$ production (or ring decay) was about 3 to 35 d depending on the soil and initial LAS concentration but irrespective of the homologue used (Ward and Larson 1988).

Larson and De Henau (1988) also reported the half-lives of LAS homologues (C 10-C 14) in the Rapid City and Harleysville soils; the mean half-life ($^{14}CO_2$) at Rapid City was 22 $\pm$ 3 d while at Harleysville it was 19 $\pm$ 1 d. However, much higher rates of LAS removal ($^{14}CO_2$ production) were reported by Larson and Bishop (1988) for 7 soils. The half-lives were as low as 1.5 to 3.3 d, even though the soils were collected from sites with no known history of exposure to surfactants.

In recent studies in the UK Holt et al (submitted) determined the rate of removal of LAS (by HPLC measurements) from sludge-amended soils in 5 fields at three locations and for three different methods of application. The half-lives of LAS ranged from 7 to 22 days (for initial concentrations of LAS from 40.3 mg/kg to 2.5 mg/kg) which agreed well with laboratory findings and monitoring studies. Changes in the distribution of the C 10-C 14 homologues were said by the authors to indicate that removal was due primarily to microbial activity rather than to leaching.

Confirmation of the above half-lives of LAS added to soils is given by Litz et al (1987). In spring and summer, primary LAS removal from plots of various German soils amended with 5 g LAS/m^2 and 50 g LAS/m^2 occurred with half-lives of 5 to 12 d and 11 to 16 d respectively, as determined by the Azure A method. The lower rate of application represents a normal rate and is equivalent to about 30 mg LAS/kg soil, while the higher rate of application is much higher than normal. In winter the half-lives were higher, at 68-117 d.

In laboratory tests with ^{14}C-labelled LAS, mineralisation occurred at similar rates; in 7 d 4-52% of ^{14}C-LAS was removed aerobically in various soils and in 14 d the removal was 15-73% at 24 °C, but at 5 °C or with water saturation (anaerobic) <1% was removed in 14 d. It was also shown that <1% LAS was lost by volatilisation under all test conditions.

4.2.3 Subsurface sediment

Subsurface soil, collected aseptically from two sites in Ontario, made into slurries with sterile groundwater taken from the same sites, were incubated with 5 µg ^{14}C-labelled C 13-LAS/l. LAS was mineralised to a high degree, ~80% with an estimated half-life for ultimate biodegradation ($^{14}CO_2$ production) of 27 h (see Table 4.7). This indicates that the microbial population in the subsurface soil was highly adapted to LAS (Larson 1984).

Federle and Pastwa (1988) determined the LAS removal in sub-surface sediments beneath a laundromat waste treatment pond and a similar control pond. LAS was mineralised at all depths tested in the laundromat sediment profile without lag and with half-lives ranging from 3.2 to 16.5 d. The rate of mineralisation in the control profile was slower with half-lives of 5.2 to 1540 d after lags of 2 to 40 d. The average $^{14}CO_2$ yield for all experiments was 33% and the fate of the remaining

^{14}C was not investigated. It is of interest that ^{14}C-benzylamine, tested at the same time, yielded only 31% CO_2 but had a half-life of 3.3 d.

Further work by Procter and Gamble is in hand on the kinetics of ^{14}C-LAS removal in subsurface soils and sediments and its relationship with soil properties and microbial population. This is expected to substantiate and extend the kinetic data already obtained and may throw further light on the mechanism of degradation.

4.3 Removal of LAS in sewage treatment

A selection of the data available on the removal of LAS by sewage treatment is given in Table 4.11. Provided the activated sludge plants are operated effectively and are not overloaded, more than 95% of MBAS is removed whereas the removal of LAS (measured by DS-GC or HPLC) is even higher at 95 to 99%. Biological (trickling or percolating) filters are more variable in performance, with percentage removed dependent on factors such as loading rate, retention time and temperature. Data for full-scale filters indicate 67-85% removal of MBAS at UK plants and 73-87% removal of LAS at some treatment works in the USA, although removals as high as 96% MBAS have been observed in larger scale pilot filters (Painter and King 1979b). Sludge digestion does not degrade LAS under anaerobic conditions; there is evidence, however, that intermediates will degrade further during anaerobic digestion.

4.3.1 Activated sludge treatment

In four field studies, Weaver (1965) observed a rough correlation between removal of BOD and that of MBAS; in nearly all cases more MBAS than BOD removal was recorded. Later, Tang (1974) reported a "significant relationship" between removal of BOD and MBAS for both activated sludge and filters.

Table 4.11. Removal of LAS in sewage treatment

Type of treatment	% Removal MBAS	LAS	Reference
Activated sludge	95	-	Klein & McGauhey (1965)
Trickling filter	85	-	
Activated sludge	93-98	-	Painter & King (1979a,b)
Large pilot filters	85-96	-	
Filters & activated sludge	85-98	-	STCSD (1967-1978)
Filters & activated overloaded problems	67-85	-	
Extended aeration	96	95	Simko et al (1965)
Small scale activated sludge	>97(20 °C)	-	Stiff & Rootham (1973)
Sludge	>90 (5-8 °C)	-	
Sludge	87	-	Janicke & Niemitz (1973)
Activated sludge - 1 yr	95	-	Wagner (1978)
Activated sludge	94-95	99	Sedlak & Booman (1986a,b)
Activated sludge			
USA	-	98	De Henau et al (1986b)
Canada	-	96	
Germany	-	99	
Filters (USA)	-	73-87	Woltering et al (1987)
Activated sludge 5 plants	-	99.5	Giger et al (1987)

However, the relationship is probably not very precise as shown, for example, by the results of Sedlak and Booman (1986a,b). They obtained about the same removal of MBAS (94, 95%) for two activated sludge units, at a municipal treatment works, run in parallel at widely different operating conditions (sludge retention times of 0.8 and 3.2 d), but the removals of BOD were different (83 and 91% respectively).

During a 1 year study in W Germany, the performance of an activated sludge plant at Büsnau-Stuttgart, serving about 3100 inhabitants was investigated (Wagner 1978). A special heavy-duty detergent was distributed and the average removals over the year were 95% MBAS, 96% BOD and 81% COD.

The effectiveness of German sewage treatment plants in degrading LAS, among other surfactants, has been evaluated in other studies (Gerike 1987) and in general the actual performance in treatment plants correlated well with the expected results based on laboratory tests.

The effect of temperature was determined by Stiff and Rootham (1973) for the removal of MBAS in laboratory scale activated sludge units treating sewage prepared from human excreta containing added LAS, starch etc, at normal rates. At 5 to 8 °C the MBAS removal was >90% which increased to >97% at 20 °C.

Some workers report MBAS removals of <90%; for example, Janicke and Niemitz (1973) using model activated sludge units recorded variable results. At 10 mg LAS/l MBAS removal was 87% whereas at 20 mg/l the MBAS removal varied between 37 and 89%. The authors attributed the variability to inhibition by LAS, beginning at about 20 mg LAS/l, although the "synthetic sewage" they used may have been deficient in phosphate. Painter and King (1978) also have shown that the recipe used by Janicke and Niemitz (1973) is deficient in phosphate when prepared from some peptones and that poor and variable removals of LAS were remedied by the addition of a phosphate salt (phosphate is now incorporated in the OECD "synthetic sewage").

The removal of LAS specifically has been reported by a number of authors. Sedlak and Booman (1986a,b) found as much as 99% LAS removed in the two activated sludge units referred to above; 96% was biodegraded and ~3% was removed on the surplus activated sludge. For three other USA plants, De Henau et al (1986b) reported an average of 98% removal of LAS and for three activated sludge plants in Canada, 96% was removed, while one plant in Germany eliminated 99% LAS.

4.3.2 Biological

In Switzerland the performance of eight activated sludge plants has been reported by Giger et al (1987). The range of LAS removal was 65 to 99.8% with a median of 99.5%. Two of the three plants with relatively low removals were overloaded and the third was a small plant without preliminary sedimentation. The authors also conducted 24 hour surveys on two occasions at the Zürich Blatt installation and obtained LAS removals of 99.3 and 98.7%; 83 to 86% was biodegraded, while 13 to 16% was adsorbed on the sludge. The proportion adsorbed in this case was much higher than the 3% reported by Sedlak and Booman (1986a,b).

A few Japanese papers on this topic have been published, but only abstracts have been consulted so that it is difficult to draw valid conclusions from the few results given. Sekiguchi et al (1975b, abstract only) investigated the performance of a municipal treatment plant (activated sludge?) over the course of a year. Although 90% BOD was removed, the removal of LAS was only 85%. The removal of MBAS at two municipal plants was only 77%; while that for LAS was 82% (Ohba et al 1975). On the other hand, Higashi et al (1976) reported 97% removal of LAS from a "synthetic wastewater" containing the surfactant. In an "activated sludge test" >90% MBAS was removed in 7 d (Yagi 1980, abstract only), but the removal of LAS-carbon was lower. Finally, Utsunomiya et al (1986, abstract only) determined "free" and "complex" (undefined) LAS in sewage and effluent from an activated sludge plant by the methylene blue and HPLC methods. In the primary sedimentation tank 58% of the "free" and 0% of the "complex" LAS was removed, while in the aeration and secondary settlement tanks the removals were 16 and 87% respectively.

In the UK, removal of MBAS in large pilot-scale filters ranged from 85 to 96% over the period 1966 to 1979; there was an increase in removal over the period as the prospects of ABS decreased (Painter and King 1979b). Data from a larger number of treatment works in the UK are recorded in the Appendices to the Progress Reports of the Standing Technical Committee on Synthetic Detergents (STCSD) 9th to 18th Reports (STCSD 1967 to 1978). While values as low as 67% MBAS removal are recorded, the large majority of values are between 87 and 98%. Some of the low values were known to be associated with overloading or operational problems.

In the USA high rates of sewage are often applied to filters compared to those in Europe. Rates in the USA are usually greater than 200 US gallons/sq ft.d (= 4.5 m^3/m^3.d; = 0.4 Kg BOD/m^3.d)* while in the UK, for example, common rates are 50 to 100 imperial gallons/cu yd.d (= 0.3 to 0.6 m^3/m^3.d = 0.075 to 0.15 Kg BOD/m^3.d)*.

The difference in rates is not solely because of the difference in the strengths of USA and UK sewage, but also because of different operating practice.

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* Assumptions made are that filters are 6 ft deep and that BOD = 100 mg/l in USA and 250 mg/l in UK.

As a consequence the percentage removal of LAS by trickling filters in the USA is not as high as in Europe. For example, Woltering et al (1987) found only 73 to 87% removal of LAS for several trickling filters in the USA and Klein and McGauhey (1965) reported 95% removal of MBAS for activated sludge units but only 85% for trickling filters in the USA. They also recorded high removal rates for septic tank-percolating field systems (97% MBAS) and for lagoon treatment (93%), but for high-rate lagoons only 56% was removed.

.3.3

:ffect of retention time

The 7th Report of the Standing Technical Committee on Synthetic Detergents (STCSD 1964) illustrates the effect of sewage retention time in laboratory activated sludge units and in biological filters on MBAS removal. For example:

Activated sludge:	Retention (h)	BOD (mg/l)	Percentage removal of MBAS
	2	31	88
	8	5	95
Filters:	Application rate $m^3/m^3.d$		
	0.72	16	91
	0.36	3	96

.4

)il

In assessing the ability of soils to treat wastewaters, Tanimoto et al (1983) found that "clinker"-type soil was able to remove LAS when dosed intermittently or continuously, while three other types did so only in the intermittent mode.

3.5

aerobic treatment

Early work, reviewed by Gledhill (1974), indicated little or no LAS (as MBAS) removal during anaerobic digestion (retention time, 25 to 30 d) and there are other early studies to support this (eg Bruce et al 1966, Swanwick et al 1969). Indeed, the concentration in digested sludge (expressed on a dry weight basis) often showed an increase over that of the fresh sludge from which it was derived during digestion since there is an overall loss of organic matter. Also, concentrations of LAS over about 10 g/kg begin to cause inhibition of methane production.

Recent work has confirmed the lack of degradation, using specific methods of analysis (Sedlak and Booman 1986a,b).

The biodegradation of ^{14}C-labelled LAS homologues and model hydrocarbon and ring compounds under aerobic followed by anaerobic conditions was studied by Ward (1986, abstract only) in a batch system developed for the purpose. The half-life for $^{14}CO_2$ production aerobically for LAS homologues was 1.5-3 d and 0.9-2 d for the model substrates. In the subsequent anaerobic period, the half-life for both the LAS homologues and model substrates was 3-4 d, indicating that, since intact LAS does not degrade anaerobically, the pre-aerobic treatment causes changes in the LAS molecule which permitted subsequent degradation under anaerobic conditions.

Only one set of data has been published on the removal in treatment plants of LAS and any intermediates.

Leidner et al (1976) reported the presence of a number of sulphophenylakanoic acids in effluents from four treatment works in Switzerland. These acids which had also been found in die-away tests, were not apparently determined quantitatively, and only the concentration of "LAS" in effluents was reported. However, recently Gilbert and Kleiser (in press) have re-examined the Leidner et al (1976) data and have found from the authors that the concentration of "LAS" given included the concentrations of the intermediates in the effluents. This arose because, after de-sulphonation, the phenylalkanes, from LAS and the phenylcarboxylic acids, from the intermediates, were all revealed as peaks on the chromatogram and the authors calculated the concentration of "LAS" by estimating the area of all the peaks on the chromatogram. Thus, the concentrations given for "LAS" included all the intermediates and these were called by Gilbert and Kleiser (in press) "LAS and residues". By making assumptions about the concentration of LAS in three of the four sewages - only one was given by Leidner et al (1976) - Gilbert and Kleiser (in press) calculated the percentage removals of "LAS and residues" to be as high as 92, 99.7 and >99.7%.

The removal of the benzene ring of LAS cannot easily be ascertained in full scale sewage treatment plants, but several investigations on this topic have been made on the laboratory-scale, see Section 4.1.3.

.4 Removal by physico-chemical processes

.4.1 hlorination

The possibility of the formation of chloroform and other chlorinated byproducts in the disinfection by chlorine of waters containing LAS was investigated by Itoh et al (1985). Separate solutions of glucose and LAS, at 6.2 mg C/l, were treated with various concentrations of chlorine and stored for 24 h at 20 °C. Glucose produced no chloroform but LAS did, when the chlorine concentration was greater than 3 mg/l. The yield of chloroform at 5 mg Cl_2/l was 0.004% (based on C), or about 1.5 µg $CHCl_3$/l from 6.2 mg LAS-C/l. Samples taken from a biodegradation test of LAS (6.2 mg C/l) gave, when chlorinated, higher yields of chloroform rising to a maximum of 0.066%, or about 25 µg/l, after 4 d storage. Similar samples taken from a biodegradation test of glucose produced much higher concentrations of chloroform, 200 µg $CHCl_3$/l after only 1 d storage. The known aromatic intermediates, sulphophenylundecanoic acid and sulphophenylbutyrate, when chlorinated, produced only about the same amount of chloroform as LAS. Other compounds were tested and it was found that acetoacetic acid, an intermediate of fatty acid metabolism, gave much higher yields of chloroform, of up to 13.9% on a carbon basis, than any other compound tested. Kosaka et al (1983) (abstract only) state that the production of trihalomethanes from LAS by chlorination is higher at higher temperatures and at higher rates of degradation; they also state that the chloroform production is higher with the degradation products of soap than of LAS.

On the other hand, no observable reaction occurred between 9.3 mg Cl_2/l and an isomer of dodecylbenzenesulphonate (SDBS) at 0.1 mg/l, as measured by HPLC; neither were any by-products observed by HPLC or by FAB-MS analysis. However, it is claimed that the small changes in concentrations reported by Itoh et al (1985) would not have been detected by the analytical techniques employed (Haley J, WRc, unpublished).

