Human & Environmental Risk Assessment
on ingredients of
European household cleaning products

Alcohol Sulphates
Human Health Risk Assessment

Draft

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Further information on the HERA Project including the HERA Methodology document and other risk assessments can be found at this website (www.heraproject.com)

2. Executive Summary

The use of household laundry and cleaning products containing Alcohol Sulphates (Alkyl Sulphates, AS) can result in exposure to AS. The skin is the predominant route of exposure to AS, however exposure from oral intake and inhalation are also considered in this risk assessment. Direct skin exposure occurs mainly in hand-washed laundry, laundry pre-treatment, hand dishwashing and surface cleaning tasks and to a smaller extent also from residues in fabric after the washing cycle. Consumers may be indirectly exposed to low levels of AS via the drinking water and food due to the potential environmental presence of AS. Residues deposited on eating utensils and crockery after hand dishwashing may be another source of oral exposure. The use of spray cleaners is also a potential source of exposure to AS through inhalation of aerosols generated by the sprayer. The calculated body burden of AS taking into account all routes of exposure and using highly conservative or worst-case assumptions is 5.93 µg/kg bw/day.

Based on an extensive database, it has been shown that the toxicological properties of AS of different chain length covered in this risk assessment are qualitatively and quantitatively similar, justifying the decision to consider AS as a single category.

AS are of a low order of acute oral and dermal toxicity. AS are not genotoxic, mutagenic or carcinogenic, and there was no evidence of adverse effects on fertility, reproduction and development. AS are irritant to skin and eyes when applied neat or as a concentrated solution, however AS concentrations below 1% were essentially non-irritating to the human skin. The repeated-dose toxicity of AS was evaluated in several sub-acute, sub-chronic and chronic toxicity studies. In dermal and oral gavage studies AS caused local irritation at the site of first contact. The target organs for the systemic toxicity of AS are the liver and the kidney. The lowest NOAEL of AS was observed in a 90-day feeding study in the rat at a dose level of 61 mg/kg/day and was based on liver toxicity.

The comparison of the aggregate exposure and the systemic NOEL results in a Margin of Exposure of 10,100. Local dermal effects due to direct or indirect skin contact with AS containing solutions in hand-washed laundry, hand dishwashing or hard surface cleaning tasks are not of concern because AS is not a contact sensitiser and not expected to be irritating to the skin at in-use concentrations.

In summary, the human health risk assessment has shown that the use of AS in household laundry and cleaning detergents is safe and that consumer exposures are not of concern.

Note that Section 4 Environmental Risk Assessment (which includes Section 3 Substance Characterisation) was published at www.heraproject.com in March 2002. These assessments will be merged and a single comprehensive document produced at a later date.

5.1 Exposure Assessment

The substances included in this assessment are listed in Appendix I and are considered as a single category of Alcohol Sulphates (Alkyl Sulphates, AS). Appendix II provides a complete discussion of the rationale for applying data on AS as a single category, based on similarities in: a) structure, b) mechanisms of toxic action, c) toxicokinetics, d) pathways of metabolism, and e) toxicological profiles.

5.1.1 Product Types

AS is used in many household detergents including laundry powders and liquids (maximum concentration in regular and compact formulations 10%), fabric conditioners (maximum concentration: 1.3%), hand dishwashing liquids (maximum concentration 16.5%; not present in machine dishwashing products) and hard surface and bathroom cleaners (maximum concentration: 8%), soap bars (maximum concentration 5%), toilet blocks (maximum concentration 20%) and surface wipes (maximum concentration 0.1%).

5.1.2 Consumer Contact Scenarios

Based on the product types, the following consumer task and the related exposure scenarios were identified:

Fabric Washing:

- Direct skin contact with neat (laundry pre-treatment) or diluted consumer product (hand-washed laundry)
- Indirect skin contact via release from clothes fibers to skin
- Inhalation of detergent dust

Dishwashing

- Direct skin contact (hand dishwashing)
- Oral ingestion of residues deposited on dishes

Surface cleaning

- Direct skin contact with diluted consumer product
- Inhalation of aerosols generated by spray cleaners

Other scenarios

- Oral ingestion of residues in drinking water and food
- Accidental or intentional overexposure

Overall, exposures routes can be categorised as

1. Skin contact – direct and indirect
2. Inhalation - direct
3. Oral – indirect

5.1.3. Consumer Exposure Estimates

There is a consolidated overview concerning habits and practices of use of detergents and surface cleaners in Western Europe which was tabulated and issued by the European Soap and Detergent Industry Association, AISE [AISE/HERA Table of H&P (2002)]. This table reflects consumers' use of detergents in g/task, use frequency, duration of task and other uses of products and is largely the basis for the exposure estimates in the following paragraphs. In some instances, e.g. habits & practices of pre-treatment of clothes, the information provided by the AISE/HERA table was not detailed enough for a targeted exposure assessment and the H&P information was directly provided by the member companies of AISE.

5.1.3.1. Direct skin contact

A. Hand-wash Laundry. Hand-washing of laundry is a common consumer habit. During this procedure, the AS-containing laundry solution comes in direct contact with the skin of hands and forearms. A hand-washing task typically takes 10 minutes [AISE/HERA Table of H&P (2002)]. The exposure to AS is estimated according to the following algorithm from the HERA guidance document:

$$Exp_{sys} = F_1 \times C \times Kp \times t \times S_{der} \times n / BW$$

For this exposure estimate, the terms are defined with following values for the calculation considering a worst-case scenario:

F_1	percentage weight fraction of substance in product	***10%*** (0.1) [AISE Internal data]
C	product concentration in mg/ml:	***10 mg/ml*** [AISE/HERA Table of H&P (2002)]
Kp	dermal penetration coefficient	***3.9x 10^{-5} cm/h*** [Prottey, 1975)
t	duration of exposure or contact	***10 min (0.167h)*** [AISE/ HERA Table of H&P (2002)]
S_{der}	surface area of exposed skin	***1980cm^2*** [TGD (1996)]
n	product use frequency (tasks per day)	**3** [AISE/HERA Table

		of H&P (2002)]
BW	body weight	***60 kg*** [TGD, (1996]

Exp_{sys} = [(0.1) x (10 mg/ml) x (3.9×10^{-5} cm/h) x (0.167h) x 3 x (1980 cm²)] / 60 kg=

0.64 µg/kg

B. Laundry Pre-treatment. Consumers typically spot-treat stains by hand with the help of either a detergent paste (i.e. water/laundry powder = 1:1) or a laundry liquid that is applied directly on the garment. In this exposure scenario, only the skin surface of both hands (~ 840 cm^2) is exposed and the treatment time is typically less than 10 minutes [AISE/HERA Table of H&P (2002)]
The exposure calculation is conducted by using the algorithm described in chapter 5.1.3.1. The following assumptions are considered to represent a realistic reflection of this scenario.

F_1	percentage weight fraction of substance in product	***10% (0.1)*** (laundry liquid) [AISE Internal data]
C	product concentration in mg/ml:	***1000 mg/ml*** [AISE/HERA Table of H&P (2002)]
Kp	dermal penetration coefficient	***3.9×10^{-5} cm/h*** [Prottey, 1975]
t	duration of exposure or contact	***5 min (0.083h)*** [reasonable worst case]
S_{der}	surface area of exposed skin	***840cm^2***, [TGD, 1996]
n	product use frequency (tasks per day)	***0.5*** [AISE/HERA Table of H&P (2002)]
BW	body weight	***60 kg*** [TGD, 1996]

Exp_{sys}= [(0.1) x (1000 mg/ml) x (3.9×10^{-5}cm/h) x (0.083h) x (840cm²) x 0.5]/ 60kg

2.27 µg/kg/day

This exposure estimate can be regarded to be very conservative in many respects. To note are the assumptions related to neat product use and the surface area of exposed skin. Typically, consumers pre-wet the laundry before applying the detergent for pre-treatment or conduct the pre-treatment under running tap water. Both practices lead to a significant dilution that is not reflected in this exposure estimate. It should also be considered that only a fraction of the two hands' surface skin would actually be exposed. The assumption that both hands will be fully immersed leads to a likely overestimate of the true exposure.

C. Hand dishwashing. The determination of AS exposure from hand dishwashing using an AS containing product can be estimated using the following algorithm:

$$Exp_{sys} = F_1 \times C \times Kp \times t \times S_{der} \times n / BW$$

For a reasonable worst-case scenario, the following assumptions have been made:

F_1	percentage weight fraction of substance in product	***16.5%*** (0.165) [Internal AISE Data]
C	product concentration in mg/ml:	***1 mg/ml*** [AISE/HERA Table of H&P (2002)]
Kp	dermal penetration coefficient	***3.9 x 10^{-5} cm/h*** [Prottey, 1975]
t	duration of exposure or contact	***45 min (0.75h)*** [AISE/HERA Table of H&P (2002)]
S_{der}	surface area of exposed skin	***1980 cm^2*** [TGD, 1996; area of hands and forearms]
n	product use frequency (tasks per day)	***3*** [AISE/HERA Table of H&P (2002)]
BW	body weight	***60 kg*** [TGD, 1996]

$$Exp_{sys} = [(0.165) \times (1\ mg/ml) \times (3.9 \times 10^{-5}\ cm/h) \times (0.75h) \times (1980\ cm^2) \times 3] / 60\ kg =$$

0.47 µg/kg/day

D. Hard surface cleaning. For this scenario it is assumed that the solution of the hard surface cleaning product containing AS comes into direct contact with the skin of the hands. The dermal exposure to AS can be estimated using an algorithm similar to that used for hand dishwashing (See scenario C).
The assumptions below are considered representative of a reasonable worst case:

F_1	percentage weight fraction of substance in product	***8% (0.08)*** [Internal AISE data]
C	product concentration in mg/ml:	***12 mg/ml*** [AISE/HERA Table of H&P (2002)]
Kp	dermal penetration coefficient	***3.9 x 10^{-5} cm/h*** [Prottey, 1975]
t	duration of exposure or contact	***20min (0.334h)*** [AISE/HERA Table of H&P (2002)]
S_{der}	surface area of exposed skin	***1980 cm^2*** [TGD, 1996;area of hands and forearms]

n	product use frequency (tasks per day)	***1*** [AISE/HERA Table of H&P (2002)]
BW	body weight	***60 kg*** [TGD, 1996]

$$\mathbf{Exp_{sys}} = [(0.08) \times (12\ mg/ml) \times (3.9 \times 10^{-5}\ cm/h) \times (0.334h) \times 1 \times (1980\ cm^2)] / 60\ kg =$$

0.41 µg/kg/day

E. Other direct skin contact scenarios. Other scenarios for potential direct dermal exposures may include activities such as filling laundry tablets or the use of toilet cleaners. These are not considered here because the short contact time and the small skin surface area involved or the low frequency (once weekly) combined with a short duration (< 1 minute). Exposure resulting from such activities is considered to be negligible.

5.1.3.2. Indirect skin contact

Wearing clothes. Residues of components of laundry detergents may remain on textiles after washing and can transfer from the textile to the skin. There are no data available showing how much AS is deposited on the fabric following a wash process. This value has, however, been determined for LAS, an anionic surfactant that is widely used in laundry detergents. Rodriguez et al (1994) determined that after a typical washing process with a laundry detergent containing linear alkylbenzene sulphonate (LAS), 2.5 g LAS per kilogram wash resided on the fabric. LAS is present in laundry detergents at levels higher than AS (18% LAS versus 10% AS) [Rodriguez, C., et al. (1994)]. Given the physico-chemical and structural similarity of these two surfactants, it is assumed that these data on LAS represent a worst-case assumption for the remaining amounts of AS on fabric.

The following algorithm was recommended in the HERA guidance document to estimate the dermal exposure to detergent residues in the fabric:

$$\mathbf{Exp_{sys} = F_1 \times C` \times S_{der} \times n \times F_2 \times F_3 \times F_4 / BW}$$

For the AS exposure estimate, the terms are defined with the following values for the calculation:

F_1	percentage weight fraction of substance in product	***Not used, = 1***
C'	product (AS) load*:	***2.5 x 10^{-2}*** mg/cm^2 [Rodriguez et al., 1994]
S_{der}	surface area of exposed skin	***17600 cm^2*** [TGD, 1996]
n	product use frequency (tasks per day)	***Not used, = 1***
F_2	percent weight fraction transferred to skin	***1% (0.01)*** [Vermeire (1993)]

F_3	percent weight fraction remaining on skin	***100% (1)*** (worst case)
F_4	percent weight fraction absorbed via skin**	***1% (0.01)*** [Schaefer, H. (1996)]
BW	body weight	***60 kg*** [TGD, 1996]

* C' was determined by multiplying the experimental value of the amount of AS deposited on fabric after a typical wash (2.5 g/kg [Rodriguez et al., 1994]) times an estimated value of the fabric density (FD = 10 mg/cm^2 [Internal P&G data]).
**. Schaefer and Redelmeier (1996) reported that the dermal penetration of ionic substances is very low; experimental animal data on AS indicate that the fraction absorbed via the skin is ca. 0.3% (Prottey and Ferguson, 1975)

$$\mathbf{Exp_{sys\ (indirect\ skin\ contact)}} = [(2.5 \times 10^{-2}\ mg/cm^2) \times (17600\ cm^2) \times (0.01) \times (0.01)] / 60kg$$

$$= \mathbf{0.73\ \mu g\ /kg/day}$$

5.1.3.3. Exposure by Inhalation

A. Dust Charging a washing machine with laundry powder may lead to dustiness and could potentially result in the inhalation of these dust particles. Van de Plassche, et al. (1998) determined an average release of ca. 0.27 µg dust per cup of product (i.e. laundry powder) used for machine laundering. AS is present in laundry powder detergents at a maximum level of 10% (or 0.027 µg AS/use). Taking the worst case assumption that all released dust is inhaled and a frequency of product use of 3 times daily, the exposure of an adult with an body weight of 60kg to AS is estimated to be

$$\mathbf{Exp_{sys\ (inhalation\ of\ detergent\ dust)}} = (0.27\ \mu g) \times 0.1 \times 3 / 60\ kg =$$

$$\mathbf{0.001\ \mu g\ /kg/day}$$

B. Aerosols. The use of surface cleaning sprays can result in aerosol formation. AS may be present in these products at a maximum concentration of 8%. The HERA guidance document specifies the algorithm to be used for calculation of consumers' worst-case exposure to AS-containing aerosols generated by the spray cleaner:

$$\mathbf{Exp_{sys} = F_1 \times C` \times Q_{inh} \times t \times n \times F_7 \times F_8 / BW}$$

F_1	percentage weight fraction of substance in product	***8% (0.080)*** [AISE Internal data]
C`	product concentration in air:	***0.35 mg/m^3*** * [P&G, internal data]
Q_{inh}	ventilation rate	***0.8 m^3/h*** [TGD, 1996]
t	duration of exposure	***10 min (0.17h)*** [AISE/HERA Table of H&P (2002)]

n	product use frequency (tasks per day)	***1*** [AISE/HERA Table of H&P (2002)]
F_7	weight fraction of respirable particles	***100% (1)*** [worst case]
F_8	weight fraction absorbed or bioavailable	***100% (1)*** [worst case]
BW	body weight	***60 kg*** [TGD, 1996]

* This value was obtained by experimental measurements of the concentration of aerosol particles smaller than 6.4 microns in size which are generated upon spraying with typical surface cleaning spray products. It is assumed that these particles are fully respirable and bio-available.

$$\mathbf{Exp_{sys\ (inhal.\ of\ aerosol)}} = [(0.08) \times (0.35\ mg/m^3) \times (0.8\ m^3/h) \times (0.17\ h) \times (1)] / 60\ kg =$$

0.06 µg/kg/day

5.1.3.4. Oral Exposures

A Indirect exposure via the environment The presence of AS in the environment can lead to indirect exposure through the intake of drinking water and food. The Environmental Risk Assessment of AS has estimated that drinking water and food may contain residual amounts of AS. In Chapter 4 of this document the Environmental Risk Assessment of AS has estimated a total intake of .6 x 10^{-5} mg/kg via drinking water and a total exposure of 5.3 x 10^{-4} mg/kg/day via drinking water and food, based on a regional scenario.

$$\mathbf{Exp_{sys\ (oral\ via\ drinking\ water\ and\ food)} = 0.53\ \mu g/kg/day}$$

The exposure estimate of the exposure via the environment should be regarded as highly unrealistic and conservative as it assumes that all food and all drinking water contains AS. This scenario does not take into account that AS will be removed in drinking water treatment plants; also, the assumption that all food is grown locally is highly unrealistic.

B. Indirect exposure via dishwashing residues. Oral exposure to AS can originate from residues present on eating utensils and crockery washed in hand dish-washing detergents. The daily exposure to AS from eating with utensils and dishware that have been washed in hand dish-washing detergents can be estimated according to the following algorithm from the HERA guidance document:

$$\mathbf{Exp_{sys\ (oral\ from\ dish\ washing\ residues)} = F1 \times C` \times Ta' \times Sa / BW}$$

For this exposure estimate, the terms are defined with following values for the calculation considering a worst-case scenario:

F_1	percentage weight fraction of substance in product	***16.5% (0.165)*** [AISE Internal data]

C`	concentration of product in dish wash solution:	***1 mg/cm^3*** [AISE/HERA Table of H&P (2002)]
Ta'	amount of water left on dishes after rinsing	***5.5 x 10^{-5} ml/cm^2*** [O. J. France, 1990; Schmitz (1973)]
Sa	area of dishes in daily contact with food	***5400cm^2*** [O. J. France, 1990]
BW	body weight	***60 kg*** [TGD, 1996]

Exp$_{sys\ (oral\ dish\ deposition)}$ = *[(0.165) x (1 mg/cm^3) x (5.5 x 10^{-5} ml/cm^2) x (5400 cm^2)] /60 kg =*

0.82 µg/kg/day

5.1.3.5 Aggregate Exposure

The overall body burden of consumers to AS through the use of AS containing house hold laundry and cleaning products by combining all scenarios and all exposure routes is calculated to be:

Exp$_{sys}$ = 5.94 µg/kg/day

Considering the contribution of the different routes of exposure, the exposure via the skin represents the major route of exposure (*ca*. 76% of the total systemic exposure), and the oral route being less prominent (*ca*. 22% of the total systemic exposure). Exposure to AS by inhalation is of minor (*ca*. 1%) importance in the use of household laundry and cleaning products.

The aggregate exposure estimate is an unrealistic, worst-case estimate of the body burden of AS. It combines several scenarios; each using highly conservative or worst-case assumptions and it is virtually impossible that each of these conservative input parameters will apply concurrently in all cases for this overall exposure estimate.

5.1.3.6. Accidental or Intentional Exposure

Accidental or intentional exposure to AS can occur through splashing, spilling or ingestion of household detergent products.

There are no reports of fatal cases or serious injuries arising from accidental ingestion of AS via household detergent products, which may contain levels of up to 20% AS. The German Federal Institute for Health Protection of Consumers and Veterinary Medicine [BgVV (1999)] has published a report on the products involved in poisoning cases. No fatal case of poisoning with detergents was reported in this report. Detergent products were not listed as dangerous.

AS in concentrated form has a potential for irritation of skin and eyes (see section 5.2.3). Inadvertent skin or eye contact of consumers with household cleaning and laundry products containing AS involves only formulated products. Therefore the potential for skin or eye irritation should be assessed taking into account formulated product instead of AS.

5.2 Hazard Assessment

The substances included in this assessment are listed in Appendix I and are considered as a single category of AS. Appendix II provides a complete discussion of the rationale for applying data on AS as a single category, based on similarities in:

a) structure,
b) mechanisms of toxic action,
c) toxicokinetics,
d) pathways of metabolism, and
e) toxicological profiles.

5.2.1 Acute Toxicity

The acute toxicity profile of AS is largely a function of their surfactant properties, and therefore very similar acute effects are observed. AS displayed a low order of toxicity when tested in rodents. Oral LD_{50}s for AS ranged from 1.4 to 7.8 g/kg in the rat and 2.6 to greater than 8 g/kg in the mouse, depending on carbon chain length.

The clinical symptoms of acute oral exposure to rodents, such as reduced activity, tremors and diarrhea, were indicative of gastrointestinal distress. Pathological changes from acute exposure to high doses of the surfactants included fluid distention and irritation of the forestomach, irritation of the small intestine, and pale livers and kidneys in decedents. Survivors displayed thickening of the stomach wall, a response to stomach injury.

Table 1 summarizes selected studies that illustrate the relationship between acute oral toxicity and carbon chain length. For the AS, shorter chain lengths are more acutely toxic. A homologous series of sodium salts of AS, ranging in chain length from C_8 to C_{18}, showed decreasing toxicity with increasing chain length, particularly for carbon chain lengths of 16 and higher. Evaluations of test materials comprising mixtures of chain lengths also showed a trend to lower toxicity as the proportion of longer chain lengths in the test material increased.

Since the surfactants were administered by gavage, the acute oral effects were predominantly due to local GI tract irritation. Therefore, trends in acute oral toxicity of AS surfactants by chain length parallel a similar trend in the relative irritant potencies.

Table 1
Acute Toxicity of AS in rodents[1]

Surfactant[2]	Species	Oral LD_{50} (g/kg)	References[3]
C_8 AS Na	Mouse	2.9	Gloxhuber, 1974
C_{10} AS Na	"	2.2	"
C_{12} AS Na	"	2.7	"
C_{14} AS Na	"	3.0	"
C_{16} AS Na	"	>8	"
C_{18} AS Na	"	>8	"
C_{12-15} AS Na Distribution: C_{12}: 18% C_{13}: 28% C_{14}: 30% C_{15}: 20%	Mouse	2.9 to 3.8	Unilever, 1975 unpublished data
C_{13-15} AS Na Distribution: C_{13}: 62% C_{15}: 37%	"	2.9	Unilever, 1975 unpublished data
C_{15-16} AS Na Distribution: C_{15}: 51% C_{16}: 49%	"	6.8	Unilever, 1976 unpublished data
C_{12} AS Na	Rat	1.4 - 2.7	Henkel, 1983 (unpublished data)
C_{12} AS K		3.4	P&G 1978 (unpublished data)
C_{14-18} AS Na Distribution: C_{14} <1% C_{16}: 30% C_{18}: 69%	" “	4.01	P&G 1974 (unpublished data)
≤C_{14}: 1% C_{16}: 65.5% C_{18}: 31.6% C_{20}: 1.6	“	5.24	"
≤C_{14}: 0.4% C_{16}: 57% C_{18}: 33.4% C_{20}: 9.16	“	7.84	"

[1] Selected studies shown for illustrative purposes
[2] Chemical shorthand nomenclature used. See Appendix I.
[3] For complete data set, including unpublished data, see Appendix III.

Acute dermal toxicity studies with C_{12-13} AS (potassium salt), C_{10-16} AS (magnesium salt) and C_{10-16} AS (ammonium salt) illustrated the skin irritation potential of the materials. Application of 0.5 g/kg to rabbit skin induced local skin irritation, eschar formation and necrosis, with sloughing at days 6-21, and residual hyperpigmentation. There was no evidence of systemic toxicity when applied to intact or abraded skin, consistent with the fact that dermal penetration of the surfactants is low. (See Appendix III.)

Inhalation of aerosolized solutions of C_{12} AS (sodium [Na], ammonium [NH_4], triethanolamine [TEA] salts) showed that these materials caused irritation of the respiratory tract in mice. After 2 minute exposures, a 50% reduction in respiratory rate occurred at concentrations of 88, 114 and 135 µg/L for the Na, NH_4 and TEA salts, respectively. Evidence of systemic toxicity was not reported (A.D. Little, 1991; Ciuchta and Dodd, 1978 and BUA Report 189, 1996)

In summary, the acute toxicity studies demonstrate that category of AS are of a low order of acute toxicity by the oral and dermal route and display toxic effects related to their surfactant properties. Their potency is explained by mechanistic trends in solubility and phase behavior by chain length as well as the contribution of the counter ions to overall irritation potential.

5.2.2. Irritation

Skin Irritation

AS produce dermal effects in a dose dependent manner that is consistent with the surfactant properties of these materials. Surfactants are designed to solubilize hydrophobic materials such as lipids. Direct application of high concentrations of anionic surfactants to mammalian tissue causes disruption of cellular bilipid membranes, increased cellular and tissue permeability, tissue edema, and damage to tissue integrity (which may be accompanied by denaturation of proteins and other biological macromolecules).

