201-16316

Stepan

Stepan Company

Northfield, Illinois 60093

Telephone 847 446 7500

RECEIVED
2006 JUL 18 AM 7:37

July 12, 2006

Honorable Stephen Johnson, Administrator
U.S. Environmental Protection Agency
P.O.Box 1473
Merrifield, VA 22 116

Attention: Chemical Right-to-Know

Subject: High Production Volume Chemical (HPVC) Program

Dear Administrator Johnson:

Stepan Company is pleased to submit the Test Plan document along with the robust summaries for the chemical, Sodium 2-(2-dodecyloxyethoxy) ethyl sulfate, CAS# 3088-3 1- 1 under the US EPA HPVC Program.

Lela Jovanovich, Ph.D. is our contact for the HPVC Program and can be reached at (847) 50 l-2272 or ljovanovich@stepan.com.

This submission is being sent electronically to the following e-mail addresses:

Oppt.ncic@epa.gov
Chem.rtk@ epa.gov
Sheridan.diane@epa.gov
Townsend.mark@epa.gov

Sincerely,

Blaine Byers, Ph.D.
Sr. Manager, Product Safety and Compliance

201-16316

RECEIVED
OPPT CBIC

2006 JUL 18 AM 7:37

SODIUM 2-(2-DODECYLOXYETHOXY)ETHYL SULPHATE (SODIUM LAURETH SULFATE; CAS NO. 3088-3 l-l): TEST PLAN

Submitted to the US Environmental Protection Agency

By

Stepan Company

DATE: May 2006

SUMMARY

Stepan Company (Stepan) has sponsored Sodium Laureth Sulfate (CAS No. 3088-3 1- 1) under the EPA's High Production Volume (HPV) Program. This document provides the Test Plan and summaries of existing data in support of this substance.

1.0 INTRODUCTION

Stepan has voluntarily committed to participate in the Environmental Protection Agency's (EPA) high production volume chemicals (HPV) challenge program, to assess the health and environmental hazards, including selected physical chemical characteristics of sodium laureth sulfate (CAS No. 3088-3 l-3). Sodium laureth sulfate is an alcohol ethoxysulfate (AES).

An evaluation of the available data and proposed test plan are included in this document. As part of this evaluation, data from structurally analogous substances (other AES) were used to fulfill some endpoints for the sponsored substance (SDA, 1991; HERA, 2003). The HERA has defined the AES family *"to encompass commercial grades of linear-type primary alcohol ethoxysulphates containing AES components of basic structure* $C_nH_{2n+}O(C_2H_4O)_mSO_3X)$ *where n=IO-18 and m = 0-8 and X = sodium, ammonium or triethanolamine (TEA)"*. Although sodium laureth sulfate was not included in this assessment, it is clearly within the bounds of the HERA definition. In addition to using data from AES, one specific AES (sodium lauryl ether sulfate, SLES, CAS No 9004-82-4) is also used in this assessment. As shown in Figure 1, SLES is a close structural analog of sodium laureth sulfate. Robust summaries for sodium laureth sulfate are provided in Appendix 1. The HERA AES documents are provided in Appendix 2.

The objective of this test plan is to evaluate the available data and determine what additional data, if any, are needed to adequately characterize the physical properties, environmental fate, and human health and environmental effects of sodium laureth sulfate. All endpoint have been fulfilled either with testing completed on the sponsored substance or through the use of testing with structurally analogous AES. No additional testing has been proposed.

Table 1: AVAILABLE DATA FOR SODIUM LAURETH SULFATE

Endpoint	
Physical Chemical Properties	
Melting Point	A (calc)
Vapor Pressure	A (calc)
Boiling Point	A (calc)
Partition Coefficient	A (calc)
Water Solubility	A (calc)
Environmental Fate	
Hydrolysis	A
Photodegradation	A (calc)
Biodegradation	A
Environmental Transport	A (calc)
Ecotoxicity	
Acute/Chronic Fish	R
Acute/Chronic Daphnia	R
Acute/Chronic Algae	R
Mammalian toxicity	
Acute Oral	A
Repeated Dose	R
Genotoxicity (*in vitro* -bacteria)	R
Genotoxicity (*in vivo*)	R
Reproductive/Developmental	R

A= Adequate data
Calc = Modeling data
R = Read across to AES

2.0 USE

Sodium laureth sulfate is used in variety of shampoos, bath products, and hand soaps with its good foaming, viscosity building, and low irritation properties (Stepan, 2006a). SLES is used as a scouring, leveling, coupling and foaming agent for textile applications.

3.0 EVALUATION OF EXISTING DATA AND PROPOSED TESTING

Chemical/Physical Prouerties:

Sodium laureth sulfate is a solid. Estimated physical chemical properties are provided in Table 2. The melting point of sodium laureth sulfate is 286 °C (EpiWin v3.11); this is in good agreement with an expected value of greater than 200 2006b). The boiling point is 659 °C (EpiWin v3.11). A vapor pressure has not been determined but it is expected to be negligible. The estimated vapor pressure is $3.4E^{-15}$ hPa (EpiWin v3.11). The estimated partition coefficient for sodium laureth sulfate is 1.14 (EpiWin v3.11). The estimated water solubility of sodium laureth sulfate is 452 mg/L (EpiWin v3.11), which is in good agreement with the expected value as this substance is very water soluble (Stepan, 2006b).

The physical and chemical properties of analogous AES are summarized in Table 2. The physical chemical properties of sodium laureth sulfate are similar to analogous AES, sodium lauryl ether sulfate (SLES, CAS No 9004-82-4; EpiWin v3.11) and AES(C12) (HERA, 2003).

Table 2: Summary of Physical and Chemical Property Data for Sodium Laureth Sulfate and AES

Compound	Physical Chemical Properties				
	Melting Point (°C)	**Boiling Point (°C)**	**Vapor Pressure (hPa)**	**Partition Coefficient**	**Water Solubility**
Sodium laureth sulfate	4.4 (freezing point) 287 (estimated)	100 659 (estimated)	3.4E- 15 (estimated)	1.14 (estimated)	452 (estimated)
Supporting data					
AES (as SLES)	290 (estimated)	666 (estimated)	2.01 E-15 (estimated)	1.62 (estimated)	187 (estimated)
AES (C12)	298 (estimated)	684 (estimated)	1.2E-13 (estimated)	0.95 (estimated)	425 (estimated)

Recommendation: No additional testing is proposed.

Environmental Fate.

Environmental fate properties of sodium laureth sulfate and AES/SLES or C 12 are presented in Table 3. Sodium laureth sulfate undergoes 10% decomposition at 1 OOC between 30 and 40 days (Roberts et al.). This is to be expected as an increased rate of hydrolysis is proportional to increases in temperature. Therefore, as temperature decreases, the rate of hydrolysis greatly slows. Under normal use and typical environmental conditions (approximately 25C at non-catalyzed conditions), this chemical is expected to be resistant to hydrolysis.
Sodium laureth sulfate has been shown to be readily biodegradable (65% after 28 days; AnaylCen, 2003). Photodegradation and fugacity modeling has been conducted for sodium laureth sulfate (EpiWin v3.11). The photodegradation half-life is 0.2 days. Level III fugacity modeling indicates sodium laureth sulfate will partition primarily to soil and water (Air = 0.355%; Water = 49.4%; Soil = 50.1% and Sediment = 0.103%).