No by-products were observed by FAB-MS after ozonation of LAS; the effect of ozone on the concentration of LAS has not yet been determined (Haley, J, WRc, unpublished).

degradation

In a study of the kinetics of the photodecomposition of C 12-LAS (primary or secondary not stated), Matsuura and Smith (1970), using a continuous flow reactor, observed complete conversion to intermediate products in 1 min and the production of 7 moles of CO_2/mole LAS in 20 min. The initial concentration of LAS was 60 to 182 mg/l and the radiation applied was in the range 2000 to 4500 A°. (It was not possible to discern the quantity of energy used.) The authors assumed that the seven C atoms originated from the alkylchain. The reaction rate was increased by 2 orders of magnitude in the presence of ferric perchlorate.

The action of UV light from a mercury lamp (10^{16} ml/quanta.ml.s) on aqueous solutions of sodium salts of benzene sulphonate, and C 8, C 9 and C 10-LAS was determined by Tchekulaev (1979). The rate of disappearance of LAS decreased with increased number of C atoms in the alkyl chain, from 59 mg/l.h for benzene to 19 mg/l.h for C 10 LAS; desulphonation accounted for a large portion of the total degradation. The results were used in interpreting the self-purification of wastewater lagoons.

Hidaka et al (1985) reported a rapid (1 to 2 d) photodegradation of Na C 12-LAS in aqueous TiO_2 suspensions, in the absence of noble metal catalysts. Suspensions containing 50 mg LAS/l and 2000 mg TiO_2/l, in special vessels holding 25 ml aliquots, were irradiated for 5 d by the output of a 450 W Xe lamp with wavelengths >33 nm. The reduction in UV absorbance indicated that the benzene ring degraded faster than the alkyl chain. It was thought that the adsorption of the surfactant on to the TiO_2 surfaces made the primary step efficient. It was considered that the rate of reaction would increase with a noble metal catalyst and that the detoxification of waste streams using artificial light or sunlight could have important applications.

Decomposition of LAS dissolved in distilled water and irradiated with ^{60}Co depends on the total amount of energy absorbed and not on the dose rate. The γ-rays react with water to yield O_2, H_2O_2 and HO_2, which are powerful oxidising agents. The absorption of 10K rads lowered the concentration of LAS from 10 mg/l to 7.8 mg/l, while 100K rads reduced the concentration to 0.9 mg/l (Rohrer and Woodbridge 1975).

Kubodera et al (1978) irradiated 530 mg primary C 12-LAS/l with 500 Ci (^{60}Co source) at a dose rate of 1.3×10^5 R/h; the concentration after 18 h was 208 mg/l. After desulphonation the sample was subjected to GC and MS and, as expected, the main product was dodecylbenzene from the unchanged LAS, with one other major and two minor peaks, none of which was identified.

An understanding of adsorption of LAS is of importance for the interpretation of biodegradation data, especially when suspended solids are present at high concentration, and for predicting the fate of LAS in the environment. For example, in the 1960s it was claimed that ABS including LAS was removed in activated sludge processes mainly by adsorption on the sludge, which is continuously wasted from the process to maintain the desired sludge retention time, rather than by biodegradation. However, Sweeney and Foote (1964) found that an insignificant amount (2 to 3%) was removed by adsorption and Sweeney (1966), using ^{35}S-labelled LAS, observed that only 1.4% LAS left the unit bound to sludge.

In common with other surfactants, LAS adsorbs to surfaces and to varying degrees to suspended particles in sewage, sludge, sediments and soil. The nature of the adsorbent plays an important part with inorganic substances generally adsorbing much less than organic. Adsorption on to biomass is high and appears to be related to the ability of LAS to interact with proteins. Many reported results illustrate this ability, the most common being good correlations between the organic carbon content of a sediment and its adsorptive power, although there are other, complicating factors.

Results on adsorption in the literature are not always presented in a way suitable for incorporation into the Freundlich equation; often only the percentage adsorbed is given. Some results are presented in Table 4.12, which include values calculated by Swisher (1987) and by the reviewer (HAP). The results are expressed as the Freundlich isotherm constant, or the adsorption partition coefficient (Kd) if only one concentration of LAS was reported.

The Kd values for primary sewage sludge range from 590 to 1440 l/kg and were obtained at LAS concentrations of 10 to 20 mg/l; primary sludge is usually treated by anaerobic digestion. For activated sludge Kd varied between 890 and 5200 l/kg and, as for primary sludges, the Kd values were obtained at realistic LAS concentrations for sewage treatment of 1 to 30 mg/l, except one concentration which was 80 mg/l. The Freundlich equation was linear, at least up to 50 mg LAS/l, indicating that the adsorption sites were not saturated (Bruce et al 1966). The data of Yoshimura (1984), calculated by Swisher (1987), for mixed C 10 - C 13-LAS and two isomers, show that the shorter chain length (C 10) is less adsorbed

Table 4.12. Adsorption partition coefficients of LAS

Adsorbent	Adsorption partition coefficient Kd(l/kg)	Concentration in liquid phase (mg/l)	Reference
Primary sludge			
	590 - 1400	10 - 20	Bruce et al (1966)
	900	10 - 20	Swanwick et al (1969)
	1000	10 - 20	Osborn (1969)
Activated sludge			
	5200	-	Sweeney and Foote (1964)
	3800	1 - 20	Water Pollution Research (1965)
	940	-	Oba (1971)
	3000 - 5000	3 - 20	Janicke and Niemitz (1973)
	660 - 1100	4 - 80	Brüschweiler and Gämperle (1982)
	2500 - 4000	3 - 30	Urano and Saito (1984)
2-phenyl C 10	47	0.6	Yoshimura (1984)
2-phenyl C 12	555	0.1	
mixed C 10-C 13	565	12	
River sediments			
	60 - 280	0.06 - 0.4	Hon-nami and Hanya (1980b)
	37	0.5	Miura and Nishizara (1982)
	300	-	Larson and Vashon (1983)
	6 - 91	5 - 40	Urano et al (1984)
	237 (138-360)	0.25 - 15	Matthijs and de Henau (1985)
Soils			
	2	45	Mansell et al (1970)
	2 - 4.5	15 - 200	Acher and Yaron (1977)
	20	-	Inoue et al (1978)
Zeolite A			
	5	5	Savitsky et al (1981)

than the longer chain (C 12). Generally the Kd values for river sediments are lower than for sewage sludges and those for soils are still lower.

Bruce et al (1966) reported that the effect of temperature on Kd was small and that varying the contact time between 1 min and 6 h, had negligible effect. Yoshimura and Nakae (1982b) also found that temperature had little effect, but that equilibrium was reached within 21 h. Lowering the pH value increased Kd until the isoelectric point was reached. Hon-nami and Hanya (1980b) found that Kd values for three river sediments increased exponentially with the number of C atoms in the alkyl chain; log Kd for C 10 was 1 to 1.4, and for C 13 it was 2.7 to 3.1.

Larson and Payne (1981) showed that adsorption of ^{14}C ring-labelled C 12-LAS to the clay silt fraction of river sediments was low, most of the ^{14}C being bound to biomass. Sediments containing about 30 times more biomass adsorbed about 15% of the ^{14}C compared with 5% for the low biomass sediment.

Using activated sludge and pure bacterial cultures, Urano and Saito (1984) reported that sterilisation by autoclaving or formaldehyde, or growth on glucose, had no effect on Kd but that the adsorption was lower for cells in the endogenous phase for 1 or 2 d; no explanation was given.

In their study of seven sediments from four rivers, Urano et al (1984) found Kd values of 6 to 91 l/kg and were able to demonstrate a good correlation between Kd and the organic content of the sediment. For example, the sediment with the lowest C content (0.7%) had a Kd of only 6 l/kg whereas the sediment with the highest C content (6% C) had the highest Kd of 91 l/kg. Based on the C content rather than on total weight of sediment, Kd on average was 1900 l/kg. They also concluded that most of the adsorption probably occurred on the biomass rather than on the humic acid fraction of the sediments.

Matthijs and de Henau (1985) used ^{14}C-labelled C 13-LAS for detecting and quantifying the fate of LAS in their study of sediments from seven Belgian rivers. They equilibrated the samples for 4 h, but their data suggest that 1 h would have been sufficient. Both the Langmuir and Freundlich equations were obeyed over the experimental concentration range of 0.25 to 15 mg LAS/l. As with most other studies the values of n in the Freundlich equation was around 1:

$$x/m = Kd\ C^{1/n}$$

where x/m = concentration of LAS in the sediment and C = equilibrium concentration of LAS in the liquid phase. The values of Kd obtained were within relatively narrow limits (233 ± 77 l/kg), but despite this there was still a positive correlation between Kd and the organic C (0.34 to 3.4%) and clay contents, but not with the Fe and Al oxide concentrations of the sediments. Desorption, rarely studied, was also investigated since it is important in the interpretation of adsorption phenomena in the environment. Sediment containing ^{14}C-labelled C 13-LAS was washed in three successive steps with KCl solution, and the ^{14}C was determined in the aqueous and solid phases. The Kd value was lower than for adsorption, namely 93 ± 34 l/kg for the four sediments tested; it was calculated that, under the conditions employed, 57% of the added ^{14}C was adsorbed in total of which 31% was irreversibly adsorbed. Thus, argued the authors, the sediment is not just an intermediate compartment for accumulation but acts as a sink for LAS. The authors concluded that the degradation rate of LAS adsorbed on the sediment should be investigated aerobically and anaerobically, together with the toxicity of LAS to organisms in river water and in the sediment.

In contrast to the results obtained for sediments, Litz et al (1987) found adsorption to soils to be directly related to the content of iron oxide as well as organic C, and inversely related to the pH of the soil.

In a validation of EXAMS (Exposure analysis modelling system) Games (1982) reported the effect of chain length on Kd but no details of concentration of LAS were given. Table 4.13 shows the progressive increase in adsorption onto activated sludge and sediments of representative phenyl isomeric mixtures of the C 10 to C 14 homologues. Sludge had about ten times the adsorptive capacity of sediments and Kd for each homologue was about 3 times that for the homologue with one less C atom in the chain. Games (1982) concluded that the success of the model depended largely on two least-understood parameters - sediment/water interface dynamics and biodegradation in sediments. The value of Kd (330 l/kg) for the C 12 homologue adsorbed onto sediment was selected by Holysh et al (1986) in successfully assessing the environment fate of LAS by their fugacity models.

Table 4.13. Effect of chain length on the adsorption of isomeric mixtures of LAS homologues - Games (1982)

Homologue	Adsorption partition coefficient Kd (l/kg)	
	Activated sludge	River sediment
C 10	220	41
C 11	1000	100
C 12	3070	330
C 13	9330	990
C 14	-	2950

The first systematic study of the structure-activity relationship for adsorption of LAS on river sediments at relevant environmental concentrations has been made by Hand and Williams (1987). They used ten ^{14}C-labelled isomers or mixed isomers of LAS at 10 to 1000 µg/l, usually 100 µg/l, and sediments from four rivers. Adsorption equilibrium was reached in less than 3 h and desorption took less than 8 h; biodegradability was thought to be insignificant. Table 4.14 gives the results for the ten products tested for the sediments with the highest and lowest adsorptive capacity. Kd increased by a factor of about 2.8 for each added methylene group, similar to the 3-fold increase found by Games (1982), and it also increased by a factor of about 2 from the 5-phenyl to the 2-phenyl isomer. The value of Kd varied by two orders of magnitude for a given sediment from C 10 to C 14 and by four orders of magnitude - from 3 to 26 000 l/kg - as sediment type, chain length and phenyl position were varied. Adsorption correlated well with silt content but not with organic carbon content which is different from the results obtained by Urano et al (1984) who found good correlation with carbon content. The use of different natural waters had no effect on the Kd value for a given sediment nor was the Kd value of 2-phenyl C 12-LAS influenced by the use of five source waters of different hardness.

Hand and Williams (1987) found that under the conditions used, single-step desorption was rapid and almost complete (Table 4.15), which is in contrast with the findings of Matthijs and de Henau (1985). The authors suggested that, since adsorption increased with increasing LAS hydrophobicity, adsorption is a hydrophobic mechanism.

Table 4.14. Adsorption of LAS at low concentrations

LAS homologue/isomer		Log Adsorption partition coefficient (log Kd)+ Sediment EPA-B1*	Sediment RC 3*
	(5-phenyl	0.5	2.2
C 10	(mixed phenyl	0.6	2.3
	(2-phenyl	0.7	2.3
C 11	(mixed phenyl	1	2.7
	(6-phenyl	1.2	3.1
C 12	(mixed	1.6	3.2
	(2-phenyl	1.7	3.5
C 13	mixed	2	3.7
C 14	(mixed	2.2	4.1
	(2-phenyl	2.6	4.3

Kd expressed as l/kg
+ Values of log Kd read from graph in Hand and Williams (1987)

* Composition of sediments (%)

Sediment	Sand	Silt	C	Clay (%)
EPA-B1	62.6	14.9	0.9	22.5
RC 3	0.2	97.4	3.5	2.4

Table 4.15. Adsorption and desorption of LAS*

LAS	Sediment	Log Kd Adsorption	Desorption
C10	RC4	1.65	1.70
C14	RC4	3.38	3.58
		3.32	2.58
		3.48	3.70
C15	EPA 5	2.67	3.07

* Hand and Williams (1987)

Miura and Nishizawa (1982, abstract only) found that LAS adsorbed on river and lake sediments but did not accumulate, since it was biodegraded at concentrations of dissolved oxygen less than 1 mg/l. Good correlations were observed (Tanaka <u>et al</u> 1983, abstract only) between C 12 LAS adsorbed onto river sediments and the weight loss on ignition of the sediment. Sediment contained relatively more C 12 and C 14-LAS than the river water in contact with it. In a series of papers, Abe and Seno (1983b,c,d, abstracts only, and 1987) studied the adsorption of LAS on soils and humic acid. Adsorption was found to be higher for the longer chain homologues, and higher for the 2- and 3-phenyl isomers than for the internal isomers; it was considered that adsorption is followed by biodegradation on the soil. Sandy soils adsorbed less than humus soils, which in turn adsorbed less than clay soils. Temperature had only a small effect on adsorption. Using UV spectra and electrophoresis, Watanabe and Kishi (1986, abstract only) followed the removal of LAS from water by natural humic substances; humic acids had a high affinity for LAS.

5. EFFECTS

In the reporting of toxicity tests (algae, invertebrates, fish etc) few authors (<15%) state categorically whether they actually determined the concentrations of LAS used in their test or whether the values stated are concentrations based on dilutions of stock solutions. Where information is given on this point it has been included in the text or in tables. Apart from any experimental errors that may have been made in preparing the test media, the possibility exists that during the toxicity tests the concentration of LAS may have decreased because of biodegradation; the longer the duration of the test, the greater chance of loss of LAS. In cases where this happens the reported EC or LC50 values will tend to be higher than they really are - that is, LAS will appear to be less toxic than it is - since it is well established that the biodegradation products of LAS are much less toxic than the intact surfactant.

1

wage treatment

1.1

ysico-chemical effects

1.1.1

aming

One problem associated with the use of detergents has been foaming at sewage works and in rivers. However, this problem has disappeared with the introduction of the straight-chain alkyl varieties of alkyl benzene sulphonates.

1.1.2

ration

It has long been known that the re-aeration rate of water bodies is lowered by the presence of surfactants. However, there is no significant effect on the performance of the activated sludge process when diffused air is used, provided that there is a normal supply of compressed air, but if the supply is only just sufficient the degree of nitrification is reduced (DSIR 1961). With mechanical aerators the aeration rate is not lowered; indeed, in some cases it may actually be increased. Since LAS is readily biodegradable the effect in practice in diffused air systems will be less than the effect of less degradable ABS. Apart from these physico-chemical effects, there was no definite evidence that LAS in the concentrations normally encountered were harmful to either the activated sludge process or percolating (trickling) filters (DSIR 1961), and no effects of LAS have been reported.

.1.3

pended solids

The addition of ABS to initially detergent-free sewage reduced slightly the settling rate of the suspended solids (Swanwick and Davidson 1961). Furthermore, when the whole detergent formulation was added much more sludge was produced, especially in hard water areas, because of the precipitation of calcium phosphate and the entrainment of flocculated organic matter and similar effects would be expected with LAS formulations.