The skin irritant properties of AS are concentration-dependent in animal models and humans (Arthur D Little, 1991; Arthur D Little, 1993; IPCS, 1996). They cause delipidation of the skin surface, elution of natural moisturizing factors, denaturation of stratum corneum protein, increased permeability, epidermal swelling, and inhibition of enzyme activities in the epidermis (Imokawa, Sugura, et al., 1975; Prottey and Ferguson, 1975; IPCS, 1996). Acute and repeated dermal application of surfactants causes dose-dependent irritation, inflammation, edema or ulceration of the skin, the severity of which is highly dependent on concentration, duration of exposure and occlusion.

Rabbit skin irritation tests on five different materials were reviewed for this report, including two dose-response studies; one on C_{13-15}AS and one on C_{16-18}AS. Findings from these studies, along with selected findings from studies summarized in a recent review (BUA Report, 1996) are summarized in Table 2 (See also Appendix III).

Overall, AS with longer carbon chains (C_{16-18}) tend to be less irritating than AS with carbon chains of C_{12} to C_{15}. Test results indicate that for the shorter carbon chain length materials, (C_{12} and C_{13-15}) concentrations of 10% or greater consistently produce moderate to severe irritation reactions. Responses to lower concentrations (1-7%) tend to vary from none to severe. The longer carbon chain length materials (C_{16-18}) and broader cut materials (C_{12-18}) produce strong

reactions at concentrations above 25%, and none or slight reactions at lower concentrations. The test conducted with 5% C_{12-18} AS produced responses that would not result in classification as an irritant under EU criteria[1].

Reports from controlled human exposures to AS confirm that low concentrations are non-irritating. It was stated in a recent review (A.D. Little, 1991) that, while concentrations of 10% are moderate to strong irritants, concentrations of 1% are only slightly irritating. In a Burckhardt Test (for details of the test method see Burckhardt, 1970), 1% AS was applied repeatedly to the skin of human volunteers. There were no adverse reactions (Henkel, 1978).

Eye Irritation

Summaries of two eye irritation studies on AS were reviewed for this report; one on a C_{13-15} AS, Na salt, and one on C_{16-18} AS, Na salt. In both studies, the materials were tested at 5% in a rabbit eye irritation test method that was a modification of the method described by the U.S. EPA (Federal Hazardous Substance Act, 16CFR1500.42). Both materials would be classified as substantial eye irritants based on test results, however, the longer carbon chain material appeared to produced qualitatively less severe symptoms than the shorter chain length (Appendix III).

When C_{13-15} AS was tested at 5%, five of six test animals exhibited slight to severe conjunctivitis, and moderate corneal lesions. One animal was euthanized on day 3 of observation due to the severity of the response. Four of the five effected animals showed discharge and iritis. All four surviving animals were fully recovered on day 8. One animal was unaffected by treatment.

When C_{16-18}AS was tested at 5%, all six test animals demonstrated slight to moderate conjunctivitis, and slight to moderate corneal lesions. Five of the animals exhibited iritis. All animals were fully recovered on day 12.

Results of these tests were consistent with eye irritation test results reported in a previous review (BUA Report 189, 1996). Selected studies from this publication are presented in Table 3. Concentrations of C_{12}AS greater than 2% produced moderate to severe irritation. Some reported that symptoms persisted for as long as 21 days. Testing with lower concentrations, i.e., 2%, resulted in only slight irritation. As with skin irritation (see previous section), the broader cut (C_{12-16}) and longer carbon chain length materials (C_{16-18}) tended to produce less irritation. Tests with 5% dilutions produced only slight eye irritation.

[1] For skin irritation tests using less than 6 animals, test results are interpreted, as follows: Calculate mean scores across three scoring time (24, 48 and 72 hours) for each animal for erythema and edema, separately. An animal is positive when the mean score for either edema or erythema are greater than 2. The test is positive for irritation if the majority of test animals are positive for the same endpoint (erythema or edema).

Table 2
Skin Irritation of AS in Rabbits (4 hour patch test)

Substance CAS RN	Test conc.	Finding	Reference
C_{12} alkyl sulphate, Na salt 151-21-3	25% 5% and 1%	Strong erythema and edema Slightly irritating	Henkel Study No. 870150; 1987 unpublished Davies et al., 1972[1]
C_{12} alkyl sulphate, TEA salt 90583-18-9	25%	Strong erythema and edema	Henkel Study No. 870150; 1987 unpublished
Sulfuric acid, mono-C_{13-15}-alkyl esters, Na salts 86014-79-1	15% 10% 7% 5% 3% 1%	Moderate to severe reactions Moderate to severe reactions Slight to severe reactions Slight to severe reactions Slight to severe reactions None to moderate reactions	Unilever Study No. CPS 83.25; 1983 unpublished
C_{16-18} alkyl sulphate, Na salt 68955-20-4	31.5% 25% 20% 15% 10% 5%	None to slight reactions Marginal to moderate reactions Slight to moderate reactions Slight to moderate reactions Marginal to moderate reactions None to slight reactions	Unilever Study No. CPS 83.45; 1983 unpublished
C_{12-18} alkyl sulphate, Na salt 68955-19-1	88.7% 43-46% 5%	Strong erythema and edema Moderate erythema, slight edema Slight erythema and edema	Henkel Study No. R9400996; 1994 unpublished Henkel, 1994[1], and personal communication Henkel Study No. TBD810154, 1980 unpublished

[1] Cited in BUA Report 189 (1996).

Table 3
Eye Irritation of AS in Rabbits
Selected from studies summarized in BUA Report 189 (1996)

Substance CAS RN	Test conc.	Finding	Method	Study conducted by:
C_{12} AS, Na salt 151-21-3	20% 10%	Strongly irritating Moderately irritating	Draize	Henkel (1977) unpublished
	20% 10% 2%	Moderately irritating Moderately irritating Slightly irritating	Draize	Ciuchta and Dodd (1978)
C_{12} AS, TEA salt 139-96-8	20% 10% 2%	Moderately irritating Moderately irritating Slightly irritating	Draize Draize Draize	Ciuchta and Dodd (1978)
	2.5%	Moderately irritating	Modified Draize	Serrano, et al. (1977)
C_{12-16} AS, Na salts 73296-89-6	20% 10% 5%	Strongly irritating Moderately irritating Slightly irritating	Draize	Henkel (1977) unpublished
C_{16-18} AS, Na salts 68955-20-4	25%	Irritating	OECD 405	Henkel (1987) unpublished
	5%	Slightly irritating	Draize	Henkel (1981) unpublished

5.2.3. Skin Sensitization

AS are universally considered non-sensitizing skin irritants. The C_{12} AS, Na salt (sodium dodecyl sulphate, or SDS) is broadly used as an irritant control in various types of studies involving contact sensitization and irritation. It is widely used in the standard Maximization test to establish a background level of skin irritation during the induction patch if a non-irritating material is being tested (as specified in OECD Guideline 406).

Two sensitization studies were reviewed for this assessment: a Maximization Assay and a Buehler Test. In addition, a summary of a third study (Maximization) was reviewed. All three tests were negative for sensitization (Appendix III).

The Maximization Study, 20 test animals were treated at induction with 5% C_{12-14} AS, Na salts. This concentration was used for both the induction injections (alone and mixed with Freund's Complete Adjuvant) and the induction patch applied 1 week after the injections. A challenge

patch with 1% test material was applied 2 weeks after the induction patch. No skin reactions were observed after challenge in any of the 20 test animals or the 10 control animals.

In the Buehler study, three induction patches were applied (6 hours, occluded) with 12.5% C_{12-14} AS, Na salts. Challenge was with 6.25% material. This challenge concentration produced slight erythematous responses in both the test and control group that were consistent with irritation responses. Four of the 20 animals in the test group, and 2 of 10 animals in the control group exhibited scores of "1" (slight erythema) at 24 hours. By 48 hours, the reactions had resolved on both control animals and on two of the four test animals. Such a rapid resolution to erythema reactions is typical of irritation rather than sensitization.

A summary of a third study was reviewed. In this Maximization Study using C_{12-14} AS, Na salts animals were treated with 0.08% at the induction injection, 0.5% at the induction patch and 0.1% at the challenge patch. There were no positive responses among the 10 test animals.

The results of these studies are in agreement with a large data base of negative sensitization data presented in a recent review (BUA, 1996).

Recently, sodium dodecyl sulphate has shown equivocal results in a recently established test for contact sensitization, the Local Lymph Node Assay (LLNA) (Basketter, et al., 1996). However, these reactions are not believed to indicate that these materials are sensitizers. Irritants can produce false positive reactions in the LLNA due to irritant induced migration of the antigen-presenting cells important in contact sensitization, Langerhans cells (Basketter, et al., 1998; Cumberbatch, et al., 2000). Further, cell typing studies on lymph node changes induced by AS indicate that the cell changes are characteristic of irritation, not sensitization (Sikorski, et al., 1996; Gerberick, et al, in press).

There have been rare reports of human subjects reacting to diagnostic patch tests with C_{12}AS, Na salt. The known irritancy potential of high concentrations of AS can easily confound the reading of diagnostic patch tests. The rare reports of human subjects reacting to SDS are almost certainly irritant reactions (Dooms-Goosens and Blockeel, 1996; Reitschel and Fowler, 2001). Further, there are no chemical structural alerts for SDS for sensitization (Barratt, et al., 1994). This further supports the conclusion that these materials are non-sensitizers.

5.2.4. Repeated Dose Toxicity

Oral exposure:

Available studies on a range of surfactant chain lengths have been reviewed to characterize the repeated dose toxicity of AS following oral administration. The test materials included C_{12}, C_{12-15}, C_{13-15}, C_{14-18} AS (sodium salts) and C_{12-14} AS (TEA complex). Both the gavage and dietary routes of administration were used. In addition to standard 28-day and 90-day studies, 21-day and 2-year dietary studies were also available. The 2-year dietary studies will be discussed in a subsequent section on carcinogenicity. (Brief summaries of all repeat dose studies are compiled in Appendix III.)

AS of different carbon chain lengths displayed remarkably similar oral toxicity profiles in repeated dose studies. (For illustrative purposes, selected oral studies, compounds evaluated, and

salient results are summarized in Table 4). For the oral route, values for the NOEL (61-252 mg/kg/day) and LOEL (123-503 mg/kg/day) were comparable, regardless of the mode of administration (dietary or gavage) or exposure duration. Gastrointestinal irritation (particularly of the forestomach) was the primary effect when the AS surfactants were administered by gavage, while liver and, in some cases, kidney effects were more noteworthy when the dietary route of exposure was employed. This shift in primary target organs with the mode of administration is readily explained. Given that the surfactants are primary irritants, local irritation predominated when the test material was delivered into the stomach as a bolus dose, but was mitigated by dietary admixture of the test materials. Furthermore, dietary administration allowed substantially higher doses to be administered. As a result, compound-related effects were more apparent in the liver and kidneys, systemic organs that are central to the metabolism and clearance of these materials.

Minor differences in potency among the AS surfactants were consistent with what is known about toxicity mechanisms. For example, 28-day gavage studies on the C_{12} AS Na and C_{12-14} AS TEA resulted in dose-dependent irritation, inflammation, edema, and ulceration of the forestomach as the primary toxic effects. The changes were partially reversible for C_{12} AS Na and fully reversible for C_{12-14} AS TEA. This finding is consistent with relative irritancy of the salts observed in mechanistic studies, TEA salts being less irritating than sodium salts. In a 90-day, oral gavage study on the C_{16-18} AS Na, partial recovery from forestomach effects was also observed, despite the longer exposure time compared to its C_{12} homolog. This is consistent with the lower irritancy potential of the longer chain length AS.

AS evaluated in both 21-day and 90-day dietary studies included C_{12}, C_{12-15}, C_{13-15}, and C_{16-18} AS (sodium salts). The liver was the primary target organ by this mode of administration, and histopathological changes in this organ were consistent among the compounds tested. All induced dose-dependent, zonal and diffuse hypertrophy of the liver. Zonal hypertrophy was often pronounced in the periportal region, which receives compounds absorbed from the GI tract into the liver for processing before they enter the systemic circulation. Histopathological findings in the liver occurred in both sexes at the highest dose, but appeared to be more frequent in females at lower doses. Fat-containing vacuoles and cytoplasmic neutral fat content in the liver were reduced (markedly so at high doses). Possible hypotheses for the latter may be that the surfactants facilitated mobilization of lipid out of the liver, or that glycogenic metabolism was disturbed in some fashion, and/or that food utilization was compromised. At high doses of surfactant, animals (particularly males) ate less, gained less weight, and notably, had very little abdominal fat. Hepatic hypertrophy was accompanied by an increase in the liver-to-body weight ratio. Changes in serum concentrations of liver enzymes were also found, a sign of alterations in hepatic function. The most consistent effects across the chain lengths studied were observed in serum alkaline phosphatase (AP), serum glutamic-pyruvic transaminase (GPT) and serum glutamic-oxalacetic transaminase (GOT). At the 90-day exposure duration, clear, dose-dependent elevations in serum AP were observed in males and females treated with the C_{12}, C_{12-15}, C_{13-15}, or C_{16-18} AS salts, over the full dose range studied. By contrast, changes in this enzyme were not consistently observed with all chain lengths at the 21-day exposure. When alterations occurred at the 21-day exposure duration, they were limited to the highest dose group. Significantly elevated levels of serum GPT were observed in males and females treated with C_{12}, C_{12-15}, C_{13-15}, or C_{16-18} AS salts. These effects were observed at the high dose only in 21-day studies and at the two highest doses in 90-day studies. Significantly elevated levels of serum GOT occurred in males treated with the highest dose of C_{12}, C_{12-15}, C_{13-15}, or C_{16-18} for 90-days,

and in males and females treated with the highest dose of the C_{13-15} AS salt for 21 days. Elevations of serum AP, GOT and GPT at high doses may be indicative of alterations in biliary clearance in response to high doses of surfactant; serum AP which showed the more clearly dose dependent changes, is a more sensitive indicator of effects on biliary clearance changes than serum GOT or GPT. The observed elevations serum GOT and GPT may also be indicative of a degree of parenchymal injury occurring at the highest doses.

A secondary target organ was the kidney. An increase in the kidney weight-to-body weight ratio, particularly in females, was a consistent finding at the higher dietary doses of surfactant. Histopathological alterations in the kidneys were also observed with certain higher chain length AS (C_{13-15} AS Na and C_{16-18} AS Na) and appeared to be related to strain of rat employed. Specifically, female rats of the Wistar strain are prone to nephrocalcinosis; the incidence and severity of this background lesion was reduced in females fed high doses of C_{13-15} AS Na and C_{16-18} AS Na, particularly at longer exposure durations.

Another high-dose effect common to AS surfactants evaluated in dietary studies was an increase in the testicular weight-to-body weight ratio with no change in absolute testicular weights. This was observed with C_{12} As Na (90-day exposure), C_{12-15} AS Na and C_{13-15} AS Na (90-day exposure), and C_{16-18} AS Na (21-day and 90-day exposure). The increase in relative testicular weights, without a concomitant change in absolute organ weight, is linked to the observed reductions in body fat and body weight induced by these materials and is not an indicator of organ toxicity. Changes in the relative weights of other organs, such as the heart, brain, adrenals, thymus and spleen, occurred in some studies but were not consistently observed. Reduced endometrial hyperplasia and ovarian activity was observed at a low incidence in a single, 21-day dietary study with C_{16-18} AS Na, at the highest dose only.

Summaries of two lifetime feeding studies with C_{12-15} AS Na were also reviewed. These studies will be discussed in more detail in a later section of this report (Section 5.2.6). The doses employed were comparable to the 21-day and 90-day dietary studies reviewed above. Pathological findings from these studies were consistent.

Table 4
Repeated dose toxicity profile following oral administration of AS (selected studies)

Surfactant	Species	Route	Exposure duration	Dose/ Concentration	NOEL/LOEL	Dose-dependent Target Organ Effects (males: m; females f)	References (App. III)
C_{12} AS Na	Rat	Oral (gavage)	**28-days** (29-day post exposure observation period)	0, 30, 100, 300, 600 mg/kg/day	NOEL= 100 mg/kg/day LOEL = 300 mg/kg/day	Forestomach (irritation, ulceration, partially reversible (both sexes). Organ weight/body weight increases: liver (f); kidneys (m); testes.	Henkel, 1987 (unpublished, TRS 16)
C_{12-14} AS TEA	Rat	Oral (gavage)	**28-days**	0, 70, 250, 750 mg/kg/day	NOEL= 70 mg/kg/day LOEL = 250 mg/kg/day	Forestomach (inflammation, edema and ulceration); reversible.	Henkel 1988 (unpublished TRS 15,)
C_{16-18} AS Na	Rat	Oral (gavage)	**90-days** (33-day post exposure observation period)	0, 100, 300, 900 mg/kg/day	NOEL= 100 mg/kg/day LOEL = 300 mg/kg/day	Some deaths at high dose. Forestomach (inflammation, ulceration, both sexes, partially reversible). Organ weight/body weight increases: liver (m, f). Organ weight-body weight decreases: thymus, adrenals (f); (reversible).	Henkel 1987 (unpublished, HESA 1)
C_{12} AS Na	Rat	Oral (dietary)	**21-days**	0, 0.023%, 0.047%, 0.094%, 0.188%, 0.375%, 0.75%, 1.5% in diet (0, 25, 52, 108, 208, 423, 830, 1643 mg/kg/day)	NOEL= 109 mg/kg/day LOEL = 208 mg/kg/day	Liver: hypertrophy, reduced cytoplasmic fat and glycogenic vacuolation (especially in f). Liver enzyme changes. Organ weight/body weight increases: liver (especially in f); kidneys: (f); brain (f). Decreased weight gains (m). Depleted body fat.	Unilever, 1976 (unpublished study L35,)
C_{12} AS Na	Rat	Oral (dietary)	**90-days**	0, 0.07%, 0.14%, 0.28%, 0.56%, 1.13%, 2.25%, in diet (0, 59, 116, 230, 470, 950, 1900 mg/kg/day)	NOEL= 116 mg/kg/day LOEL = 230 mg/kg/day	Liver: hypertrophy, reduced cytoplasmic fat and glycogenic vacuolation (especially in f); liver enzyme changes. Organ weight/body weight increases: liver (m, f); kidneys (f); adrenals (f); brain (m, f); testes. Decreased weight gains (m, f). Depleted body fat.	Unilever, 1977 (unpublished study, L 36)

Table 4 - continued
Repeated dose toxicity profile following oral administration of category surfactants (selected studies)

Surfactant	Species	Route	Exposure duration	Dose/ Concentration	NOEL/LOEL	Dose-dependent Target Organ Effects (males: m; females f)	References (App. III)
C_{12-15} AS Na	Rat	Oral (dietary)	**21-days**	0, 0.047%, 0.094%, 0.188%, 0.375%, 0.75%, 1.5% in diet (0, 60, 117, 252, 503, 1010, 1956 mg/kg/day)	NOEL= 252 mg/kg/day LOEL = 503 mg/kg/day	Liver: hypertrophy, reduced cytoplasmic fat and glycogenic vacuolation (especially in f). Organ weight-body weight increases: liver (m, f); brain (m); testes. Decreased body weight gains and abdominal fat (m).	Unilever, 1976 (unpublished study, L6)
C_{12-15} AS Na	Rat	Oral (dietary)	**90-days**	0, 0.07%, 0.14%, 0.28%, 0.56%, 1.13%, 2.25%, in diet (0, 62, 122, 245, 488, 1016, 2081 mg/kg/day)	NOEL= 122 mg/kg/day LOEL = 245 mg/kg/day	Liver: hypertrophy, reduced cytoplasmic fat and glycogenic vacuolation (m, f). Liver enzyme alterations. Organ weight/body weight increases: liver (m, f); kidneys (f); testes (m). Decreased weight gain and abdominal fat (m).	Unilever, 1976 (unpublished study, L 8)
C_{13-15} AS Na	Rat	Oral (dietary)	**21-days**	0, 0.047%, 0.094%, 0.188%, 0.375%, 0.75%, 1.5% in diet (0, 51, 97, 199, 384, 784, 1566 mg/kg/day)	NOEL= 199 mg/kg/day LOEL = 384 mg/kg/day	Liver: hypertrophy reduced cytoplasmic fat and glycogenic vacuolation (especially in f). Alterations in liver enzymes (m, f). \Organ weight/body weight increases: liver (m, f); kidney (f); heart (m). Decreased weight gains (m).	Unilever, 1976. (unpublished study, L22)
C_{13-15} AS Na	Rat	Oral (dietary)	**90-days**	0, 0.07%, 0.14%, 0.28%, 0.56%, 1.13%, 2.25%, in diet (0, 64, 134, 253, 512, 1007, 2096 mg/kg/day)	NOEL= 134 mg/kg LOEL = 253 mg/kg	Liver: hypertrophy educed cytoplasmic fat and glycogenic vacuolation (m, f). Alterations in liver enzymes (m, f). Kidney: reduced severity of nephrocalcinosis (f). Organ weight/body weight increases: liver (m, f); kidney (f); brain (m, f); testes. Decreased body weight gains (m, f). Greatly reduced abdominal fat (m).	Unilever, 1977 (unpublished study; L 23)

Table 4 - continued
Repeated dose toxicity profile following oral administration of category surfactants (selected studies)

Surfactant	Species	Route	Exposure duration	Dose/ Concentration	NOEL/LOEL	Dose-dependent Target Organ Effects (males: m; females f)	References (App. III)
C_{16-18} AS Na	Rat	Oral (dietary)	**21-days**	0, 0.047%, 0.094%, 0.188%, 0.375%, 0.75%, 1.5% in diet (0, 50, 103, 202, 417, 796, 1660 mg/kg/day)	NOEL= 202 mg/kg/day LOEL = 417 mg/kg/day	Liver: hypertrophy, reduced cytoplasmic fat and glycogenic vacuolation (m, f). Liver enzyme alterations (especially m). Kidney: reduced severity of renal lesions (f). Spleen: reduced hematopoiesis. Uterus: diminished ovarian activity and endometrial hyperplasia (highest dose). Organ weight/body weight increases: liver (m, f); brain (m); testes; adrenals (f). Reduced weight gains (highest dose m, f). Greatly reduced abdominal fat.	Unilever, 1976 (unpublished study, L31)
C_{16-18} AS Na	Rat	Oral (dietary)	**90-days**	0, 0.07%, 0.14%, 0.28%, 0.56%, 1.13%, 2.25%, in diet (0, 61, 123, 230, 482, 970, 2067 mg/kg/day)	NOEL= 61 mg/kg/day LOEL = 123 mg/kg/day	Liver: hypertrophy, reduced cytoplasmic fat and glycogenic vacuolation (m, f). Liver enzyme alterations (m, f). Kidneys: Reduced incidence, severity of nephrocalcinosis. Organ weight/body weight increases: liver (m, f); kidneys (m, f); brain (m, f); testes. Reduced body weight gains and food intake. Greatly reduced abdominal fat.	Unilever, 1977 (unpublished study, L32)

Dermal exposure:

Four studies with C_{12-15} AS Na assessed repeated dose toxicity in mice by the dermal route. Exposure durations included 21 days, 90 days and 2 years. (See Appendix III for a more extensive summary of the study designs and results.)

The 21-day study employed doses of 0%, 5%, 10%, 15% and 18% test material concentrations with twice weekly application to the shaved backs of mice. The results were consistent with the irritant properties of the surfactants. All mice in the highest dose group died due to dehydration caused by fluid loss through skin lesions. At the 10% concentration, edema, hyperkeratosis and acanthosis of the epidermis were observed at the site of application. Responses increased in severity at 15% to include ulceration and necrosis with inflammatory exudate in decedents, and epidermal thickening due to hyperkeratosis and acanthosis in survivors. These were adaptive responses to sustained skin irritation induced by the test materials. There were no systemic histopathological changes on other organs or tissues.

Dose levels employed in the 90-day study were 0%, 5%, 10%, 12.5% and 15%. As with the 21-day study, effects at the site of application were consistent with the irritant properties at the test material. Dose-related ulceration of the epidermis with inflammatory exudate was observed at the 12.5% and 15% concentrations. Dose-dependent increases in edema, vascular dilatation, epidermal acanthosis, hyperkeratosis and hypergranulosis were prominent at the 10% treatment level and above. Hemoglobin levels were reduced and white blood cell counts increased in males of the high dose group. No clinical chemistry measurements were performed. Other noteworthy systemic effects included increases in liver-to-body weight ratios in both sexes at the 15% concentration, and in females at the 12.5% concentration. Absolute kidney weights increased in males and kidney weight-to-body weight ratios increased in females at the 15% treatment level. These target organs are consistent with those observed in the oral studies. Effects at these more distant organs suggest that a higher level of percutaneous absorption of the test material may have occurred at high doses with the longer duration of exposure in this study.