AES have been shown to be readily biodegradable, regardless of chain length (HERA, 2003).

Table 3 Summary of Environmental Fate Data for Sodium Laureth Sulfate and AES

Compound	Environ		ental Fate	
	Stability in Water	**Photodegradation**	**Level III Fugacity Model (%)**	**Biodegradation**
Sodium laureth sulfate	Stable	Rate constant: .000000000045 cm³/(molecule*sec) Half-life: .2 days	Air = 0.355 Water = 49.4 Soil = 50.1 Sediment = 0.103	65% after 28 days
SLES	Not applicable	Not applicable	Not applicable	8 1% after 26 d S)
AES (C12)	Not applicable	Not applicable	Not applicable	Readily biodegradable

Recommendation: No additional testing is proposed.

Aquatic Toxicity.

Aquatic toxicity data are not available for sodium laureth sulfate. Extensive acute and chronic toxicity data are available for AES (SDA, 1991; HERA, 2003; Warne and Schifko, 1999). As reported in HERA (2003), there appears to be no difference in sensitivity of fish and invertebrates to AES. Similar effects are expected for the sponsored substance.

Recommendation: No additional testing is proposed.

Table 4 Chronic Aquatic Toxicity Data for Sodium Laureth Sulfate and AES/SLES

Compound	Environmental Effects		
	Fish (mg/L)	**Daphnia (mg/L)**	**Algae (mg/L)**
Sodium laureth sulfate	14-day LC50 = 2836 (estimated): read across to AESISLES	No data: read across to AES/SLES	No data: read across to AESISLES
	Supporting data		
AES (C12)	*Saccobranchus fossilis 60* d *> 2.24*	*C. dubia 7* d NOEC = .34, .88, *1.2,* 2.7, 6.3 *Brachionus calyciflorus* 2 d EC20 = 0.97-1.1, 2.3	*S. capricornutum 96* hr NOEC growth = 12 River water 'community' Chlorophyll a NOEC 3 weeks = 70 (enhancement at *5)*
AES (C12-14)	*Pimephales promelas 96* hr LC50 = 13 (ammonium salt) *Lepomis macrochirus 96* hr LC50 = 24 (ammonium salt) *Cyprinodon variegates 96* hr LC50 = 2.3 (ammonium salt)	No data	*Selenastrum capricornutum* 5 d algistatic = 101, algicidal > 1000
C12-13AE2S	*Salmo gairdneri* 96 hr LC50 = 28	No data	No data
C12AE2.1S	*Lepomis macrochirus 24* hr LC50 = 87	No data	No data
C12AE2S	*Pimephales promelas 48 hr* LC50 = 1.5	No data	No data
C12-14AE2.2S	No data	*Daphnia magna 24* hr EC50 = 21	No data
ammonium C12-14AES	No data	*Daphnia magna 96* hr EC50 = 5.7	No data
SLES	No data	*Ceriodaphnia dubia* 48 hr EC50 = 3.12	No data

Acute Mammalian Toxicity:

Sodium laureth sulfate has a low acute oral toxicity in rats, with an LD50 greater than 5000 mg/kg bw (Hill Top Research, 1982a) (Table 5). The acute oral toxicity of AES is low, with LD50's ranging from 1700 to greater than 5000 mg/kg bw (HERA, 2003). The acute oral toxicity of SLES is also low, with an LD50 of 1600 mg/kg bw (JACT, 1983).

In a semi-occlusive skin irritation study in rabbits, the application of sodium laureth sulfate caused evidence of tissue damage in two of 6 animals (Hill Top Research, 1982b). Atonia, blanching discoloration and spreading of irritative effects was also noted during this study. Application of undiluted sodium laureth sulfate to the right eye of each of 6 rabbits caused evidence of significant corneal, iris and conjunctival changes (Hill Top Research, 1982c).

Recommendation: No additional testing is proposed.

Reueated Dose Toxicity:

Repeated dose toxicity studies have not been conducted with sodium laureth sulfate. Extensive testing has been conducted with AES. One of these studies is summarized here. NaC 12-14AE2S was tested for systemic toxicity at repeated doses by oral gavage of 0, 25, 75, and 225 mg/kg bodyweight (HERA, 2003). The compound was administered by gavage for 90 days. Recovery group animals were included in the 0, 75 and 225 mg/kg dose groups. There were no clinical signs, effects on body weight, food or water consumption or body weight gain. The forestomach of the animals of the treated group showed some lesions. A NOEL was not determined, although these effects are not relevant to human health. Based on systemic toxicity, behavioural and clinical abnormalities and other general or specific toxic effects, a no adverse effect level (NOAEL) of 225 mg/kg was established. Additional studies are described in HERA, 2003 (provided as Appendix 2). Overall, these studies indicate no adverse effects, behavioral or clinical abnormalities of AES were observed up to a dose level of 250 mg/kg body weight per day (HERA, 2003). Similar effects are expected for the sponsored substance.

Recommendation: No additional testing is proposed.

Reproductive/Developmental Toxicity:

Reproductive toxicity studies have not been conducted with sodium laureth sulfate. Studies with AES show no reproductive effects. Specifically, a key study has been identified (HERA, 2003). A two generation reproduction study in rats was conducted with NaC12-14AE2S following OECD guideline 4 16. There were no treatment-related reproductive effects at any dose level on the parents or offspring (NOAEL > 3 300 mg/kg/day). The NOAEL for systemic effects of 0.1 % (86.6 mg/kg bw) for the FO generation and a NOAEL of 0.1 % (149.5 mg/kg bw) for the Fl generation was reported (HERA, 2003).

Studies assessing the teratogenic potential and developmental toxicity of AES have been described (HERA, 2003). It was concluded in HERA (2003) "that there is sufficient evidence that AES is not teratogenic or a developmental toxicant under the conditions described. A NOAEL greater than 1000 mg/kg bw/day can be estimated for teratogenicity and embryotoxicity on the basis of the segment II embryotoxicity study which is judged to be of highest reliability. The NOAEL for developmental toxicity appears to be greater than 750 mg/kg bw/day." Similar effects are expected for the sponsored substance.

Recommendation: No additional testing is proposed.

Mutagenicity Assays:

No genetic toxicity testing is available for sodium laureth sulfate. AES have been tested extensively for bacterial and mammalian genotoxicity; results of this testing are consistently negative. It was concluded in HERA (2003) "that there is no evidence that AES are either mutagenic or genotoxic." Similar effects are expected for the sponsored substance.

Recommendation: No additional testing is proposed.