In a survey of about 400 septic tanks in the UK (Min Housing and Local Govt 1960) no significant effects attributable to ABS were found, except that more solids accumulated in hard water areas. (Of course, had synthetic detergents not been invented, more soap would have been used and the calcium and magnesium salts of the soap acids - stearic, palmitic etc - would have been precipitated.)

5.2 Biological activity

5.2.1 Sewage treatment

There is much evidence to show that LAS has no effect on sewage treatment. An example is the treatment of "artificial" sewage, prepared from human excreta and other components, with and without LAS at 20 mg/l in the OECD confirmatory test (Stiff and Rootham 1973). Table 5.1 gives the percentage removals of MBAS, BOD and COD compared with the controls (values in brackets) at three temperatures.

The results indicate that the addition of 20 mg LAS/l had no effect on the performance of the units. However, in these particular tests the initial degree of nitrification was high (normal nitrifying activated sludge was initially added to the units) but decreased throughout the test period in all vessels, including the controls. In other tests, concentrations up to 30 mg LAS/l allowed full nitrification provided that the units had reached a steady state. Baillod and Boyle (1968) reported an apparent increase in nitrification, especially in the proportion of nitrate produced,

Table 5.1. Effect of LAS on performance of porous pot activated sludge units at three temperatures*

Parameter	Concentration in influent (mg/l)	% Removal in effluent at (°C)		
		19.5	12	8
MBAS	20±2 (0)	97 (-)	97 (-)	97 (-)
BOD	200-300	95 (95)	97 (96)	93 (91)
COD	500-600	85 (86)	85 (86)	83 (81)

* Values in brackets refer to controls without LAS.

when up to 23 mg LAS/l was added to semi-continuous activated sludge units treating a synthetic waste water.

Janicke and Niemitz (1973) and Gilbert and Pettigrew (1984) also reported no adverse effects on performance of laboratory-scale activated sludge units by up to 20 mg LAS/l. Above this concentration Janicke and Niemitz (1973) reported decreased nitrification in their extended aeration units which treated a synthetic sewage. Earlier, McClelland and Mancy (1969) had found interference in COD removal from a powdered milk wastewater at concentrations of LAS >10 mg/l; their sludge became less easily settleable and they had other difficulties with their Husmann units. It is common experience that, in laboratory-scale units such as Husmann units and Porous Pots, the microbial population and the physical properties of the sludge often change markedly in the first weeks of an experiment especially if the activated sludge used has not already been acclimatised to the influent wastewater. These artefacts do not truly represent what happens in practice and the test should either continue until a steady-stage is reached or be abandoned.

Haberl et al (1980) successfully operated, at a treatment works, a pilot plant fed with municipal sewage containing 30 to 40 mg MBAS/l. Addition of 24 to 30 mg LAS/l, to a total of about 70 mg MBAS/l, for 24 h periods did not harm the performance of the aeration unit, but did cause the sludge to be less readily settleable.

Okada and Sudo (1983) and Inamori et al (1983) used a synthetic waste water (not deficient in P) to determine the effect of adding LAS on the performance of a submerged filter in an activated sludge unit. At 10 mg LAS/l the units performed well, provided that the BOD loading was regulated at 0.8 kg BOD/m^3.d or less. When the concentration of LAS was raised in one step to 50 mg/l the removal of COD decreased but returned to normal values after about two weeks. (Soap added at only twice the normal concentration caused bulking of sludge.)

In inhibition tests, Harrison et al (1976) found that oxygen uptake by a Pseudomonad sp, which could degrade but not grow on LAS, was completely inhibited by 30 mg LAS/l at cell concentration in suspension of 250 mg/l but that 150 mg LAS/l was needed for complete inhibition at cell concentrations of 1000 mg/l. Using the OECD activated sludge respiration inhibition method (1500 mg activated sludge solids/l plus nutrient solution), neither Marlon A nor Dobane JNQ sulphonate showed inhibition at 100 mg LAS/l (Painter 1986). The presence of 15 mg LAS/l in die-away tests using air-dried sludge (30 mg/l) did not affect the removal of glucose or sucrose; in some cases, however, LAS did not degrade until the sugar had been removed (Painter and Durrant 1976). In tests with 3000 mg activated sludge/l, no effect of LAS on sugar removal was observed and LAS was degraded without lag at the same rate as in the absence of the sugars (~1 mg LAS/g dry sludge h).

Shitana and Suzuki (1981) also reported that LAS concentrations of ⩽10 mg/l had no effect on the oxygen uptake of glucose/glutamic acid solutions and that concentrations of ⩽20 mg LAS/l did not effect sewage treatment.

Anaerobic digestion of sewage sludge was not inhibited by LAS concentrations in sludge of up to about 15 g/kg dry solids; this is equivalent to a concentration of about 25 mg LAS/l in crude sewage (eg Bruce et al 1966; Pitter et al 1971 and Gilbert and Pettigrew 1984). Between 15 and 20 g/kg, reliable digestion is sometimes impaired and above 20 g/kg a more serious inhibition of gas production has been observed especially if other potentially inhibitory compounds are present. However, if acclimatisation is allowed over a sufficiently long period with gradual, stepwise increases in concentrations, successful digestion could be obtained with concentrations as high as 30 g/kg dry solids (Bruce et al 1966). Current concentrations of LAS are such that inhibition by LAS alone is extremely unlikely. To overcome inhibition caused by anionic surfactants, stearine amines have been used (Swanwick et al 1969).

.2.2
tural waters
d sewage effluents

Larson and Maki (1982) determined the effect of two concentrations (0.5 and 5.0 mg/l) of C 12-LAS on the structure and function of microbial communities in model ecosystems (lake sediment plus overlying flowing water) fed with either well water or sewage effluent. The sewage effluent would have contained partial breakdown products since the continuous activated sludge unit from which it had been obtained was deliberately operated to give only 50% LAS removal. With well water, LAS had no effect on the microbial structure of the aqueous phase at either 0.5 or 5.0 mg LAS/l and microbial function was unimpaired at the lower dose. At 5 mg/l both glucose and LAS degradation were inhibited. However, the microbial activity of water taken from a river exposed to sewage effluent was not affected by 5 mg LAS/l. When sewage effluent was fed to the ecosystem, the activity of the microbial population was also not decreased at either level. The authors concluded that function measurements were better than determination of structure in characterising microbial populations and that the activity of microbial communities in relatively simple model ecosystems closely approximated that of natural populations in surface waters.

Larson and Ventullo (1987, submitted to Water Research) followed up this study by determining, in the field, the effect of a range of concentrations of C 13-LAS on the activity of epilithic bacterial communities grown on plates suspended in a river. They also measured the chronic effect (21 d) of LAS on these communities in bioassay tubes suspended in the river, both above and below an effluent outfall. Heterotrophic activity was determined as the turnover time of ^{14}C-labelled glucose used as test substrate. LAS had little effect on the total biomass, as determined by acridine orange direct counts. The results, in Table 5.2, show that the microbial activity was higher below the outfall than above it (turnover times of 4.2 h and 12.9 h, respectively) and at neither site did concentrations up to 0.1 mg LAS/l affect the activity. Concentrations of 1 mg LAS/l significantly lowered the activity at both sites in the acute tests, and the effect was progressively more pronounced up to 100 mg LAS/l. However, chronic exposure to LAS for 21 d brought about a complete recovery of microbial activity and in some cases even an increase.

Table 5.7. Effects of acute and chronic exposure to LAS on the activity of epilithic bacteria - Larson and Ventullo (1987) (Submitted to Water Research)

Study site	Concentration of C 13-LAS (mg/l)	Glucose turnover time Day 0 (acute)	Day 21 (chronic)
Above outfall	0	12.9	6.4
	0.01	11.0	7.7
	0.1	11.6	4.2
	1	21.4	3.2
	2	-	1.3
	10	114	-
	100	510	-
Below outfall	0	4.2	3.0
	0.01	5.0	3.9
	0.1	5.4	3.7
	1	10.7	2.6
	2	-	1.8
	10	142	-
	100	390	-

The same authors (Ventullo et al 1988b, in press), using ^{14}C-labelled C 13-LAS to study biodegradation, found similar effects in a lake ecosystem. Again, bacterial numbers were not affected (acridine orange direct counts) by any concentration of LAS added to lake water in bottles or carboys suspended in the lake for 3 h or 21 d. Acute exposure to concentrations above 0.5 mg/l gave higher glucose turnover times (46 to 556 h) than the controls or concentrations up to 0.1 mg/l (10 to 17 h).

As before, chronic exposure for 21 d eliminated the effect of increased turnover times, with the control and 100 mg LAS/l giving turnover times of 6 h and 3 h respectively. Thus, chronic exposure of lake microbial communities to C 13-LAS had no long-term detrimental effects on bacterial activity and resulted in increased biodegradation of LAS (see Section 4).

5.2.3

Sea water

The microbial activity of sea water, as determined by ^{3}H-thymidine incorporation and ^{14}C-labelled glucose utilisation, was inhibited by the addition of LAS of unstated type and nominal concentration. The EC 50 values (the concentration producing 50% inhibition) over 30 min - 1 h were 5.1 and 0.056 mg/l respectively (Vives-Rego et al 1986). Nonylphenol ethoxylate (13 EO) also showed a wide disparity (2.9 and 0.0003 mg/l respectively) but the authors made no comment on the disparities.

5.2.4

Soils

In soils, Vandoni and Goldberg-Federico (1973a) reported that LAS and 3-sulphophenyl C 12-LAS inhibited nitrification but that inhibition fell to zero as the compounds were degraded, eg if a pre-incubation period was allowed. About 50% inhibition occurred with 500 mg LAS/kg, but at 100 mg/kg there was only about 10% inhibition. Since the monitored concentrations of LAS in soils are 1 to 45 mg/kg it is very unlikely that LAS would inhibit the oxidation of ammonia in soils.

Welp and Brümmer (1985) used a reduction test - Fe III to Fe II - to determine the effect of chemicals on the microbial activity of soils. The 'no effect level' for LAS was about 250 mg/kg and the EC 50 about 500 mg/kg for a strongly adsorbing soil, but only 33 and 55 mg/kg respectively for a poorly adsorbing soil.

Litz et al (1987) reported that the addition to soil of 5 g LAS/m^2 - yielding about 30 mg LAS/kg dry matter - and even the 10-fold application rate had no effect on soil respiration.

Sebastiani _et al_ (1981a,b) reported that the penetration through soil (in columns) of _Staphylococci_ was enhanced by the presence of surfactants, including LAS, but could detect no effect of LAS metabolites.

Oda _et al_ (1977; abstract only) reported no effect by LAS on the DNA in pure cultures of _Bacillus subtilus_ and _Escherichia coli_; neither did LAS induce mutations. Two Russian reviews on the effect of surfactants on micro-organisms have been published (Braginskii _et al_ 1983; Krivets 1984).

5.3 Virus removal

The removal of enteroviruses on sand columns was said to be substantially diminished in the presence of LAS and other surfactants (Dizer _et al_ 1984). The count of adsorbed viral particles was reduced almost to zero by 100 mg LAS/l (the only concentration tested), however, this concentration is very much greater than would be present in effluent or ground water. Viruses added to ground water or to tertiary effluent were removed much more readily than those added to sewage effluents.

Ward and Ashley (1980) reported that heat de-activation for 15 minutes was more effective in removing a rotavirus from anaerobically digested sludge than from water; the same removal took place at 50 °C in sludge as at 55 °C in water. The active agent was identified as ionic surfactant and it was found that with LAS at 1000 mg/l, the only concentration tested, which again is very much higher than found in rivers or sewage effluent, recovery of the rotavirus was <0.025% after 60 minutes at 21 °C compared with 100% recovery in a tris-buffer.

5.4 Aquatic plants

5.4.1 Freshwater algae

Algae, together with Daphnia magna and fish, have conventionally been used to assess the toxicity of chemicals to aquatic organisms and values reported for the EC 50 for a number of freshwater algal species are given in Table 5.3. In some cases the effect on amount of biomass produced was used as the end-point, while in other cases the effect on kinetic growth rate was determined.

The range of values producing an effect (EC 50 or chronic) for the eight species is very wide, 0.9 to 500 mg LAS/l (Table 5.3), which is similar to that (0.05 to 500 mg/l) reported by Lewis (1986) from a review of the work of a number of authors. Even with a single species there were widely different reported EC 50 values, for example, Microcystis aeruginosa 0.9 to 56 mg LAS/l, Selenastrum capricornutum 29 to 100 mg/l. One factor influencing the values could have been the particular type of LAS tested.

The very high value of 500 mg LAS/l for Scenedesmus quadricauda reported in Table 5.3 is not an EC 50 value and needs some explanation. Chawla et al (1986) reported no effect on growth rate below 500 mg/l but found that 100, 200 and 500 mg/l gave lowered survival rates when the organisms were sub-cultured into fresh medium after 7 d growth. At 500 mg/l there was reported to be a decrease in nutrient uptake as well as in protein and DNA syntheses.

Chattopadhyay and Konar (1985b) subjected ecosystems to 0.25 to 1.1 mg LAS/l (Parnol J of unstated composition) and found no change over 90 d in species composition compared with controls.

le 5.3. EC 50 values of LAS for various algae

cies	EC 50 (mg/l)				
	Canton & Slooff (1982)	Yamane (1984)	Lewis & Hamm (1986)	Chawla et al (1986)	Matulova (1979)
posure time, d] surement	[4] biomass	[2-3] growthrate	[4] growth rate	[→15] biomass	[?] ?
rocystis uginosa	32-56 (11.1)	10-20 (11.6)	0.9 (12) 5.0 (13)	-	-
nedesmus dricauda	-	-	-	500*	10+ (NS)(NS)
enastrum ricornutum	-	50-100 (11.6)	29 (12) 116 (13)	-	-
orella garis	18-32 (11.1)	-	-	-	-
amydomonas ra/moewusii	-	-	-	-	10+ (NS)
zschia ticula	-	20-50 (11.6)	-	-	-
icula liculosa	-	-	1.4	- (13)	-

No effect below this concentration
Inhibitory, but degree not stated; lethal at 500 mg/l
Not stated
Values in brackets are average chain lengths of C.
All EC50 values are based on nominal concentrations, except for Lewis & Hamm (1986) who measured LAS concentrations.

(Fresh LAS was added at 15 day intervals over the period, but determinations of LAS were not made). However, they reported that at 0.51 and 1.1 mg/l primary productivity and chlorophyll-a production was significantly lower than in the control. Kondo et al (1984, abstract only) found Chlorella pyrenoidosa to have lowered photosynthetic activity, ATP and chlorophyll content and to be severely inhibited by 10 mg C 12-LAS/l. Kikuchi (1979, abstract only) reported lowering of cell division and chlorophyll content with some cell death in colonies of Pleodorina californica exposed to low, unstated concentrations of LAS for an unstated period; at higher, unstated concentrations organelles disintegrated and most colonies died.

Lewis (1986), besides reporting the 96 h EC 50 values for Selenastrum capricornutum and Microcystis aeruginosa given in Table 5.3, also indicated for these species "first effect" concentrations of between 0.5 and 1.0 and between 0.05 and 0.1 mg C 11.8-LAS/l, respectively. (The concentrations of LAS added were confirmed by analysis at the beginning and end of the test.) These results were compared with those from field tests in which enclosed natural phytoplankton assemblages, suspended in a lake, were exposed for 10 d to a range of C 11.8-LAS concentrations (0.01 to 108 mg/l). After 10 d the number of species and diversity and similarity indices were determined; the first concentration to alter the community structure was between 27 and 108 mg/l (no intermediate concentrations were tested). Thus, the "first effect" levels, from the laboratory test, are 27 to 108-fold lower than the lowest concentration found to alter the community structure in lake water. However, if the EC 50 values are compared, there is essentially no difference for S. capricornutum (29 compared with 27 to 108 mg/l) but there is still a large difference (~30-fold) for M. aeruginosa. Later, Lewis and Hamm (1986) extended the field study by measuring the in situ effects of LAS on ^{14}C assimilation by photosynthesis by phytoplankton in lake water several times over a period of months. The assimilation test lasted 3 h at ambient temperature (18 to 28 ^{o}C) and the results are given in Table 5.4.