Summary

The repeated dose studies reveal striking commonalities in toxicity among AS, which can be related to their mechanisms of action and common pathways of metabolism. Their primary effects and relative potencies at the site of application (gavage dosing and skin painting studies) were consistent with surfactant-mediated irritant effects. Secondly, AS displayed common target organs of toxicity following oral exposure: the forestomach in gavage studies, and the liver and kidneys in dietary studies. All materials tested displayed similar NOEL's. Thirdly, the histopathological findings in the primary target organs induced by high oral doses of the materials were consistent across chain lengths. The observed toxicological profile of this category reflect the similarities in structure, systemic disposition, and metabolic transformation.

The lowest NOAEL for this category observed following repeated administration was established in rats receiving C_{16-18} AS in the diet at concentrations of 0.07 % (equivalent to 61 mg/kg/day) for 90 days. The NOAEL was based on liver toxicity.

5.2.5 Genotoxicity (*in vitro*):

Category surfactants were consistently negative when tested *in vitro* (Ames tests). A total of 16 Ames tests were reviewed to support the assessment, covering a comprehensive range of relevant

chain lengths and counter ions. The test materials and results are described in Appendix III. The general lack of mutagenic activity for anionic surfactants is corroborated in the published scientific literature (Yam, Bomman et al., 1984).
These results are predictable based on a consideration of the surfactant structures and mechanisms of mutagenesis. Mutagens are chemicals that either contain, or can be metabolized to, highly reactive electrophiles capable of modifying nucleophilic sites on DNA. AS do not possess electrophilic functional groups or functional groups capable of being metabolically activated to electrophiles with the requisite reactivity. Specifically, AS with fully saturated carbon chains are not metabolized to reactive electrophilic intermediates. The consistent lack of mutagenic activity with AS is consistent with these mechanistic predictions.

The ability to predict *in vitro* genotoxicity of these materials based on their structures further underscores the significance of structure-activity relationships among these materials and the appropriateness of treating them as a single chemical category.

Genotoxicity (*in vivo*):

The database of *in vivo* genotoxicity studies to support the category assessment is summarized in Appendix III. The database includes mammalian bone marrow chromosome aberration tests, mammalian erythrocyte micronucleus tests, and rodent dominant lethal mutation assays. The reliable studies performed to evaluate chromosomal effects (mammalian bone marrow chromosome aberration tests and mammalian erythrocyte micronucleus tests) showed consistently negative results. Notably, two of the reliable, mammalian bone marrow chromosome aberration tests (on C_{12} AS Na and C_{12-15} AS Na) were 90-day repeated dose studies conducted at the maximum tolerated dose (MTD).

Rodent dominant lethal assays were conducted on C_{12} and C_{12-15} AS salts. The results of these studies were negative overall, which further supports the conclusion that this category of surfactants shows no significant mutagenic potential, either *in vitro* (see above) or *in vivo*.

Equivocal results were reported in a bone marrow cytogenetics test and a dominant lethal assay. These data were considered to be of a low reliability and not supported by similar or more reliable studies for the same endpoint. Overall, the weight of the evidence clearly supports a lack of adverse effects for these endpoints.
.
Based on the chemical structures of the category surfactants, no specific affinity for, or reactivity with, chromosomal DNA or structural proteins is anticipated. It has been suggested in the literature that cellular toxicity correlates with lipophilicity, and that lipophilic materials that are cytotoxic are more likely to show positive results in *in vivo* assays for genotoxicity based simply on generalized cytotoxic effects (Rosenkranz and Klopman, 1983). If this is the case, chromosomal damage in response to high surfactant doses, if observed, would likely be secondary to surfactant-induced cytotoxicity and a generalized disruption of cellular integrity.

Some *in vivo* assays assess the bone marrow, a site of erythropoiesis that might be vulnerable to generalized cellular toxicity during the process of cell division. However, since metabolism of the surfactants curtails and destroys their surfactant properties, cytogenetic effects secondary to generalized cytotoxicity should be limited, unless the dose or mode of administration is such that the metabolic capacity of the test animals is overwhelmed. The oral, repeated dose studies conducted at the MTD indicate that such conditions are not likely to be achieved in a properly conducted study. Therefore, since the surfactant structures are not expected to modify genetic macromolecules, and the materials are rapidly metabolized to short-chained, polar compounds in mammals, direct or indirect *in vivo* cytogenetic effects are not expected.

Summary: AS show a consistent absence of mutagenic activity when tested in *in vitro* tests. Neither AS nor its metabolites possess electrophilic functional groups or functional groups associated with mutagenic activity. In addition, AS is consistently negative in the reliable studies performed to evaluate chromosomal effects (mammalian bone marrow chromosome aberration tests and mammalian erythrocyte micronucleus tests). These points support the conclusion that AS are not mutagenic.

5.2.6. Carcinogenicity

Brief summaries of two, lifetime feeding studies with C_{12-15} AS Na were reviewed for this report. The test materials used in the individual studies were prepared by two different production methods (high conversion bleached or HCB; and low conversion, unbleached or LCU). They differed slightly in chain length distribution, the latter having a slightly higher proportion of the C_{15} AS. In both studies, the test material was dosed at 0, 0.015, 0.15 and 1.5% in the diet. Each dosage group contained 45 males and 45 females. Animals were evaluated for survival, growth, and changes in hematology and biochemistry. Histopathology was conducted on a number of tissues, including the heart, liver, spleen, kidneys, brain, adrenal and testes.

There was no increase in tumor incidence, nor any impact on tumor type in either study. For both studies, approximately 70% of animals survived to study termination. Mortality was similar across dosage groups and controls. Animals in the 1.5% dose groups in both studies exhibited reduced food and water consumption, and slower growth rates. Within these high dose groups, there was a decreased number of total tumors and tumor-bearing animals. This decrease was probably due to a decreased caloric intake in these animals (Kritchevsky, D., 1993, 1995; Weindruch, R., 1989). Other pathological findings in both studies were similar to those in the 21-day and 90-day dietary studies discussed in Section 5.2.4. Increased absolute liver weights and liver to body weight ratios, hypertrophy of the hepatic parenchyma, increased relative testicular weights, reduced incidence and severity of chronic nephropathy and nephrocalcinosis, and reduced arterial medial hypertrophy were among the findings at the higher dose levels.

Based on these 2-year feeding studies, and the absence of any tumor effects of the test materials, the NOAEL and LOAEL for carcinogenicity is greater than the highest dose of 1.5%. Other pathology findings in the high dose groups indicate a NOAEL for non-carcinogenic effects of 0.15%, and a LOAEL of 1.5%.

Other reports of carcinogenicity studies have been mentioned in a previous review of surfactants (A.D. Little, 1991). A 1-year oral feeding study in rats with C_{12}AS dosed at levels of 0.25, 0.5 and 1.0% was negative for tumorogenesis. In addition, no increase in tumors was found in a 2-year skin painting study. Other studies mentioned in this review were flawed by either inadequate numbers of animals or confounding factors.

Overall results of the chronic feeding and skin painting study summaries reviewed for this report, together with the absence of a mutagenic response in *in vitro* and reliable *in vivo* tests (Section 5.2.5) support the conclusion that AS are not carcinogenic.

5.2.7. Reproductive Toxicity:

No reproductive studies were available for review on AS. However, a reproductive toxicity study on a structurally similar surfactant material, alpha olefin sulfonate (AOS), was reviewed. (Appendix II provides a complete discussion of the rationale for applying data on AOS to the assessment of AS based on similarities in: a) structure, b) mechanisms of toxic action, c) toxicokinetics, d) pathways of metabolism, and e) toxicological profiles.)

A 1:1:1 mixture of C_{14}, C_{16}, and C_{18} AOS (magnesium salts), administered continuously in the diet at 1250, 2500 and 5000 ppm, was evaluated in a 2-generation reproductive toxicity study in rats. The protocol used was comparable to the OECD 416 guideline. Males and females of the F_0 parental generation were treated for 13 weeks prior to mating, then through gestation and lactation of two successive litters, F_{1A} and F_{1B}. Using selected animals of the second litter as the parental base, the process was repeated to produce two further litters, F_{2A} and F_{2B}. Animals from the latter were selected to form an F_2 generation, which was treated for 13 weeks and subjected to a histopathological examination. For the F_0 and F_1 generations, 12 males per dose group were each paired with 2 females for mating (24 females per group), yielding a approximately 20 pregnant females per dose group. The results showed no adverse effects upon growth, reproductive performance or litter responses in rats exposed to the test material mixture for two successive generations (see Appendix III). General health, food and water intake, food utilization, weight gain, reproductive performance and fertility were not adversely affected. Terminal necropsy of adults and offspring revealed no treatment-related abnormalities, and histopathological examination of tissues from the F_2 generation revealed no adverse treatment-related effects. Test material consumption in the F_0, F_1 and F_2 generations over the course of 13 weeks of exposure is shown in Table 5.

The dietary repeated dose studies on C_{12}, C_{12-15}, C_{13-15}, and C_{16-18} AS (sodium salts) are particularly relevant for comparison with the dietary, 2-generation reproductive toxicity study on AOS described above. When administered continuously in the diet at doses comparable to those employed in the reproductive toxicity study on AOS, the AS surfactants showed no adverse histopathological effects on reproductive organs. The NOELs in 21-day and 90-day repeated dose studies on these AS surfactants ranged between dietary concentrations of 700 ppm to 1888 ppm, which corresponded to mean daily intakes in the range of 61 mg/kg/day to 252 mg/kg/day. These doses are within the same order of magnitude as the test material consumption in the reproductive toxicity study on the AOS mixture (Table 5), which likewise showed no adverse histopathological effects on systemic organs and no effects on reproductive function.

Table 5
Test material consumption by successive generations in a 2-generation reproductive toxicity study in rats of AOS
[diet contained 5000 ppm of a 1:1:1 mixture of, C_{14}-, C_{16}-, and C_{18} AOS (magnesium salts)]

		Test material consumption (mg/kg/day)	
Generation	**Sex**	**Week 1**	**Week 13**
F_0	Males	727	250
	Females	679	320
F_1	Males	891	261
	Females	871	338
F_2	Males	1040	266
	Females	961	370

Furthermore, no significant histopathological effects on the reproductive organs were observed in dietary, repeated dose studies of a range of AS surfactants at dietary concentrations that exceeded

those employed in the 2-generation reproductive study on AOS, even though the administered doses of AS were high enough to adversely affect the liver, the site of metabolism of the compounds. Histopathological findings in the reproductive organs of AS-treated rodents represented background lesions and were similar to historical rates for the strains employed. Testicular effects were limited to relative weight changes, with no concomitant adverse histopathological findings. Absolute testicular weights were unchanged by treatment with AS surfactants, although relative weights of the testes increased at doses high enough to retard body weight gains in males (Table 4). This result is consistent with the preservation of the male reproductive organs even when overall growth of the animals was adversely affected. In the uteri of AS-treated animals, the incidence and severity of background lesions such as endometrial hyperplasia, neutrophil infiltration of the submucosa, submucosal edema, catarral endometritis, and stromal pigmentation were unaffected (data not shown). In a 21-day day study on C_{16-18} AS (sodium salt), diminished ovarian activity and endometrial stimulation were observed in a single rat at the highest dose only (1.5% dietary level of the detergent) and minimal endometrial hyperplasia was observed two more females of this dose group. These observations were not reproduced in a subsequent 90-day study that employed up to 2.25% dietary concentrations of the same surfactant. It is unlikely that the endometrial changes observed at the highest dose with a single surfactant (C_{16-18} AS, sodium salt) in a single study is toxicologically significant, since these findings were not reproducible at longer exposures to the same chemical and were not observed with any other test material in this category.

Summary: The 2-generation reproductive study on the AOS mixture showed a complete absence of treatment-related effects on reproductive capacity or systemic organ pathology at systemic doses ranging from approximately 1000 - 250 mg/kg/day based on food intake, similar to the NOELs in repeated dose studies on AS (Table 4). The lack of reproductive organ toxicity in dietary, repeated dose studies on various AS surfactants, even at doses in excess of the NOELs, provides further corroboration for the absence of specific, surfactant-mediated effects on the reproductive organs. The comparable toxicokinetic and metabolic profiles of category surfactants, as well as their the toxicological similarities for this and other toxicological endpoints, support the conclusion that insights from the reproductive toxicity study on AOS are applicable to AS.

Developmental Toxicity:

A published developmental toxicity study assessed the teratogenic potential of AS in rats, mice and rabbits following oral (gavage) administration (Palmer et al, 1975 a b). The dose range was 0, 0.2, 2, 300 and 600 mg/kg/day during the appropriate days of gestation. The protocol was comparable to OECD 414 guidelines. Details are described in Appendix III.

The chain length of the AS evaluated for teratogenicity was not specified in the study report (it is believed to be the C_{12} sodium salt, most commonly used as a surrogate for the category). Mice and rabbits were more sensitive to maternal toxicity induced by the AS test material. At the 600 mg/kg dose, marked maternal toxicity was evident in mice and rabbits, while slight to moderate toxicity was observed in rats at the same dose. An increased incidence of total litter loss occurred at doses that caused frank maternal toxicity; when dams showing total litter loss were excluded from the analysis, litter parameters were unchanged by treatment. In mice, a higher incidence of minor skeletal anomalies occurred at 600 mg/kg of AS; however, in all species, the incidence of major or minor visceral or skeletal anomalies was unaffected by treatment at non-maternally toxic doses. The NOEL for maternal toxicity for all species was 2 mg/kg/day; for litter and pregnancy data a NOEL of 2 mg/kg/day was established. For foetal data and NOEL of 600 mg/kg/day was observed for rats; in rabbits and mice the NOEL was 300 mg/kg/day. The spread in the dose levels selected for this study resulted in an apparent low NOEL for maternal and litter effects, however the severity of the effects at 300 mg/k/day suggested that the true NOEL for these effects is between 2 and 300mg/kg.

The AOS test material evaluated was a mixture of 60.4%:39.5% alkenyl sulfonate and hydroxyalkane sulfonate prepared from C14-18 α-olefin. As with AS, pregnant mice and rabbits were more sensitive to toxic effects of the test substance. The incidence of major malformations, minor visceral and skeletal anomalies in rats was unaffected by treatment. In rabbits, the incidence of minor skeletal anomalies and the proportion of pups with extra ribs was observed at the 300 mg/kg dose; total litter loss occurred at 600 mg/kg. In mice, a higher incidence delayed ossification was generally observed in all treated groups; at 600 and 300 mg/kg/day cleft palate was noted in some mouse pups. Because the incidence in controls was uncharacteristically low, no biological significance can be ascribed to these observations.

These two studies underscore the similarities in species toxicity and potency of the AS and AOS surfactants when assessed for developmental toxicity, and support assessing AS and AOS as members of the same chemical category. Given the similarities in structure, toxicokinetics and biotransformation of category surfactants, results from these studies provide adequate insight on the developmental toxicity profile of the category as a whole.

Several additional developmental toxicity studies in the rat have been reported [Appendix III]. Although the reliability score of these studies [cat. 2/3] is lower than those reported above, mainly due to the group sizes, these studies are consistent with the previous studies. They confirm that AS does not induce malformations, however in some cases slight delayed development [weight depression and/or local irritation] was observed at levels inducing significant maternal toxicity body. The lowest LOEL for maternal toxicity was 195 mg/kg/day; for developmental effects a NOAEL of 390 mg/kg/day was reported.

Summary. Developmental toxicity studies have consistently shown that AS is without major skeletal or visceral effects on the developing foetus. In some studies there was evidence of slightly delayed foetal development, however this effect was observed only at dose levels inducing toxicity in the maternal animals. In the rat the lowest LOEL for maternal effects, based on depression of body weight and/or local irritation was *ca*. 300 mg/kg/day; for developmental effects NOEL's were *ca*. 300 mg/kg/day.

5.3 Risk Characterisation

5.3.1 Hazard Summary

The category of the AS under consideration in this risk assessment is of a low order of acute toxicity by the oral and dermal route. Acute oral LD50 values generally exceeded 2000 mg/kg; acute dermal LD50 values were > 500 mg/kg, usually based on the absence of mortality at the highest dose tested. Administration of a single dose caused a significant irritant response at the site of first contact.

Neat or concentrated solutions of AS are skin irritants. Longer carbon chains materials (C_{16-18}) are generally less irritating than AS in the C_{12}-C_{15} range. The shorter chain materials consistently produced moderate to severe irritation reactions at concentrations of *ca*. 5% or greater, but responses at lower concentrations (*ca*. 1 - 5%) were variable. Human data suggest that concentrations of AS at levels below 1% are essentially non-irritating, however concentrations of 10% cause moderate to strong irritation of the skin.
Neat or concentrated solutions of AS are irritant to the eye. As with the skin irritation the shorter chain material tend to give a stronger response that the longer chain materials. Concentrations of 2% resulted in only slight irritation, but higher levels produced moderate to severe irritation.

AS do not have a skin sensitisation potential.

Repeated administration showed a low potential for cumulative toxicity, with effects in sub-acute, sub-chronic and chronic studies qualitatively and quantitatively similar for the category of AS. Repeated oral or dermal administration resulted in significant local effects in the fore-stomach or the skin, associated with the significant irritant properties of AS. Target organs for the systemic toxicity of AS are the liver and the kidney. The range of NOAEL's recorded for systemic (non-local) toxicity are in the range of 61 – 252 mg/kg/day with the majority of the NOAEL's recorded at *ca*. 100 mg/kg/day. Excluding the local effects associated with application of material directly onto the skin or the stomach, evidence of slight liver toxicity, usually consisting of liver weight increases, biochemical changes or hepatocellular hypertrophy was observed at the lowest observed effect level (LOEL).

AS are not mutagenic and lack a carcinogenic potential.

Repeated exposure to AS did not adversely affect the reproductive organs and a structurally related material (alpha-olefin Sulphonate, AOS) was without adverse effects on reproduction and fertility when administered over two successive generations; a NOAEL was established at dietary concentrations of 5000 ppm (250 mg/kg/day; highest dose tested). In developmental toxicity studies AS and AOS were without major (skeletal and visceral) effects on the developing foetus, even in the presence of toxicity in the maternal animal. There was evidence of slightly delayed development, however this effect was observed only at doses levels inducing toxicity in the maternal animals. In the rat LOEL's for maternal effects were *ca*. 300 mg/kg or higher, based on depression of body weight gain and/or local irritation; for developmental NOAEL's were recorded at *ca*. 300 mg/kg and above.

5.3.2 Exposure summary

Based on the information from the Habits and Practices tables it can be concluded that skin exposure resulting from the use of AS in household laundry and detergent products is the major route of exposure to AS. Using the algorithms recommended in the HERA methodology document it has been estimated that *ca.* 76% body burden results from dermal absorption, resulting mainly (*ca.* 60%) from direct skin contact of concentrated or diluted detergent products; indirect skin exposure -from laundered clothing- represents *ca*. 15% of the overall dermal exposure.
Use of household laundry and cleaning products may result in presence of residual amounts of AS in surface water and food. Intake of drinking water and food and from residues present on eating utensils and crockery there is a potential for exposure via the oral route. The estimated oral intake represents *ca.* 22% of the total body burden.
Inhalation of AS from dusts or aerosols formed from detergents products represents a minor fraction (*ca*. 1%) of the overall systemic exposure.

5.3.3 Endpoints Selected for Risk Assessment

Single exposures/Local Effects. AS are of a low order of acute toxicity. Acute systemic toxicity is not of concern for household detergents and will not be considered further. Single exposures cause irritation at the site of first contact and irritation is regarded as a key effects following exposure to AS. Human data suggest a threshold for skin irritation at 1%. For eye irritancy dilutions of alkyl sulphate at levels of 2% appear to be essentially non-irritating.

Repeat Exposures/Systemic effects. Studies investigating the dermal route, the most relevant for human exposure, resulted in significant local irritation. Some of the repeated dose toxicity studies using the dermal route of exposure provided some evidence of systemic toxicity however there is insufficient information to determine if these effects represented a direct toxic effect from systemic exposure to AS or if the response was associated with the significant dermal inflammation. For this reason studies based on dietary exposure appears to be more appropriate to assess potential systemic toxicity resulting from repeated exposures to AS. Based on liver toxicity, the lowest NOAEL established for repeat dose toxicity was 61 mg/kg. For reasons of simplicity a value of 60 mg/kg/day has been used in this risk assessment.
This value is based on a 90-day study toxicity study in the rat; this study is regarded to be of acceptable reliability (score 2C). Comparison of this NOAEL with that from other repeat dose toxicity studies in the rat, including chronic/2-year studies, indicate that the selected value used in the risk assessment is the lowest value within the significant data base for this repeated dose toxicity.

The toxicology database for AS has not identified a potential for adverse reproductive and developmental effects. Also, there is no evidence to suggest that pregnant or developing animals are more sensitive to the toxic effects of AS than non-pregnant animals. Reproductive and developmental endpoints will therefore not be assessed separately in this risk assessment. Similarly, there is no need for a further assessment of the mutagenicity and carcinogenicity endpoints.

5.3.4 Margin of Exposure Calculation

The margin of exposure (MOE), i.e. the ratio of the No Observed Adverse Effect Level (NOAEL) and the estimated human exposure has been calculated for the scenarios assessed in section 5.1.

5.3.4.1. Exposure scenario: direct skin contact

A. Hand-wash laundry. The NOEL of 60 mg/kg bw/day was divided by the daily systemic dose of 0.64 µg/kg bw/day which was estimated for the dermal exposure to AS from hand-washed laundry.

$$MOE_{direct\ skin\ \ hand\text{-}washed\ laundry} = 60{,}000/0.64\ [\mu g/kg\ bw/day] = \mathbf{93{,}750}$$

B. Laundry Pre-treatment. The MOE was calculated by dividing the NOEL of 60 mg/kg bw/day by the estimated exposure from pre-treatment of clothes of 2.27 µg/kg bw/day.

$$MOE_{direct\ skin\ \ pre\text{-}treatement} = 60{,}000/2.27\ [\mu g/kg\ bw/day] = \mathbf{26{,}431}$$

C. Hand dishwashing. The MOE was calculated by dividing the NOEL of 60 mg/kg bw/day by the estimated exposure from hand dishwashing of 0.47 µg/kg bw/day

$$MOE_{direct\ skin\ \ hand\ dishwashing} = 60{,}000/0.47\ [\mu g/kg\ bw/day] = \mathbf{> 100{,}000}$$

D. Hard surface cleaning. The MOE was calculated by dividing the NOEL of 60 mg/kg bw/day by the estimated exposure from hand dishwashing of 0.41µg/kg bw/day

$$MOE_{direct\ skin\ \ hard}\ \text{surface cleaning} = 60{,}000/0.41\ [\mu g/kg\ bw/day] = \mathbf{> 100{,}000}$$

5.3.4.2. Exposure scenario: Indirect skin exposure

Wearing clothes. The systemic dose from indirect skin exposure to AS residues on washed fabric was estimated to be 0.73 µg/kg bw/day. The calculated Margin of Exposure is:

$$MOE_{indirect\ skin}\ \text{surface cleaning} = 60{,}000/0.73\ [\mu g/kg\ bw/day] = \mathbf{82{,}191}$$

5.3.4.3. Exposure scenario: Inhalation

A. Dust. The systemic dose of AS via inhalation via detergent dust during the washing process was estimated to amount 0.001 µg/kg bw/day. The MOE that could be calculated from this low exposure is >> 100,000. The described exposure does not significantly add to the overall AS exposure and will therefore not be considered further in this risk assessment.

B. Aerosols. For calculation of the MOE, the NOEL of 60 mg/kg bw/day was divided by the daily systemic dose of 0.06 µg/kg, estimated for the inhalation of AS-containing aerosols in spray cleaning applications.

$$\mathbf{MOE}_{\text{aerosol inhalation}} = 60{,}000/0.06\ [\mu g/kg\ bw/day] = \mathbf{>> 100{,}000}$$

5.3.4.4. Exposure scenario: Oral route

A. Indirect exposure via the environment. For calculation of the MOE, the NOEL of 60 mg/kg bw/day was divided by the daily systemic dose of 0.53 µg/kg estimated for the uptake of AS from drinking water and food.

$$\mathbf{MOE}_{\text{oral route}} = 60{,}000/\ 0.53\ [\mu g/kg\ bw/day] = \mathbf{>100{,}000}$$

B. Indirect exposure via dinnerware. The MOE was calculated by dividing the NOEL of 60 mg/kg bw/day by the estimated oral exposure from AS residues left on eating utensils and dinnerware of 0.82 µg/kg bw/day.

$$\mathbf{MOE}_{\text{oral route}} = 60{,}000/0.82\ [\mu g/kg\ bw/day] = \mathbf{73{,}170}$$

5.3.4.5 Aggregate exposure

The consumer exposure calculated by summation of the all routes of exposure, including direct and indirect skin contact of neat or diluted AS containing product, inhalation of AS containing aerosols from spray cleaner applications and dusts from powdered laundry products and from the oral route via the environment (in food and drinking water) and residues on eating utensils and crockery, results in an estimated systemic AS exposure of 5.94 µg/kg bw/day. As discussed previously (see 5.1.3.5), the calculated aggregate exposure is based on a combination of scenarios, and is considered to be highly unrealistic and an extreme worst case for the total consumer exposure.