Table 5 Mammalian Toxicity Data for Sodium Laureth Sulfate and AES

Compound	Mammalian Toxicity				
	Oral LD50 (mgikg)	Repeat Dose Toxicity	Repro. Effects	Develop. Effects	Genetic Toxicity
Sodium laureth sulfate	>5000	No data: read across to AES	No data: read across to AES	No data: read across to AES	No data: read across to AES
Supporting data					
AES	1700 - >5000	Rat, 90 day, diet, NOAEL = 225 mg/kg/bw/d *	Rat, two-generation, drinking water, NOAEL (reproduction parent and offspring) > 3% (> 300 mg/kg/bw); NOAEL (FO, systemic) = 0.1 % (86.6 mg/kg bw); NOAEL (F1, systemic) = 0.1 % (149.5 mg/kg bw)	Rat, Segment II, gavage, NOAEL (teratogenicity and embryotoxicity) > 1000 mg/kg bwlday; NOAEL (developmental) > 750 mg/kg bw/day*	Bacterial: negative Mammalian: negative
SLES	1600				

* = AES (NaC1: 4AE2S)

REFERENCES

AnalyCen ecotox (2003) Biodgradation (OECD 306) Test Results for the Test Substance Agent 2527-68ES-7.

EPIWIN (v3.11)

Hill Top Research, Inc (1982a) Acute Oral Toxicity Screen in Rats of 9 10- 11 for Stepan Chemical Company. Project No. 82-082 1-2 1.

Hill Top Research, Inc (1982b) Skin Irritation Study in Rabbits of 910-12 for Stepan Chemical Company. Project No. 82-0822-21.

Hill Top Research, Inc (1982c) Primary Eye Irritation Study in Rabbits of 91 O-13 for Stepan Chemical Company. Project No. 82-0823-2 1.

HERA (2003) Human & Environmental Risk Assessment on ingredients of European household cleaning products: Alcohol Ethoxysulphates. Human Health Risk Assessment Draft January 2003.

Journal of the American College of Toxicology (JACT) (1983) (Mary Ann Liebert, Inc., 1651 Third Ave., New York, NY 10128) Vol 2(5): 1.

Roberts D.W., R Alvarez, D. Bethel1 and R. Fessy. HYDROLYSIS KINETICS OF PRIMARY ALCOHOL SULPHATES AND PRIMARY ALCOHOL ETHOXY SULPHATES

Unilever Research Port Sunlight SDA (199 1) Environmental and Human Safety of Major Surfactants: Volume 1. Anionic Surfactants Part 2. Alcohol Ethoxy Sulfates

Stepan Company (2006a) Products: STEOL®-270.
http://www.stepan.com/en/products/product_detail.asp?id=520

Stepan Company (2006b) Personal Communication

Warne, M.S.T. and A.D. Schifko (1999) Ecotoxicology and Environmental Safety, 44(2): 196-206

Sodium laureth sulfate, CAS no 3088-31-l:

$CH_3 - (CH_2)_{11} - 0 - [CH_2 - CH_2 - O]_2 - SO_3H$ ·Na

SLES, CAS no 9004-82-4:

$CH_3 - (CH_2)_{11} - 0 - [CH_2 - CH_2 - O]_n - SO_3H$ ·Na, n = moles of EO

FIGURE I: Structures of Sodium Laureth Sulfate and Sodium Lauryl Ether Sulfate

APPENDIX 1
DOSSIER
SODIUM LAURETH SULFATE

APPENDIX 2

HUMAN & ENVIRONMENTAL RISK ASSESSMENT ON INGREDIENTS OF EUROPEAN HOUSEHOLD CLEANING PRODUCTS: ALCOHOL ETHOXYSULPHATES

201-16316

RECEIVED
OPPT CBIC

2006 JUL 18 AM 7:37

IUCLID

Data Set

Existing Chemical	:	ID: 3088-31-1
CAS No.	:	3088-31-1
EINECS Name	:	sodium 2-(2-dodecyloxyethoxy)ethyl sulphate
EC No.	:	221-416-0
Molecular Formula	:	C16H34O6S.Na

Producer related part

Company	:	Epona Associates, LLC
Creation date	:	26.01.2006

Substance related part

Company	:	Epona Associates, LLC
Creation date	:	26.01.2006
Status	:	
Memo	:	Stepan
Printing date	:	23.05.2006
Revision date	:	
Date of last update	:	23.05.2006
Number of pages	:	27
Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 APPLICANT AND COMPANY INFORMATION

Type	:	
Name	:	Stepan Company
Contact person	:	Lela Jovanovich
Date	:	
Street	:	
Town	:	Northfield, Illinois
Country	:	
Phone	:	
Telefax	:	
Telex	:	
Cedex	:	
Email	:	
Homepage	:	

26.01.2006

1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

1.0.3 IDENTITY OF RECIPIENTS

1.0.4 DETAILS ON CATEGORY/TEMPLATE

1.1.0 SUBSTANCE IDENTIFICATION

IUPAC Name	:	Ethanol, 2-[2-(dodecyloxy)ethoxy]-, hydrogen sulfate, sodium salt
Smiles Code	:	S(=O)(=O)(O[Na])OCCOCCOCCCCCCCCCCC
Molecular formula	:	C16 H33 O6 S1 Na1
Molecular weight	:	376.49
Petrol class	:	

26.01.2006

1.1.1 GENERAL SUBSTANCE INFORMATION

1.1.2 SPECTRA

1.2 SYNONYMS AND TRADENAMES

2-(2-Dodecyloxyethoxy)ethyl sodium sulfate

26.01.2006

Diethylene glycol monododecyl ether sulfate, sodium salt

26.01.2006

Diethylene glycol monolauryl ether sodium sulfate

26.01.2006

Diethylene glycol monolauryl ether sulfate, sodium salt

26.01.2006

Ethanol, 2-[2-(dodecyloxy)ethoxy]-, hydrogen sulfate, sodium salt

26.01.2006

Lauristyl diglycol ether, sulfate sodium salt

26.01.2006

Lauryl diethylene glycol ether, sulfonate sodium

26.01.2006

Sodium diethylene glycol dodecyl ether sulfate

26.01.2006

Sodium dioxyethylenedodecyl ether sulfate

26.01.2006

Sodium lauryl alcohol diglycol ether sulfate

26.01.2006

Sodium lauryl di(oxyethyl) sulfate

26.01.2006

Sodium lauryloxyethoxyethyl sulfate

26.01.2006

Sodiumlaurylglycolether sulfate

26.01.2006

Sulfuric acid mono[2-[2-(dodecyloxy)ethoxy]ethyl] ether sodium salt

26.01.2006

1.3 IMPURITIES

1.4 ADDITIVES

1.5 TOTAL QUANTITY

1.6.1 LABELLING

1.6.2 CLASSIFICATION

1.6.3 PACKAGING

1.7 USE PATTERN

1.7.1 DETAILED USE PATTERN

1.7.2 METHODS OF MANUFACTURE

1.8 REGULATORY MEASURES

1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES

1.8.2 ACCEPTABLE RESIDUES LEVELS

1.8.3 WATER POLLUTION

1.8.4 MAJOR ACCIDENT HAZARDS

1.8.5 AIR POLLUTION

1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES

1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS

1.9.2 COMPONENTS

1.10 SOURCE OF EXPOSURE

1.11 ADDITIONAL REMARKS

1.12 LAST LITERATURE SEARCH

1.13 REVIEWS

2.1 MELTING POINT

Value	:	286 °C
Sublimation	:	
Method	:	other: calculated
Year	:	2006
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	MPBPWIN (v1.41) Program Results: ========== Experimental Database Structure Match: no data SMILES : S(=O)(=O)(O[Na])OCCOCCOCCCCCCCCCCC CHEM : Ethanol, 2-[2-(dodecyloxy)ethoxy]-, hydrogen sulfate, sodium salt MOL FOR: C16 H33 O6 S1 Na1 MOL WT : 376.49
Remark	:	The substance is a solid at room temperature. The melting point will be >200 C, and is in good agreement with the modeled value.
Result	:	Melting Point: 349.84 deg C (Adapted Joback Method) Melting Point: 271.16 deg C (Gold and Ogle Method) Mean Melt Pt : 310.50 deg C (Joback; Gold,Ogle Methods) Selected MP: 286.89 deg C (Weighted Value)
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