Table 5.4. Effect of LAS on photosynthesis - comparison of laboratory and field results

LAS	3h EC 50 in lake water (mg/l)	Variation of the short-term tests	Laboratory Growth (96 h) (mg/l)	EC 50	
			SC	MA	NP
C12	3.4 ± 2.4 (0.5 - 8.0)	16x	29	0.9	-
C13	1.9 ± 2.7 (0.2 - 8.1)	41x	116	5.0	1.4

SC <u>S. capricornutum</u>
MA <u>M. aeruginosa</u>
NP <u>Navicula pelliculosa</u>

Although the laboratory tests showed that C 13-LAS was less inhibitory than C 12-LAS to the two species tested, C 13-LAS was slightly more toxic in terms of ^{14}C photosynthetic assimilation by the mixed lake population. Also, photosynthesis was more sensitive to LAS, at least under the conditions of the test, than the community structure (Lewis 1986). The short-term photosynthetic response was highly variable, 16 to 41-fold. This variation was attributed largely to seasonal changes in the ecosystem, such as the chemical composition of lake water, composition of phytoplankton population, age of community, effect of grazers, availability of nutrients and temperature. For instance the EC 50 value for phytoplankton exposed to C 13 LAS varied from 8.1 mg/l at 16.5°C to 0.2 mg/l at 28°C. The growth response of the three single algal cultures also varied from time to time. The authors concluded, in agreement with other workers, that in order to define the effect of a chemical several algal species should be used in the standard laboratory toxicity tests as, for instance for LAS, <u>Microcystis</u> seems to be more sensitive than

Selenastrum which is often used as the "standard" test species to predict chemical hazard to aquatic life.

Despite the variability of the test results and the differences in the field and laboratory responses standard laboratory tests were able to provide estimates of "safe" concentrations for short-term photosynthesis.

5.4.2 Marine algae

Hitchcock and Martin (1977) reported that, in batch tests, 24-hour exposure to 0.025 mg C 13-LAS/l was almost 100% lethal to the "red tide" alga, Gymnodinium breve; this concentration was said to be non-toxic to other marine organisms. When 0.0125 mg LAS/l was added to the top of a cylinder containing the alga G. breve, cells migrated to the bottom where they were found to be intact but without activity; the cells recovered after 4 d in solutions containing 0.0125 mg/l but not in those containing 0.025 mg/l. (All concentrations were nominal).

In a study of the effect on G. breve of a sterol isolated from a cyanobacterium (Eng-Wilmot et al 1979), it was found that C 12-LAS was 25% toxic to the dinoflagellate at 0.08 mg/l in 20 h at 25 °C and 100% at 0.1 mg/l whereas C 13-LAS had no effect up to 0.1 mg/l but was toxic at 0.15 mg/l (all concentrations are nominal).

Using C 11.8-LAS, it was found that the marine diatom, Dunaliella tertiolecta, was killed at concentrations >10 mg/l; algistatic effects were observed at 2-4 mg/l and below 2 mg/l there was no effect (Proctor and Gamble, unpublished).

Takimoto *et al* (1982, abstract only) investigated the effects of LAS and its proposed metabolites on the growth of two marine algae, *Skeletonema costatum* and *Heterosigma sp*. Growth of the latter was inhibited at all concentrations tested (unstated), while that of the former was stimulated at lower (unstated) concentrations and inhibited at higher concentrations (period unstated). Kondo *et al* (1984, abstract only) reported the depression of photosynthetic activity, ATP and chlorophyll content, as well as growth inhibition of *Thallassiosira pseudonana* at concentrations above 10 mg LAS/l (period unstated).

Takita (1985, abstract only) reported the inhibition by LAS (at unstated concentrations) of sporelings of *Porphyra yezoensis*, and that the longer alkyl chain homologues and the outer phenyl isomers showed higher degrees of toxicity than shorter chain homologues and inner phenyl isomers. GC revealed that the more toxic components decreased more rapidly than others during biodegradation in sea water.

Only few data have been published on the effect of LAS on aquatic macrophytes. In controlled, flow-through tests, with constant light and nutrient concentrations, Bishop and Perry (1981) reported that the results on the effects on frond counts of the aquatic macrophyte duckweed (*Lemna minor*) were more consistent than those on dry weight, root length and growth rate. The EC50 values for these four parameters were, respectively 2.7, 3.6, 4.9 and 4.8 mg LAS/l. Labus and Kohler (1981, abstract only) state that aquatic macrophytes were more sensitive to LAS than were terrestrial plants. The role of synthesis was reduced in a 20 day continuous flow test at concentrations as low as 0.5 mg LAS/l.

rtebrates

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1.1

ercial LAS

A summary of the acute and chronic toxicity data of LAS (commercial product) to the common test organism, Daphnia magna, are given in Table 5.5. In general, the 48 h LC 50 ranged from about 1 to 10 mg LAS/l. There are two values which are significantly outside this range, namely, the 24 h LC 50 of 18 to 32 mg/l (Canton and Slooff 1982) and an exceptionally low 48 h value of 0.013 mg/l (Lal et al 1983, 1984a). Whereas the higher value (18 to 32 mg/l) may be explained by the shorter exposure time used in this test (24 compared with 48 hours used in some of the other tests) no satisfactory explanation can be provided for the 2 to 3 orders of magnitude lower value of 0.013 mg/l reported by Lal et al (1983, 1984a). There was 40% mortality in the control population which vitiates the results. It also appears that LAS in the free acid form could have been used which would have contributed to the low LC50 value.

The effect of some of the test conditions on the toxicity results has been investigated. Lewis (1983), using LAS with an average chain length of 11.8, found that the density of daphnids between 1 animal per 2 ml down to 1 animal per 50 ml of test solution had little effect on the LC 50 values obtained which were in the range 3.5 to 5.1 mg LAS/l. Similarly, the age of animals at the time of testing had little effect for animals aged between 6 and 48 h (LC 50 = 2.2 to 2.6 mg LAS/l) but as the age increased beyond 48 h the LC 50 also increased until at 216 h a value of 10.1 mg/l was reached (Barera and Adams 1983). Taylor (1985)

Table 5.5 Acute and chronic toxicity of LAS to Daphnia magna

Type of LAS Av. chain length	Duration of test h	Hardness of water (mg $CaCO_3$/l)	LC 50 (mg/l)	Reference
		Acute		
13.3	48	250	2.3 (2.6)	+Kimerle & Swisher (1977)
11.7	24	not given	~10	Petresa (1987)
not given	24	not given	~10	Moreno Danvila (1979)
11.8	48	35,181,340	5.6,2.97,2.71	+Lewis & Perry (1981)
11.1	24	138	18-32	Canton & Slooff (1982)
11	48	182-307	2.2-2.6	Barera & Adams (1983)
11.8	96	120	2.8-6.8	+Maki (1979)
11.8	48	131	3.5-5.1	+Lewis (1983)
11.8	48	165	1.8-5.6	Lewis & Suprenant (1983)
C10 85%) C12 10%) C14 5%)	48	22	6.7 [3.3]	Taylor (1983)
C10 30%) C12 10%) C14 60%)	48	22	1.0 [0.64]	Taylor (1983)
not given	48	120	0.013	(Lal et al (1983) (Lal et al (1984a)
13	96	120	1.8-2.8	+Maki (1979)
11.8	48	295-310	3.6-4.6 (unfed) 4.1-8.1 (fed) 2.3-10.4	(Taylor (1985) (((
not given	24	not given	5.5	Gard-Terech & Palla (1986)
		Chronic		
11.8	21 days		2.2-4.1	Taylor (1985)
11.8	21 days	120	1.3-2.2	+Maki (1979)

Table 5.5 Continued

Type of LAS Av. chain length	Duration of test h	Hardness of water (mg $CaCO_3$/l)	LC 50 (mg/l)	Reference
12.6	14 days	22	1.5	(+ Woltering & Ritchie
13.05		22	2.6	((1984)
13	21 days	120	1-1.4	+Maki (1979)

Values in round brackets for 24 h
Values in square brackets are predicted from values for individual homologues
+ These authors monitored the concentration of LAS in the tests

investigated, using C 11.8-LAS, the effect of feeding five diets to the animals before testing but found little effect (LC 50 = 3.6 to 4.6 mg/l). The addition of food during the test generally increased the LC 50 (LC 50 = 4.4 to 8.1 mg/l). A larger effect of diet was observed in chronic tests (21 d), the range of LC 50 values being 2.2 to 4.7 mg LAS/l. The 21 d "no observed effect concentration" (NOEC) for the five diets ranged from 1.2 to 3.2 mg LAS/l.

The hardness of the water used in the test was found by Maki and Bishop (1979) and by Lewis and Perry (1981) to have a minor effect on the toxicity of LAS. At a hardness of about 25 mg $CaCO_3$/l the LC 50 was 5 to 7 mg LAS/l; as the hardness increased the LAS became more toxic and by about 150 mg $CaCO_3$/l the LC 50 was 2.5 to 4 mg/l but no further increase in toxicity was observed with further hardness increases. The hardness of the culture medium in which the daphnids were grown prior to the test also had an effect, but this was less than that of the test medium. For example, animals grown in hard water (225 to 350 mg $CaCO_3$/l) were more susceptible to LAS when tested subsequently in waters with low hardness (~25 mg $CaCO_3$/l; LC 50 = 1.8 to 2.9 mg/l) than with high hardness (~350 mg $CaCO_3$/l; LC 50 = 3.2 to 4.0 mg/l). Maki and Bishop (1979) also showed that the acute toxicity of LAS to D. magna was not affected by previous exposure to LAS (at 0.4 mg/l) for periods extending up to 7 generations.

In addition to the data given in Table 5.5, Canton and Slooff (1982) reported the 21 d LC 50 (mortality) to be as high as 18 mg LAS/l, and a NOEC of 10 mg/l. (The effect on reproduction was less clear.) On the other hand, Woltering and Ritchie (1984) reported the 14 d LC 50 to be 1.5 to 2.6 mg LAS/l and Taylor (1985) reported 21 d LC 50 of 2.2 to 4.1 mg/l. All three groups of workers used the "static renewal" method, the renewals being made every 48 h or 3 times per week. Only Woltering and Ritchie (1984) determined actual concentrations of LAS in the test solutions and for this reason the LC 50 values obtained may be lower than those reported in the other two studies. In chronic tests (21 d) Maki (1979) found the NOEC value for C 11.8 to be 1.18 mg/l and for C 13 0.57 mg/l. At the other extreme, Lal et al (1984a) found very toxic effects in chronic tests. At only 0.005 mg/l, equivalent to 40% of their 48 h LC 50, the number of hatches was reduced by 80% and the number of neonates by over 90%.

Hatori et al (1984) (seen only in abstract), found that 30 mg C 12-LAS/l had a significant effect on reproduction of D. magna. Braginskii and Shcherban (1985) reported that low, unstated concentrations of LAS lowered the resistance of D. magna to attack by filamentous green algae, which overgrew daphnids in water polluted by detergents but not in clean water.

1.2
Homologues

Many studies have shown that the higher homologues of LAS are more toxic to D. magna than the lower homologues, Table 5.6. The 48 h LC 50 for the C 10 homologue was 8 to 30 mg/l, while for the C 14 the LC 50 was 0.4 to 0.8 mg/l. The C 16 and C 18 homologues were even more toxic. No doubt some of the differences between individual values of LC 50 for given homologues are due to such factors as hardness; although the values of Taylor (1983) who used a water of low hardness are lower than other corresponding values. The Table also shows that D. pulex was about as sensitive as D. magna to the LAS homologues.

'able 5.6. Acute and chronic toxicity of LAS homologues to Daphnia magna

lard-ness mg aCO$_3$/l)	Duration of test (h)	C 10	C 11	C 12	C 13	C 14	C 16	C 18	Reference
				LC 50 (mg/l)					
				Acute					
50	48	12.3	5.7	3.5	2.0	0.7	-	-	Kimerle & Swisher
	24	53	15.8	10.7	2.7	1.2	-	-	(1977)
20	48	-	-	6.8 (8.6)*	-	0.8	0.2 (0.6)	- (0.15)	Maki & Bishop (1979)
20	48	29.6	21.2	5.9	2.6	0.68	0.11	0.12	Maki & Bishop (1979)
-	24	55	31	8.4	5.0	2.4	-	-	Moreno Danvila (1987)
-	24	55	30	8	2	1	-	-	Moreno Danvila (1983)
2	48	7.7	4.5	1.4	1.2	0.41	-	-	Taylor (1983)
				Chronic					
2	14 d	32.1	-	6.4	-	0.9	-	-	Woltering & Ritchie (1984)

Values in round brackets refer to D. pulex

In chronic tests using river water, lasting 14 d, Woltering and Ritchie (1984) reported the NOEC and first observed effect concentrations (FOEC) for some LAS homologues and commercial products; Table 5.7.

able 5.7. NOEC and FOEC values for LAS homologues and commercial products

	Homologues			Commercial Products			
iain length	10	12	14	11.8	12.6	13.05	13.3
)EC (mg/l)	9.8	4.9	0.1	1.2-6.0	0.9	0.8	0.6
'EC (mg/l)	19.7	9.8	0.2	-	1.9	1.6	-

The authors thus confirmed that the higher homologues are more toxic. Woltering and Ritchie (1984) concluded from these results and other unquoted data that the toxicity of LAS to D. magna is essentially the same in laboratory media and in surface waters, except for the effect of water hardness.

[.3
ıres of LAS with
r substances

Taylor (1983) tested two known mixtures of LAS homologues to D. magna (Table 5.5) and reported that the predicted LC 50 values were about half of the measured values. Lewis and Perry (1981), measured the toxicity of binary and ternary mixtures of LAS with cationic, non-ionic and anionic surfactants. They found that response addition was more applicable than concentration addition in describing the toxicities of the mixtures, but that the toxicity of about a quarter of the mixtures could not be accounted for by either model. Moreno Danvila (1983) reported that in most cases, binary and ternary mixtures of LAS and other anionic and nonionic surfactants gave the expected LC 50 value; no explanations were offered for the exceptional cases. Waters (1982) showed that the addition of stoichiometric amounts of LAS to distearyldimethylammonium chloride (DSDMAC) decreased sixfold the toxicity of the cationic surfactant, that is, the LC 50 increased from 0.1 to 0.6 mg DSDMAC/l.

In the presence of clay, the toxicity of two homologues of LAS was slightly decreased; the LC 50 for C 14 rose from 1.0 to 1.4 mg/l and for C 18 from 0.09 to 0.18 mg/l. There was no decrease in toxicity for C 11 (Maki and Bishop 1979).

The observed acute toxicity of equimolar mixtures of LAS and DTDMAC towards the mysid shrimp was found to be as predicted by simple addition. The 96 h LC 50 was 3.6 mg/l and the separate contributions of the two surfactants were calculated to be 1.4 and 2.2 mg/l, respectively (Proctor and Gamble unpublished).

Panigrahi and Konar (1986) determined the effect of 1.01 mg LAS/l alone and in the presence of a petroleum refinery effluent but not the effect of the effluent alone on aquatic ecosystems. The test vessels contained 60 l borehole water plus 5 kg soil (5 replicates for each condition). Twelve additions of LAS were made over the 90 days duration of the test. LAS alone had no effect on the population of chironomid larvae but reduced both the zooplankton and phytoplankton populations, namely from 328/l to 131/l and 1065/l to 718/l, respectively. Addition of refinery effluent (RE) to the systems containing LAS caused further reduction in the populations. The presence of 0.4% RE reduced the zooplankton count to 53/l whereas 6.4% RE decreased the phytoplankton count to 243/l. The numbers of chironomid larvae were significantly reduced in the presence of 3.2% RE from 1541/m^2 to 984/m^2 and of 12.7% RE to 383/m^2. The authors concluded from these results, and others not included in the paper, that LAS and refinery effluent enhance the toxicity of each other.