The MOE can be calculated by dividing the NOEL of 60,000 µg/kg bw/day by the total exposure:

$$\mathbf{MOE}_{\text{total}} = 60{,}000/5.94\ [\mu g/kg\ bw/day] = \mathbf{10{,}100}$$

5.3.4.6. Accidental and intentional exposure

Accidental ingestion of a few milligrams of AS as a consequence of accidental ingestion of laundry and cleaning products is not expected to result in any significant adverse health effects given the low acute toxicity profile of laundry and cleaning products in general, and AS in particular. This view is supported not only by available toxicological information from animal studies, but also by the fact that national poison control centers have not reported a case of lethal poisoning or severe health effects with detergents containing AS.

Accidental eye contact and accidental or intentional skin contact with undiluted laundry or cleaning products containing AS at a concentration up to 16.5 % might potentially cause irritation. However if the material is rinsed off immediately after skin or eye contact, the effects are reversible shortly after the accidental exposure. Nevertheless, in case of accidental eye contact, immediate rinsing with plenty of water is recommended. In animal experiments such immediate action has been shown to minimize irritation effects.

Skin or eye contact with AS containing solutions under typical usage conditions (e.g., in hand-washed laundry or hand dishwashing) is not expected to cause significant irritation.

5.3.5. Risk Assessment

5.3.5.1. Systemic Effects

Consumers are exposed to AS through its use in laundry and cleaning products. All potential exposure scenarios were identified, quantified and assessed by comparing the estimated systemic exposure values with the systemic NOAEL determined in repeated dose toxicity studies. The MOE for the systemic dose resulting from the total consumer exposure is > 100,000. This MOE calculation reflects the summation of all exposure scenarios using mostly worst-case assumptions; it is highly unlikely that all these worst-case scenarios would apply simultaneously.

The MOE as calculated in 5.3.4.5 is very large and more than adequate to account for the inherent uncertainty and variability of the hazard data. The NOAEL is appropriate as it represents the lowest NOAEL for the repeat dose toxicity studies, including chronic (2-year) studies. The available toxicology database for AS is complete and it has shown that AS is not mutagenic, genotoxic or carcinogenic, nor was there any evidence for reproductive toxicity, or adverse developmental or teratogenic effects. Based on the toxicological profile of AS a minimum value of 100 for the MOE would be required to account for inter and intra-species variability.

In summary, the use of AS in consumer products such as laundry and cleaning detergents does not raise any safety concerns with regard to systemic toxicity.

5.3.5.2 Local Effects

AS is not a contact sensitiser and its irritation potential is concentration dependent. Under normal use conditions with direct skin contact (in hand laundering, hand dishwashing or hard surface cleaning) the consumer is exposed to detergent solutions containing up to 1 mg/mL (*ca*. 0.1%). At these concentration levels, AS is virtually non-irritating to the skin. Brief exposure to neat or concentrated detergent formulations (e.g., pretreatment of clothes) may result in minor signs of superficial irritation, but is generally not a cause of concern. This assessment is supported by many consumer surveys conducted by AISE member companies.

AS is present in liquid or powdered laundry and cleaning products at concentrations up to 16.5%. Accidental eye contact with undiluted detergent product, may cause irritation. This assessment is supported by poison control center data demonstrating that accidental eye spillage of AS containing products will at worst result in a transient irritation which heals within a few days with no irreversible effects to the eye. Nevertheless, in case of such an accident, the eyes should be rinsed immediately with plenty of water.

Accidental ingestion of an AS containing detergent product is not expected to result in any significant adverse health effect. This assessment is based on toxicological data demonstrating the low acute oral toxicity of AS and AS containing laundry and cleaning products. National poison control centers have not reported a case of lethal poisoning or severe health effects associated with accidental ingestion of detergents containing AES.

5.3.6. Summary and Conclusion

Consumer use of AS containing household laundry and cleaning products can result in exposure to AS. The skin is the predominant route of exposure to AS, however exposure from oral intake and inhalation are also considered in this risk assessment. Direct skin exposure occurs mainly in hand-washed laundry, laundry pre-treatment, hand dishwashing and surface cleaning tasks and to a smaller extent also from residues in fabric after the washing cycle. Consumers may be exposed to AS due to its potential environmental presence and presence of AS at low levels in drinking water has been taken into account and food. Residues deposited on eating utensils and crockery after hand dishwashing may be another source of oral exposure. The use of spray cleaners is also a potential source of exposure to AS through inhalation of aerosols generated by the sprayer. The calculated body burden of AS taking into account all routes of exposure and using highly conservative or worst-case assumptions is 5.94 µg/kg bw/day.

Based on an extensive database, it has been shown that the toxicological properties of AS covered in this risk assessment are qualitatively and quantitatively similar, justifying the decision to consider AS as a single category.

AS are of a low order of acute oral and dermal toxicity. AS are not genotoxic, mutagenic or carcinogenic, and there was no evidence of adverse effects on fertility, reproduction and development. These endpoints were therefore not carried forward for risk assessment. AS is irritant to skin and eyes when applied neat or as a concentrated solution, however AS concentrations below 1% were essentially non-irritating to the human skin. The repeated-dose toxicity of AS was evaluated in several sub-acute, sub-chronic and chronic toxicity studies. In dermal and oral gavage studies AS caused local irritation at the site of first contact. The target organs for the systemic toxicity of AS are the liver and the kidney. The lowest NOAEL of AS was observed in a 90-day feeding study in the rat at a dose level of 61 mg/kg/day and was based on liver toxicity.

The comparison of the aggregate exposure and the systemic NOEL results in a Margin of Exposure of 10,100. Local dermal effects due to direct or indirect skin contact with AS containing solutions in hand-washed laundry, hand dishwashing or hard surface cleaning tasks are not of concern because AS is not a contact sensitiser and not expected to be irritating to the skin at in-use concentrations.
In summary, the human health risk assessment has shown that the use of AS in household laundry and cleaning detergents is safe and that consumer exposures from these uses are not of concern.

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5.5. Contributors to this Risk Assessment

This risk assessment was developed by experts from the following companies :

Shell Chemicals Ltd. (lead), Procter &Gamble, Cognis, Unilever, The Health & Environmental Safety Alliance, Inc. (consultant)

The HERA Human Health Task Force gave additional input.

K. Berthold, BASF - J. Boyd, Colgate-Palmolive - Ph. Carthew, Unilever - O. Grundler, BASF - S. Jacobi, Degussa - S. Kirkwood, McBride - B. Kleber, Cognis - R. Kreiling, Clariant - G. Moran, Unilever - J. R. Plautz, CIBA - M. Rios-Blanco, Colgate-Palmolive - C. Rodriguez, P&G (Chair) - Th. Roth, Clariant - G. Veenstra, Shell Chemicals - F. Wiebel, Henkel.

Appendix I. Substances Included in the Human Health Assessment

CAS Number	Chemical name	Synonyms
139-96-8	Sulfuric acid, monododecyl ester, compd. W/ 2,2',2''-nitrilotriethanol (1:1)	C_{10} AS-TEA
142-31-4	sodium octyl sulphate	Sulfuric acid, mono-octyl alkyl ester, sodium salt Sulfuric acid, monooctyl ester, sodium salt Sulfuric acid, octyl ester,sodium salt sodium octyl sulphate C_8AS-Na
142-87-0	sodium decyl sulphate	Sulfuric acid, monodecyl ester, sodium salt C_{10}AS-Na Sulfuric acid, monodecyl ester, sodium salt Texapon 1030 Emal 3F
151-21-3	Sulfuric acid, monododecyl ester sodium salt	Dodecyl alkyl sulphate C_{12} AS, Na Lauryl sulphate, sodium salt Sodium lauryl sulphate Sodium dodecyl sulphate X0025.11 Sulfuric acid, mono-C_{12}-alkyl ester, sodium salt Radiolabeled (^{14}C) C_{12} Alkyl Sulphate
1120-01-0	1-Hexadecanol, hydrogen sulphate, sodium salt	Hexadecyl sulphate sodium salt C_{16} AS-Na C_{16} alkyl sulphate, Na salt Sodium hexadecyl sulphate
1120-04-3	Sulfuric acid, monooctadecyl ester, sodium salt	C_{18} alkyl sulphate, Na salt Sodium octadecyl sulphate C_{18}AS-Na

CAS Number	Chemical name	Synonyms
1191-50-0	1-Tetradecanol, hydrogen sulphate, sodium salt	C_{14} alkyl sulphate, Na salt Sodium tetradecyl sulphate Tetradecyl sodium sulphate Tetradecyl sulphate C_{14} AS-Na Radiolabeled C_{14} sodium tetradecyl sulphate-1-^{14}C Radiolabeled (^{14}C) C_{14} Alkyl Sulphate X0556.03R
2235-54-3	Sulfuric acid, monododecyl ester, ammonium salts	Stepanol AMV
68081-96-9	Sulfuric acid, mono-C10-16-alkyl esters, ammonium salts	Ammonium neutralized coconut alkyl sulphate Ammonium coconut alkyl sulphate
68081-98-1	Sulfuric acid, mono-C14-18-alkyl esters, sodium salts	Tallow alkyl sulphate from Alfol 1618x, 1618 and 1620
68130-43-8	C8-18 alkyl sulphate, sodium salt	
68140-10-3	Sulfuric acid, monotallow alkyl esters, sodium salts	
68412-83-9	Sulfuric acid, mono-C8-30-alkyl esters, compds. with triethanolamine	
68585-47-7	Sulfuric acid, mono-C10-16-alkyl esters, sodium salts	Sodium coconut alkyl sulphate Sodium Alkyl Sulphate ($C_{12/14}$)
68611-55-2	Sulfuric acid, mono-C10-16-alkyl esters	C_{14-15} alkyl sulphate, Na salt $C_{14/15}$ Sodium Alcohol Sulphate Dobanol 45 S [Alkyl Sulphate derived from] Neodol 45 P2722.02 P2101.01
68890-70-0	C12-15 alkyl sulphate, sodium salt	Dobanol 25 sulphate HCB Dobanol 25 sulphate HCU Dobanol 25 sulphate LCB Dobanol 25 sulphate LCU

CAS Number	Chemical name	Synonyms
68955-19-1	Sulfuric acid, mono-C12-18-alkyl esters, sodium salts	Tallow alkyl sulphate C_{12-18} alkyl sulphate, sodium salt C_{12-18} AS-Na Tallow alkyl sulphate from Henkel natural alcohols Collector FAS T 30
68955-20-4	C16-18 alkyl sulphate, sodium salt	Alfol 16-18 sulphate C_{16-18} alcohol sulphate Sulfuric Acid, mono C_{16-18}-alkyl esters, sodium salts C_{16-18} AS-Na Sulfopon T 55 (contains 3-7% C_{14}) Lanette E (contains no C_{14})
73296-89-6	C12-16 alkyl sulphate, sodium salt	Sulfuric acid, mono-C_{12-16}-alkyl esters,sodium salts C_{12-16} AS-Na Texapon V needles
85338-42-7	Sulfuric acid, mono-C8-10-alkyl esters, sodium salts	
85586-07-8	C12-14 alkyl sulphate, sodium salt	Sulfuric acid, mono-C_{12-14}-alkyl esters, sodium salts C_{12-14} AS-Na Emal 10 N (Needle) from Kao Corporation S.A.
85586-38-5	Sulfuric acid, mono-C8-18-alkyl esters, magnesium salts, compds. with triethanolamine	
85665-45-8	Sulfuric acid, mono-C8-14-alkyl esters, compds. with triethanolamine	C_{8-14} AS-TEA TEA lauryl sulphate Texapon TH
85681-68-1	Sulfuric acid, mono(C14-18 and C16-18-unsatd. alkyl) esters, sodium salts	
86014-79-1	Sulfuric acid, mono-C13-15-alkyl esters, sodium salts	Acropol 13-15 sulphate Synprol 13-15 sulphate Sulfuric acid, mono-C_{13-15}-alkyl esters, sodium salt

CAS Number	Chemical name	Synonyms
90583-10-1	Sulfuric acid, mono-C8-14-alkyl esters, ammonium salts	C_{8-14} AS-NH_4 Texapon A Texapon A 400
90583-12-3	Sulfuric acid, mono-C12-16-alkyl esters, ammonium salts	
90583-13-4	Sulfuric acid, mono-C12-18-alkyl esters, ammonium salts	C_{12-18} AS-NH_4
90583-16-7	Sulfuric acid, mono-C12-14-alkyl esters, compds. with ethanolamine	C_{12-14} AS-MEA C_{12-14} alkyl sulphate, MEA salt Texapon MLS
90583-18-9	Sulfuric acid, mono-C12-14-alkyl esters, compds. with triethanolamine	Triethanolamine lauryl sulphate
90583-19-0	Sulfuric acid, mono-C8-14-alkyl esters, lithium salts	
90583-23-6	Sulfuric acid, mono-C12-14-alkyl esters, magnesium salts	C_{12-14} AS-Mg Magnesium coconut alkyl sulphate Sulfuric acid,mono-C_{10-12}-alkyl esters,magnesium salts
90583-27-0	Sulfuric acid, mono-C8-16-alkyl esters, sodium salts	C_{8-16} AS-Na Sulfopon HS
90583-31-6	Sulfuric acid, mono(C14-18 and C18-unsatd. alkyl) esters, sodium salts	C_{14-18} AS-Na and C_{18} unsaturated AS-Na C_{14-16} and C_{18} unsaturated AS-Na Oleyl-Cetylsulphate,Na-salt C_{16} and $C_{18:1}$ AS-Na
91648-54-3	Sulfuric acid, mono-C14-C16-alkyl esters, sodium salts	
91783-23-2	Sulfuric acid, mono-C12-C13-alkyl esters, sodium salts	
92797-61-0	Sulfuric acid, mono(C13-15-branched and linear alkyl) esters, sodium salts	
96690-75-4	Sulfuric acid, mono-C12-14-alkyl esters, ammonium salts, compds. with triethanolamine	
117875-77-1	Sulfuric acid, mono-C10-16-alkyl esters, compds. with triethanolamine	C10-16-Alcohol sulfuric acid, triethanolamine salt C10-16-alkyl alcohol sulfuric acid, triethanolamine salt

Appendix II. Rationale for the Structure-Based Category*

AS are evaluated as a single chemical category for the HERA hazard evaluation, based on:

- Structural similarity
- Shared mechanisms of toxic action derived from their physico/chemical properties
- Similar toxicokinetics in mammals
- Common pathways of metabolism in mammals
- Comparable toxicological profiles in mammals

In addition, for some toxicological endpoints where the data on AS were not entirely comprehensive, data on the related surfactant chemicals, C_8 alkyl sulphonate, and C_{14-16} alkyl olefin sulphonate were also included. Data on these related chemicals provided additional or supporting information on specific toxic endpoints to allow completion of the assessment.

A. Surfactant Structure

The molecules have three functional moieties: 1) the aliphatic hydrocarbon chain, 2) the polar, terminal anion, and 3) the countervalent cation or amine. The most salient structural feature, the presence of an aliphatic hydrocarbon chain bearing a polar, anionic terminus, confers surfactant properties. Surfactant properties play a central role in the toxicity of these materials. A detailed discussion of surfactant effects on mammalian tissues, including the impact of chain length, anionic polar terminus and positive counterion, will be described in the next section.

Besides the effects of surfactancy, the potential independent contribution of the counter-ion to overall toxicity has been considered. The HPV surfactants are neutralized with a sodium cation, an ammonium cation or triethanolamine. Certain related chemicals (included in the category to provide supporting toxicological data) bear counterions such as magnesium or potassium. The nature of the counter-ion influences solubility and relative irritancy of the surfactants. However, because of dissociation, the counter-ions do not fundamentally alter pathways of mammalian tissue disposition, metabolism, excretion, or target organs of toxicity. This conclusion is substantiated by the research summarized in parts C and D below, which employed surfactant salts with a variety of counterions.

The mammalian toxicity profile of the counter-ions themselves is a function of their basicity. Triethanolamine is a weaker base than ammonia and less irritating to skin and mucous membranes than most amines (Gosselin, Smith et al, 1984). Its acute toxicity is low, as reflected by high oral LD_{50} values in rats of 4.2 to 11.3 g/kg (Hathaway, H. et al., 1996). In rats fed 0.73 g/kg daily, the major effect was fatty degeneration of the liver. The No Observable Effect Level (NOEL) was 0.08 g/kg (Hathaway, H. et al., 1996). Drinking water administration to rats at 1% or 2% concentration continuously for 2 years was toxic to the kidneys, especially in females. Triethanolamine was not mutagenic in bacterial assays (Hathaway, H. et al, 1996). Therefore, counter-ions derived from the relevant bases modulate tissue irritation potency but do not contribute any unique systemic toxicity.

B. Mechanisms of surfactant action

Surfactant properties play a salient role in mediating mammalian health effects for this category of chemicals. Surfactants are designed to solubilize hydrophobic materials such as lipids. Direct application of high concentrations of anionic surfactants to mammalian tissue causes disruption of

* The references cited in Appendix II are incorporated in section 5.4 (References) in the main body of this document.

cellular bilipid membranes, increased cellular and tissue permeability, tissue edema, and damage to tissue integrity (which may be accompanied by denaturation of proteins and other biological macromolecules). These effects are illustrated by considering the impact of high concentrations of surfactant on the skin and gastrointestinal mucosa.

Like other anionic surfactants, AS and AOS are concentration-dependent primary skin irritants in animal models and humans (Arthur D Little, 1991; Arthur D Little, 1993; IPCS, 1996). They cause delipidation of the skin surface, elution of natural moisturizing factors, denaturation of stratum corneum protein, increased permeability, epidermal swelling, and inhibition of enzyme activities in the epidermis (Imokawa, Sugura, et al., 1975; Prottey and Ferguson, 1975; IPCS, 1996). Acute and repeated dermal application of surfactants causes dose-dependent irritation, inflammation, edema or ulceration of the skin, the severity of which is highly dependent on concentration, duration of exposure and occlusion. A comprehensive consideration of skin effects of surfactants is beyond the scope of this discussion, but typical effects are illustrated in the SIDS acute and repeated dose studies involving dermal application (See human health endpoints summary tables in Appendix III and the individual robust summaries; available on request).

Not surprisingly, analogous irritant effects are observed when high doses are applied to the gastrointestinal mucosa. For example, irrigation of the GI tract of cats with 10% and 20% sodium lauryl sulphate caused dose-dependent loss of superficial epithelial layers, intramucosal edema and congestion of the esophagus, while loss of surface mucosal cells, vascular congestion, submucosal edema, and focal ulceration were observed in the stomach (Berenson and Temple, 1975). Similarly, irritant sequelae observed in acute oral toxicity studies (gavage mode of administration) included hemorrhage, edema and congestion of the stomach walls, with necrosis of the intestinal villi and mucosa (See human health endpoints tables in Appendix III and individual robust summaries in Appendix V; available on request) Although no direct experimental comparisons have been performed, the gastrointestinal mucosa may be more susceptible to surfactant-induced injury than the skin, which is protected by the keratinized, horny layer of the stratum corneum.

Influence of the polar head group

The presence of the anionic polar head group plays a role in mediating lipid solubilization and tissue penetration. For example, when pure surfactants with C_{12} chain length, but different functional groups, were assessed for their ability to disrupt cellular membranes (as measured by lytic ability against rabbit polymorphonuclear leukocytes), lysis increased with the polarity of the head group (Gibson, 1980). Skin penetration, as evidenced by the ability to elute proteins from the skin, is a function of the presence of both the polar head group and the length of the lipophilic chain. However, the presence of a polar head group is a critical factor, as anionic surfactants of various structures are more potent at eluting protein and amino acids from the skin than non-ionics (Prottey and Ferguson, 1975).

Influence of hydrocarbon chain length

Tissue irritation potency is a function of chain length for both AS and AOS. Mechanistic studies with varying chain lengths of AS and AOS indicate that irritation potential first rises with chain length, becomes maximal at about 12 carbons, and then decreases for longer chain lengths. This behavior has been postulated to be a function of two competing forces. The initial rise is thought to be a function of increasing hydrophobicity (oil/water partitioning), which facilitates insertion into lipid membranes; the decline at chain lengths higher than 12 is thought to be related to phase behavior, particularly the increased capacity for micelle formation at lower concentrations. Micelle formation may compete with cellular lipid interaction and reduce the concentration of individual molecules in solution that available to insert into cellular membranes (Schott, 1973; Rhein, Robbins, et al., 1986).

This pattern of activity with chain length has been consistently observed in mechanistic studies that examined surrogate measures of irritancy, such as cell lysis, protein denaturation and extraction, tissue swelling, and human forearm skin roughening by homologous series of AS and AOS surfactants. For example, when a series of sodium AS of chain lengths between C_9 and C_{15} was studied, maximum amino acid and protein extraction from guinea pig skin occurred with a carbon chain length of 12 (Prottey and Ferguson, 1975). (Table AII.1 provides an illustrative example). Similarly, cell lysis, as measured by the erythrocyte hemolytic activity of a homologous series of AS (C_8 to C_{15}), increased with chain length to maximal activity at 12 carbons, then showed no marked changes in activity at higher chain lengths (Zaslavsky, Ossipov et al., 1978).

AOS homologs showed similar trends in activity by chain length. For example, maximal swelling of isolated stratum corneum by homologous series of AS and AOS occurred at 12 or 14 carbon atoms (Rhein, Robbins et al., 1986). Swelling was concentration-dependent and saturable. Using C_{12} AS Na, saturation was shown to occur near the critical micelle concentration (Rhein, Robbins et al., 1986). In clinical studies involving application of 1% aqueous solutions of C_{12}, C_{14}, C_{16}, and C_{18} AOS to human forearm skin for 10 minutes, the C_{12} AOS induced more skin roughening than compounds with longer chain lengths. The relative degree of skin roughening *in vivo* correlated with the extent of protein denaturation measured *in vitro* (Imokawa, Sumura et al., 1975)

Table AII.1

Extraction of Protein and Amino Acids from Guinea Pig Skin by 25 mM AS

	% increase in extraction relative to washing with water	
Alkyl Sulphate chain length	**Soluble protein**	**Total Amino Acids**
9	50.8	62.7
10	166.1	84.2
11	119.5	100.4
12	238.9	194.8
13	198.5	141.7
14	163.9	110.3
15	77.9	41.3

From Prottey and Ferguson, 1975

Mechanistic studies of protein denaturation, as an indicator of surfactant irritant potency, demonstrated that AOS were less potent than AS at liberating sulfhydryl groups or at enzyme inhibition (Imokawa and Katsumi, 1976; Imokawa and Mishima, 1976). The studies examined skin keratin as a filamentous protein, serum albumin as a globular protein, acid phosphatase as an enzyme protein and membrane lysozyme as membrane protein. Alkyl chain length correlated with denaturing potency and irritation potential.

Influence of the counterion

The impact of the positively charged counter-ion on irritancy has also been investigated. Studies on broad cut C_{12} AS showed that the sodium salts of AS were more irritating to rabbit skin than ammonium or triethanolamine salts (Chiuta and Dodd, 1978) (see Table AII.2).

Other mechanistic studies demonstrated that swelling of isolated stratum corneum by C_{12} AS was reduced when either magnesium cation or triethanolamine were substituted for sodium cation (Blake-Haskins, 1986; Rhein, Robbins et al., 1986). The lower irritation potential of the triethanolamine salt is consistent with the fact that triethanolamine is a weaker and less irritating base.

Table AII.2
Counter ion effects on primary skin irritation by C_{12} AS

	Primary skin irritation score in rabbits after 24 hour occlude patch		
Surfactant concentration (%)	Sodium salt	Ammonium salt	Triethanolamine salt
2	>5 to <5.5	>5 to <5.5	3.5
10	6	5-6	5
20	6	6	5.2

(Chiuta and Dodd, 1978)

AS and sulfonates are, by and large, used in products that are ultimately discharged down-the-drain and enter the environment via the sewer system. From there onwards, the medium is aqueous. Regardless of the cation present in the product, new anion/cation equilibria are re-established in the environment. Sodium salts are most commonly used for aquatic toxicity assays in the laboratory. In the test vessels, new equilibria will be established as is the case in the environment. The observed toxicity is a result of the surfactant in its newly equilibrated state, regardless of the exact nature of the original cation in the raw material.