14.02.2006 (3) (15)

2.2 BOILING POINT

Value	:	659 °C at
Decomposition	:	
Method	:	other: calculated
Year	:	2006
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	Adapted Stein and Brown Method MPBPWIN (v1.41) Program Results: ========== Experimental Database Structure Match: no data SMILES : S(=O)(=O)(O[Na])OCCOCCOCCCCCCCCCCC CHEM : Ethanol, 2-[2-(dodecyloxy)ethoxy]-, hydrogen sulfate, sodium salt MOL FOR: C16 H33 O6 S1 Na1 MOL WT : 376.49
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

14.02.2006 (3) (15)

2.3 DENSITY

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value	:	ca. 0 hPa at 25 °C
Decomposition	:	
Method	:	other (calculated)
Year	:	2006
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	MPBPWIN v1.41; Modified Grain Method
Result	:	Selected VP: 2.57E-015 mm Hg = 3.4 E-15 hPa
Reliability	:	(2) valid with restrictions Data were obtained by modeling

26.01.2006 (3)

2.5 PARTITION COEFFICIENT

Partition coefficient	:	octanol-water
Log pow	:	1.14 at 25 °C
pH value	:	
Method	:	other (calculated)
Year	:	2006
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	WSKOW v1.41
Reliability	:	(2) valid with restrictions Data were obtained by modeling
Flag	:	Critical study for SIDS endpoint

26.01.2006 (3)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in	:	Water
Value	:	ca. 452 at 25 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	
Stable	:	
Deg. product	:	
Method	:	other: calculated
Year	:	2006
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	WSKOW v1.41 Equation Used to Make Water Sol estimate: Log S (mol/L) = 0.796 - 0.854 log Kow - 0.00728 MW + Correction (used when Melting Point NOT available)

Correction(s):	Value
No Applicable Correction Factors	

Remark	:	The modeled results are in good agreement with the expected water solubility of this substance.
Result	:	Log Water Solubility (in moles/L) : -2.921 Water Solubility at 25 deg C (mg/L): 451.6
Reliability	:	(2) valid with restrictions Data were obtained by modeling
Flag	:	Critical study for SIDS endpoint

26.01.2006 (3) (15)

2.6.2 SURFACE TENSION

2.7 FLASH POINT

2.8 AUTO FLAMMABILITY

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2.12 DISSOCIATION CONSTANT

2.13 VISCOSITY

2.14 ADDITIONAL REMARKS

3.1.1 PHOTODEGRADATION

INDIRECT PHOTOLYSIS

Sensitizer	:	
Conc. of sensitizer	:	
Rate constant	:	.000000000045 cm^3/(molecule*sec)
Degradation	:	50 % after .2 day(s)
Deg. product	:	
Method	:	other (calculated)
Year	:	2006
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4

Result :

```
AOP Program (v1.91) Results:
============================
SMILES : S(=O)(=O)(O[Na])OCCOCCOCCCCCCCCCCC
CHEM   : Ethanol, 2-[2-(dodecyloxy)ethoxy]-, hydrogen
sulfate, sodium salt
MOL FOR: C16 H33 O6 S1 Na1
MOL WT : 376.49
----------------- SUMMARY (AOP v1.91): HYDROXYL RADICALS
-----------------
Hydrogen Abstraction      = 45.4767 E-12 cm3/molecule-sec
Reaction with N, S and -OH =  0.0000 E-12 cm3/molecule-sec
Addition to Triple Bonds  =  0.0000 E-12 cm3/molecule-sec
Addition to Olefinic Bonds =  0.0000 E-12 cm3/molecule-sec
Addition to Aromatic Rings =  0.0000 E-12 cm3/molecule-sec
Addition to Fused Rings   =  0.0000 E-12 cm3/molecule-sec

  OVERALL OH Rate Constant =  45.4767 E-12 cm3/molecule-sec
  HALF-LIFE =    0.235 Days (12-hr day; 1.5E6 OH/cm3)
  HALF-LIFE =    2.822 Hrs
----------------- SUMMARY (AOP v1.91): OZONE REACTION
--------------------

        ****** NO OZONE REACTION ESTIMATION ******
        (ONLY Olefins and Acetylenes are Estimated)

Experimental Database:  NO Structure Matches
```

Reliability	:	(2) valid with restrictions Data were obtained by modeling
Flag	:	Critical study for SIDS endpoint

26.01.2006 (3)

3.1.2 STABILITY IN WATER

Type	:	abiotic
t1/2 pH4	:	at °C
t1/2 pH7	:	at °C
t1/2 pH9	:	at °C
Deg. product	:	
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4

Method : Reaction kinetics were followed by sampling. In some cases acid-base

titration was used to measure the increase in acidity as the reaction proceeds, in some cases titration with lead nitrate was used to determine the bisulphate formed in the reaction, and in other cases the traditional Epton 2-phase titration was used to determine the concentration of surfactant remaining.

Remark : SLES would undergo 10% decomposition at 100C between 30 and 40 days (see results for Linear E1 AES and Linear E3 AES in Table 1). This is to be expected as an increased rate of hydrolysis is proportional to increases in temperature. Therefore, as temperature decreases, the rate of hydrolysis greatly slows. Under normal use and typical environmental conditions (approximately 25C at non-catalyzed conditions), we would expect this chemical to be resistant to hydrolysis.