5.1.4

LAS intermediates

Kimerle and Swisher (1977) showed that three known intermediates of LAS were very much less toxic than intact LAS. The 48 h LC 50 of sulphophenylbutyrate, - valerate and -undecanoate were 6000, 5000 and >1000 mg/l respectively. Gard-Terech and Palla (1987) found that the toxicity of LAS (commercial product) fell from 24 h LC 50 of 4.4 mg/l to the equivalent of 21 mg/l after 60 h and to 50 mg/l after 80 h in biodegradability tests, and suggested that this was evidence that LAS intermediates were less toxic than LAS.

5.5.1.5
LAS impurities

Kimerle and Swisher (1977) reported that the toxicities of the sulphonates of dialkyltetralins (DATS) and indane were between one half and one tenth of that of the corresponding LAS homologues. This was confirmed in another study (Procter and Gamble, unpublished). The 48 h LC 50 for C 10-DATS was 139 mg/l compared to 30 mg/l for C 10-LAS and 5.6 mg/l compared with 0.7 mg/l for C 14-DATS and C 14-LAS, respectively. Moreno Danvila (1987) showed the 24 h LC 50 for the C 16 dialkyltetralin sulphonate to be 420 mg/l and that of the C 20 homologue to be 27 mg/l, with the C 17 to C 19 homologues having intermediate toxicities.

5.5.2
Other freshwater invertebrates

Ceriodaphnia sp has been suggested as an alternative to D. magna because of a number of advantages;

- widely distributed
- only 7 d needed for chronic tests
- has equal or greater sensitivity to chemicals as D. magna

Taylor (1984) found that Ceriodaphnia sp had similar sensitivity to D. magna; for example, in well water the 48 h LC 50 was 4.8 mg LAS/l and in Ohio river water 6.0 mg LAS/l. The 7 d LC 50 in Ohio river water was 5.3 mg/l, with the NOEC and FOEC being 1 to 2 mg/l.

Lewis and Suprenant (1983), using an LAS of average chain length of 11.8, determined the toxicity to six species of invertebrates and found a range of LC 50 values of about 150-fold, as shown in Table 5.8.

Chattopadhyay and Konar (1985b) reported little effect on chironomids exposed to 0.25 mg LAS/l after 90 d in 60 l outdoor vats but numbers

Table 5.8. Acute Toxicity of LAS to Different Invertebrates (Lewis and Suprenant (1983)

Organism	48 h LC 50 (mg/l)
Dero sp (oligochete)	1.7 (1.3-2.1)
Asellus sp (isopod)	270 (180-400)
Gammarus sp (amphipod)	3.3 (2.8-4.0)
Paratanytarsus parthenogenica (midge)	23 (18-30)
Rhabditis sp (nematode)	16 (14-19)
Dugesia sp (flatworm)	1.8 (1.4-2.1)

Values in brackets are 95% confidence limits

were reduced by 62% at 0.38 to 1.1 mg LAS/l. Pittinger et al (submitted) examined the effect of LAS, of average chain length 11.8, on the midge Chironomus riparius in partial life-cycle bioassays. There was no reduction in midge egg-hatching during 72 h exposure to 18.9 mg LAS/l, the highest concentration tested, but LAS was more toxic to post-hatch larvae, in the absence of sediments, the 17 d EC 50 - pupal development or emergence of adults - being between 2.4 (no effect) and 3.7 mg/l (virtually no emergence). In the presence of sediments there was no effect on pupal development or adult emergence at 319 mg LAS/kg dry sediment and the first observed effect occurred at 993 mg/kg, the next concentration tested. The NOEC of sorbed LAS fractions was about 100-fold greater than those calculated from soluble fractions for the overlying or interstitial waters, indicating that adsorbed LAS was not bio-available to the midge.

Lal et al (1983) reported on D. magna and five other aquatic species, including fish fingerlings, using an LAS in the crude form of an "acid slurry" obtained from the manufacturer, presumably in India. The results (Table 5.9) show the median tolerance limit (= LC 50) and the "presumed harmless" concentration. As noted with D. magna earlier, Lal et al (1984a) found very much higher toxicity for LAS compared with other workers. The LC 50 values ranged from 0.013 to 0.08 mg/l and the "presumed harmless" concentrations from 0.0007 to 0.0075 mg/l. The tests seemed to have been conducted under normal conditions - temperature 25 ^oC, pH 7.2 to 7.5, dissolved oxygen 6.7 to 7.2 mg/l and water hardness 120 mg $CaCO_3/l$. The survival in the various controls after 48 h was 100%, except for D. magna, for which survival was only 60%. However, as the toxicities reported for LAS by Lal et al (1983, 1984a,b) are so much higher than those obtained by other workers, the data should be treated with some reservations.

Table 5.9. Toxicity of commercial LAS to various species (Lal et al 1983)

Organism	LC 50 (mg/l)	Presumed harmless concentration (mg/l)
Daphnia magna (water flea)	0.013	0.0007
Culex pipieus (mosquito)	0.08	0.0075
Tubifex rivulorum (slug worm)	0.059	0.0047
Lymnaea vulgaris (snail)	0.06	0.0052
Rana cynophlyctis (tadpole)	0.049	0.0038
Cirrhina mrigala (fish)	0.022	0.0015

(Test concentrations were nominal)

5.5.3

Marine invertebrates

5.5.3.1

Bivalve molluscs

For most marine organisms (as for freshwater species) adults are less sensitive to LAS than the early life stages; exceptions are the cockle (Cardium edule) and the scallop (Pecten maximus) (Swedmark et al 1971). The 96 h LC 50 values for adult stages of eight species of bivalves and crustaceans were >50 mg C-12 LAS/l; LAS concentrations were monitored.

Only 64% of embryos of the American oyster, Crassostrea virginica, developed when exposed to 0.1 mg LAS/l and many which developed were abnormal (Calabrese 1971). It was also shown that LAS as present in sewage effluent was less toxic than untreated material. The 6 h LC 50 of 1-day old oyster larvae was 1 mg C 12-LAS/l; also 0.05 mg/l had serious effects on growth in 24 h, although all survived (Renzoni 1971). Eight to ten-day old larvae exposed to 1 mg LAS/l for 6 h experienced a reduction in numbers; the survivors settled and underwent metamorphosis. The same author in a later study confirmed the 6 h LC 50 of 1 mg LAS/l for 1-day old larvae but found that exposure to 0.05 mg LAS/l for 1 week caused death of the total population (Renzoni 1974). The concentrations of LAS do not appear to have been monitored during the tests.

Exposure of clams, Tapes philippinarum, for 9 d to 2 mg LAS/l or more caused 100% mortality, while no deaths occurred at 1 mg LAS/l or less in the same period. The 96 h LC 50 was 10.5 mg LAS/l (Seko and Ishii 1981; abstract only).

Fertility of the mussel, Mytilus edulis, was decreased by 39% after exposure to 0.05 mg LAS/l for 260 h and larval development was inhibited when exposed to LAS concentrations > 0.3 mg/l (Granmo 1972). It is unclear whether LAS was monitored, but the water was changed daily.

5.5.3.2 Crustaceans

The 96 h LC 50 for the pink shrimp, Penaeus duororum was found to be 66 mg LAS/l and the NOEC 10 mg/l. The mysid shrimp, Mysidopsis bahia, was much more sensitive having a 96 h LC 50 of 1.42 mg LAS/l, with an NOEC of 0.93 mg/l. Other species were of intermediate sensitivity, namely, oyster larvae LC 50 of 7.4 mg/l (48 h), grass shrimp 13.8 mg/l (96 h) and blue crab 29.9 mg/l (96 h). (Procter and Gamble, unpublished).

In a longer term test (28 d) the highest no-observed effect concentration for mysid shrimp was between 0.16 and 0.38 mg/l (Procter and Gamble, unpublished).

5.6 Fish

5.6.1 Freshwater fish - acute toxicity

5.6.1.1 Commercial LAS

Acute toxicity values for various freshwater species of fish are summarised in Table 5.10. Most tests lasted 96 h but in many cases other details of the tests, including the nature of the LAS used, are often not stated. For a given species, the LC 50 values obtained are usually in reasonable agreement, especially considering the differences that could exist because of different test conditions used. For example, the individual values for fathead minnow (3.4 - 4.2 mg/l), and rainbow trout (1.7-5 mg/l) were, respectively, within about a threefold range of each other, while

Table 5.10 Acute toxicity of LAS to various fish species (in decreasing order of toxicity

LC 50 (mg/l)	Exposure Period (h)	Hardness (mg $CaCO_3$/l)	Species	Reference
0.022 (-)	48	120	Cirrhina mrigala (Nain - Indian carp)	Misra et al (1985)
0.06 (-)	48	120	Cirrhina mrigala (Nain - Indian carp)	Lal et al (1984a)
0.72 (AV.12)	96	76	Lepomis macrochirus (bluegill)	Dolan & Hendricks (1976)
1 - 2 (AV 11.8, 12.7)	24	ns	Lebistes reticulatus (guppy)	Van Emden et al (1974)
1.3 (-)	24	ns	Chrystiptera hollisi	Iimori & Takita (1979)
1.5 (AV 13)	96	76	Tilapia mossambica	Chattopadhyay & Konar (1985a)
1.67 (11.8)	96	137	Lepomis macrochirus (bluegill)	Lewis & Perry (1981)
1.7 (-)	96	ns	Salmo gairdneri (rainbow trout)	Gilbert & Kleiser (in press)
1.9 (12)	96	ns	Lebistes reticulatus (guppy)	Procter & Gamble (unpubl)
2.2+ (AV.12)	24	290	Salmo gairdneri (rainbow trout)	Vailati et al (1975)
2.4 (12)	96	ns	Fundulus heteroclitus (mummichog)	Procter & Gamble (unpubl)
3.0+ (SDA lot 1-1)	96	50	Notropis atheroides (emerald shiner)	Thatcher & Santner (1966)
3.4+ (-)	96	ns	Pimephales promelas (fathead minnow)	McKim et al (1975)
3.5 (-)	96	ns	Lepomis macrochirus (bluegill)	Maki & Bishop (1985)
3.7+ (-)	96	43	Esox lucius (northern pike)	McKim et al (1975)
3.7+ (-)	96	43	Micropterus dolomieu (smallmouth bass)	McKim et al (1975)
4.0+ (SDA lot 1-1)	96	50	Lepomis macrochirus (bluegill)	Thatcher & Santner (1966)
4.0+ (-)	96	43	Catostomus cornumensoni (white sucker)	McKim et al (1975)

Table 5.10 (continued)

LC 50 (mg/l)	Exposure Period (h)	Hardness (mg $CaCO_3$/l)	Species	Reference
4.0 (-)	24	ns	<u>Oryzias latipes</u> (killifish)	Iimori & Takita (1979)
4.2+ (SDA lot 1-1)	96	50	<u>Pimephales promelas</u> (fathead minnow)	Thatcher & Santner (1966)
4.5 (12)	96	ns	<u>Lepomis macrochirus</u> (bluegill)	Procter & Gamble (unpubl)
4.9+ (SDA lot 1-1)	96	50	<u>Notropis cornutis</u> (common shiner)	Thatcher & Santner (1966)
~5 (AV.12)	96	ns	<u>Salvelinus alpinus</u> (arctic charr)	Olsen & Höglund (1985)
~5 (AV.11.5)	96	ns	<u>Salmo gairdneri</u> (rainbow trout)	Pohla-Gubo & Adam (1982)
5.0*) 5.4+) (-)	96	ns	<u>Cyprinus carpio</u> (carp)	Lopez-Zavala <u>et al</u> (1975)
5.6-10 (AV 11.1)	96	138	<u>Poecilia reticulata</u> (guppy)	Canton & Slooff (1982)
6.4+ (SDA lot 1-1)	96	50	<u>Ictalurus melas</u> (black bullhead)	Thatcher & Santner (1966)
6.2+ (-)	96	52	<u>Carassius auratus</u> (goldfish)	Tsai & McKee (1980)
7.2 (12)	96	ns	<u>Carassius auratus</u> (goldfish)	Procter & Gamble (unpubl)
9.8 (-)	96	160	<u>Heteropneustes fossilis</u> (catfish)	Zaccone <u>et al</u> (1985)
10-18 (AV.11.1)	96	138	<u>Oryzias latipes</u> (killifish)	Canton & Slooff (1982)
23.0*) 4.8°) 12.0+) (-)	96	ns	<u>Tilapia melanopleura</u> (white tilapia)	Lopez-Zavala (1975)
3-10 (AV.11.6)	96	ns	Fishes	Gerike (1987)
0.1-7.6	96	ns	Fishes	Reiff <u>et al</u> (1979)

* Technical LAS
\+ Commercial LAS
° commercial LAS + enzymes

\+ measured concentrations in tests
ns not stated.

Values in brackets denote chainlength or average chain length those for bluegill (0.72-4.5 mg/l), killifish (10 to 4-18 mg/l) and guppy (1-10 mg/l) were more disparate.

Mention needs to be made of the data reported from Lal's group for Cirrhina mrigala (Nain - an Indian carp) (Lal et al 1984b, Misra et al 1985). The two 48 h LC 50 values are extremely low (0.022 and 0.06 mg LAS/l) and are about two orders of magnitude lower than the average for all other species. This large difference was also reported for other organisms, eg Daphnia, by Lal's group (see Section 5.5.2). Examination of the published experimental details of the tests reveals no reason for these low values: neither do the authors comment on the apparent discrepancy.

Apart from these exceptionally low values, the acute LC 50 values for freshwater fish are generally in the range 3 to 10 mg LAS/l as stated by Gerike (1987). The range of 0.1 to 7.6 mg LAS/l given by Reiff et al (1979) for a number of species seems to be too low at the lower limit.

The only marine species listed, Fundulus heteroclitus (mummichog), had a lower than average 96 h LC 50 of 2.4 mg LAS/l.

Abel (1974) has reviewed the factors which can affect the results of laboratory toxicity tests and drew attention to such parameters as water hardness, temperature, etc. Marchetti (1968) and Swedmark et al (1971) reported increased toxicity of LAS with increasing temperature but Hokanson and Smith (1971) found no effect. Eyanoer et al (1985) also reported very little change in the toxicity of LAS to Puntius gonionotus, in medium hard water between 28 °C (96 h LC 50 = 11.8 mg LAS/l) and 35 °C (11.5 mg/l). Gerike (1987) reported a decrease in 96 h LC 50 for goldfish from ~16 mg

LAS/l at 15 °C to ~11 mg/l at 20 °C; rainbow trout showed over the same temperature range a decrease in LC 50 from ~6 mg LAS/l to ~4 mg/l whereas the LC 50 for golden orfe was little affected.

As with <u>Daphnia</u>, increasing the hardness increases the toxicity of LAS to fish; for example, the LC 50 for goldfish decreased from 15 mg LAS/l in very soft water, 0 °F, to 5.7 mg/l in harder water, 50 °F (Gafa 1974). Wakabayashi <u>et al</u> (1975) (abstract only) found a 24 h LC 50 for <u>Oryzias latipes</u> in distilled water of 23 mg LAS/l compared with 13 mg LAS/l in soft water; the toxicity increased still further as increasing amounts of salt were added to the soft water. The same authors (Wakabayashi <u>et al</u> 1978) reported that average survival time of carp exposed to 18 mg LAS/l (av. chainlength 11.7) decreased from 47 h at 0 mg $CaCO_3$/l to 1.9 h at 200 mg $CaCO_3$/l. Using 8 mg C 12-LAS/l the corresponding values were >72 h to 16 h.

Eyanoer <u>et al</u> (1985) reported the 96 h LC 50 for <u>P. gonionotus</u> to be 13.6 mg LAS/l in soft water, 11.8 mg LAS/l in medium-hard water and 11.5 mg LAS/l in hard water; the waters contained 50, 110 and 260 mg $CaCO_3$/l, respectively.

Organic matter present in the test water appears to affect toxicity. Iimori and Takita (1979; abstract only) found that the toxicity of LAS to <u>Oryzias latipes</u> and <u>Chrystiptera hollisi</u> was lowered in the presence of gluten, which also decreased the uptake of ^{14}C by fish when exposed to ^{14}C-labelled LAS.