Mechanism of action in aquatic organisms

The mechanism of toxic action of surfactants in aquatic organisms is attributed to their narcotic action, which is described as a "nonspecific and reversible disturbance of the functioning of the membrane, caused by accumulation of the pollutants in the hydrophobic phases within the organism" (Van Wezel and Opperhuizen, 1995). Surfactants do not have chemically reactive groups that may cause direct, specific toxicity. Rather, their toxicity is due to physical interactions with cell membranes. It follows, then, that there will exist a certain relationship between the toxicity of the surfactant and the properties that will determine how effectively it can insert itself into the lipid bilayer, which is the cell membrane. Both the nature of the hydrophobe (the alkyl chain) and the nature of the hydrophilic head group, in other words the factors which together define the interfacial properties of the surfactant - play a role in this (Rosen et al., 1999). The hydrophobe determines the ease with which the surfactant will insert itself into the bilayer and the amount of disturbance due to hydrophobic interactions it will cause once it is in place. The hydrophilic head group contributes to toxicity by disrupting membrane-associated functions due to electrostatic interactions. For the same hydrophobe, N-containing surfactants (amines and quaternary ammonium compounds) are the most toxic, followed by the neutral surfactants. The anionic surfactants are the least toxic (Versteeg et al., 1997). Given our understanding of the mechanism of toxic action of surfactants, the grouping of the anionic surfactants of interest [AS and sulfonates with hydrophobes that differ in the length of the alkyl chain] for the purpose of risk assessment, is justified.

C. Mammalian Absorption, Distribution and Excretion

The mammalian absorption, tissue disposition and excretion of AS and AOS are very similar. AS and AOS are rapidly absorbed from the GI tract, and readily metabolized and excreted by mammals and humans (Denner, Olavesen et al., 1969; Burke, Olavesen et al., 1975; Merits, 1975; Burke, Olavesen et al., 1976; Inoue, O'Grodnick et al., 1982).

Plasma concentrations of AS are maximal within 30 minutes to 2 hours of oral administration (Denner, Olavesen et al., 1976; Merits, 1975). Studies with radiolabeled AS (ranging in chain length from C_{10} to $C_{18)}$ showed that, irrespective of chain length or counter ion, over 80% to 90% of the administered dose was excreted in the urine in rats, pigs and humans (Denner, Olavesen et al., 1969; Burke, Olavesen et al., 1975; Merits, 1975; Burke, Olavesen et al., 1976). However, in the dog, a significant proportion of the administered AS dose was excreted unchanged in the feces (Merits, 1975). Studies with C_{10}, C_{11}, C_{12}, and C_{18} AS indicate that the proportion of the dose excreted in urine and feces is not significantly altered by the route of administration (oral, intravenous, or intraperitoneal) (Denner, Olavesen et al., 1969; Burke, Olavesen et al., 1975; Merits, 1975; Burke, Olavesen et al., 1976). The distribution of label in urine and feces from orally administered potassium dodecyl ^{35}S-sulphate (C_{12} A^{35}S-K) was similar in both antibiotic-treated and untreated rats, indicating that the intestinal flora do not play a significant role in the metabolism of this compound (Denner, Olavesen et al., 1969).

A similar toxicokinetic profile was observed with ^{14}C-labeled C_{14} AOS in rats (Inoue, O'Grodnick et al., 1982). The radioalabeled compound was a mixture of the sodium 3-hydroxy alkane sulfonate and the sodium alken-2-yl sulfonate. After oral administration to rats, 80% of the dose was rapidly absorbed from the GI tract. Blood concentrations peaked at 3 hours; 4.3% of the dose was excreted in bile by 12 hours, by 24 hours, 72% of the dose was excreted in the urine and 22% in feces. Parenteral administration led to excretion of half the dose within 1 hour, and 90% excretion by 6 hours.

Tissue disposition studies with C_{12} AS in rats indicated that 36% of an i.v. dose reached the liver within 15 minutes, followed by the intestine, the kidney and the blood (Greb and Wingen, 1980). Studies with potassium salts of ^{35}S-labeled C_{10} and C_{18} AS in the rat also indicated that liver and kidney were early sites of labeling. The shorter chain length surfactant was cleared from tissues more rapidly: after 6 hours, only traces of the C_{10} salt remained in the kidney, whereas it took 12 hours for the C_{18} salt to be cleared from the kidney (Burke, Olavesen et al., 1972; Burke, Olavesen et al., 1975). The systemic disposition of percutaneously absorbed surfactants was similar to other routes of administration, with the highest percentage of the dose recovered in the liver and kidneys.

Absorption by the percutaneous route is limited, however, since anionic surfactants tend to bind to the skin surface (Howes, 1975; Black and Howes, 1980). Early studies with isolated human skin were unable to detect penetration of a homologous series of AS, ranging from C_8 to C_{18} (Blank and Gould, 1961). Animal studies confirmed a low level of percutaneous absorption of AS, alkyl sulfonates and AOS. Less than 0.4% of a 3 μmol dose of ^{35}S-labeled C_{12} AS-Na was percutaneously absorbed in guinea pigs, based on recovery of the radiolabel in urine, feces and expired air (Prottey and Ferguson, 1975). Interestingly, the sodium salt of C_{12} alkyl sulfonate showed slightly less penetration than the corresponding sulphate (Prottey and Ferguson, 1975). Studies with rats indicated that pre-washing of the skin with surfactant enhanced AS skin penetration (Black and Howes, 1980). About 0.6% of 0.5 ml of 0.2% aqueous ^{14}C-labeled C_{14} AOS was absorbed through rat skin in 24 hours, based on recovery in urine, bile and major organs (Minegishi, Osawa et al., 1977). However, when skin integrity was damaged, about 50% of the same dose was recovered in urine, bile and major organs 30 hours after dermal application.

D. Metabolism

AS and alkyl sulfonates

The metabolism of saturated AS and alkyl sulfonates is similar. Studies of radiolabeled, even-chained AS (specifically, C_{12}, C_{16} and C_{18}) indicated that they are extensively metabolized in rats, dogs and humans to yield a sulphate ester as the final product of degradation (Denner, Olavesen et al., 1969; Burke, Olavesen et al., 1975; Merits, 1975). Both ^{35}S- and ^{14}C-labeled AS were employed in the metabolic studies.

The major metabolite was identified as the 4-carbon compound, butyric acid 4-sulphate; this short-chained metabolite is highly polar and excreted in the urine. When ^{35}S-labeled butyric acid sulphate was injected into rats, 80% was eliminated unchanged and 20% was eliminated as inorganic sulphate (probably through internal nucleophilic displacement of the sulphate anion to form the 4-butyrolactone) (Denner, Olavesen et al., 1969). This reaction occurs non-enzymatically *in vitro* when the pH is lowered to 5 (Ottery, Olavesen et al., 1980). The 4-butyrolactone has been found as a minor metabolite. Dog urine also contained one other minor metabolite, glycolic acid sulphate.

$HOOC\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}OSO_3H$	$OC\text{-}CH_2\text{-}CH_2CH_2$	$HOOC\text{-}CH_2OSO_3H$
butyric acid-4-sulphate	4-butyrolactone (ring structure)	Glycolic acid sulphate
Major Metabolite	Minor metabolite	Minor metabolite
Found in rats, dogs, humans	Found in rats, dogs, humans	Found in dogs

The postulated mechanism for even-carbon AS is degradation by a common pathway involving ω-oxidation, followed by β-oxidation, to yield metabolites with chain lengths of C_2 and C_4. Metabolism of odd numbered chains (specifically, C_{11}) in rats was postulated to follow a similar ω, β degradation pathway: proprionic acid-3-sulphate was the major urinary metabolite and pentanoic acid-5-sulphate and inorganic sulphate were minor metabolites (Burke, Olavesen et al., 1975; Burke, Olavesen et al., 1976).

The biotransformation of alkyl sulfonates (C_{12}, C_{16}, and C_{11} have specifically been studied) also involves ω-oxidation, followed by β-oxidation. The major metabolites are the analogous sulfonates (Taylor, Olavesen et al., 1978; Black and Howes, 1980).

This body of research indicates that the biotransformation of the hydrocarbon chain of AS and alkyl sulfonates proceeds via the normal metabolic pathway of cytochrome P450 dependent ω-oxidation of aliphatic fatty acids (Klassen, Amdur et al., 1996). Furthermore, a common Phase II mammalian detoxification pathway for aliphatic fatty alcohols involves enzymatically-mediated sulphate conjugation, followed by urinary excretion of the resulting polar, sulphate esters. When one considers AS (and alkyl sulfonates), it is apparent that the "polar conjugation" of an aliphatic alcohol to a sulphate (or sulfonate) ester has been accomplished by virtue of the parent compound structure. Therefore, it is follows that the anionic terminus of AS and alkyl sulfonates is retained in the major mammalian metabolites, and that urinary excretion is the dominant elimination pathway. Since the biotransformation of the surfactants involves shortening of the hydrophobic chain to yield more polar, excretable end-products, metabolism is expected to curtail the effects of surfactancy on systemic tissues distant from the site of application, thereby reducing toxicity.

Alkyl olefin sulfonates

The metabolism of AOS is less well defined. The urinary metabolites are more polar, alcoholic, unsaturated, and of sulfonic functionality. Their chemical structures have not been elucidated, but it has been speculated that the metabolites are hydroxylated or polyhydroxylated sulfonic acids of a shorter chain length (Inoue, O'Grodnick et al., 1982). Hence, some common elements between the metabolism of AOS and AS may be a degree of sequential shortening of the carbon chain and retention of the terminal anion. However, the presence of functional groups such as the alkene double bond and the hydroxyl group in AOS mixtures may provide sites for further conversion. Other pathways for metabolic transformation may include adding a water molecule across the carbon double bond (through epoxidation/hydrolysis) to yield polyhydroxylated products. This could be

followed by conjugation of hydroxyl groups to yield more polar final end products. Glucuronide conjugates would excreted via the bile: the low level of biliary excretion for AOS provides circumstantial evidence for hydroxylation and conjugation as a potential, minor metabolic pathway. Whatever the combination of pathways involved, the biotransformation of AOS to shorter, more polar metabolites enhances clearance, degrades the hydrophobe, and would be expected to reduce surfactant-mediated systemic effects.

F. Toxicological Similarities

Similarities in toxicological behavior among the category surfactants further substantiate the validity of treating them as a single category of chemicals for the health effects assessment. This is illustrated by considering the toxicity profiles, as discussed in detail in the Hazard Assessment portion of this report (Section 5.2.)

APPENDIX III*

THE ALKYL SULPHATES

DETAILED SUMMARY OF MAMMALIAN TOXICOLOGY END POINTS

* The references cited in this Appendix refer to the Robust Summary Identifier. The Robust Summaries are not part of this document (available from AISE upon request).

TOXICOLOGY END POINTS - Data Availability and Quality

Chemical Class	Alkyl sulphate (AS) neutralized with a metal cation.
Health Effect End Point:	**5.2.1 Acute oral toxicity**
Conclusion: (data availability and quality)	**This end-point has been adequately characterized.** See attached tables for summary of supporting data.
Rationale:	This acute toxicity profile of the alkyl sulphate (AS) has been adequately characterized. Reliable, acute oral studies ar available covering representative hydrocarbon structures. The data are consistent in showing a low order of acute oral toxicity (LD_{50} > 2 g/kg in most cases), and some evidence of decreasing toxicity with increasing chain length. Most available studies fall into Klimisch reliability categories 1 (reliable without restriction) and 2 (reliable with restriction). Some studies rated category 2 predated the establishment of GLP or OECD guidelines. However, their conduct was scientifically sound, with methodology comparable to today's standards. A subset of studies on alkyl sulphate salts with chain lengths of C_{12}, C_{12-14}, C_{12-15}, and C_{15-16} were rated Klimisch category 3. These non-guideline studies were conducted prior to the establishment of GLPs. They differed from guideline protocols primarily in the use of mice (rather than the preferred rodent, the rat) as test species, 3 rather than 5 animals per dose group, and a 21-day post observation period. The results from these studies were consistent with studies rated Klimisch category 1 and 2: they corroborate a low order of acute toxicity, with LD_{50} values in the same order of magnitude as those found for similar chemicals tested in the rat.
Summary of Test Results	The acute toxicity data for this class of chemicals generally shows a low order of acute toxicity (LD_{50} > 2 g/kg) and some evidence of decreasing toxicity with increasing chain lengths.
Additional Data Needs	None.

Toxicology End-Point

5.2.1 Acute oral toxicity

Chemicals	GLP	OECD	Other	Species	Result	Reliability	Refs*
C_{12} As NH_4	Not stated (1980)		Comparable to OECD 401	Rat	LD_{50} > 0.5 ml/kg	**2C**	TRS 21
C_{12-13} AS K	No (1978)		Comparable to OECD 401	Rat	LD_{50} = 3.4 g/kg (95% C.I.: 2.6 - 4.2 g/kg)	**1B**	TRS3
C_{12} AS-Na	Not stated		Comparable to OECD 401	Rat	LD_{50} = 1427 mg/kg (males) LD_{50} = 977 mg/kg (females)	**2D**	TRS 2
C_{12} AS-Na	No (1975)		Not stated	Mouse	LD_{50} = 1.9 g/kg (95% C.I.: 1.5 - 2.3 g/kg)	**2D**	L34
C_{10-16} AS-Na	No (1974)		Comparable to OECD 401	Rat	LD_{50} = 2.7 g/kg (95% C.I.: 2.41 - 2.95 g/kg)	**2B**	TRS 5
C_{12-14} AS Mg C_{11}<1; C_{12}: 42%-54%; C_{13}: 37%-45%; C_{14+} <2	No (1975)		Comparable to OECD 401	Rat	LD_{50} = 2.71 g/kg active (95% CI 2.3-2.7 gkg)	**2B**	TRS 7
C_{12-14} AS Na	No (1977)		Not stated	Mouse	LD_{50} = 2.6 g/kg (95% C.I.: 2.0 - 3.3 g/kg) Irritation of the stomach, small and large intestines, pale livers and kidneys in decedents. Thickening of stomach wall and enlargement of the mesenteric lymph node in all survivors.	**2D**	L29
C_{12-15} AS Na C_{12}: 18%; C_{13}: 28% C_{14}: 30%; $C_{15:}$ 20%	No (1975)		Not stated	Mouse	LD_{50} = 3.5 g/kg (95% C.I.: 2.8 - 4.5 g/kg) Irritation of stomach and intestine and pale liver and kidneys in decedents; thickening of stomach wall in surviving animals	**2D**	L1

*Codes refer to Robust Summary identifier

Toxicology End-Point

5.2.1 Acute oral toxicity (continued)

Chemicals	GLP	OECD	Other	Species	Result	Reliability	Refs
C_{12-15} AS Na C_{12}: 18%; C_{13}: 28% C_{14}: 30%; $C_{15:}$ 20%	No (1975)		Not stated	Mouse	LD_{50} = 2.9 g/kg (95% C.I.: 2.5 - 3.4 g/kg) Gaseous/fluid distention and irritation the stomach, irritation of small intestine, pale liver and kidneys in decedents. Thickening of stomach wall in survivors.	**2D**	L2
C_{12-15} AS Na C_{12}: 18%; C_{13}: 28% C_{14}: 30%; $C_{15:}$ 20% (bleached)	No (1975)		Not stated	Mouse	LD_{50} = 4.3 g/kg (95% C.I.: 3.6 - 5.1 g/kg) Gaseous/fluid distention and irritation the stomach, irritation of small intestine, pale liver and kidneys in decedents. Thickening of stomach wall in survivors.	**2D**	L4
C_{12-15} AS Na C_{12}: 18%; C_{13}: 28% C_{14}: 30%; $C_{15:}$ 20% (unbleached)	No (1975)		Not stated	Mouse	LD_{50} = 3.8 g/kg (95% C.I.: 3.1 - 4.6 g/kg) Gaseous distention and irritation the stomach, irritation of small intestine, nasal excretion of protoporphyrin. Gaseous distention and irritation the stomach, irritation of small intestine, pale liver and kidneys in decedents. Thickening of stomach wall in survivors.	**2D**	L5
C_{12-15} AS Na C_{12}: 17%; C_{13}: 30% C_{14}: 31%; $C_{15:}$ 18% (unbleached)	No (1975)		Not stated	Mouse	LD_{50} = 2.8 g/kg (95% C.I.: 2.3 - 3.3 g/kg) Fluid distention and irritation the stomach, irritation of small intestine, pale kidneys in decedents. Thickening of stomach wall in survivors.	**2D**	L3

Toxicology End-Point

5.2.1 Acute oral toxicity (continued)

Chemicals	GLP	OECD	Other	Species	Result	Reliability	Refs
C_{13-15} -AS Na C_{13}: 62%; C_{15}: 37%	No (1975)		Not stated	Mouse	LD_{50} = 2.9 g/kg (95% C.I.: 2.2 - 3.8 g/kg) Gaseous/fluid distention of the stomach, irritation of small intestine, pale liver and kidneys in decedents. Thickening of stomach wall in survivors.	**2D**	L19
C_{15-16} AS C_{15}: 51% C_{16}: 49%	No (1976)		Not stated	Mouse	LD_{50} = 6.8 g/kg (95% C.I.: 5.7 - 8.2 g/kg) Gross distension of the stomach and irritation of the stomach and small intestine, cyanosis and diarrhea in decedents. Thickening of stomach wall in survivors.	**2D**	L26
C_{10-16} AS-NH_4	No (1975)		Comparable to OECD 401	Rat	LD_{50} =1.83 g/kg (95% C.I. 1.6-2.1 g/kg)	**2B**	TRS 6
See also data on Na salts and TEA complex							
C_{12-14} AS TEA	Not stated		Not stated	Rat	LD_{50} = 3.9 g/kg	**4**	TRS 4
C_{10} AS TEA	yes	401		Rat	LD_{50} >2 g/kg	**1A**	HESA 1
See also data on Na salts above							

Toxicology End-Point

5.2.1 Acute oral toxicity (continued)

Chemicals	GLP	OECD	Other	Species	Result	Reliability	Refs
C_{12-18} AS Na (tallow AS) C_{14}: <1% C_{16}: 30% C_{18}: 69%	No (1974)		Comparable to OECD 401	Rat	LD_{50} = 4.01 g/kg (25% active) (95% C.I.: 3.39- 4.74 g/kg)	**2B**	TRS 5
C_{16-18} AS Na C_{14}: 3-7% C_{16}: 25-35% C_{18}: 60-67%	Yes		791831/ EWG Annex V part B	Rat	LD_{50} > 2 g/kg	**1B**	HESA 3
C_{12-18} AS Na (tallow AS) (C_{10}: 0.02%; C_{12}: 0.3%; C_{14}: 0.8%; C_{16}: 65.5%; C_{18}: 31.6% C_{20}: 1.6%)	No (1972)		Not stated	Rat	LD_{50} = 5.24 g/kg (95% C.I.: 4.6-5.96 g/kg)	**2D**	TRS 11
C_{12-18} AS Na (tallow AS) (C_{10}: 0.04%; C_{12}: 0.05%; C_{14}: 0.3%; C_{16}: 57%; C_{18}: 33.4% C_{20}: 9.16%)	No (1972)		Not stated	Rat	LD_{50} = 7.84 g/kg	**2D**	TRS 11
C_{12-18} AS Na C_{12}: 12% C_{14}: 4% C_{16}: 19% C_{18}: 64%	Yes		791831/ EWG Annex V part B	Rat	LD_{50} > 2 g/kg	**1B**	HESA 20

Toxicology End-Point

5.2.1 Acute oral toxicity (continued)

Chemicals	GLP	OECD	Other	Species	Result	Reliability	Refs
C_{12-18} AS Na (tallow AS) (C_{12}: 6.7%; C_{14}: 5%; C_{16}: 26.7%; C_{18}: 61%)	No (1972)		Comparable to OECD 401	Rat	LD_{50} = 7.64 g/kg (95% C.I.: 6.61-8.82 g/kg)	**2D**	TRS 8
C_{12-18} AS Na (tallow AS)	No (1974)		Comparable to OECD 401	Rat	LD_{50} = 4.01 g/kg (95% C.I.: 3.39- 4.74 g/kg)	**2B**	TRS 5
C_{12-18} AS Na	No (1954)		Not stated	Rat	LD_{50} = 4.16 g/kg	**2D**	TRS 9
C_{12-18} AS Na	No (1954)		Not stated	Rat	LD_{50} = 2.4 g/kg	**2D**	TRS 10
C_{12-18} AS Na	No (1954)		Not stated	Rat	LD_{50} = 2.34 g/kg	**2D**	TRS 10
C_{12-18} AS Na	No (1954)		Not stated	Rat	LD_{50} = 0.5 g/kg	**2D**	TRS 10
C_{14-15} AS Na;	No (1974)		Comparable to OECD 401	Rat	LD_{50} =1.78 g/kg (95% C.I.: 1.47 - 2.16 g/kg,)	**2B**	TRS 5
C_{14-16} AS Na and C_{18} unsaturated AS Na	yes	401		Rat	LD_{50} >2 g/kg	**1A**	HESA 2

TOXICOLOGY END POINTS
Data Availability and Quality

Chemical Class	Alkyl sulphate (AS) neutralized with a metal cation.
Health Effect End Point:	**5.2.1. Acute dermal toxicity**
Conclusion: (data availability and quality)	**This end-point has been adequately characterized.** See attached table for summary of supporting data.
Rationale:	This acute dermal toxicity profile of this chemical class has been adequately characterized. Studies are available on three alkyl sulphates, C_{10}-AS (potassium salt) and C_{10-16}-AS (magnesium and ammonium salts). The results are consistent in showing skin irritation at the site of surfactant application but limited systemic toxicity by the percutaneous route. The studies fall into Klimisch reliability categories 1 (reliable without restriction) and 2 (reliable with restriction). Some studies rated category 2 predated the establishment of GLP or OECD guidelines. Their conduct was scientifically sound, with methodology comparable to today's standards except for a smaller group size (3 animals/sex/dose). Mechanistic studies reported in the literature demonstrate a low order of percutaneous absorption through intact skin. An extensive data base exists showing a low order of acute oral toxicity for this class of materials, and most dermal studies included application to abraded skin to enhance potential skin penetration. Therefore, based on the available data, no significant percutaneous toxicity is expected for this class of materials beyond local irritant effects. Therefore, no additional studies are recommended.
Summary of Test Results	The chemicals showed no deaths or evidence of systemic effects when administered at doses ranging from 0.5 g/kg to 2 g/kg, depending on the active concentration of the raw material.
Additional Data Needs	None recommended. The dermal studies employed lower doses (0.5 g/kg) than are usually administered to classify a material as non-hazardous (2000 mg/kg). However, a substantial database exists showing a low order of toxicity by the oral route. Given the extensive data base of oral studies and the limited absorption of this class of surfactants by the dermal route, no unique toxicological insights beyond the severity of skin effects would be gained by additional percutaneous testing.