Result : Table I Uncatalysed hydrolysis rate constants for PAS and AES
All at 100°C unless otherwise stated

Surfactant	k1 (sec-1 x 10-8)	t (10% decomp.)
Linear E1 AES	4.1	30 days
Linear E3 AES	3.1	40 days

Table II. Acid catalysed hydrolysis rate constants for PAS and AES

Surfactant	K2 (M-1sec-1) x 10-6 100 oC
Linear E1 AES	9
Linear E3 AES	9

Conclusion : Stable
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
23.05.2006 (13)

3.1.3 STABILITY IN SOIL

3.2.1 MONITORING DATA

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type : fugacity model level III
Media :
Air : % (Fugacity Model Level I)
Water : % (Fugacity Model Level I)
Soil : % (Fugacity Model Level I)
Biota : % (Fugacity Model Level II/III)
Soil : % (Fugacity Model Level II/III)
Method : other: calculated
Year : 2006

Result : Level III Fugacity Model (Full-Output):
==========
Chem Name : Ethanol, 2-[2-(dodecyloxy)ethoxy]-, hydrogen sulfate, sodium sal

Molecular Wt: 376.49
Henry's LC : 4.45e-011 atm-m3/mole (Henrywin program)
Vapor Press : 2.57e-015 mm Hg (Mpbpwin program)

Liquid VP : 1e-012 mm Hg (super-cooled)
Melting Pt : 287 deg C (Mpbpwin program)
Log Kow : 1.14 (Kowwin program)
Soil Koc : 5.66 (calc by model)

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	0.355	5.64	1000
Water	49.4	900	1000
Soil	50.1	900	1000
Sediment	0.103	3.6e+003	0

	Fugacity (atm)	Reaction (kg/hr)	Advection (kg/hr)	Reaction (percent)	Advection (percent)
Air	4.43e-018	754	61.4	25.1	2.05
Water	5.06e-016	659	856	22	28.5
Soil	1.31e-014	668	0	22.3	0
Sediment	4.65e-016	0.344	0.0358	0.0115	0.00119

Persistence Time: 577 hr
Reaction Time: 832 hr
Advection Time: 1.89e+003 hr
Percent Reacted: 69.4
Percent Advected: 30.6

Half-Lives (hr), (based upon Biowin (Ultimate) and Aopwin):
Air: 5.645
Water: 900
Soil: 900
Sediment: 3600
Biowin estimate: 2.648 (weeks-months)

Advection Times (hr):
Air: 100
Water: 1000
Sediment: 5e+004

Reliability : (2) valid with restrictions
Data were obtained by modeling

Flag : Critical study for SIDS endpoint
26.01.2006 (3)

3.3.2 DISTRIBUTION

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type : anaerobic
Inoculum : other: microorganisms present in seawater
Concentration : 2.3 mg/l related to Test substance

related to
Contact time : 28 day(s)
Degradation : 65 (±) % after 28 day(s)
Result : readily biodegradable
Kinetic of testsubst. : 0 day(s) 0 %
7 day(s) 50 %
14 day(s) 53 %
21 day(s) 57 %
28 day(s) 65 %
Control substance : Benzoic acid, sodium salt
Kinetic : 28 day(s) 92 %
%
Deg. product :
Method : OECD Guide-line 306
Year : 2006
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Reliability : (1) valid without restriction
Guideline study
Flag : Critical study for SIDS endpoint
16.02.2006 (1)

Type : aerobic
Inoculum :
Contact time : 26 day(s)
Degradation : 81 (±) % after 26 day(s)
Result : readily biodegradable
Deg. product :
Method : other: Sturms evolved CO2 procedure
Year :
GLP : no data
Test substance : other TS

Test substance : NaC12AE2.1S
Reliability : (2) valid with restrictions
16.02.2006 (11)

Type : aerobic
Inoculum :
Contact time : 20 day(s)
Degradation : 100 (±) % after 20 day(s)
Result : readily biodegradable
Deg. product :
Method : other: BOD
Year :
GLP : no data
Test substance : other TS

Result : Total depletion of oxygen at 20 days. After 5 days the BOD was 58%.
Test substance : NaC12AE2.1S
Reliability : (2) valid with restrictions
16.02.2006 (11)

3.6 BOD5, COD OR BOD5/COD RATIO

3.7 BIOACCUMULATION

3.8 ADDITIONAL REMARKS

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type : static
Species : Pimephales promelas (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
LC50 : = 13
Method : other
Year :
GLP : no data
Test substance : other TS

Method : Static
22 deg C
pH 7.3
Hardness: 50-52 mg/L CaCO3
Result : 96 hr LC50 = 13 mg/L (95% Confidence limits: 10-18)
Test substance : C12-14AES (ammonium salt)
Reliability : (2) valid with restrictions
13.02.2006 (11)

Type : static
Species : Salmo gairdneri (Fish, estuary, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
LC50 : = 28
Method : other
Year :
GLP : no data
Test substance : other TS

Method : Static
15 deg C
pH 8.2-8.6
Hardness 260 mg/L
Result : 96 hr LC50 = 28 mg/L (95% confidence limits: 23-35)
Test substance : C12-13AE2S (Dobanol 23-2S/28)
Reliability : (2) valid with restrictions
13.02.2006 (14)

Type : static
Species : Lepomis macrochirus (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
LC50 : = 24
Method : other
Year :
GLP : no data
Test substance : other TS

Method : Static
22 deg C
pH7.2
Hardness 42-44 mg/L CaCO3
Result : 96 hr LC50 = 24 mg/L (95% Confidence limits: 18-32)
Test substance : C12-14AES
Reliability : (2) valid with restrictions
13.02.2006 (11)

Type : static
Species : Cyprinodon variegatus (Fish, estuary, marine)
Exposure period : 96 hour(s)
Unit : mg/l
LC50 : = 2.3
Method : other
Year :
GLP : no data
Test substance : other TS

Method : Static
22 deg C
pH 8.0
Salinity: 32 parts per thousand
Result : 96 hr LC50 = 2.3 mg/L (95% confidence limits: 1.3-3.7)
Test substance : C12-14AES
Reliability : (2) valid with restrictions
13.02.2006 (11)

Type : static
Species : Lepomis macrochirus (Fish, fresh water)
Exposure period : 24 hour(s)
Unit : mg/l
LC50 : = 87
Method : other
Year :
GLP : no data
Test substance : other TS

Method : Static
21 deg C
pH 7.1
Hardness 35 mg/L CaCO3
Test substance : C12AE2.1S
Reliability : (2) valid with restrictions
13.02.2006 (11)

Type : static
Species : Pimephales promelas (Fish, fresh water)
Exposure period : 48 hour(s)
Unit : mg/l
LC50 : = 1.5
Method : other
Year :
GLP : no data
Test substance : other TS

Method : Static
21 deg C
pH 7.0-7.2
Hardness 100 mg/L CaCO3
Result : 24 hr LC50 = 1.5 mg/L
48 hr LC50 - 1.5 mg/L
Test substance : C12AE2S
Reliability : (2) valid with restrictions
13.02.2006 (10)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : static

Species : Daphnia magna (Crustacea)
Exposure period : 24 hour(s)
Unit : mg/l
EC50 : = 21
Method : other
Year : 1972
GLP : no data
Test substance : other TS

Test substance : C12-14AE2.2S (natural-alcohol derived)
Reliability : (2) valid with restrictions
13.02.2006 (9)

Type : static
Species : Daphnia magna (Crustacea)
Exposure period : 30 hour(s)
Unit :
Method : other
Year : 1976
GLP : no data
Test substance : other TS

Remark : In a test with Daphnia, the toxicity of C12aveAES (lauryl ether sulfate) decreased steadily with time as a result of biodgradation. After 30 hours in static conditions, the solution was virtually non-toxic. No toxicity values were reported.
Test substance : C12aveAES (lauryl ether sulfate)
Reliability : (2) valid with restrictions
13.02.2006 (8)

Type : static
Species : Daphnia magna (Crustacea)
Exposure period : 96 hour(s)
Unit : mg/l
EC50 : = 5.7
Method : other
Year :
GLP : no data
Test substance : other TS