5.6.1.2 Homologues and isomers

As has been observed with invertebrates (Section 5.5) the toxicity of LAS to fish also increases as the chainlength of the homologues increases, (Table 5.11).

Table 5.11. Acute toxicity LC 50 (mg/l) of LAS homologues to fish

Duration of test (h)	°	10	11	Chain length 12	13	14	(Other)		Reference
Pimephales promelas (fathead minnow)									
48		43	16	4.7	0.4	0.4	1.7 (Commercial LAS)		Kimerle & Swisher (1977)
96		57.5	27.9	6.6	2.0	0.5	4.5 (C11-14)		Macek & Sleight (1977)
[NOEC*		49	18	4.9	1.6	0.49	4.2]		
96		-	12.3, 4.1 (11.2) (11.7)	-	0.86 (13.3)	-			Holman & Macek 1980
96#		100	28	6	2.4	0.6			Taylor (1983)
Carassius auratus (goldfish)									
6									Divo (1976)
	6	-	46.5	20.5	7.2	-			
	5	76	39.5	16.5	5.8	-			
	4	66	30.5	12.5	4.6	-			
	3	56	22.5	8.5	3.3	-			
	2	36	14.5	4.5	2.0	-			
	mixture	-	-	-	-	1.6			
Lepomis macrochirus (bluegill)									
96		21- 47	11.6	1.2-6.5	1.11	0.25-0.42	0.087 (16)	0.38 (18)	Little (1977)

Duration of test (h)	10	11	LC 50 (mg/l) Chain length 12	13	14			Reference
Oryzias latipes (killifish)								
24	-	-	70	-	5.9	0.78 (16)	23 (LAS)	Wakabayashi et al (1975)

Values or words in round brackets describe the LAS used.
* NOEC = No observed effect concentration.
° Position of the $SO_3-C_6H_4$ group.
\# Used river water

Differences between the LC 50 values for a given homologue are due not only to species difference but also to differences in test parameters such as duration, hardness of water, temperature, etc. There was approximately a 10-fold difference in toxicity between homologues separated by two carbon atoms, ie C 10 - C 12, C 11 - C 13. The C 16 homologue appears to be most toxic, with the C 14 and C 18 homologues having about equal toxicity.

Wakabayashi et al (1975) (abstract only) reported that the relative toxicity of the homologues to Oryzias latipes was unaffected by the water used for the tests (river or laboratory water). Kikuchi and Wakabayashi (1984) (abstract only), working with the same species, reported that "the lipophilic chainlength affected surfactant (LAS) toxicity" but no further details are given in the abstract. Oba et al (1977, abstract only) made a similar qualitative statement in their abstract. Divo (1976) reported that mixtures of homologues gave observed toxicities which agreed well with values calculated assuming simple addition of toxicities with no synergism or antagonism and this has been confirmed by Macek and Sleight (1977) and Taylor (1983) for the fathead minnow, Table 5.12.

The effect of continuous exposure for 30 d to homologues of LAS on eggs and fry of the fathead minnow also confirmed that the longer the chain-length (up to C 14) the higher the toxicity (Procter and Gamble 1976, unpublished), Table 5.13.

The study by Divo (1976) shows that the outer isomers (SO_3-C_6H_4- group on positions 2 and 3), which have been found to be more biodegradable than the inner isomers (4, 5 and 6), (see Section 4) were also more toxic to Carassius auratus. Similarly the toxicity towards Carassius auratus of the 1,4-dialkyltetralinsulphonates, present in LAS

Table 5.12. Effect of mixtures of different LAS homologues (chainlength C) on the toxicity to fathead minnow Taylor (1983)

Composition of mixture %					96h LC 50 (mg/l)	
					Observed	Predicted
C 10	11	12	13	14		
85	0	10	0	5	10.0	9.2
30	0	10	0	60	1.0	0.98
8	29	34	29	0	4.6	4.4

Table 5.13. Effect of chainlength of LAS homologue on the minimum threshold concentration (MTC) for fathead minnow eggs and fry

Chainlength	10	11	12	13	14	11-14
MTC (mg/l)	14-28	7-14	1-2.5	0.12-0.28	0.05-0.1	1.02-2.05

as impurity, also increased with increasing chainlength (Divo 1976). The 6 h LC 50 values were about 190 mg LAS/l (C 10), 90 mg/l (C 11), 40 mg/l (C 12) and 17 mg/l (C 13). These were of the same order of magnitude as the 24 h LC 50 values for the LAS homologues for fathead minnow, namely, 87 mg LAS/l (C 10), 25 mg/l (C 12) and 9 mg/l (C 14) (Kimerle and Swisher 1977).

The toxicity of LAB, also present as impurity, was less than the corresponding LAS. The 96 h LC 50 (bluegill) for C 11.8-LAB was 55.9 mg/l and for a mixture of the LAB and C 11.8-LAS (at equal weights) was 17.7 mg/l (Procter and Gamble, unpublished).

5.6.1.3 Degradation intermediates

Early studies investigated the toxicity of LAS before and after biodegradation; either by assessing the toxicity of effluents from model activated sludge units; or by testing methylene blue-reacting substances and alkanoic acid intermediates isolated from similar effluents; or by testing known or putative intermediates synthesized for the purpose. There are, however, very few new studies on the effect of degradation of LAS on its toxicity. Swisher _et al_ (1964), Kimerle and Swisher (1977), Dolan and Hendricks (1976) and Brown _et al_ (1978) all demonstrated the lowering of the toxicity of LAS in effluents with increased degradation when expressed in terms of the MBAS content. For example, Brown _et al_ (1978) showed that fresh surfactant was 3 to 4 times more toxic to rainbow trout on the basis of MBAS content, than when present in treated effluents. Schöberl and Kunkel (1977) isolated various chemical fractions from activated sludge effluents and tested them against golden orfe. No deaths were recorded in 48 h with isolated MBAS at up to 20 mg MBAS/l, while untreated Marlon A at 4 to 5 mg MBAS/l killed all the fish in 48 h. The non-surfactant intermediates were non-toxic at 200 mg/l in 48 h.

An intermediate formed early in the degradation of LAS - sulphophenylundecanoic acid - was found to have a 96 h LC 50 of 75 mg/l for bluegill (Swisher _et al_ 1964) and about the same value for the fathead minnow (Kimerle and Swisher 1977). Subsequently Swisher _et al_ (1978) published a corresponding value for the acid for fathead minnow of 1200 mg/l but the material tested was a mixture of two much less toxic fractions (representing different isomers) obtained by further purification of the material used in the earlier studies. Sulphophenylbutyric and -valeric acids were much less toxic than the undecanoic acid(s); namely LC 50 values of ~10 000 to 28 000 mg/l (Kimerle and Swisher 1977; Swisher _et al_ 1978) and ~10 000 mg/l (Kimerle and Swisher 1977).

Divo and Cardini (1980) isolated almost in pure form the alkanoic acid derivatives of 2-sulphophenyl C 13-LAS and 4-sulphophenyl

C 13-LAS, and tested them against Carassius auratus. The 6 h LC 50 values for the two LAS isomers were 2.0 and 4.6 mg/l respectively whereas the corresponding alkanoic acids caused no deaths at 800 mg/l, the highest concentration tested, even after 48 h. The authors concluded that the toxicities of the two intermediates were at least 500 and 200 times respectively less than those of the two original LAS homologues.

In chronic tests in which eggs and fry of fathead minnow were exposed for 30 d, the minimum threshold concentration (MTC) of LAS was 2.05 mg/l, while that for sulphophenylundecanoic acid was >52 mg/l and for the butyric acid derivative it was >1400 mg/l (Swisher et al 1978).

Oba et al (1977, 1978, abstracts only) found that the mortality and hatchability of killifish (Oryzias latipes) was less inhibited by biodegraded LAS than intact LAS.

5.6.1.4 Mixtures of LAS with other substances

Solon et al (1969) reported synergism between LAS and parathion but none with endrin or DDT when tested on fathead minnow. Four other phosphorus-containing insecticides also exhibited synergism with LAS, but three others did not (Solon and Nair, 1970). Using the goldfish, Tsai and McKee (1978) found that the toxicities of LAS and chloramine were additive at toxicity ratios of 1:1, while at unequal ratios synergism occurred. With copper at 1:1 and 2:1 (LAS:Cu) an additive response was observed only at higher concentrations. With time (24-96 h) the effect became more nearly additive for the 1:1 and 2:1 (LAS:Cu) ratios, while the 1:2 (LAS:Cu) mixtures became more synergistic.

As with D. magna, Lewis and Perry (1981) found the toxicity to bluegill of mixtures of LAS and other surfactants to be generally additive or less than additive. However, mixtures of LAS and the cationic surfactant MDAC were more acutely toxic (ie synergistic) than predicted. For example, equitoxic mixtures of LAS and MDAC, expected to have a 96 h LC 50 of 2.77 mg/l, were found to have an LC 50 of 1.54 mg/l.

5.6.2
Freshwater fish - chronic effects

Table 5.14 summarises the results of chronic tests for six species but most data have been obtained with the fathead minnow.

The most sensitive stage was usually the survival of fry; total length or growth of fish surviving exposure to LAS was not greatly affected. The chronic effects concentrations given in Table 5.14 tend to be lower than corresponding 96 h LC 50 values for adult fish. Pickering and Thatcher (1970) noted that the NOEC (no observed effect concentration which is more or less equivalent to the minimum threshold concentration, MTC) in their study with the fathead minnow was between 14 and 28% of the 96 h LC 50 value. In another study, also with fathead minnow (Procter and Gamble, unpublished), the ratios for MTC/LC 50 for five LAS homologues (C 10 - C 14) were 36, 50, 26, 10 and 15% respectively; for a commercially blended C 10 - C 14 mixture the proportion was 33%. There are insufficient data to calculate the proportion for other species.

The 28-d NOEC value for death of adults (Poecilia reticulata) was reported by Canton and Slooff (1982) to be 3.2 mg LAS/l.

The NOEC values for the fathead minnow in Table 5.14 suggest that the conclusion of Gerike (1987), that the "toxic limit" (taken to imply the highest acceptable concentration) for the life cycle of this species is 0.25 mg LAS/l, is

Table 5.14: Chronic effects of LAS on developmental stages of fish

Species	Developmental stage	Effect-level: LAS (mg/l)	Type of LAS	Hardness (mg $CaCO_3$/l)	Reference
Fathead minnow	egg hatching	(9 d LC 50 2.3-3.6	SDA	170	‡Pickering (1966)
		(1 d LC 50 3.6	lot 1-1		
	survival of hatched fry	NOEC 0.9			
Fathead minnow	survival of fry	7-14 d NOEC 0.63-1.2	"	200	‡Pickering & Thatcher (1970)
	growth, egg production, hatching	35 d - NOEC 2.7			
Fathead minnow	survival of fry, etc	30 d MTC 1.02-2.05	C10-C14	-	‡Procter & Gamble (unpubl)
		" 14-28	C10		
		" 0.05-0.10	C14		
Fathead minnow	survival of fry	UDA* NOEC >52 BA+ NOEC >1400	-	41	Swisher <u>et al</u> (1978)
Fathead minnow	larval survival	NOEC 1.09	AV.11.8	200	‡Maki (1979)
		NOEC 0.74	AV.11.8	39	‡Maki (1979)
		NOEC 0.15	13		‡Maki (1979)
Fathead minnow	life cycle: embryo larvae	NOEC 5.1	AV.11.2	39	‡Holman & Macek (1980)
		" 0.48	AV.11.7	39	
		" 0.11	AV.13.3	39	
Fathead minnow	survival of larvae	NOEC 0.5	not stated	-	Gilbert & Kleiser (in press)
Rainbow trout	lethality to eggs	14 d LC 50 0.12	AV.12	290	‡Vailati <u>et al</u> (1975)
	lethality) eggs	24 h LC 50 10.8	"		
	lethality) adults	24 h LC 50 2.2	"		
	lethality) fry	24 h LC 50 1.0	"		
Fathead minnow	standing crop	30 d LOEC 0.5	not stated	43	‡McKim <u>et al</u> (1975)
White sucker		<0.5			
Northern pike		0.5			
Smallmouth bass		2.3			

* sulphophenylundecanoic acid + sulphophenylbutyric acid ‡ These authors measured concentrations in the test

NOEC = no observed effect concentration

MTC = minimum threshold concentration, ie, an estimate of the lowest toxicant concentration causing a significant effect, or LOEC reasonable. Rainbow trout are more sensitive and the toxic limit would be less than 0.12 mg/l, Vailati _et al_ (1975).

Table 5.14 also shows that two intermediates of LAS, sulphophenylundecanoic acid and sulphophenlybutyric acid, have very much less effect than LAS on fathead minnow in chronic tests (Swisher _et al_ 1978).

Other effects have been observed; for example, Chattopadhyay and Konar (1985a) showed that exposure for 90 d to 0.25 mg LAS/l, equivalent to about 20% of the 96 h LC 50, caused _Tilapia mossambica_ to have slightly reduced fecundity, about 40% reduced feeding rate and much reduced growth. Misra _et al_ (1985) found damaged gill epithelium in _Cirrhina mrigala_ exposed for 30 d to 0.005 mg LAS/l, equivalent to 25% of the 48 h LC 50. Arima _et al_ (1981, abstract only) determined the LC 50 for eggs, larvae and fry of carp and found that eggs were more susceptible than fry, which in turn were more susceptible than larvae. Yamamoto (1986, abstract only) treated twin and double embryo of killifish, derived from the blastomeres partially separated at the two-cell stage, with 10 mg LAS/l for 3 h. Some teratological malformation occurred.

5.6.3 Effects on gills and perfused gills

Immersion of catfish in LAS solutions, containing 1.5 to 2.5 mg LAS/l, for up to 8 d led to injury to gills, namely separation of lamellae from their vascular components (Zaccone _et al_ 1985). Also, there was decreased activity of enzymes of aerobic pathways, eg succinate dehydrogenase, and increased activity of enzymes of anaerobic pathways, eg lactic acid dehydrogenase. Misra _et al_ (1985) found similar damage to the gills of _Cirrhina mrigala_ after immersion in a concentration as low as 0.005 (or 0.015 based on data quoted in Lal et al (1984a)) mg LAS/l for 30 d. However, it has to be stressed (as pointed out in Sections 5.5.1.1 and 5.5.2) that the toxicities reported for LAS by Lal et al (1983) and Misra et al (1985) are very much higher than those obtained by other workers and that therefore these results should be treated with reservations. The effects on the epidermis of the heads of Salmo gairdneri of keeping the fish in 1.0 to 2.5 mg LAS/l for up to 8 d were determined by electron microscopy. There was cytoplasmic degeneration and vacuolization followed by death of the cell. At 1 mg LAS/l regeneration took place after 8 d in LAS-free water (Pohla-Gubo and Adam 1982).

Using perfused isolated gill arches, Jackson and Fromm (1977) found that exposure to LAS at 5 to 100 mg/l for 65 min showed a dose-related exponential increase in tritiated water uptake. Bolis and Rankin (1978) used isolated gills of 3 species of salmon - Oncorhynchus gorbuscha, O. kisutch and O. keta. Perfusion with 0.6 to 3 mg LAS/l produced concentration-dependent vasodilation, which was partially blocked by propranolol. The maximum responses with LAS were much greater in seawater or pre-spawning freshwater fish than in spawning fish. Nor-adrenaline-induced vasodilation in perfused isolated gills from eels or brown trout was inhibited in fish which had been kept in 1 mg LAS/l but not in 0.1 mg LAS/l (Bolis and Rankin 1980). Later the same authors (Bolis et al 1984) reported that, contrary to its positive effect on nor-adrenaline-induced vasodilation, LAS had little effect on the response to a vasoactive intestinal polypeptide. In the presence of 10 µg endorphin/l, LAS inhibition of the nor-adrenaline response was much less pronounced. The viability of isolated gills of rainbow trout deteriorated markedly when perfused with 3.5 mg LAS/l (Pärt et al 1985). The same concentration of LAS added with 0.9 mg Cd/l reduced the transfer of the metal. However, at lower concentrations - 0.1 mg Cd/l and 0.05 mg LAS/l - the presence of LAS more than doubled the transfer of Cd.