Mammalian Toxicology End-Point

5.2.1 Acute dermal toxicity

Chemicals	GLP	OECD	Other	Species	Result	Reliability	Refs
$C_{12\text{-}13}$ AS K	No (1978)		Comparable to OECD 402 except 3 animals/sex/dose	Rabbit	LD_{50} > 0.5 g/kg Skin erythema, edema, eschar formation, desquamation by day 6. Some residual skin irritation at 14 days. No systemic toxicity.	**1B**	TRS13
$C_{10\text{-}16}$ AS Mg	No (1975)		Comparable to OECD 402 except 3 animals/sex/dose	Rabbit	LD_{50} > 2 ml/kg (intact and abraded skin) equivalent to >0.5 ml/kg (active) Skin erythema, eschar formation, necrosis through day 14, sloughing by day 21, leaving skin hyper-pigmented. No systemic toxicity.	**2B**	TRS12
$C_{10\text{-}16}$ AS-NH_4	No (1975)		Comparable to OECD 402 except 3 animals/sex/dose	Rabbit	LD_{50} > 0.5 g/kg (intact and abraded skin) Severe erythema and slight eschar formation at 24 hrs. Necrosis by day 2, with sloughing by day 14 and hyper-pigmentation of new skin. No systemic toxicity.	**2B**	TRS 14

TOXICOLOGY END POINTS
Data Availability and Quality

Chemical Class	AS neutralized with a metal cation.
Health Effect End Point:	**5.2.2. Skin irritation**
Conclusion: (data availability and quality)	**This end-point has been adequately characterized.** See attached table for summary of supporting data.
Rationale:	The skin irritant properties of AS have been adequately characterized. Rabbit skin irritation studies on 3 different materials were reviewed for this report: C_{12} AS Na, C_{12-14} AS TEA, C_{12-18} AS Na (2 studies). These studies fall into Klimisch reliability category 1 (reliable without restriction). In addition, summaries of two dose-response studies were also reviewed; one on C_{13-15}AS and one on C_{16-18}AS. These studies fall into Klimisch reliability category 3 (not reliable) since details are not available on key aspects of the protocol. Alkyl sulphates produce skin irritation in a dose dependent manner that is consistent with the surfactant properties of these materials. Overall, alkyl sulphates with longer carbon chains (C_{16-18}) tended to be qualitatively less irritating than alkyl sulphates with carbon chains of C_{12} to C_{15}. Test results indicate that for the shorter carbon chain length materials, (C_{12} and C_{13-15}) concentrations of 10% or greater consistently produce moderate to severe irritation reactions. Responses to lower concentrations (1-7%) tend to vary from none to severe. The longer carbon chain length materials (C_{16-18}) and broader cut materials (C_{12-18}) produce strong reactions at concentrations above 25%, and none or slight reactions at lower concentrations. The test conducted with 5% C_{12-18} AS produced responses that would not result in classification as an irritant under EU criteria Results are consistent with literature reports indicating that AS produces skin irritation in a dose response manner that is consistent with the surfactant properties of these materials. No additional studies are recommended.
Summary of Test Results	Moderate to strong irritant at concentrations above 5%. Lower concentrations produce sllight to moderate irritation.

Additional Data Needs	None recommended.

Mammalian Toxicology End-Point

5.2.2 Skin Irritation

Chemicals	GLP	OECD	Other	Species	Result	Reliability	Refs
C_{12} AS Na	Not stated, but study conducted by OECD guidelines, which specify GLP	Yes OECD 404	Acute Dermal Irritation/ Corrosion Dose level; 25% via 4 hour patch 5 animals	Rabbit	Strong erythema and edema Mean erythema for the test animals over three scoring time points (24, 48 and 72 hours) was 2.0. Mean edema was 0.7	**1A**	Henkel study no. 870150
C_{12-14}AS TEA	Not stated, but study conducted by OECD guidelines, which specify GLP	Yes OECD 404	Acute Dermal Irritation/ Corrosion Dose level; 25% via 4 hour patch 5 animals	Rabbit	Strong erythema and edema Mean erythema for the test animals over three scoring time points (24, 48 and 72 hours) was 3.7. Mean edema was 2.1.	**1A**	Henkel study no. 870150
C_{12-18} AS Na	Yes	Yes OECD 404	Acute Dermal Irritation/ Corrosion Dose level; 88.7% (undiluted) via 4 hour patch 3 animals	Rabbit	Strong erythema and edema Mean erythema for the test animals over three scoring time points (24, 48 and 72 hours) was 3.0. Mean edema was 1.4	**1A**	Notox study no. 130793
C_{12-18} AS Na	Not stated, but study conducted by FHSA guidelines, which specify GLP	No FHSA protocol	Acute Dermal Irritation/ Corrosion Dose level; 5% 4 hour patch 5 animals	Rabbit	Slight erythema and edema Mean erythema for the test animals over three scoring time points (24, 48 and 72 hours) was 0.5. Mean edema was 0.1. Material would not be classified as an irritant at 5%.	**1A**	Cognis study no. 372
C_{13-15} AS Na	No	No Similar to OECD 404	Acute Dermal Irritation/ Corrosion Dose levels; 15%, 10%, 7%, 5%, 3%, and 1% via 4 hour patch 8 animals per dose group	Rabbit	Irritant Moderate to strong irritant reactions at 3% -15% doses. Slight to moderate irriant at the 1%.	**3B** Grading scale and evalution of results not explained.	Unilever study no. CPS 83.25

Mammalian Toxicology End-Point

5.2.2 Skin Irritation (continued)

Chemicals	GLP	OECD	Other	Species	Result	Reliability	Refs
$C_{16\text{-}18}$AS Na	No	No Similar to OECD 404	Acute Dermal Irritation/ Corrosion Dose levels; 31.5% (undiluted), 25%, 20%, 15%, 10%, and 5% via 4 hour patch 8 animals per dose group	Rabbit	Moderately Irritating Sllight to moderate reactions at 25%, 20% and 15%. Marginal to moderate at 10% and 5%. Undiluted material produced marginal to slight reations.	**3B** Grading scale and evalution of results not explained.	Unilever study no. CPS 83.45
C_{12}AS Na	No	No	Burckhardt Test Dose levels; 5% and 1% 1% Repeated open application of test solutions	Rabbits Humans	Mild irritation at 5%. No reactions at 1% No reactions at 1%	**4C** Not assignable, only brief summary available	Henkel study no. 148.

TOXICOLOGY END POINTS
Data Availability and Quality

Chemical Class	Alkyl sulphate (AS) neutralized with a metal cation.
Health Effect End Point:	**5.2.2. Eye irritation**
Conclusion: (data availability and quality)	**This end-point has been adequately characterized.** See attached table for summary of supporting data.
Rationale:	The eye irritant properties of AS have been adequately characterized. Summaries of rabbit eye irritation studies on 2 different materials were reviewed for this report: $C_{13\text{-}15}$ AS Na and $C_{16\text{-}18}$ AS Na. These studies fall into Klimisch reliability category 3 (not reliable) since details are not available on key aspects of the protocol. Both materials produced eye irritation when tested at 5%. As with skin irriation (see previous section), the longer chain ($C_{16\text{-}18}$) material tended to be less irritating. Animals dosed with this material exhibited what were classified as slight to moderate responses. Whereas, animals treated with the $C_{13\text{-}15}$ material exhibited moderate to severe responses. Although both test are classified as reliability catergory 3, the data are sufficient to conclude that the materials are eye irritants. Further, the test results are consistent with published reports on AS, indicating the material produces eye irritation in a manner that is consistent with the surfactant properties of these materials. No additional studies are recommended.
Summary of Test Results	These materials are eye irritants at concentrations of 5%.
Additional Data Needs	None recommended.

Mammalian Toxicology End-Point **5.2.2 Eye Irritation**							
Chemicals	GLP	OECD	Other	Species	Result	Reliability	Refs
C_{13-15} AS Na	No (1975)	No (FHSA Protocol)	Draize Eye Irritation Dose level; 5% 6 animals	Rabbit	Moderate to severe eye irritant Moderate corneal lesions in 5 animals. Four animals demonstrated discharge and iritis. All the lesions healed after 8 days. A sixth animal was unaffected	**3**	Unilever study no. RE 75/49C
C_{16-18} AS Na	No (1975)	No (FHSA Protocol)	Draize Eye Irritation Dose level; 5% 6 animals	Rabbit	Slight to moderate eye irritant All animals exhibited slight to moderate corneal lesions and conjunctivitis. Five animals demonstrated iritis. All the lesions had healed within 12 days.	**3**	Unilever study no. RE 75/56C

TOXICOLOGY END POINTS
Data Availability and Quality

Chemical Class	Alkyl sulphate (AS) neutralized with a metal cation.
Health Effect End Point:	**5.2.3 Skin Sensitization**
Conclusion: (data availability and quality)	**This end-point has been adequately characterized.** See attached table for summary of supporting data.
Rationale:	This sensitization properties of AS have been adequately characterized. Sensitization studies on 2 materials were reviewed for this report: a Maximization Test on C_{12-14} AS TEA and a Buehler Test on C_{12-18} AS Na. These studies fall into Klimisch reliability categories of 2 (reliable with restriction) and 1 (reliable without restrictions), respectively. In addition, a summary of a Magnusson - Kligman study on C_{12-14} AS Na was also reviewed (reliability rating 4). All three tests were negative for sensitization. Alkyl sulphates are universally considered non-sensitizing skin irritants. The C_{12} AS, Na salt is broadly used as an irritant control in various types of studies involving contact sensitization and irritation. It is widely used in the standard Maximization test to establish a background level of skin irritation during the induction patch if a non-irritating material is being tested (as specified in OECD Guideline 406). There have been occasional reports of positive responses for sensitization in predictive tests (Local Lymph Node Assay) and diagnostic patch tests. However, these reactions are undoubtedly due to the irritating nature of these materials rather than a true sensitization. In addition, AS has no chemical structural alert for sensitization. No additional studies are recommended.
Summary of Test Results	Alkyl sulphates are non-sensitizing.
Additional Data Needs	None recommended.

Mammalian Toxicology End-Point

5.2.3 Skin sensitization

Chemicals	GLP	OECD	Other	Species	Result	Reliability	Refs
C_{12-14} AS TEA	No (1977)	No (Similar to OECD 406)	Guinea Pig Maximization Dose levels; Induction injections, 5% Induction patch, 5% Challenge patch, 1% 20 test and 10 control animals	Guinea Pig	No evidence of sensization Irritant reactions were present at the treatment sites aafter induction. However, these had resolved prior to challenge	**2B** Method is similar to OECD 406. Controls not treated during induction. Some details missing.	Henkel study no. 80
C_{12-18} AS Na	Yes	Yes	Buehler Test Dose levels; Induction: 12.5% Challenge: 6.25% 20 test and 10 control animals	Guinea Pig	Reactions not indicative of sensitization. At 24 hours, both the test and control groups had responses of "1" for 20% of the animals (4 of 20 in test group, 2 of 10 in control group). There were no responses greater than "1" in either test or control. By 48 hours, the test group showed reactions of "1" in 10% of animals (2 of 19). The control group showed no reactions.	**1A**	Scantox study no. RE 02156
C_{12-14} AS Na	No (1977)	No	Magnusson - Kligman Maximization Comparable to OECD 406 Dose levels; Induction injections, 0.08% Induction patch, 0.5% Challenge patch, 0.1% 10 test animals	Guinea Pig	Negative for sensitization 0/10 guinea pigs sensitised.	**4** Key information on protocol and test substance missing. No controls	Unilever study no. SSM 77.402

TOXICOLOGY END POINTS - Data Availability and Quality

Chemical Class	Alkyl sulphate (AS) neutralized with a metal cation.
Health Effect End Point:	**5.2.4 Repeated Dose Toxicity (oral)**
Conclusion: (data availability and quality)	**This end-point has been adequately characterized.** See attached tables for summary of supporting studies.
Rationale:	The repeated-dose toxicity profile of the category surfactants has been well characterized. Twenty eight-day and/or 90-day studies rated Klimisch reliability categories 1 or 2 are available. A series of non-guideline, 21-day studies is available on several chemicals that have also been tested in 90-day studies. Although these 21-day protocols do not meet current guidelines, the studies show consistent results with longer, guideline studies and lend additional perspective on the effects of duration and route of exposure. Additional perspective is also provided by two 2-year feeding studies on $C_{12\text{-}15}$ AS Na.
Summary of Test Results	The forestomach, GI tract and liver were the primary target organs for category surfactants, with clear, dose-dependent effects. GI tract Irritation, particularly of the forestomach, was more pronounced for gavage administration compared to dietary exposure. A higher degree of local irritation by gavage is expected, since dietary admixture mitigates the bolus dose effect. At high surfactant doses, liver effects were evidenced by altered serum concentrations of liver enzymes, liver cell hypertrophy, and decreases in liver fat content. At the highest doses, animals also ate less, gained less weight, and had significantly less abdominal fat. Liver effects were more apparent in dietary studies, partly because these allowed administration of higher doses of the test material with less GI tract injury. Notably, gavage studies that included recovery groups indicated that systemic effects other than forestomach irritation were fully reversible. All studies gave clear NOEL and LOEL values. In comparable study protocols, the degree of toxicity decreased with increasing surfactant chain lengths.
Additional Data Needs	None. The metabolic and repeated dose toxicity profiles demonstrate a strong structure-activity relationship among category surfactants. No significant further insight would be gained by additional testing.

Toxicology End-Point 5.2.4 Repeated Dose Toxicity (oral)							
Chemicals	GLP	OECD	Other/ Protocol Details	Species	Result	Reliability	Refs
C_{12} AS Na	Yes		28 day oral study (gavage). 79/831/EC Annex V, Part B.7 . 10 rats /sex/group Dose levels: 30, 100, 300, 600 mg/kg Vehicle control. 29-day observation period post-dosing.	Rat	NOEL= 100 mg/kg LOEL= 300 mg/kg. Two deaths at 600 mg/kg. High dose reduced to 300 mg/kg after 10 treatments. Decreased food consumption and body weight gains at high dose. In 600 mg/kg group, there was an increase in relative liver, kidney, brain, and gonad weights. Highest dose caused ulceration of stomach, partially reversible. White deposits were found in stomach and intestines at 100-mg/kg dose, but no adverse histopathologic findings.	**1A**	TRS 16
C_{12} AS-Na **(99% pure)**	No (1976)		21-day oral study. Similar to OECD 407 but 21 day dietary exposure; 3 animals/sex/ group (test) and 6 rats/ sex/group (controls) Dose levels: 0, 0.023, 0.047, 0.094, 0.188, 0.375, 0.75, 1.50 % in diet (25, 50, 110, 200, 420, 830, 1600 mg/kg/day Standard diet control.	Rat	NOEL= 0.094% in diet (108 mg/kg/day) LOEL= 0.375% in diet (207 mg/kg/day), (Increased weight, liver cell hypertrophy and elevated serum GPT) High dose animals (1600 mg/kg/day) showed decreased food consumption. Male rats in high dose group gained less weight than controls. Relative liver weights of high dose males and females fed 0.188-1.50% in diet were increased. Relative kidney weights and brain weights were higher in high dose females. Body fat was depleted in high dose animals. The livers of high dose rats (1.5% in diet) showed diffuse periportal hypertrophy, reduced cytoplasmic fat content, and reduced glycogenic vacuolation. Periportal parenchymal hypertrophy and reduced cytoplasmic fat and glycogenic vacuolation were also seen in 5/6 rats fed 0.75% and 3/6 rats fed 0.375% and 0.188% (8/11 were female).	2D (**NON-standard protocol**) 21-day vs 28-day exposure duration Not GLP. Meets basic scientific principles	L35

Toxicology End-Point

5.2.4 Repeated Dose Toxicity (oral) (continued)

Chemicals	GLP	OECD	Other/ Protocol Details	Species	Result	Reliability	Refs
C_{12} AS-Na (99% pure)	No (1976)		13-week (90 day) feeding study in rats. Similar to OECD 408 10 rats/sex/dose (test groups); 20 rats/sex/dose (controls) Dose levels: 0, 0.07%, 0.14%, 0.28%, 0.56%, 1.3% and 2.25% in diet (59, 116, 230 470, 950, 1900 mg/kg/day on average. Standard diet control.	Rat	NOEL= 0.14% in diet (116 mg/kg/day) LOEL= 0.28% in diet (230 mg/kg/day) (Liver cell hypertrophy, elevated serum GPT and AP) One female fed 0.28% was sacrificed at week 4 due to poor body weight gain and deformity of the thorax. High dose groups ate less. High dose females drank more. Serum protein decreased in high dose males. Serum cholesterol decreased in the high dose groups. Serum triglyceride decreased in the three highest dose groups. Males of the high dose group and both sexes of the two highest dose groups had elevated serum GOT and GPT, respectively. Serum AP increased in the three highest dose groups. Serum creatine phosphokinase was elevated in males of the high dose group and females of the two highest dose groups. Relative liver weights increased in males of the two highest dose groups and females of the three highest dose groups. Relative kidney weights increased in females at three highest doses. Relative weights of the testes increased at highest dose. High dose animals had significantly less abdominal fat. Livers of rats in the two highest dose groups showed prominent periportal and diffuse hypertrophy, reduced glycogenic vacuolation and cytoplasmic neural fat. Haemosiderin content of parenchymal and Kupfer cells was reduced. Periportal and diffuse parenchymal hypertrophy, reduced cytoplasmic fat and glycogenic vacuolation. in females at four highest doses.	**2C**	L36

Toxicology End-Point

5.2.4 Repeated Dose Toxicity (oral) (continued)

Chemicals	GLP	OECD	Other/ Protocol Details	Species	Result	Reliability	Refs
C_{12-15} AS Na C_{12}: 17%; $C1_3$: 30.4%; C_{14}:30.8%; C_{15}: 17.7% (bleached)	No		21-day oral study. Comparable to OECD 407 but 21 day dietary exposure; 3 animals/sex/ group (test) and 6 rats/ sex/group (controls) Dose levels: 0, 0.047%, 0.094%, 0.188%, 0.375%, 0.75% and 1.50% in diet (60, 117, 252, 503, 1010, 1956 mg/kg/day on average. Standard diet control.	Rat	NOEL= 0.188% in diet (252 mg/kg/day) LOEL= 0.375% in diet (503 mg/kg/day) (increased liver weight, liver cell hypertrophy, elevated serum GPT) Males of the three highest dose groups gained significantly less weight. Males of the high dose group ate less. High dose females drank less water. Both sexes of the high dose group (1.50% in diet) and males of the second highest dose group (0.75%) had higher levels of serum GPT. Relative liver weights of both sexes increased in the two highest dose groups. Relative weights of the testes increased at the high dose. Relative brain weights were increased in males of the three highest dose groups. There were no remarkable macroscopic findings. Prominent periportal and diffuse hypertrophy, markedly reduced cytoplasmic (glycogenic) vacuolation and reduced cytoplasmic neural fat were seen at the high dose (1.5%) (4/6 diffuse; 2/6 periportal). Diffuse parenchymal or periportal hypertrophy was seen in 1(male) of 6 rats (diffuse) and 3/5 rats (periportal) of the second highest dose group (0.75%). Periportal hypertrophy was present in 2/6 rats at the third highest dose level (0.375%). Four of 6 rats affected at the 0.75% and 0.375% dose levels were female.	2D (**NON-standard protocol**) 21-day vs 28-day exposure duration Not GLP. Meets basic scientific principles	L6

Toxicology End-Point

5.2.4 Repeated Dose Toxicity (dermal) (continued)

Chemicals	GLP	OECD	Other/ Protocol Details	Species	Result	Reliability	Refs
C_{12-15} AS Na C_{12}: 17%; $C1_3$: 30.4%; C_{14}:30.8%; C_{15}: 17.7% (bleached)	No		21-day dermal (percutaneous) study. Similar to OECD 410 but smaller group sizes (3 mice /sex/group), shorter duration (21 days of exposure) and no clinical biochemistry assessments. Dose groups: 0%, 5%, 10%, 15%, 18% in water; vehicle control. 2x weekly application with 3-day interval between treatments.	Mouse	NOEL = 5% concentration LOEL = 10% concentration (epidermal hyperplasia) All mice at 18% died or were sacrificed because or dehydration caused by fluid loss through skin lesions. The 15% dose group ate significantly more food and drank more water than controls. Increased relative liver weights (males) and relative kidney weights (females) were found at the 15% dose level. Exudate adherent to damaged skin (2/6) and dry white scales (5/6) observed at the 15% dose level. Extensive ulceration and necrosis of the epidermis with infllammatory exudate in decedents. Ulceration (3/6) and inflammatory exudate of the epidermis, epidermal thickening due to hyperkeratosis (6/6), hypergranulosis (6/6) and acanthosis (6/6) with a high degree of pleomorphism in the skin (5/6), and epidermal edema with changes to the dermis at the epidermal junction were found at the 15% concentration. Edema (2/6), hyperkeratosis (2/6) and acanthosis (2/6) observed at the 10% concentration. No systemic histopathological effects on other organs and tissues.	2D (**NON-standard protocol**) 21-day vs 28-day exposure duration Not GLP. Meets basic scientific principles	L7

Toxicology End-Point

5.2.4 Repeated Dose Toxicity (oral) (continued)

Chemicals	GLP	OECD	Other/ Protocol Details	Species	Result	Reliability	Refs
C_{12-15} AS Na C_{12}: 17%; $C1_3$: 30.4%; C_{14}:30.8%; C_{15}: 17.7% (bleached)	No (1976)		13-week (90 day) feeding study in Wistar rats, Similar to OECD 408 10 rats/sex/dose (test groups); 20 rats/sex/dose (controls) Dose levels: 0, 0.07%, 0.14%, 0.28%, 0.56%, 1.13% and 2.25% in diet (62, 122, 245 488, 1016, 2081 mg/kg/day on average. Standard diet control 13-week (90 day) feeding study in Wistar rats. Similar to OECD 408. 10 rats/sex/dose (test groups); 20 rats/sex/dose (controls) Dose levels: 0, 0.07%, 0.14%, 0.28%, 0.56%, 1.13% and 2.25% in diet (62, 122, 245 488, 1016, 2081 mg/kg/day on average. Standard diet control..	Rat	NOEL= 0.14% in diet (122 mg/kg/day) LOEL= 0.28% in diet (245 mg/kg/day) (Liver cell hypertrophy, elevated serum GPT and AP) The two highest dose groups gained less weight. The high dose groups ate less. High dose females drank less water. Serum protein decreased at the highest dose (males). Serum Mg, protein, cholesterol, decreased at the highest dose (males). Serum GOT was elevated in high dose males. Serum GPT, was elevated in males of the two highest dose groups (1.13% and 2.25%) and females of the second highest dose group (1.13%). Serum AP was increased in the 1.13% and 2.25% dose groups (females) and 1.13% group (males). Relative liver weights (both sexes) increased in the three highest dose groups (0.56%-2.25%). Absolute spleen weights increased in males at the two highest doses and females at the highest dose. Absolute kidney weights decreased in high dose males. Relative kidney weights increased in females at the two highest doses. Relative weights of the testes increased at the two highest doses. High dose males had virtually no abdominal fat and changes in color and consistency of intestinal contents. These findings were less frequently noted in high dose females. Diffuse (6 females, 3 males) and periportal (11/20) hypertrophy, reduced cytoplasmic (glycogenic) vacuolation, reduced cytoplasmic neutral fat and hemodiderin content prominent at high dose. Periportal hypertrophy also observed at nest two highest doses (1.13% and 0.56%). Periportal parenchymal hypertrophy was observed in 4 females at the 0.28% level. reduced cytoplasmic (glycogenic) vacuolation, reduced cytoplasmic neutral fat and hemodiderin conten were observed at the 0.56%-2.25% dose levels. Nephrocalcinosis is freqently observed in untreated females of the Wistar strain: the incidence and severity was reduced in the highest dose group. Lymphatic dilation of the small intestine was more prominent at the high dose.	**2C**	L8

Toxicology End-Point

5.2.4 Repeated Dose Toxicity (dermal) (continued)

Chemicals	GLP	OECD	Other/ Protocol Details	Species	Result	Reliability	Refs
C_{12-15} AS Na C_{12}: 17%; C_{13}: 30.4%; C_{14}:30.8%; C_{15}: 17.7% (bleached)	No		13 week dermal (percutaneous) study. Similar to OECD 411. 10 mice/sex/dose. Dose groups: 0%, 5%, 10%, 12.50%, 15% in water; vehicle control. 2x weekly application (M/Th or Tu/F) for 13 weeks Clinical observations and macroscopic and microscopic assessment of organs and tissues. No clinical biochemistry assessments.	Mouse	NOEL = 5% concentration LOEL = 10% concentration (epidermal hyperplasia) One mouse treated with 12.5% died after 1 week due to dehydration and anorexia The two highest concentration groups drank more than controls. Hemoglobin levels reduced and wbc cunts increased in high dose males. Absolute and relative heart weights were higher in high dose females. Relative liver weights were increased in botjh sexes at the 15% and in females at the 12.5% concentration. Increased absolute kidney weights (males) and relative kidney weights (females) were found at the 15% dose level. Exudate adherent to skin (4/20) was observed at the 15% dose level. Loss of hair color lateral and ventral to application site observed at all treatment levels. Extensive ulceration and necrosis of the epidermis of the decedent at the 12.5% treatment level. Dose-related ulceration of the epidermis (4/20) with inflammatory exudate (11/20) observed at 15% and 12.5% treatment levels. Epidermal acanthosis, hyperkeratosis and hypergranulosis were prominent at treatment levels of 10% and above. Dose dependent increases in cellularity, edema, and vascular dilatation were also prominent at the 10% treatment level and above. Dose dependent increase in splenic hematopoiesis was also observed.	**2D** no clinical chemistry	L9