Result : 96 hr LC50 = 5.7 mg/L under nominal concentrations of the active ingredient
Test substance : ammonium C12-14AES
Reliability : (2) valid with restrictions
13.02.2006 (11)

Type :
Species : other: Ceriodaphnia dubia
Exposure period : 48 hour(s)
Unit : mg/l
EC50 : 3.12
Limit Test : no
Analytical monitoring : no
Method : other
Year : 1999
GLP : no data
Test substance : other TS

Result : Water Parameters:
Temperature: 23 C (mean value)
Conductivity: 500 umhos/cm (mean value)

		Effect Concentration #1: 3.12 mg/l (mean); 2.43 mg/l (minimum); 4.01 mg/l (maximum)
Test substance	:	CAS Registry Number (CAS) : 9004-82-4 Chemical Name (NAM) : .alpha.-Sulfo-.omega.-(dodecyloxy)poly(oxy-1,2-ethanediyl), Sodium salt
Reliability	:	(2) valid with restrictions Peer reviewed published data
Flag	:	Critical study for SIDS endpoint

16.02.2006 (2) (16)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species	:	Selenastrum capricornutum (Algae)
Endpoint	:	other
Exposure period	:	5 day(s)
Unit	:	mg/l
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	other TS
Result	:	The 5-day algistatic concentration for C12-14AES in S. capricornutum was 101 mg/L (95% confidence limits: 42-312 mg/L), while the 5-day algicidal concentration was > 1000 mg/L.
Test substance	:	C12-14AES
Reliability	:	(2) valid with restrictions

13.02.2006 (11)

Species	:	other algae: Laminaria saccharina
Endpoint	:	other
Exposure period	:	
Unit	:	mg/l
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	other TS
Remark	:	The author hypothesized that the detergent mixture attacked the proteinaceous flagella on the zoospores; this would account for the loss of mobility.
Result	:	In a toxicity test with the alga, Laminaria saccharina, concentrations between 5 x 10E-5 mg/L and 5 x 10E4 mg/L of a detergent containing C12(ave)AES (sodium lauryl ether sulfate), sodium dodecyl benzene sulfonate, and lauric diethanolamide were used. In 50 mg/L, zoospores of L. saccharina were inhibited from swimming in 7 minutes, and in 500 mg/L, swimming ceased in 15 seconds. A concentration of 0.1 mg/L prevented the zoospores from settling (an action which normally precedes development in sporophytes).
Test substance	:	C12(ave)AES (sodium lauryl ether sulfate)
Reliability	:	(2) valid with restrictions

13.02.2006 (12)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS

4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Value	:	> 5000 mg/kg bw
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male/female
Number of animals	:	10
Vehicle	:	
Doses	:	5 g/kg
Method	:	other
Year	:	1982
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	Five male and 5 female rats were administered by gavage 5 g/kg of the undiluted test substance. Animals were observed for 14 days for signs of toxicity and mortality. All animals were weighed and sacrificed at the end of the 14 day observation period and subjected to a gross necropsy.
Result	:	There were no deaths. There were no clinicla signs in male rats. Two female rats exhibited diarrhea and on efemale rat exhibited central nervous system depression. There were no gross pathological alterations.
Reliability	:	(1) valid without restriction Similar to guideline study
Flag	:	Critical study for SIDS endpoint

16.02.2006 (4)

Type	:	LD50
Value	:	1600 mg/kg bw
Species	:	rat
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Method	:	other
Year	:	1983
GLP	:	no data
Test substance	:	other TS
Test substance	:	CAS Registry Number (CAS) : 9004-82-4 Chemical Name (NAM) : .alpha.-Sulfo-.omega.-(dodecyloxy)poly(oxy-1,2-ethanediyl), Sodium salt
Reliability	:	(2) valid with restrictions Peer reviewed published data

16.02.2006 (2) (7)

5.1.2 ACUTE INHALATION TOXICITY

5.1.3 ACUTE DERMAL TOXICITY

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.2.1 SKIN IRRITATION

Species	:	rabbit
Concentration	:	undiluted
Exposure	:	Semiocclusive
Exposure time	:	24 hour(s)
Number of animals	:	6
Vehicle	:	
PDII	:	4
Result	:	
Classification	:	
Method	:	other
Year	:	1982
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	0.5 mL of the test substance was applie dto the intact and abraded skin of 6 rabbits and allowed to remain in contact with the skin for 24 hours. The sites were scored for erythema and edema and checked for tissue damage at the end of the application peroid and again at 72 hours.
Result	:	The PII was 4.0. Evidence of tissue damage in the form of coriaceousness was found in two animals. Atonia, blanching discoloration and spreading of irritative effects was also noted during the study.
Reliability	:	(1) valid without restriction Similar to guideline study

26.01.2006 (6)

5.2.2 EYE IRRITATION

Species	:	rabbit
Concentration	:	undiluted
Dose	:	.1 ml
Exposure time	:	24 hour(s)
Comment	:	not rinsed
Number of animals	:	6
Vehicle	:	
Result	:	
Classification	:	
Method	:	other
Year	:	1982
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	The test substance was applied to the right eye of each of 6 rabbits. The eyes were examined prior to treatment. Examinations for gross signs of eye irritation were made at approximately 24, 46 and 72 hours following application. Additional readings were made at 4 and 7 days after treatment. Scoring of irritative effects was performed according to the method of Draize. An irritation score was

calculated for each rabbit on a basis of 0-110.

Result : The eyes of all 6 rabbits were found to show evidence of significant corneal, iris and conjunctival changes. Mean irritation scores ranged from 34.8 at 24 hours to 10.2 after 7 days.

Reliability : (1) valid without restriction
Similar to guideline study

26.01.2006 (5)

5.3 SENSITIZATION

5.4 REPEATED DOSE TOXICITY

5.5 GENETIC TOXICITY 'IN VITRO'

5.6 GENETIC TOXICITY 'IN VIVO'

5.7 CARCINOGENICITY

5.8.1 TOXICITY TO FERTILITY

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9 SPECIFIC INVESTIGATIONS

5.10 EXPOSURE EXPERIENCE

5.11 ADDITIONAL REMARKS

6.1 ANALYTICAL METHODS

6.2 DETECTION AND IDENTIFICATION

7.1 FUNCTION

7.2 EFFECTS ON ORGANISMS TO BE CONTROLLED

7.3 ORGANISMS TO BE PROTECTED

7.4 USER

7.5 RESISTANCE

8.1 METHODS HANDLING AND STORING

8.2 FIRE GUIDANCE

8.3 EMERGENCY MEASURES

8.4 POSSIB. OF RENDERING SUBST. HARMLESS

8.5 WASTE MANAGEMENT

8.6 SIDE-EFFECTS DETECTION

8.7 SUBSTANCE REGISTERED AS DANGEROUS FOR GROUND WATER

8.8 REACTIVITY TOWARDS CONTAINER MATERIAL

(1) AnalyCen ecotox (2003) Biodgradation (OECD 306) Test Results for the Test Substance Agent 2527-68ES-7.