5.6.4 Behaviour and other effects

Adverse effects on the swimming patterns of goldfish by LAS were reported by Marchetti (1968). The concentrations of C 12-LAS and C 14-LAS required to reduce the activity in 6 h to zero were 4.7 and 3.2 mg/l respectively, both being about 50% of the 6 h LC 50 values. Although the author implies that effects on swimming activity were found at concentrations close to those present in Italian surface waters, he does not give surface water concentrations found. Tatsukawa and Hidaka (1978, abstract only) studied avoidance reactions in ayu (Plecoglossus altivelis) induced by two forms of LAS, carried out in an apparatus based on the Scherer model. The estimated threshold concentrations (ETC) of avoidance were as low as 0.11 µg/l for "formulation" LAS and 1.5 µg/l for "pure reagent" LAS. (Soap had an ETC of 31 ug/l.) Later, the same authors (Hidaka and Tatsukawa 1985) studied avoidance reactions in Oryzias latipes and expressed results as the Avoidance Ratio (the percentage of time spent in clean water in the test trough). The AR_{65} - the concentration giving 65% avoidance ratio - was as low as 13 µg LAS/l. No detailed information was given for either study on the LAS used or whether exposure concentrations were monitored.

No change in the schooling pattern of fingerlings of Cirrhina mrigala over 30 d in a solution containing 0.015 mg LAS/l was found by Lal et al (1984b), but they did find an increased rate of opercular movement and an increase in lactic acid (18%) and lactic acid dehydrogenase (23%) in the gills. Increases in enzymes - glucose-6-phosphate dehydrogenase and lactic acid dehydrogenase - were also reported by Zaccone et al (1985, abstract only) in catfish exposed to sub-lethal concentrations of LAS. They also found increased numbers of mucous cells which produced acidic glycoproteins and more O-acetylated sialic acids.

Effects on young carp kept for up to 125 d in 5 mg LAS/l were reported by Walczak et al (1983). Small reductions in red blood cell count (13%) and haemoglobin content (8%) were found after 125 d, but not after 62 d, while the mean corpuscular volume decreased (8-12%) by as early as 7 d. Up to 50% decrease in white blood cells was noted after 14 to 125 d. The fish lost their appetite, swam lower and became disinterested - they did not attack the submerged hand of the feeder, as did control carp. There was little recovery when the fish were returned to an LAS-free environment.

Olsen and Höglund (1985) reported that LAS reduced the olfactory-mediated attraction between juveniles of arctic charr (Salvelinus alpinus). The effect was produced either by pre-exposing the fish to 1 to 2 mg LAS/l for 4 days or by adding 0.02 to 0.2 mg LAS/l to the appropriate test vessels. In the test, the attraction was completely quenched by 1 mg LAS/l, which also affected locomotor activity.

5.6.5 Bioaccumulation

The degree to which LAS is accumulated in fish and fish tissues is usually expressed by the bioconcentration factor (BCF) defined as the concentration of LAS in the tissue or whole body divided by the concentration in the aqueous phase with which the fish is in equilibrium. BCF values for whole body and for some tissues of five species are given in Table 5.15. However, it must be pointed out that in four of the references cited

Table 5.15: Bioaccumulation of LAS and alkyl-benzenes in fish

Species	LAS	Exposure Period (h)	Tissue	Bioconcentration Factor	Reference
Carp	^{35}S-n-lauryl* 1.1 mg/l	2	gill	4.0	Kikuchi et al (1978)
			hepatopancreas	1.7	
			gall bladder	0.5	
		24	gill	13	
			hepatopancreas	97	
			gall bladder	1000	
Carp	^{35}S n-lauryl* 0.5 mg/l	24-72	hepatopancreas	30	Wakabayashi et al (1978)
			gall bladder	9000	
Carp	^{14}C & ^{35}S n-lauryl*	72			Wakabayashi et al (1981)
	9.1 ug/l		whole body	16	
	300 ug/l		whole body	400	
Bluegill	^{14}C LAS 0.5 mg/l	-	whole body	104	Kimerle et al (1981)
			gall bladder	-5000	
Blue gill	^{14}C 12 LAS	120			Bishop & Maki (1980)
	0.064 mg/l		whole body	220 (280)	
	0.68 mg/l		whole body	94 (120)	
Blue gill	^{14}C alkylbenzene	-	whole body	35 (6300)	Werner & Kimerle (1982)
Fathead minnow	various	<50 d	whole body	270-1200	Comotto et al (1979)
			gall bladder	20 000 - 70 000	
Golden orfe	^{14}C dodecyl*	72	whole body	130	Freitag et al (1985)

values in brackets are calculated values
* These LAS were probably 1-sulphophenylalkane

the radio-labelled compound used was probably a 1-sulphophenyl alkane and therefore not truly representative of commercial LAS which contains no 1-phenyl isomers.

It is normally found that for LAS after 24 h, and certainly after 72 h, immersion equilibrium has been reached. The highest BCF values are observed for the gall bladder which seems to be the main receptical of the LAS. The difference between the BCF values for gall bladder of the carp in Table 5.15 (Kikuchi et al 1978, Wakabayashi et al 1978) was suggested by the authors to be due to the use of fish of lower age in the second study. Wakabayashi et al (1981) also observed that the BCF decreased when aqueous solutions of LAS greater than 300 µg LAS/l were used and that values increased with increasing hardness of the test solution. They also studied the route by which LAS was absorbed (Kikuchi and Wakabayashi 1979, abstract only).

For bluegill, the "whole body" BCF values obtained in the different tests agree fairly well and are also in good agreement with the values calculated from the octanol/water partition coefficients (Kimerle et al 1981, Bishop and Maki 1980). For alkylbenzene in the bluegill, however, the "whole body" BCF was much less than that calculated (Werner and Kimerle 1982) and the difference was attributed to metabolism within the fish. Using fathead minnows, Comotto et al (1979) showed that the BCF was a function of chain length of the LAS used, as well as of time of exposure. From an analysis of the tissues, which showed that between 2 and 75% of the ^{14}C present was intact LAS, the authors presumed that LAS intermediates were present, formed during metabolism by the fish.

In depuration tests with bluegill, it was found that the time for 50% removal of ^{14}C was 2 to 5 d (Kimerle *et al* 1981) or 30 h (Bishop and Maki 1980). With fathead minnows >85% was depurated in 3 to 4 d and approximately 100% after 10 d (Comotto *et al* 1979).

LAS has been reported to increase the bioaccumulation of other chemicals. Topcuoglu and Birol (1982) found that the LAS significantly increased the accumulation of zinc in young goby, *Proterorhinus marmoratus*, Pall and similarly Nakanishi *et al* (1985, abstract only) found that LAS (dodecylbenzenesulphonate) increased the BCF of PCB and two nitrophenylethers by about 1.5-fold. In the latter case fish pre-exposed to LAS showed a reduced uptake of the other chemicals.

5.6.6

Marine fish - acute toxicity

Swedmark *et al* (1971), using a C 12-LAS, determined the 96 h LC 50 for three marine species to be about 1 mg/l (cod, *Gadus morrhua*), 1.5 mg/l (flounder, *Pleuronester flesus*) and 1 to 5 mg/l (plaice, *P. platessa*).

5.6.6.1

Mixtures of LAS and other substances

The threshold concentrations for cod (*Gadus morhua* L) measured in terms of mortality of eggs and larvae, decreased hatching frequency, larval viability and normal development, were <0.01 mg LAS/1, <0.01 mg Cu/l and 1 mg Zn/l when tested separately (Swedmark and Granmo 1981). In mixtures of LAS and Cu, the threshold values were higher, indicating that the presence of Cu decreased the toxicity of LAS, while in mixtures of LAS and Zn, the threshold for Zn fell, indicating that LAS had increased the toxicity of Zn.

5.7 Terrestrial plants

Surfactants, including LAS, can reach the soil by the application of sewage sludge (raw or digested) and by the irrigation of fields by waters containing sewage or sewage effluents. There has been no systematic study to determine what effects surfactants might have on plants growing in soil containing LAS and as yet there are no standard test methods available.

Only a limited number of data have been reported on the effects of LAS on plants; most of these are listed in Table 5.16. Many studies have used aqueous solutions of LAS applied to the soil in pots and/or directly to the leaves, some were carried out under hydroponic conditions and a few were directed towards ascertaining whether LAS affected the passage of pesticides into plants. Studies on seed germination were usually carried out in Petri dishes. No studies have been published on the effect of LAS on plants grown on soil amended with LAS-containing sludge. However, in practice, this is the general exposure route of plants to LAS which will have probably less effect, as the LAS is less bioavailable, than applying the surfactant in aqueous solution.

Early work in Rumania by Popa <u>et al</u> (1967), who used hydoponics to grow tomatoes, showed that when added during the blooming and fructification stages LAS at 100 mg/l had no effect on quantity or quality, while at 5 000 mg/l there was a 50% reduction in yield. When added throughout the whole growth period (Popa and Gruia 1968), 100 mg LAS/l caused a slight reduction in yield, but concentrations up to 500 mg/l did not affect the quality of the fruit.

Table 5.16 Effects of LAS on terrestrial plants

Plant	LAS concentration (mg/l)	Effect	Reference
Avena bianca	500	50% germination 50% seedling growth	Vandoni & Goldberg-Federico (1973b)
Barley	10-40 (C10-C13)	None on growth	Lopez-Zavala et al (1975)
Barley	100 (=22 mg/kg)	None	Gilbert & Kleiser (in press)
Barley	1000	Retarded growth	
Beans	25	Stimulation of growth	Lopez-Zavala et al (1975)
Chinese cabbage	10	Critical concentration	Takita (1982)
Clover	100 (C12-LAS)	Reduced germination	Parr (1968)
Coleus dwarf salicifolius	1000	Spindly seedlings from seeds	Farone (1979)
Crotolaria juncia	100 1000 10 000	94% germination 93% germination 76% germination	Sharma et al (1985)
Cucumber	10 100	No effect Retarded growth	Gilbert & Kleiser (in press)
Festuca pratensis	500-1000 250-500	EC 50 - germination EC 50 - seedling growth	Vandoni & Goldberg-Federico (1973b)
Kentucky blue grass	1000	About 50% germination	Farone (1979)
Lettuce	100 1000	None Retarded growth	Gilbert & Kleiser (in press)
Lolium italicum	1000 250-500	About 25% less germination About 50% less seedling growth	Vandoni & Goldberg-Federico (1973b)
Onions	100	Complete inhibition of germination	Farone (1979)
Orchids (seedlings)	10 100	30% reduced growth 60% reduced growth	Ernst et al (1971)
Orchids (seedlings)	1000	Extensive cellular changes	Healey et al (1971)

Table 5.16 continued

Plant	LAS concentration (mg/l)	Effect	Reference
Peas	50	Inhibited growth by 50%	Lichtenstein *et al* (1967)
Peas	100	Low germination; spindly seedlings	Farone (1979)
Peas	100 1000	None Retardation of growth	Gilbert & Kleiser (in press)
Pelargonium	1000	Little effect on germination	Farone (1979)
Pisum sativum	100 1000	92% germination 75% germination	Sharma *et al* (1985)
Rice	5 40	Promoted growth Inhibited growth	Wakiuchi *et al* (1977)
Rice	1, 10 50	None Marked decrease in yield	Taniyama (1978)
Rice	10	Critical concentration	Takita (1982)
Soybean	1000	Little effect on germination	Farone (1979)
Sunflower	1000	about 40% less germination	Farone (1979)
Tomatoes	100 1000 5000	(Hydroponics) None Some inhibition 50% inhibition	Popa *et al* (1967)
Tomatoes	40	Stimulation of growth	Lopez-Zavala *et al* (1975)
Tomatoes	100 1000	None Retarded growth	Gilbert & Kleiser (in press)
Trees (5 spp hardwood)	5000	No increase in absorption of two pesticides	Hall (1973)
- pines	100 1000 10 000	Reduced growth. more browning of shoots than seawater alone	Dowden *et al* (1979)
Trifolium repens (Clover)	1000 500	20% inhibition of germination EC 50, growth of seedlings	Vandoni & Goldber Federico (1973b)

Germination and growth of four species of plant were unaffected by LAS up to 100 mg/l (Vandoni and Goldberg-Federico 1973b); branched chain ABS was more toxic, while 3-sulphophenyl C 12-LAS was less inhibitory.

In Mexico, Lopez-Zavala et al (1975) reported no adverse effects of C 10 to C 13-LAS on the growth of barley, beans and tomatoes at 10 to 40 mg/l.

Farone (1979), in search of a surfactant which would shorten the germination time of seeds which took longer than 5 d to germinate, reported that LAS lowered the percentage of seeds which germinated and sometimes resulted in spindly seedlings.

Sharma et al (1985) reported the effects of soaking seeds of two plants in solutions of various concentrations of LAS which were then allowed to germinate in Petri dishes. Little effect was seen on seeds of Pisum sativum up to 1000 mg LAS/l, when 25% inhibition was recorded. Crotolaria juncea tolerated 1000 mg LAS/l (7% inhibition) but showed 24% inhibition at 10 000 mg/l, although the pH value of this latter solution was as high as 10.5.

A branched-chain ABS was not inhibitory up to 250 mg/l in pot tests with oats, red clover and alfalfa (Luzzati 1981a,b). In field tests there was no effect on the yield of peas up to 8 quintals/hectare ($\equiv 80 g/m^2$) and up to 4 quintals/hectare ($\equiv 40 g/m^2$) for potatoes.

In a recent study, Gilbert and Kleiser (in press) applied solutions containing 0, 0.1, 10, 100 and 1000 mg LAS/l to the soil and leaves of a range of crop plant types. There were no effects at 10 mg/l, and none at 100 mg/l except for cucumber. Visible and retardation effects were observed at

1000 mg/l on the five other species, barley, radish, pea, tomato and lettuce. De Henau et al (1986b) calculated that, under the conditions of the test used by Gilbert and Kleiser (in press), 10 mg LAS/l was equivalent to 2.2 mg LAS/kg soil, or 0.4 g LAS/m² soil.

The effects of adding LAS to soil equivalent to 1, 10, 100 amd 1000 mg/kg dry soil on the growth of Sorghum bicolor (sorghum), Helianthus annuus (sunflower) and Phaseolus aureus (mung bean) were recently assessed (Unilever 1987, unpublished). The LAS contained C 11, C 12 and C 13 homologues and had a MW of 343. There was no growth reduction over 21 d by 100 mg LAS/kg for any of the species and the 21 day EC 50 (growth) values were:

sorghum	167 mg/kg
sunflower	289 "
mung bean	316 "

At 1000 mg/kg still fairly high degrees of emergence of shoots occurred (69,91 and 95% respectively) but in each case several emerged shoots were dead.

The general opinion (Sivak et al 1982; De Henau et al 1986b) seems to be that LAS, with few exceptions, is not inhibitory to most plants at concentrations below 10 mg/l. This concentration is nominally equivalent to 2.2 mg LAS/kg which is typical of values so far reported in soils; however, in practice, this is probably equivalent in effect to higher soil concentrations.

A review, with 176 references, discussing the characteristics of surfactants and their effect on plants, as well as the influence of structure on phytotoxicity, has been presented by Sugimura et al (1984), in Japanese.

More recently Litz et al (1987) have studied in field tests the uptake of LAS by rye grass grown in plots of various German soils dosed with 5 g LAS/m^2 applied to the soil as an aqueous solution. This rate is equivalent to normal practice yielding a soil concentration of about 30 mg/kg but the method of application makes LAS more bio-available than the normal method of applying LAS as present in sewage sludge. The concentration of LAS in the whole plant after 45-54 d was 130-230 µg/g, the LAS being expressed as the non-specific "Azure A active substances" (AzAAS), which is roughly equivalent to MBAS. At a ten-fold higher rate of application - 50 g LAS/m^2 - a rate very unlikely to be found in practice, the concentration in rye grass after 45 to 54 d was 470 to 1000 µg AzAAS/g. In considering these high concentrations in grass, it must be borne in mind that the rate of LAS application to soil was very high, the method of application increased the bioavailability of LAS and the analytical method used was not LAS specific.