Toxicology End-Point

5.2.4 Repeated Dose Toxicity (oral) (continued)

Chemicals	GLP	OECD	Other/ Protocol Details	Species	Result	Reliability	Refs
C_{13-15} AS Na C13: 62%; C15: 37%	No (1976)		21 day oral study. Similar to OECD 407 but 21 day dietary exposure; 3 animals/sex/ group (test) and 6 rats/ sex/group (controls) Dose levels: 0, 0.047, 0.094, 0.188, 0.375, 0.75, 1.5% in diet (51, 97, 199, 384, 784 1566 mg/kg/day Standard diet control.	Rat	NOEL= 0.188% in diet (199 mg/kg/day) LOEL= 0.375% in diet (384 mg/kg/day) (Increased weight, liver cell hypertrophy and elevated serum GPT, GOT and LDH) High dose animals (1566 mg/kg/day) gained less weight and high dose females had a significantly lower terminal body weight. High dose females ate and drank less. Calcium and serum protein decreased in high dose males. Serum GPT, GOT increased at the highest dose (both sexes). LDH increased in both sexes at the two highest doses. AP and createnine decreased in high dose males. Relative liver weights increased in males and females fed the two highest doses. Relative kidney weights and brain weights were higher in high dose females. Absolute spleen weights were reduced in high dose females. Periportal (4/6) and diffuse (2/6) hepatic parenchymal hypertrophy, marked reduction in cytoplasmic (glycogenic) vacuolization and reduced cytoplasmic neural fat content prominent in the highest dose group. Diffuse hepatic parenchymal hypertrophy (1/6) and periportal hypertrophy (3/6) were onserved at the second highest dose. Periportal parenchymal hypertrophy was also identified in 2/6 rats fed the third highest dose.	2D (**NON-standard protocol**) 21-day vs 28-day exposure duration Not GLP. Meets basic scientific principles	L22

Toxicology End-Point

5.2.4 Repeated Dose Toxicity (oral) (continued)

Chemicals	GLP	OECD	Other/ Protocol Details	Species	Result	Reliability	Refs
C_{13-15} AS Na C13: 62%; C15: 37%	No (1977)		Oral study. Similar to OECD 408 13-week (90 day) feeding study in Wistar rats. 10 rats/sex/dose (test groups); 20 rats/sex/dose (controls) Dose levels: 0, 0.07%, 0.14%, 0.28%, 0.56%, 1.13% and 2.25% in diet (0, 64, 134, 253 512, 1007, 2096 mg/kg/day on average. Standard diet control.	Rat	NOEL= 0.14% in diet (134 mg/kg/day) LOEL= 0.28% in diet (253 mg/kg/day) (Increased liver weight, liver cell hypertrophy, elevated serum GPT and AP) 1 female fed 0.28% was sacrificed at week 5 due to vaginal hemorrhage and anemia. Both sexes in the highest dose group and males in the second highest dose group gained less weight. Terminal weights were reduced in high dose females and in males at the 0.28% treatment level and above. Animals in the two highest dose groups ate less. The highest dose group drank less water. drank less water. Serum glucose decreased in high dose females. Serum cholesterol decreases in the two highest dose groups in males and the three highest dose groups in females. Serum triglycerides decreased in males of the two highest dose groups. Serum GOT was elevated in high dose males. Serum GPT, was elevated in high dose females and in males at the two highest doses. Serum cholinesterase was elevated in males of the two highest dose groups. Serum AP was elevated in the three highest dose groups in males (0.56% and higher) and the four highest dose groups in females (0.28% and higher). *(continued next page)*	**2C**	L23

Toxicology End-Point 5.2.4 Repeated Dose Toxicity (oral) (continued)							
Chemicals	GLP	OECD	Other/ Protocol Details	Species	Result	Reliability	Refs
Continued from previous page	*(Continued from previous page)* C_{13-15} AS Na C13: 62%; C15: 37%		13-week (90 day) feeding study in Wistar rats. Similar to OECD 408 10 rats/sex/dose (test groups); 20 rats/sex/dose (controls) Dose levels: 0, 0.07%, 0.14%, 0.28%, 0.56%, 1.13% and 2.25% in diet (64, 134, 253 512, 1007, 2096 mg/kg/day on average. Standard diet control.	Rat	*(continued from previous page)* Absolute and relative liver weights increased in females at the 0.28%-treatment-level and above. Relative liver weights of males were elevated in males at the two highest dose levels. Absolute spleen weights increased in males fed 0.28% and above and in females at the two highest treatment levels. Absolute kidney weights decreased in males at the 0.28% treatment concentration and above. Relative kidney weights increased in high dose females. Relative brain weights increased in males at the two highest doses and in high dose females. Relative weights of the testes increased at the two highest doses. High dose males had virtually no abdominal fat, and three females fed 0.56% or 1.13% were similar in this regard. Changes in color and consistency of intestinal contents were observed in males and females at the three highest treatment levels. Diffuse hepatic hypertrophy (18/20) and periportal hepatic hypertrophy (2/20) observed at the highest dose (2.25% concentration). Diffuse hypertrophy (9/20) and periportal hypertrophy (10/20) observed at the next highest dose (1.13% conc.). Periportal hypertrophy was observed at the 0.56% concentration level (12/20) and the 0.28% concentration level (2 females/20). Nephrocalcinosis was reduced in the kidneys of high dose females. Lymphatic dilation of the intestine occurred at the high dose.	**2C**	L23

Toxicology End-Point

5.2.4 Repeated Dose Toxicity (oral) (continued)

Chemicals	GLP	OECD	Other/ Protocol Details	Species	Result	Reliability	Refs
C_{12-14} AS TEA	Yes		28-day, oral (gavage) 79/831/EC Annex V, Part B.7 10 rats /sex/group Dose levels 70, 250, 750 mg/kg	Rat	NOEL= 70 mg/kg LOEL= 250 mg/kg At 250 mg/kg: inflammation and edema of forestomach. At 750 mg/kg: Increased leukocytes, high dose females. Inflammation, edema, ulceration, acanthosis and papillomatous hyperplasia of the forestomach. Forestomach alterations were reversible.	**1A**	TRS 15 German
C_{16-18} AS Na C_{14}: 3-7% C_{16}: 25-35% C_{18}: 60-70%	Yes		79/831/EC Annex V, Part B 90-day oral, gavage. 10 rats /sex/group Dose levels 100, 300, 900 mg/kg. 33-day post exposure observation	Rat	NOAEL= 100 mg/kg LOAEL= 300 mg/kg Two females died at high dose. Body weight gains declined in a dose-dependent fashion. Leukocyte counts increased at the high dose. Serum GPT was significantly increased and serum GOT slightly increased in a dose dependent fashion. The forestomach, liver, thymus (females only) and spleen were target organs. Dose dependent changes in organ weight-to-body weight ratios were observed for the liver (increased, both sexes), thymus and adrenals (decreased, females) and spleen (increased, males). The high dose caused inflammation, ulceration and proliferative changes of the forestomach (both sexes); slight acanthosis and hyperkeratosis of the forestomach were observed at mid dose. Target organ effects were reversible except for stomach irritation, which was partially reversible.	**1B**	HESA 7 German

Toxicology End-Point

5.2.4 Repeated Dose Toxicity (oral) (continued)

Chemicals	GLP	OECD	Other/ Protocol Details	Species	Result	Reliability	Refs
C_{16-18} AS Na C_{12}: 0.7% C_{14}: 1.3% C_{16}: 65% C_{18}: 30% C_{20}: 3%	No (1976)		Oral study. Similar to OECD 407 but 21 day dietary exposure; 3 animals/sex/ group (test) and 6 rats/ sex/group (controls) Dose levels: 0, 0.047, 0.094, 0.188, 0.375, 0.75, 1.5% in diet (0, 50, 103, 202, 417, 796 1660 mg/kg/day Standard diet control.	Rat	NOEL= 0.188% in diet (202 mg/kg/day) LOEL= 0.375% in diet (417 mg/kg/day) (Increased weight, liver cell hypertrophy and elevated serum GPT; increased bulk of ileal contents and food utilization impaired) High dose animals gained less weight. Male rats in high dose group had impaired food utilization. High dose females ate less). Males of the highest dose group and females of the two highest dose groups drank less water. Serum Ca reduced in males fed 1.5% and 0.75%. Serum Mg, protein reduced in high dose males. Serum GPT elevated at the high dose (both sexes). Serum AP reduced in males at the two highest treatment levels. Absolute heart weights reduced at the high dose (both sexes). Relative liver weights reduced at the high dose (both sexes). Absolute kidney weights reduced in males at the two highest dose levels. Absolute spleen weights reduced in high dose males. Absolute adrenal weights reduced in high dose males and relative adrenal weights reduced in females at the two highest dose groups. Relative testicular weight increased at the high dose. At necropsy, high dose males were notable for their small size and reduced body fat deposits. Their Ilial contents were bulky and mucoid. Periportal/centrilobular/diffuse hypertrophy, a marked reduction of hepatic cytoplasmic (glycogenic) vacuolation and neutral fat content (more marked in females) were prominent at the high dose. Diffuse parenchymal hypertrophy (1/6) and zonal hypertrophy (5/6) observed at the 0.375% dietary concentration. Zonal hypertrophy was periportal in females and centrilobular in males. Renal lesions typical of control Wistar rats were reduced at the high dose. Splenic hematopoetic activity was reduced at the high dose. No ovarian activity or endometrial stimulation was evident in 1/10 and minimal endometrial hyperplasia was evident in 2/10 females at the high dose.	2D (**NON-standard protocol**) 21-day vs 28-day exposure duration Not GLP. Meets basic scientific principles	L31

Toxicology End-Point

5.2.4 Repeated Dose Toxicity (oral) (continued)

Chemicals	GLP	OECD	Other/ Protocol Details	Species	Result	Reliability	Refs
C_{16-18} AS Na C_{12}: 0.7% C_{14}: 1.3% C_{16}: 65% C_{18}: 30% C_{20}: 3%	No (1977)		Oral study. Similar to OECD 408 13-week (90 day) feeding study in Wistar rats. 10 rats/sex/dose (test groups); 20 rats/sex/dose (controls) Dose levels: 0, 0.07%, 0.14%, 0.28%, 0.56%, 1.13% and 2.25% in diet (61, 123, 230, 482, 970, 2067 mg/kg/day on average. Standard diet control.	Rat	NOEL= 0.07% in diet (61 mg/kg/day) LOEL= 0.14% in diet (123 mg/kg/day) (Increased liver weight , hepatic cell hypertrophy, elevated serum GPT) 1 male rat fed 1.13% in diet died week 2 due to congenital abnormality. Body weight gains and terminal body weights decreased in two highest dose groups (males) and three highest dose groups (females). Food intake reduced in high dose males and females fed 0.28% and above. Food utilization reduced in males (high dose) and females (two highest doses). Water intake lower at two highest doses. Serum Ca decreased in high dose females and males of two highest dose groups. Serum urea increased in the high dose groups. Serum protein and cholesterol decreased in high dose females. Serum protein, triglycerides decreased in males at two highest doses. Serum cholinesterase increased in males at two highest doses. Serum GOT increased at three highest doses (0.56% and above) (both sexes) AP increased at 0.56% and above (females) and 0.14% and above (males). Packed cell volumes and Hb reduced in males fed (0.56% and above). Absolute heart weights decreased at 1.13% and above. Relative liver weights increased in males fed 0.56% and above, females fed 0.28% and above. *(continued next page…)*	**2C** **Not GLP**	L32

Toxicology End-Point

5.2.4 Repeated Dose Toxicity (oral) (continued)

Chemicals	GLP	OECD	Other/ Protocol Details	Species	Result	Reliability	Refs
C_{16-18} AS Na C_{12}: 0.7% C_{14}: 1.3% C_{16}: 65% C_{18}: 30% C_{20}: 3% ***Continued from previous page***			Oral study. Similar to OECD 408 13-week (90 day) feeding study in Wistar rats. 10 rats/sex/dose (test groups); 20 rats/sex/dose (controls) Dose levels: 0, 0.07%, 0.14%, 0.28%, 0.56%, 1.13% and 2.25% in diet (61, 123, 230, 482, 970, 2067 mg/kg/day on average. Standard diet control.	Rat	*(…continued from previous page)* Absolute and relative kidney weights increased in males and females at highest dose. Relative brain weights increased in males fed 1.13% and higher, females fed 0.56% and higher. Absolute adrenal and pituitary weights decreased in high dose males. Relative testicular weights increased in males fed 1.13% and 2.25%. High dose rats (both sexes) lacked significant abdominal fat. Changes in color and consistency of intestinal contents seen in three highest dose groups. Zonal/diffuse hypertrophy, reductions in cytoplasmic (glycogenic) vacuolation, basophilia, cytoplasmic neutal fat and hemosiderin of parenchymal and Kupfer cells were prominent at the 2.25% and 1.13% dietary levels. . At 2.25% level;, diffuse hypertrophy was accompanied by intense cenrilobular acidophilia, Zonal parenchymal hyprtrophy present at 0.28% and above (periporal in females, centrilobular and pewriportal in males. Nephrocalcinosis incidence and severity (frequently seen in untreated female Wistar rats) reduced in high dose females. Lymphatic dilatation more prominent in small intestine of high dose groups.	**2C**	L32

TOXICOLOGY END POINTS
Data Availability and Quality

Chemical Class	Alkyl sulphate (AS) neutralized with a metal cation or an amine derivative.
Health Effect End Point:	**5.2.5 Genetic Toxicity - *in vitro***
Conclusion: (data availability and quality)	**This end-point has been adequately characterized.** See attached tables for summary of supporting studies.
Rationale:	A substantial database of reliable studies (Klimisch reliability category: 1A) exists on the *in vitro* genetic toxicity of alkyl sulphates. Results in the Ames reverse mutation assay were consistently negative with or without metabolic activation. No evidence of mutagenic activity was observed, irrespective of carbon chain length, unsaturation, or the nature of the countervalent moiety that neutralized the anionic surfactant. The lack of mutagenic activity for this chemical class is predictable based on structural and mechanistic considerations. Mutagens are chemicals that either 1) contain highly reactive electrophilic centers capable of interacting with nucleophilic sites on DNA (direct acting agents) or 2) can be metabolized to highly reactive electrophiles. The chemical structures represented by this chemical class do not contain electrophilic functional groups or functional groups capable of being metabolized to electrophiles. Alkyl sulphates with fully saturated carbon chains are not metabolized to reactive electrophiles. The consistent lack of mutagenic activity with AS is consistent with these mechanistic predictions.
Summary of Test Results	The alkyl sulphates showed no evidence of mutagenicity in standard *in vitro* bacterial reversion mutation assays (Ames test).
Additional Data Needs	None. The database is extensive and the conclusions are corroborated in published scientific reviews.

Toxicology End-Point **5.2.5 Genetic Toxicity - *in vitro***							
Chemicals	GLP	OECD	Other/ Protocol details	Species	Result	Reliability	Refs
C_{12} AS- Na	Yes	471	Ames Reverse Mutation Assay	S. typhimurium	Negative. No mutagenic activity with or without metabolic activation	**1A**	TRS 18
C_{12-15} AS Na *version HCB* C_{12}: 17% C_{13}: 27.8% C_{14}: 29.5% C_{15}: 19.9% C_{12-15} AS Na *version LCU* C_{12}:17.8%; C_{13}: 27.8%; C_{14}: 29.5%; C_{15}:19.9%	No (1975)		Ames et al. PNAS 70: 2281-2285((1973): In presence of post mitochondrial supernatant with or without MFO cofactors.	S. typhimurium (TA 1535 and 1538 only)	Negative (TA 1535). Equivocal in the presence of activation cofactors (TA 1538): no dose response.	**3C**	L10
C_{8-16} AS - Na C_8: 13% C_{10}: 13% C_{12}: 60-66% C_{14}: 21-25% C_{16}:10-12%	Yes	471	Ames Reverse Mutation Assay	S. typhimurium	Negative. No mutagenic activity with or without metabolic activation	**1A**	HESA 10
C_{12-14} AS - Na	Yes	471	Ames Reverse Mutation Assay	S. typhimurium	Negative. No mutagenic activity with or without metabolic activation	**1A**	TRS 20

Toxicology End-Point **5.2.5 Genetic Toxicity - *in vitro* (continued)**							
Chemicals	GLP	OECD	Other/ Protocol details	Species	Result	Reliability	Refs
C_{12-16} AS - Na C_{12}: 60-65% C_{14}: 21-25% C_{16}:10-12%	Yes	471	Ames Reverse Mutation Assay	S. typhimurium	Negative. No mutagenic activity with or without metabolic activation	**1A**	HESA 13
C_{13-15} AS Na C_{13}: 62% C_{15}: 37%	No (1977)		Ames assay (TA 1535, TA 1538) w/w-out MFO cofactors	S. typhimurium	Negative.	4 (insufficent details given)	L24
C_{15-16} AS Na $C_{15:}$ 51% $C_{16:}$ 49%	No (1977)		Ames assay (TA 1535, TA 100 TA1538; TA 98)	S. typhimurium	Negative. No mutagenic activity in the presence of Aroclor induced S9 with or without metabolic cofactors.	2 2D (no data in strain TA 1537; no info on controls)	L27
C_{12-18} AS - NH_4	Yes	471	Ames Reverse Mutation Assay	S. typhimurium	Negative. No mutagenic activity with or without metabolic activation	**1A**	HESA 14
C_{8-14} AS TEA C_8: 8.5% C_{10}: 8.5% C_{12}: 68% C_{14}:25% C_{16}:6%	Yes	471	Ames Reverse Mutation Assay	S. typhimurium	Negative. No mutagenic activity with or without metabolic activation	**1A**	HESA 9
C_{12-14} AS TEA	Yes	471	Ames Reverse Mutation Assay	S. typhimurium	Negative. No mutagenic activity with or without metabolic activation	**1A**	TRS 19

Toxicology End-Point **5.2.5 Genetic Toxicity - *in vitro* (continued)**							
Chemicals	GLP	OECD	Other/ Protocol details	Species	Result	Reliability	Refs
C_{12-14} AS MEA C_{12}: ca 70% C_{14}: ca 30% See also C_{12-14} AS Na (above)	Yes	471	Ames Reverse Mutation Assay	S. typhimurium	Negative. No mutagenic activity with or without metabolic activation	**1A**	HESA 12
C_{16-18} As Na C_{14}: 3-7% C_{16}: 25-35% C_{18}: 60-67%	Yes	471	Ames Reverse Mutation Assay	S. typhimurium	Negative. No mutagenic activity with or without metabolic activation	**1A**	HESA 16
C_{14-16} AS Na and C_{18} unsaturated AS Na	Yes	471	Ames Reverse Mutation Assay	S. typhimurium	Negative. No mutagenic activity with or without metabolic activation	**1A**	HESA 15
C_8 AS- Na	Yes	471	Ames Reverse Mutation Assay	S. typhimurium	Negative. No mutagenic activity with or without metabolic activation	**1A**	HESA 8
C_{10-12} AS- Na C_{10}: 94-100% C_{12}: 0-4%	Yes	471	Ames Reverse Mutation Assay	S. typhimurium	Negative. No mutagenic activity with or without metabolic activation	**1A**	HESA 11

TOXICOLOGY END POINTS
Data Availability and Quality

Chemical Class	Alkyl sulphate (AS) neutralized with a metal cation or an amine derivative.
Health Effect End Point:	**5.2.5 Genetic Toxicity - *in vivo***
Conclusion: (data availability and quality)	**This end-point has adequately characterized based on the available studies and structure-activity considerations.** See attached tables for summary of supporting studies.
Rationale:	Several alkyl sulphates have been evaluated in *in vivo* rodent studies for chromosomal aberrations. The most reliable studies (Klimisch reliability categories 1 and 2) gave negative results. Notably, some negative studies involved dietary administration for 90 days, at the maximum tolerated dose. (Guideline protocols involve single, acute doses). Although these repeated dose studies lacked in life, clinical observations, the extended duration of exposure provides strong reassurance for the absence of significant clastogenic effects. A small number of less reliable studies showed equivocal results at high doses; however, these studies do not meet today's scientific standards. In addition to assessments of potential induction of chromosomal aberrations, the database also includes *in vivo* dominant lethal mutation assays on to C_{12-} and C_{12-15} alkyl sulphates, which showed essentially negative results, as expected based on the *in vitro* Ames assay results for the category. Surfactants have the ability to disrupt lipid membranes and to denature macromolecules. If doses of surfactant high enough to be cytotoxic were to be achieved in cellular tissues, generalized disruption of cell membranes and cellular integrity could potentially occur, with denaturation of biological macromolecular structures, (such as proteins or chromatin) as a secondary effect. This type of non-specific effect on chromosome structure would be generic to materials with surfactant properties. Therefore, no unique effects would be anticipated from any of the structural relatives in the category.
Summary of Test Results	Linear alkyl sulphates of various chain lengths showed no evidence of adverse cytogenetic or clastogenic effects in reliable, *in vivo* assays. No unique effects would be expected for any structure in this class.
Additional Data Needs	None. Additional testing would not significantly add to our understanding of the category.

Toxicology End-Point **5.2.5 Genetic Toxicity - *in vivo***							
Chemicals	GLP	OECD	Other/ Protocol details	Species	Result	Reliability	Refs
C_{12} AS - Na	No (1977)		Mammalian bone marrow chromosome aberration test. Dose: 1.13% active in diet (MTD); 0.56% active in diet 6 rats/sex/group Protocol comparable to OECD 475 except that test substance administered in diet for 90 days. Positive controls received control diet for 90 days and 25 mg/kg cyclophosphamide 24 hours before sacrifice. Up to 360 divisions scored per test group.	Rat	No clastogenic effects	**2C** **basic data given, similar to guideline; lacking in life, clinical obser-vations.**	TRS 21
C_{12} AS - Na *99% pure*	No (1976)		Cytogenetic toxicity to rat bone marrow. Similar to OECD 475.Same protocol as study described above. Dose: 0.56% and 1.13% active in diet (MTD).	Rat	No clastogenic effects.	**2C** **basic data given, similar to guideline; lacking in life. clinical obser-vations.**	L38

Toxicology End-Point **5.2.5 Genetic Toxicity - *in vivo***							
Chemicals	GLP	OECD	Other/ Protocol details	Species	Result	Reliability	Refs
C_{12} AS Na	No (1976)		Rodent dominant lethal mutation assay. Similar to OECD 478. Fifteen males per dose in the test and negative control groups; 30 in positive control.	Mouse	Negative. No effects on frequency of pregnancy, number of implantations, or frequency of early deaths at any dose tested.	**2C**	L37
C_{12-15} AS Na C_{12}: 17.8% C_{13}: 27.8% C_{14}: 29.5% C_{15}: 19.9%	No (1977)		Mammalian *in vivo* chromosome aberration test. (single dose, oral, gavage). Similar to OECD 475. 8 hamsters/sex/dose group. Doses: 0 (saline) 1.25 and 2.5 g/kg active. (equiv. To 0.4 and 0.8 times the LD_{50}). Approx. 800 divisions scored/sex/dose	Hamster	Negative at low dose group (both sexes), high dose males. Marginal but statistically significant increase in chromatid gaps in high dose females.	**2C** **basic data given, similar to guideline; lacking in-life, clinical obser-vations.**	L11

Toxicology End-Point

5.2.5 Genetic Toxicity - *in vivo* (continued)

Chemicals	GLP	OECD	Other/ Protocol details	Species	Result	Reliability	Refs
C_{12-15} AS Na *version LCU: C_{12}: 17%* *C_{13}:30.4%* *C_{14}: 30.8%* *C_{15}: 17.7%* Version HCB *C_{12}: 17.8%* *C_{13}:27.8%* *C_{14}: 29.5%* *C_{15}: 19.9%*			Cytogenetic toxicity to rat bone marrow. Dose: 1.13% active in diet (MTD). 6 rats/sex/group. Protocol similar to OECD 475 except 90-day dietary administration of test substance. Positive controls received control diet for 90 days and 25 mg/kg cyclophosphamide 24 hours before sacrifice. Up to 360 divisions scored per sex per group.	Rat	Negative. No evidence of clastogenic effects.	**2C** **basic data given, similar to guideline; lacking in-life, clinical obser-vations.**	L13
C_{12-15} AS Na *version LCU: C_{12}: 17%* *C_{13}:30.4%* *C_{14}: 30.8%* *C_{15}: 17.7%* *Version HCB* *C_{12}: 17.8%* *C_{13}:27.8%* *C_{14}: 29.5%* *C_{15}: 19.9%*	No (1976)		Rodent dominant lethal mutation assay. Similar to OECD 478 but 15 males/ dose (test, negative control groups) 30 in positive control. Doses: 210, 980, 3050 mg/kg (unbleached): 300, 960, 3010 mg/kg (bleached)	Mouse	*LCU:* Negative. No effect on live implants, early or late embryonic death. *LCB:* Negative/equivocal. Decreased pregnancy frequency and increase in early embryonic deaths in females mated in week 4. (of 8 weeks total); the dose response relationship of the finding for animals mated at this time point was equivocal.	**2C**	L12