(2) BiblioLine(c) 1997-2006, NISC International, Inc. All Rights Reserved. www.nisc.com

(3) EpiWin v3.11

(4) Hill Top Research, Inc (1982) Acute Oral Toxicity Screen in Rats of 910-11 for Stepan Chemical Company. Project No. 82-0821-21.

(5) Hill Top Research, Inc (1982) Primary Eye Irritation Study in Rabbits of 910-13 for Stepan Chemical Company. Project No. 82-0823-21.

(6) Hill Top Research, Inc (1982) Skin Irritation Study in Rabbits of 910-12 for Stepan Chemical Company. Project No. 82-0822-21.

(7) Journal of the American College of Toxicology (JACT) (1983) (Mary Ann Liebert, Inc., 1651 Third Ave., New York, NY 10128) Vol 2(5): 1

(8) Lundahl, P. and R. Cabridene (1976) A focus on a method of study of the toxicity of pollutants during biodegradation. J. Francais d'Hydrologie 7(3) #21:143-150 (English translation) as cited in SDA (1991) Environmental and Human Safety of Major Surfactants: Volume 1. Anionic Surfactants Part 2. Alcohol Ethoxy Sulfates

(9) Lundahl, P., R. Cabridene and R. Xuereff (1972) Qualites biologiques de queloques agents de surface anioniques, 6th International Congress on Surface Active Agents as cited in SDA (1991) Environmental and Human Safety of Major Surfactants: Volume 1. Anionic Surfactants Part 2. Alcohol Ethoxy Sulfates

(10) Monsanto Company, unpublished data as cited in SDA (1991) Environmental and Human Safety of Major Surfactants: Volume 1. Anionic Surfactants Part 2. Alcohol Ethoxy Sulfates

(11) Proctor and Gamble Company, unpublished data as cited in SDA (1991) Environmental and Human Safety of Major Surfactants: Volume 1. Anionic Surfactants Part 2. Alcohol Ethoxy Sulfates

(12) Pybus, C. (1973) Effects of anionic detergent on the growth of Laminaria. Marine Pollution Bulletin 4(5):73-77 as cited in SDA (1991) Environmental and Human Safety of Major Surfactants: Volume 1. Anionic Surfactants Part 2. Alcohol Ethoxy Sulfates

(13) Roberts D.W., R Alvarez, D. Bethell and R. Fessy. HYDROLYSIS KINETICS OF PRIMARY ALCOHOL SULPHATES AND PRIMARY ALCOHOL ETHOXY SULPHATES Unilever Research Port Sunlight

(14) Shell Chemical Company, unpublished data as cited in SDA (1991) Environmental and Human Safety of Major Surfactants: Volume 1. Anionic Surfactants Part 2. Alcohol Ethoxy Sulfates

(15) Stepan Company (2006) Personal Communication

(16) Warne, M.S.T.; A.D. Schifko (1999) Ecotoxicology and Environmental Safety, 44(2): 196-206

10.1 END POINT SUMMARY

10.2 HAZARD SUMMARY

10.3 RISK ASSESSMENT

201-16316

HERA

Human & Environmental Risk Assessment

on ingredients of

European household cleaning products

2005 JUL 19 AM 7:37
RECEIVED

Alcohol Ethoxysulphates

Human Health Risk Assessment

Draft

January 2003

All rights resewed No part of this publication may be used, reproduced, copied, stored or transmitted in any form of by any means, electronic, mechanical, photocopying, recording or otherwise without the prior written permission of the HERA Substance Team or the involved company.

The content of this document has been prepared and reviewed by experts on behalf of HERA with all possible care and from the available scientific information. It is provided for information only. HERA cannot accept any responsibility or liability and does not provide a warranty for any use or interpretation of the material contained in this publication.

1. Contents

2. Executive Summary 3

3. Substance Characterisation 4

3.1. CAS No and Grouping Information 4

3.2. Chemical Structure and Composition 4

3.3 Manufacturing Route and Production/Volume Statistics 4

3.4. Use Applications Summary 7

4. Human Health Assessment 9

4.11 Consumer Exposure 9

4.2. Hazard Assessment 16

4.3 Risk Assessment 43

5. *Environmental Assessment*

Note that Section 5 Environmental Risk Assessment will published at www.heraproject.com in 2003. These assessments will be merged and a single comprehensive document produced at a later date.

6. Contributors to this Risk Assessment 47

Appendix 1. Literature Search 54

Further information on the HERA Project including the HER4 Methodology document and other risk assessments can be found at this website

2. Executive Summary

Alcohol ethoxysulphates (AES) are a widely used class of anionic surfactants. They are used in household cleaning products, personal care products, institutional cleaners and industrial cleaning processes, and as industrial process aids in emulsion polymerisation and as additives during plastics and paint production. Uses in household cleaning products, the scope of HERA, include laundry detergents, hand dishwashing liquids, and various hard surface cleaners.

Through its presence in many commonly used household detergents, consumers are exposed to AES mainly via the dermal route, but to some extent also via the oral and the inhalatory route. Skin exposure occurs mainly in hand-washed laundry, laundry pre-treatment and hand dishwashing and to a minor extent also through AES residues in the fabric after the washing cycle and skin contact during hard surface cleaning tasks. Oral exposure occurs mainly through residues deposited on eating utensils and dishes after hand dishwashing.

AES are of low acute toxicity. Neat AES are irritant to skin and eyes. The irritation potential of AES containing solutions depends on concentration. Local dermal effects due to direct or indirect skin contact with AES containing solutions in hand-washed laundry or hand dishwashing are not of concern because AES is not a contact sensitizer and AES is not expected to be irritating to the skin at in-use concentrations.

The available repeated dose toxicity data demonstrate the low toxicity of AES. Also, they are not considered to be mutagenic, genotoxic or carcinogenic, and are not reproductive or developmental toxicants.

The consumer aggregate exposure from direct and indirect skin contact as well as from the oral route via dishware residues results in an estimated total body burden of 29 µg/kg bw/day.

The comparison of the aggregate exposure and the systemic NOAEL results in a margin of exposure (MOE) of 2586. This is a very large margin of exposure, large enough to account for the inherent uncertainty and variability of the hazard database and inter and intra-species extrapolations, which are usually considered by a factor of 100 or greater.

In summary, the human health risk assessment has demonstrated that the use of AES in household laundry and cleaning detergents is safe and does not cause concern with regard to consumer use.

3. Substance Characterisation

Alcohol ethoxysulphates (AES), also known as alkyl ethersulphates, are a widely used class of anionic surfactants. They are used in household cleaning products, personal care products including toothpaste and shampoos, hand and other personal cleaning products, institutional cleaners and industrial cleaning processes, and as industrial process aids in emulsion polymerisation and as additives during plastics and paint production. Uses in household cleaning products, relevant to the HERA program of risk assessments, include laundry detergents, hand dishwashing liquids, and various hard surface cleaners.

3.1. CAS No and Grouping information

There are more than 36 CAS Numbers describing AES. A comprehensive list is presented in Appendix 1 of this document. Although clearly important from a Regulatory perspective, this assessment is not based on CAS Nos., but on a clear definition of the product family's composition.