In laboratory tests lasting 7 d, much less LAS was absorbed by the plant, namely 5 µg/g at 5 g/m^2 and 20 µg/g at 50 g/m^2. The authors concluded that degradation of LAS within the plant was slow, but the evidence for this is not clearly stated in the paper.

6. CONCLUSIONS

1. The amount of LAS currently produced in the USA, Japan and Western Europe is approximately 1.4 million tonnes/year, based on the world demand for LAB. The actual quantity used in detergents in Western Europe in 1986 was 466 000 tonnes. Calculating the amount used in Western Europe from the population (320

million) and the average per capita use of 2.5 g/d (derived from analysis of sewage) yields only a usage of 292 000 tonnes, a discrepancy of about 35%. Part of the difference can probably be explained by the loss of LAS in the sewerage system due to biodegradation.

2. The pattern of use of detergents is such that LAS ends up mainly in domestic wastewater (eg sewage). In many countries this undergoes sewage treatment with high removal of LAS prior to discharge to the aquatic environment (rivers, streams, estuaries and the sea). However, in some countries a significant proportion of sewage is discharged directly to the aquatic environment with little or no treatment. The disposal of sewage sludge (crude, digested or activated) to agricultural land or landfills and the use of LAS-contaminated water for irrigation are other routes of entry of LAS to the environment. Thus, LAS may be present not only in sewage and sewage effluents but also in streams, groundwater, estuaries and the sea, both in solution and adsorbed to suspended solids and sediments and also in sewage sludge amended soils.

3. The identification and accurate quantification of LAS in various environmental compartments have been put on a much firmer basis by the development and application of chemical analytical methods which are specific for LAS. The chromatographic methods, with or without mass spectrometry, can be modified to determine LAS in total or as individual homologues and isomers. These methods are

specific and overcome the disadvantages of the colorimetric methods (such as the MBAS method) which are non-specific and also respond to interfering substances.

4. Using these LAS-specific analytical methods, and radioactive-labelled LAS, the fate of LAS has been studied both in laboratory experiments and in the field. The overwhelming evidence from these studies is that LAS degrades extensively and is completely mineralised at relatively high rates in the aquatic environment and in soil provided aerobic conditions exist. The degradation of LAS is mainly, if not completely, by biological processes before or after adsorption on to sludge or sediment. Chemical and photo-chemical processes appear to play little part.

   Although LAS is not degraded under strictly anaerobic conditions, LAS present in anaerobically digested sludge (or in any other type of sludge) is subsequently degraded when the sludge is exposed to oxygen when applied, for example, to drying beds or agricultural land.

5. Most of the evidence shows that LAS is completely degraded (mineralised) in the environment. However, in a small number of laboratory experiments aromatic intermediates have been reported to persist for long periods, whereas environmental samples - treated sewage - contain either no intermediates or very low concentrations. The use in batch tests of C-labelled LAS has confirmed that well over 90% of both ring and

alkyl-chain carbon is mineralised or converted to biomass. Incomplete degradation reported in the small number of laboratory experiments has been attributed to factors such as the concentration and biochemical activity of the inoculum, deficiencies and imbalances in media, lack of biomass in synthetic sewage and shock caused by transfer of biomass to new environment, etc. Evidence for this conclusion is based on laboratory tests where changes in test conditions, for example increasing the concentration of biomass or using acclimatised sewage, resulted in higher degrees of removal of organic carbon and decreased concentrations of intermediates.

6. The bacteriology of LAS degradation has not been fully clarified, but this is of little practical consequence. No single species has been isolated which completely mineralises LAS. Some species are able to convert LAS to aromatic intermediates in the presence of nutrient broth, but very few have been found to attack LAS in the absence of other substrates. Only one species has been isolated which could convert the intermediates (sulphophenylalkanoic acids) to mineral products. This organism in mixture with one of the LAS attackers was able to degrade LAS completely, thus indicating that more than one species is required for the mineralisation of LAS.

7. The kinetic rates of the biodegradation of LAS to CO_2 in a variety of environmental compartments are 1st order with respect to LAS at the concentrations likely to be present. The rates have been found to increase,

sometimes by as much as 100-fold, as the microbial population becomes acclimatised to the surfactant. A summary of rates of mineralisation is given in Table 6.1. The rates are sufficiently high relative to the residence time of LAS in the specific environmental compartment to ensure that not only will LAS not accumulate, but that the ambient concentrations remain relatively low.

8. The removal of LAS in efficiently operated activated sludge systems is high (95-99%) and similar removals are obtained with biological (trickling) filters operated at European loading rates. In the USA, however, filters are operated at higher loadings and 73 to 87% LAS is removed. It is common experience that in the activated sludge process the removal of LAS proceeds at a higher rate than nitrification, thus if the sewage treatment works is designed to nitrify it will at the same time remove LAS. Similarly, if partially treated or untreated sewage is discharged to the aquatic environment LAS tends to be removed faster than ammonia which at elevated concentration in the undissociated form is toxic to aquatic life. Anaerobic digestion of sewage sludge removes very little, if any, LAS.

The average chain length of LAS tends to be shorter after aerobic sewage treatment. This may be due to the difference in the degree to which the homologues are adsorbed onto solid particles but may also be the results of preferential biodegradation.

Table 6.1: Selected rate constants and half-life periods of the mineralisation of LAS in the environment

Environmental Compartment	Rate Constant (d^{-1})	Half-life (d)
River water		
- above outfall, alone	0.05	14
- above outfall + sediment	0.26	2.8
- below outfall, alone	0.5	1.4
- below outfall + sediment	0.95	0.7
Groundwater slurry	0.63	1.1
Sub-surface sediment	0.64	1.1
Soil	0.07	10 (3-35)*
Soil and sludge	0.16	4.3

* range of a large number of soils.

9. LAS is adsorbed onto soil and sediments. The degree of adsorption of LAS onto solids is roughly proportional to the organic content of the solids.

10. Degradation studies which have been made with sludge-amended soils suggest that LAS degrades at relatively high rates. The reported half-life periods ranged from 3 to 35 days depending on the history of the soil. Thus at normal sludge application rates the concentration of LAS in the treated soil is reduced to pre-application values within 12 to 120 days. LAS is completely mineralised since 70% of the theoretical CO_2 is produced.

11. The ranges of concentrations of LAS found in the various parts of the aquatic environment are given in Tables 6.2 and 6.3; these decrease progressively from sewage to effluents, to river waters and finally to very low concentrations in estuaries and the sea.

The concentration of LAS in sewage can exceed 10 mg/l in some countries but is generally in the range 2 to 5 mg/l. The difference in concentration found is mainly associated with differences in water usage. Values quoted for LAS concentrations in the environment are generally at the upper end of the range of environmental levels likely to be present because measurements tend to be made at sites where LAS is expected to be present at elevated concentrations.

Concentrations in rivers are usually less than 0.05 mg/l; levels in some countries have been reported to be as high as 1 to 2 mg LAS/l

Table 6.2: Concentrations of LAS found in environmental compartments and concentrations affecting microbial activity in those compartments

Environmental Compartment	Units	Concentration of LAS in the compartment	Concentration of LAS* giving an effect on microbial activity
A. Sewage	mg/l	2-10	> 20: nitrification > 70: settlement of activated sludge >100: respiration rate
B. Effluent from sewage treatment	mg/l	AS 0.02-0.1 TF 0.6	
C. Rivers			
- highest probable 'unpolluted' values	mg/l	0.01-0.04	(0.5: no effect (5.0: no effect, after (acclimatisation
- just below outfall		0.1	
- severely polluted		1-2	(100 : ditto
D. Estuarine/coastal waters	mg/l	0.0008-0.008	(5.1: EC50 on ^{3}H-thymidine (incorporation
- near outfall		up to 0.03	(0.056: EC50 on ^{14}C-glucose incorporation

Table 6.2 (continued)

Environmental compartment	Units	Concentration of LAS in the compartment	Concentration of LAS* giving an effect on microbial activity
E. Sewage Sludges			
- primary/anaerobic	g/kg	5-15	15: first observed effect on methane production
- aerobic		2-5	
- activated sludge		0.1-1	
- air dried		0.15	
F. Soils			
"Normal"	mg/kg	1-2	(100: 10% inhibition (of nitrification
Just after sludge application		13-47	(500: 50% inhibition of nitrification

* = as commercial product
AS = activated sludge
TF = trickling (or percolating) filter

Table 6.3: Concentrations of LAS in environmental compartments and Likely "Safe Concentrations" in the environmental compartments

Environmental compartment	Concentration of LAS in the compartment	Organism	LC50*	Likely "Safe Concentration" (LSC) for most sensitive stage	Note
A. <u>Rivers</u>					
- highest probable "normal" values	0.01-0.04 mg/l	Algae	0.9-100 mg/l	0.05-0.1 mg/l	1
- just below outfall	0.1 mg/l	<u>Daphnia</u>	1-10 mg/l	0.6-1.2 mg/l	2
- severely polluted	1-2 mg/l				
		Fish			
		- fathead minnow	4 mg/l	0.5 mg/l	3
		- rainbow trout	2 mg/l	0.03 mg/l	4
B. <u>River sediments</u>		<u>Chironomus riparius</u>	-	no effect at >319 mg/kg	5
- above outfall	1-10 mg/kg				
- just below outfall	100-300 mg/kg				

Note 1. LSC based on "first effects" concentration for <u>Microcystis aeruginosa</u> observed between 0.05 and 0.1 mg/l C 11.8 LAS (Lewis 1986).
2. LSC based on 21 d NOEC of 1.2 for C 11.8 and 0.58 for C 13. (Maki 1979).
3. LSC based on like cycle NOEC of 0.48 mg/l for C 11.7 (Holman and Macek 1980).
4. LSC based on early life stage test, 14 d LC50 of 0.12 for egg lethality (Vailati <u>et al</u> 1975) and applying an arbitrary safety factor of 4 in the absence of a reported "no-effects" concentration.
5. LSC based on "no effects" in pupal development or adult emergence at 319 mg/kg dry sediment (Pittinger <u>et al</u> unpublished).

Table 6.3: (continued)

Environmental compartment	Concentration of LAS in the compartment	Organism	LC50*	Likely "Safe Concentration" for most sensitive stage
C. <u>Estuarine/coastal waters</u>				
- away from outfall	0.0008-0.008 mg/l	Algae+	6 h LC100** (C13) 0.025 mg/l	
- near outfall	0.01-0.03 mg/l	Oyster larvae	6-h, 1 mg/l** 1 week 0.05 mg/l - 100% kill	
D. <u>Estuarine/sea sediments</u>				
- at sea	none detected			
- near outfall	5-17 mg/kg			
E. <u>Soils</u>				
- "normal"	1-2 mg/kg	Various terrestrial plants	-	100 mg/kg, no effect (based on LAS on soil).
- just after sludge application	13-47 mg/kg			

\+ <u>Gymnodinium breve</u> * of commercial product, except where stated

** Lowest reported value for relevant species selected because the system of disposal of wastewaters allows washing water or sewage to be discharged directly to rivers. The concentrations of LAS in estuarine and coastal waters away from outfalls are extremely low at <0.008 mg/l, but just below outfalls the concentration can be as high as 0.03 mg/l (Table 6.2). No LAS could be detected in sediments taken at sea, while just below outfalls in estuaries and on the coast sediments contained 5 to 17 mg LAS/kg (Table 6.3). It is to be noted that the average chain length of LAS in effluents and rivers is less than that in the corresponding sewage, indicating that the higher homologues are removed to a greater degree than the lower homologues.

12. Because of their great variety, high metabolic potential and adaptability, heterotrophic micro-organisms exhibit more tolerance to LAS than do other organisms. The respiration rate of activated sludge has been shown to be unaffected by concentrations of LAS up to 100 mg/l, though concentrations over 20 mg/l sometimes had a slight effect on nitrification and at about 70 mg/l settlement of sludge began to be affected (Table 6.2). As the concentration of LAS in raw sewage is normally less than 10 mg/l, and as the surfactant is readily biodegradable no deleterious effects on sewage treatment occur.

The microbial activity of epilithic bacteria in rivers is unaffected by 0.5 mg LAS/l, a value well in excess of that usually present in river water. Rivers already receiving LAS were unaffected by concentrations in excess of

5 mg LAS/l and other rivers showed no adverse effects at this concentration after their microbial populations had become acclimatised to the surfactant.

13. The effect of the concentrations of LAS found in coastal waters on microbial activity is in question because of conflicting reports from assimilation tests. The EC50 for thymidine incorporation has been given as 5.1 mg LAS/l whereas for glucose incorporation an EC50 value as low as 0.056 mg LAS/l has been reported. The practical significance of such tests is uncertain but if the latter EC50 is valid, LAS could have some effect on microbial activity in coastal waters, but only very near to outfalls.

14. Even though LAS does not degrade under strictly anaerobic conditions, the concentrations currently present in sewage sludges (5 to 15 g/kg) would not affect methane production (Table 6.2). Inhibition of anaerobic digestion of sewage sludge has been observed at a concentration of about 15 g LAS/kg, although sludge containing about twice this concentration of LAS has been digested successfully by careful operation.

The concentration of LAS in soils is far lower (1 to 2 mg/kg), even after recent applications of sludge (13 to 47 mg/kg), than that required to give 10% inhibition of nitrification (100 mg/kg) (Table 6.2). Since the autotrophic nitrifying bacteria are much more sensitive to inhibition than the heterotrophic bacteria, it can safely be concluded that oxidation of organic carbon is not affected by LAS in soil.

15. The lethal effects of LAS on a large variety of organisms have been studied in the laboratory to derive acute LC50 values. In addition several chronic toxicity tests for the commonly accepted test species have been conducted using impairment of reproduction and the development of early life stages as criteria. The toxicities of individual homologues of LAS have also been studied and the higher homologues have been found to be more toxic than the lower ones (they are also removed to a greater extent in sewage treatment than the lower members). For practical reasons, only the effects of commercial mixtures of LAS are considered here, although it is recognised that the toxicity of an observed environmental concentration of LAS in effluent, river water or the sea will be overestimated if the reference toxicity values were obtained in aqueous solutions of (untreated) commercial LAS. All the metabolic intermediates tested were found to be much less toxic than the original intact LAS.

16. Table 6.3 shows that the concentrations of LAS in rivers do not reach levels harmful for algae, Daphnia and the two species of fish for which chronic tests have been reported. This conclusion is reinforced by the knowledge that the values reported for concentrations of LAS in rivers refer to those rivers where LAS was likely to be found at relatively high concentrations.

In the case of algae a greater degree of protection is afforded since, in model ecosystems containing algae, the "first effect concentrations" of LAS on various properties of the system were significantly higher than those found in laboratory tests of single algal species.

Few studies have been made on organisms inhabiting river sediments but larvae of <u>Chironomus riparius</u> were not affected by LAS in river sediment at concentrations of about 300 mg/kg. Thus, even near sewage effluent outfalls the larval stage of the midge will not be harmed by LAS.

The effect of LAS on organisms in estuaries and coastal waters is not yet fully established but for most species any effects are not likely to be serious or significant. There is conflicting or unclear evidence as to the effect on the "red-tide" alga and on the larvae of oysters (Table 6.3), which might be adversely affected at concentrations found close to outfalls.

Tests with plants grown on sludge-amended soils have indicated that the levels of LAS resulting from sewage sludge applications (13 to 47 mg/kg) are unlikely to have an effect (NOEC >100 mg/kg). There is an absence of data on possible effects on earthworms and other organisms inhabiting soil.

17. Finally, it can be concluded from the results of the literature review and from the successful use and uneventful disposal of the surfactant for the past quarter of a century, that at the current level of use LAS is unlikely to pose a hazard to the environment.

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