Toxicology End-Point **5.2.5 Genetic Toxicity - *in vivo***							
Chemicals	GLP	OECD	Other/ Protocol details	Species	Result	Reliability	Refs
C_{12-14} AS - TEA	Yes	474	Mammalian erythrocyte micronucleus test Dose: 400, 2000, 4000 mg/kg, oral, gavage.	Mouse	Negative. No statistically significant effects on micronuclei or PCE/NCE ratio at highest dose at any time point. Lower doses not examined.	**1A**	TRS 22
C_{16-18} AS - Na C14: 3-4% C16: 25-35% C18: 60-70%	Yes	474	Mammalian erythrocyte micronucleus test Dose: 400, 2000, 4000 mg/kg, oral, gavage.	Mouse	Negative. No statistically significant effects on micronuclei or PCE/NCE ratio at highest dose at any time point. Lower doses not examined. No death, decreases activity, diarrhea in first 24 hours post-dosing.	**1A**	HESA 19
C_8 AS- Na	No		Cytogenetic toxicity to mouse bone marrow cells. Non guideline study. Single dose: 1 ml, 1% soln. per 100 g body wt. Group size unspecified.	Mouse	Equivocal. Nominal increase in % chromosomal abnormalities 1-4 weeks post treatment. Protocol does not meet today's standards. No dose response, no positive controls, no assessment of systemic toxicity, no statistical analysis	**3C**	HESA 18

TOXICOLOGY END POINTS
Data Availability and Quality

Chemical Class	Alkyl sulphate (AS) neutralized with a metal cation.
Health Effect End Point:	**5.2.6 Carcinogenicity**
Rationale:	Brief summaries of two, lifetime feeding studies with C_{12-15} AS Na were reviewed for this report. The test materials used in the individual studies were prepared by two different production methods (high conversion bleached or HCB; and low conversion, unbleached or LCU). They differed slightly in chain length distribution, the latter having a slightly higher proportion of the C_{15} AS. In both studies, the test material was dosed at 0, 0.015, 0.15 and 1.5% in the diet. There was no increase in tumor incidence, nor any impact on tumor type in either study. For both studies, approximately 70% of animals survived to study termination. Mortality was similar across dosage groups and controls. Animals in the 1.5% dose groups in both studies exhibited reduced food and water consumption, and slower growth rates. Within these high dose groups, there was a decreased number of total tumors and tumor-bearing animals. Other pathological findings were similar to those in the repeated dose feeding studies summarized in Section 5.3.4. Increased absolute liver weights and liver to body weight ratios, hypertrophy of the hepatic parenchyma, increased relative testicular weights, reduced incidence and severity of chronic nephropathy and nephrocalcinosis, and reduced arterial medial hypertrophy were among the findings at the higher dose levels. Both of these 2-year studies were conducted in the mid-1970s, and importants study details are not available. Therefore, both studies are assigned a reliability rating of 4 (not assignable). However, the data are consistent with other carcinogenicity studies have been mentioned in a previous review of surfactants (A.D. Little, 1991). A 1-year oral feeding study in rats with C_{12}AS dosed at levels of 0.25, 0.5 and 1.0% was negative for tumorigenesis. In addition, no increase in tumors was found in a 2-year skin painting study. Other studies mentioned in this review were flawed by either inadequate numbers of animals or confounding factors. Alkyl sulphates show a consistent absence of mutagenic activity when tested in *in vitro* tests. Neither AS nor its metabolites possess electrophilic functional groups or functional groups associated with mutagenic activity, as discussed in the previous section of this report (Section 5.3.7). In addition, AS is consistently negative in the reliable studies performed to evaluate chromosomal effects (mammalian bone marrow chromosome aberration tests and mammalian erythrocyte micronucleus tests). These points provide further

	support the conclusion that alkyl sulphates are not mutagenic. Therefore, no additional studies are recommended.
Summary of Test Results	The material was not tumorigenic at the any dose level, including the highest dose of 1.5%.
Additional Data Needs	None recommended.

Toxicology End-Point **5.2.6 Carcinogenicity**							
Chemicals	GLP	OECD	Other/ Protocol Details	Species	Result	Reliability	Refs
C_{12-15} AS Na C_{12}: 17.8%; C_{13}: 27.8%; C_{14}:29.5%; C_{15}: 19.9%	No.		2-year feeding study. Dose levels: 0.015, 0.15, and 1.5% in diet Control: standard diet No data for dose on a body weight basis 45 rats/sex/dose Tumor incidence (Peto statistical method)	Rat	NOEL [Neoplasia] Not determined (greater than the high dose of 1.5%) NOEL [Toxicity] 0.15% LOEL [Toxicity] 1.5% (changes in liver weight and morphology) Survival was unaffected by treatment (68% - 248/360 treated rats survived to termination). Neoplasia was most common cause of death but was not enhanced by treatment. Growth retarded at high dose (both sexes). Number of tumors and number of tumor-bearing rats decreased at high dose, probably because of reduced caloric intake. Elevated serum GPT, LDH and AP in high dose males. Increased absolute and relative liver weights at high dose. Apparent, dose-related increase in parenchymal hypertrophy (0% - 5 rats; 0.015% - 6 rats, 0.15% - 8 rats; 1.5% - 67 rats); increased parenchymal lipid granulomata and focal parenchymal necrosis (males only). High dose group had reduced incidence and severity of 1) arterial medial hypertrophy and myocardial fibrosis, 2) chronic nephropathy and nephrocalcinosis and 3) focal/multifocal testicular arteritis.	**4** Not assignable only brief summary available.	L14

Toxicology End-Point

5.2.6 Carcinogenicity (continued)

Chemicals	GLP	OECD	Other/ Protocol Details	Species	Result	Reliability	Refs
C_{12-15} AS Na C_{12}: 17.0%; C_{13}: 30.4%; C_{14}: 30.8%; C_{15}: 17.7%	No (1976)		2-year feeding study. Dose levels: 0, 015, 0.15, and 1.5% in diet Control: standard diet No data for dose on a body weight basis 45 rats/sex/dose Tumor incidence (Peto statistical method)	Rat	NOEL [Neoplasia] Not determined (greater than the high dose of 1.5%) NOEL [Toxicity] 0.15% LOEL [Toxicity] 1.5% (changes in liver weight and morphology) These was no significant increase in any tumor type in any treatment group. Males in the high dose group showed a slight increase in overall pancreatic tumors, but this was not statiscally significant, when specific tumor types (exocrine and islet cell tumors) were considered separately. Survival unaffected (68% - 244/360 treated rats survived to termination). Survival highest in at high dose and lowest at low dose. Neoplasia most common cause of death, but was not enhanced by treatment. Growth retarded at high dose (particularly males). Food consumption (particularly males) and water consumption (both sexes) reduced at high dose. Reduced WBC count in high dose females. Elevated serum GPT, LDH and AP in high dose males. Reduced serum LDH a in high dose females. Increased absolute and relative liver weights at high dose (both sexes). Increased incidence and/or severity of parenchymal hypertrophy, pigmented lipid granulomata and focal parenchymal necrosis (males only). High dose group had reduced incidence and severity of 1) arterial medial hypertrophy 2) chronic nephropathy and nephrocalcinosis. Splenic erythropoiesis, myelopoiesis and stem cell hyperplasia reduced in high dose females. Red pulp hemosiderin more pronounced in same group. Total number of tumors and tumor-bearing rats decreased in high dose females, due to reduction in liver and lymphoreticular tumors.	**4** Not assignable only brief summary available.	Unilever study no. RG 954

Toxicology End-Point

5.2.6 Carcinogenicity (continued)

Chemicals	GLP	OECD	Other/ Protocol Details	Species	Result	Reliability	Refs
C_{12-15} AS Na Version HCB C12:17.0%; C13: 30.4%; C14: 30.8%; C15:17.7% C_{12-15} AS Na: Version LCU C12:17.8%; C13: 27.8%; C14: 29.5%; C15:19.9%	No (1976)		2-year skin painting study. Dose levels: 0,5% and 10% Active ingredient Control: water vehicle 50 mice/sex/dose Twice weekly treatment Tumor incidence (Peto statistical method); analysis of variance (non-neoplastic lesions)	mouse	To be completed	**To be determined**	L16

TOXICOLOGY END POINTS
Data Availability and Quality

Chemical Class	Alkyl sulphate (AS) neutralized with a metal cation or an amine derivative.
Health Effect End Point:	**5.2.7 Toxicity to Reproduction**
Conclusion: (data availability and quality)	**This end-point is adequately characterized for the category, based on structure-activity considerations.** See attached tables for summary of the supporting reproductive study on AOS.
Rationale:	The reproductive toxicity profile of category surfactants is substantiated by a reliable, two-generation, dietary study on a 1:1:1 mixture of C_{14}-, C_{16}-, and C_{18} AOS (magnesium salts) in rats, which showed no treatment-related adverse reproductive effects and no adverse histopathological effects on systemic organs. Further corroborating evidence for reproductive toxicity profile of the category surfactants derives from the absence of adverse histopathological effects on reproductive organs in 21-day and 90-day repeated dose studies on C_{12}-, C_{12-15}, C_{13-15} and C_{16-18} AS (sodium salts). No reproductive organ toxicity was observed in the latter studies, even at dietary concentrations that exceeded those administered in the 2-generation reproductive toxicity study on AOS. Representative AS and AOS surfactants show minimal systemic effects when administered to rodents by the dietary route at roughly equivalent concentrations (up to 5000 ppm). Given the similarities in metabolism and toxicokinetics of alkyl sulphates, alkyl sulfonates and alkyl olefin sulfonates, and their comparable toxicity profiles, no significant qualitative differences in reproductive toxicity are expected among category surfactants.
Summary of Test Results	No evidence of reproductive effects was observed in the available 2-generation reproductive/developmental study on a 1:1:1 mixture of C_{14}-, C_{16}-, and C_{18} AOS (magnesium salts).
Additional Data Needs	None. Based on structure activity considerations, this endpoint is adequately characterized. Additional testing would not add significantly to our understanding of the category.

Toxicology End-Point

5.2.7 Toxicity to Reproduction

Chemicals	GLP	OECD	Other	Species	Result	Reliability	Refs
C_{14} AOS Mg C_{16} AOS Mg C_{18} AOS Mg Chain length ratio: 1:1:1	Not stated (1980)		Comparable to OECD 416. 2 generation, reproductive dietary study in rats Dose groups: 0, 1250, 2500 and 5000 ppm 12 males and 24 females/sex/dose group, to achieve approx 20 pregnant animals following mating. 13 week pre-mating exposure in F_0 F_1 and F_2 generations. Two successive litters per generation (A, B). Animals form F_{1B} litters mated to form F_1 generation; Animals from F_{2B} litters selected to form F_2 generation. Macroscopic organ evaluation on parental F_0 and F_1 animals and offspring not selected for F_1 and F_2 generations. After 91 days of treatment, full macroscopic and histopathological evaluation on F_2 generation.	Rat	NOEL= 5000 ppm (general toxicity) NOEL= 5000 ppm (parental reproductive toxicity) NOEL= 5000 ppm (reproductive toxicity, F_1 generation) Equivalent to 703 -285 mg/kg/day (F_0) and 785-300 mg/kg/day (F_1) Marginally reduced viability in F_{1A} generation on lactation day 25 in 2500 and 5000 ppm groups. No effects on F_{1B}. Slightly reduced litter size for F_{2A} generation in 5000 ppm group on lactation day 1. No effects on F_{2B} No adverse macroscopic or microscopic organ effects related to treatment in any generation.	**2B**	TRS 23

TOXICOLOGY END POINTS
Data Availability and Quality

Chemical Class	Alkyl sulphate (AS) neutralized with a metal cation or an amine derivative.
Health Effect End Point:	**5.2.7 Developmental Toxicity/Teratogenicity**
Conclusion: (data availability and quality)	**This end-point adequately characterized for the category, based on structure-activity considerations.** See attached tables for summary of supporting data.
Rationale:	There are 2 reliable, published studies on the developmental toxicity of AS and AOS. The studies were perfomed with rats, mice and rabbits and employed the oral (gavage) route of exposure. The AOS was identified as a mixture of 60.4%:39.5% alkenyl sulfonate and hydroxyalkane sulfonate prepared from C_{14-18} α olefin. The AS structure and chain length was not identified, but is likely to be C_{12} AS-Na, a commonly used surrogate for the category. Both materials were consistent in showing maternal toxicity stemming GI tract irritation, and effects on litter size, litter loss and minor skeletal malformation only at doses which caused frank maternal toxicity. No significant developmental effects occrred at lower doses. Mice and rabbits were more sensitive than rats to the effects of either test material. Studies of limited reliability, conducted in rats, are available on C_{12} As Na, C_{12-15} AS-Na and C_{15-16} AS-Na. These studies were of limited reliability for a variety of reasons. However, in general, the types of effects reported, and exposures at which they were observed, were consistent with results from the two reliable studies described above.
Summary of Test Results	See description above. NOELs for teratogenic effects were 500-600 mg/kg in the rat.
Additional Data Needs	None. The two published studies on AS and AOS showed strikingly similar results and are adequate to characterize this endpoint for the category, based on structure-activity considerations. Less reliable studies, although not adequate on their own, were consistent with published studies in the types of effects observed at comparable doses. Structure-activity considerations would predict no substantial differences in toxicity among category surfactants.

Toxicology End-Point

5.2.7 Developmental Toxicity/Teratogenicity

Chemicals	GLP	OECD	Other	Species	Result	Reliability	Refs
C_{12} AS Na	No (1974)		Developmental toxicity study comparable to OECD 414. Oral (gavage) administration of test materials to pregnant animals 7 days/week on days 6-15 of gestation (rats and mice) and 6-18 of gestation (rabbits). Test duration day 0 (mating) - 20 (rats); 0-17 (mice) 0-29 (rabbits) Dose: 0.2, 2, 300 and 600 mg/kg. 20 females/group (rats.mice) and 13 females/group (rabbits) Daily maternal observations. At necropsy, examination of maternal macroscopic organ changes, dissection of uteri, implantations, viable young and embryonic deaths, examination of maternal ovaries and corpora lutea, and external and internal examination of visceral and skeletal abnormalities in the young.	Rat, Mouse, Rabbit	NOEL (maternal toxicity): 2 mg/kg (rat, mouse rabbit) In all species, toxicity of GI tract was principal maternal finding, including diarrhea, anorexia, weight loss and cachexia prior to morbidity or death. Marked toxicity occurred at 600 mg/kg in all mice and rabbits, slight to moderate (retardeed weight gain) at 300 and 600 mg/kg in rats, and 300 mg/kg in mice and rabbits. NOEL (pregnancy/litter effects) 2 mg/kg (rat, mouse rabbit) At maternally toxic doses there was increased fetal loss and reduced litter size in rabbits and mice due almost entirely to total litter loss, secondary to primary effects on the mother. When dams showing total litter loss were excluded from calculation, litter parameters were not different from controls. At nontoxic or slightly toxic doses, litter size and fetal loss were unaffected. NOEL (fetal data) Rats: 600 mg/kg Mice and rabbits: 300 mg/kg The incidence of major malformations or minor visceral and skeletal anomalies was unaffected except for a higher incidence of skeletal anomalies in mice at 600 mg/kg.	**2A**	TRS24

Toxicology End-Point

5.2.7 Developmental Toxicity/Teratogenicity (continued)

Chemicals	GLP	OECD	Other	Species	Result	Reliability	Refs
C_{12-} AS Na	No (1976)		Rat Teratology study (oral, gavage). 15 animals per group (10 dissection, 5 natural parturition) dosed days 6-15 of gestation. Doses: 0 (saline), 63, 125, 250, 500 mg/kg. Positive control: aspirin 250 mg/kgsubcutaneous).	Rat	NOAEL (maternal toxicity): 250 mg/kg LOAEL (maternal toxicity): 500 mg/kg (severe G.I. irritation, diarrhea, decreased food consumption, reduced weight gain). 5/15 rats died at 500 mg/kg; all fetusus from remaining animals examined. NOAEL (teratogenicity): not specified No significant differences in gross or skelatal anomalies. No significant difference in post partum mortalities. No skeleltal defects in rat pups. LOAEL (teratogenicity): not determined 63 mg/kg: 1 fetus with unossified thoracic centrae with branched rib 250 mg/kg: single fetus with asymmetry and reduced ossification of lumbar arch. 500 mg/kg. Mean placental weight reduced. 1 aborted fetus, day 14 or gestation. Malformations in 3 fetuses from separate pregnancies: cleft palate, edema and shortening of pubic bone; unossified metatarsus of hind foot.	**3B** Insufficient numbers of dams. No details on statistical methods	L 39
C_{12-15} AS Na *version LCU* $C_{12:}$ 17.8% C_{13}: 27.8% C_{14}:29.5% C_{15}: 19.9%	No (1976)		Rat Teratology study (oral, gavage). 15 animals per group (10 dissection, 5 natural parturition) dosed days 6-15 of gestation. Doses: 0 (water), 47, 94, 375, 563 mg/kg. Positive control: aspirin 250 mg/kg (subcutaneous)	Rat	NOAEL (maternal toxicity): 375 mg/kg (diarrhea immediately post-dosing) LOAEL (maternal toxicity): 563 mg/kg (severe G.I. irritation, diarrhea, decreased food consumption, reduced weight gain). NOAEL (teratogenicity): 563 mg/kg No differences in intrauterine mortality. No differences in number of pregnancies. No treatment related effects on gross or skeletal fetal abnormalities. LOAEL (fetal effects): Reduced mean fetal body weight at 563 mg/kg. 2 pups from 94 mg/kg group had calcified nodules of the ribs.	**3B** Insufficient numbers of dams. No detail on statistical methods. Room temp. not stablized, rose to over 80ºF	L17

Toxicology End-Point

5.2.7 Developmental Toxicity/Teratogenicity (continued)

Chemicals	GLP	OECD	Other	Species	Result	Reliability	Refs
C_{12-15} AS Na *version HCB* $C_{12:}$ 17.8% C_{13}: 27.8% C_{14}:29.5% C_{15}: 19.9%	No (1976)		Rat Teratology study (oral, gavage). 15 animals per group (10 dissection, 5 natural parturition) dosed days 6-15 of gestation. Doses: 0 (water), 63, 125, 250, 500 mg/kg. Positive control: aspirin 250 mg/kg (subcutaneous)	Rat	NOAEL (maternal toxicity): 250 mg/kg LOAEL (maternal toxicity): 500 mg/kg (diarrhea, decreased food consumption, reduced weight gain first 5 days of treatment). NOAEL (teratogenicity): 500 mg/kg No differences in number of pregnancies or live fetuses. No differences in intrauterine mortality. No effects on fetal or placental size. No effects on gross or skeletal fetal abnormalities. No effects on major or minor anomalies or malformations. No effects on post-partum mortality or skeletal effects in pups.	**2D** Insufficient numbers of dams; other aspects of the study meet basic scientific standards .	L18
C_{13-15} AS	No (1979)		Rat Teratology study (oral, gavage). 15 animals per group (10 dissection, 5 natural parturition) dosed days 6-15 of gestation. Doses: 0 (water), 49, 98, 195, 390 mg/kg. Positive control: aspirin 250 mg/kg (subcutaneous)		NOAEL (maternal toxicity): 195 mg/kg (diarrhea); 390 mg/kg (weight gain) LOAEL(maternal toxicity): 390 mg/kg (diarrhea in 5/15 after 4th dose, lasted 24 hours; lowered food consumption first 5 days of treatment.) NOAEL (teratogenicity): 390mg/kg No differences in number of pregnancies or live fetuses. intrauterine mortality. No effects on fetal or placental size. No effects on gross or skeletal fetal abnormalities. No effects on major or minor anomalies or malformations. No effects on pup viability. No adverse skeletal effects in pups.	**3B** Test material identity not docu-mented Insufficient number of dams Room temp. not stablized, rose to over 80°**F**	L25

Toxicology End-Point

5.2.7 Developmental Toxicity/Teratogenicity (continued)

Chemicals	GLP	OECD	Other	Species	Result	Reliability	Refs
C_{15-16} AS - Na C_{15}:: 51% C_{16}: 49%	No (1979)		Rat Teratology study (oral, gavage). 15 animals per group (10 dissection, 5 natural parturition) dosed days 6-15 of gestation. Doses: 0 (water), 125, 250, 500, 750, 1000 mg/kg/dy. Positive control: ethyl cellosolve 180 uL/kg/dy (subcutaneous)		NOAEL (maternal toxicity): 500 mg/kg LOAEL: 750 mg/kg (diarrhea in 4/15, rats; lowered food consumption first 6 days of treatment.; reduced body weight and body weight gain) 1000 mg/kg induced severe diarrhea in 13/15 rats within 48 hours. 4 rats died and one humanely sacrificed. Food consumption, body weights and weight gains significantly reduced. NOAEL (developmental effects):1000 mg/kg [report author] At doses of 250 mg/kg and higher, increase d incidence of fetuses with unossified cervical centra and forepaw phalanges. At 1000 mg/kg, Increase in intrauterine deaths; Decrease in post-implantation losses. No effect on number of live fetuses per pregnancy, Significant increase in skeletal abnormalities. Effects not considered biological significant [report author]	**3B** Insufficient numbers of dams	L28
C_{16-18} AS Na	No (1978)		Rat Teratology study (oral, gavage). 15 animals per group (10 dissection, 5 natural parturition) dosed days 6-15 of gestation. Doses: 0 (water), 112, 225, 450, 675, mg/kg/dy. No data on positive control	Rat	NOAEL (maternal toxicity): 225 mg/kg LOAEL: 450 mg/kg (reduced body weight gain days 10-15 of gestation) 675 mg/kg induced diarrhea in 4/15 rats after 3rd dose (day 9 of gestation). NOAEL (developmental effects): 675 mg/kg [study author]. LOAEL (developmental effects):Undetermined. No clear, dose dependent effects. Reduced mean placental weight for male fetuses in 450-mg/kg group. Intracapsular kidney hemorrhage observed in some fetuses at three lowest treatment groups only. Significant increase in skeletal abnormalities at the lowest dose only.	**3B** Insufficient numbers of dams	L33

Toxicology End-Point

5.2.7 Developmental Toxicity/Teratogenicity

Chemicals	GLP	OECD	Other	Species	Result	Reliability	Refs
C_{14-16} alkane hydroxy AOS-Na and **C_{14-16} alkene AOS-Na**	No (1974)		Comparable to OECD 414 Developmental toxicity study with oral (gavage) administration of test materials to pregnant animals 7 days/week on days 6-15 of gestation (rats and mice) and 6-18 of gestation (rabbits). Test duration day 0 (mating) - 20 (rats); 0-17 (mice) 0-29 (rabbits) Dose: 0.2, 2, 300 and 600 mg/kg. 20 females/group (rats, mice); 13/group (rabbits) Daily maternal observations. At necropsy, examination of maternal macroscopic organ pathology, dissection of uteri, implantations, viable young and embryonic deaths, examination of maternal ovaries and corpora lutea, and external and internal examination of visceral and skeletal abnormalities in the young.	Rat, Mouse, Rabbit	NOEL (maternal toxicity): 600 mg/kg (rat): < 0.2 mg/kg (mouse) < 0.2 mg/kg (rabbit) No treatment related toxicity in rats. At 600 mg/kg all rabbits and six of 20 mice died, showing reduced activity, weight gain and diarrhea before death. At 300 mg/kg one rabbit died and survivors showed weight loss. At the 2 lower doses, and initial retardation in weight gain was observed in both rabbits and mice. NOEL (pregnancy/litter effects) Rats: 600 mg/kg. Mice: 2 mg/kg. High incidence of total litter loss at 300 and 600 mg/kg, probably due to toxicity to the dam. Litter sizes were comparable to controls. Rabbits: 2 mg/kg. At 300 mg/kg, a slightly but not significantly lower mean pup weight was secondary to maternal toxicity. NOEL (fetal data) Rats: 600 mg/kg Mice: 2 mg/kg. Cleft plate in 4 pups of three litters and two pups one litter at 300 mg/kg. Higher incidence of reduced ossification at 600 mg/kg and reduced ossification of occipitals at other doses however, control incidence unusually low. Rabbits: 2 mg/kg. At 300 mg/kg, no major malformations or visceral abnormalities; minor skeletal abnormalities and pups with extra ribs observed.	**2A**	TRS 25