3.2. Chemical structure and composition

The alcohol ethoxysulphate family is defined for HERA purposes to encompass commercial grades of linear-type primary alcohol ethoxysulphates containing AES components of basic structure $C_nH_{2n+}O(C_2H_4O)_mSO_3X)$ where n=l O-l 8 and m = O-8 and X = sodium, ammonium or triethanolamine (TEA). Sodium salts of AES are by far the commonly used grades. Further detail on the structures included in the AES family are given in Section 3.3.

3.3 Manufacturing Route and Production/Volume Statistics

Three steps are involved in the manufacture of AES on a commercial scale, and each is important in understanding the composition range included in the HERA AES family.

Detergent alcohol production

Ethoxylation

Sulphation and neutralisation

The HERA AES family is derived from linear-type primary alcohols in the C_{10} to C_{18} range. As marketed, such alcohols usually contain a distribution of alkyl chain lengths. The linear-type alcohols include those which are mixtures of entirely linear alkyl chains, and those which are mixtures of linear and mono-branched alkyl chains, though still with a linear backbone. Such alcohols and their blends are substantially interchangeable as feedstocks for AES used in the major applications falling within the scope of HERA.

Excluded from the HERA AES family are alcohol ethoxysulphates derived from alcohols with other alkyl chain structures such as multi-branched alcohols, for example commercial *iso*-tridecanols. These grades of AES are not typically used in household cleaning products. Their uses are small and specialised and they are not considered further in this assessment.

The linear-type alcohols used to produce HER4 AES include those derived from vegetable or animal sources via oleochemical processes and those derived from ethylene via Ziegler chemistry. Such alcohols contain even carbon numbered alkyl chains only, and are produced in single carbon cuts or more usually wider cuts from C6 through C22+. Cl2 through Cl 8 grades are the predominant feedstocks for HERA AES.

The other essentially linear alcohols used to produce HERA AES, also known as linear oxo-alcohols, are derived from linear higher olefins via oxo-chemistry. The feedstock linear olefins are typically derived from ethylene or normal paraffins. Such alcohols contain mixtures of even/odd or odd carbon numbered alkyl chains depending on the feedstock olefin, and are produced in grades ranging from C7 through C15. Typically 90-40% of the carbon chains are linear, the remainder being mono-branched 2-alkyl isomers, predominantly 2-methyl. The mono-branched isomers thus have a linear backbone. Cl2 through Cl5 grades are the predominant feedstocks for HERA AES.

The principle structures present in HERA C_{12} AES for example are:

$$CH_3(CH_2)_{11}O(CH2CH2O)_nSO_3Na$$

$$CH_3(CH_2)_8CHCH_2O(CH2CH2O)_nSO_3Na$$
$$\quad\quad\quad\quad |$$
$$\quad\quad\quad\quad CH_3$$

where n ranges from O-8.

Ethoxylation of detergent alcohols is carried out typically by base catalysed reaction with ethylene oxide. The average value of n for the important sulphation grades is 1-3 moles EO per mole alcohol. Example distributions of EO adducts are shown in the following table. As there is substantial unethoxylated alcohol in such feedstock ethoxylates, the derived AES contains a comparable amount of alcohol sulphate.

Table 1 : Typical Distribution of Ethoxylate Adducts

Avearge EO Groups	3	2	1
Oligomer distribution, %m/m, of $RO(CH_2CH_2O)_nH$ where n=			
0	13.1	23.5	42.9
1	9.1	12.8	20.3
2	11.9	15.6	14.9
3	12.9	13.4	8.8
4	11.8	10.1	5.1
5	10.3	7.5	3.0
6	7.9	5.0	1.9
7	6.5	4.0	1.4
8	4.8	2.9	0.9
9	3.9	1.8	0.5
10	2.9	1.4	0.3
11	1.9	0.9	0.1
12	1.3	0.6	
13	0.7	0.3	
14	0.5	0.2	
15	0.3		
Average EO Number	3.1	2.1	1.0

In the final step, alcohol ethoxysulphates are produced by sulphation of ethoxylates using sulphur trioxide or chlorosulphonic acid followed by immediate neutralisation with base to produce typically a sodium salt, less commonly an ammonium salt. Minor volumes are neutralised with alkanolamines, usually triethanolamine (TEA). Most AES is produced as low or high concentration aqueous solutions e.g. 25-30% or 68-70% actives.

Many grades of AES are produced commercially. These may differ in the parent detergent alcohol, the degree of ethoxylation, the neutralising anion, the concentration of AES active matter, and whether shipped as an aqueous solution, a paste or in solid form. On an active matter basis, commercial sodium AES typically contains approximately 2-4% of unsulphated organics (alcohols and ethoxylated alcohols), 2-4% sodium sulphate or chloride depending on the sulphation process, and optionally trace amounts of inorganic pH buffering agents. As mentioned previously all AES contains alcohol sulphate, generally 15-45 % depending on the degree of ethoxylation.

Two aspects of the trace chemistry of AES production have been of concern in the past:

Traces of 1,4-dioxane are formed as a by-product during the sulphation reaction with alcohol ethoxylates. Since first being recognised in 1979, its level has been controlled by manufacturers by attention to operating conditions including SO3/feed ratios, sulphation reactor temperatures and post reactor conditions including neutralisation. It is also important to avoid excursions from normal operating conditions. Suppliers of modem sulphation equipment incorporate 1,4-dioxane reduction features in their designs. Levels of 500ppm/actives were reported in the 1980's. Levels are now controlled and monitored by in-plant analysis against user specifications and, depending on the degree of ethoxylation are typically in the range of 30-200ppm/actives.

The hazard database for AES reported in section 4.2 has largely been generated with

commercial grade materials which will have contained 1,4 dioxane levels typical of the time of production.

Acute skin sensitisers were discovered in one batch of AES in 1966 and determined to be sultone-type materials. As discussed in Section 4.2.1.3 this incident was extensively researched to discover the root cause and is now regarded as an isolated incident and a result of conditions not normally present in AES manufacture. While analytical techniques are available for the contaminants, they are research methods unsuitable for in-plant control. Recurrence is prevented by manufacturers by a) avoiding contamination of sulfation grade ethoxylates with alpha olefms or reaction conditions where alcohols could be dehydrated at a trace level to alpha olefms, and b) avoiding use of sodium hypochlorite to reduce finished product colour under inappropriate reaction conditions. Following these process changes batches of AES were extensively tested in Human Repeat Insult Patch tests and shown to be non-sensitisers (refer to Section 4.2.8).

Of the AES used in consumer cleaning applications in Europe, a preliminary estimate gives 90% derived from even carbon numbered linear alcohols (C 12-14 and Cl 6-l 8), with the remaining 10% derived from odd and even carbon numbered essentially linear-oxo alcohols.

The European (EU, CH and NO) production volume of AES surfactants on an active matter basis is estimated to be 305,000 tonnes/y (CESIO statistics for 2000; CESIO = European Committee for Surfactants and their Organic Intermediates, a sector group of the European Chemical Industry Council, CEFIC). About 261,000 tonnes/y are estimated to remain in Europe, the remainder is exported. The imported volume is thought to be negligible.

3.4. Use applications summary

Tonnage used in HERA applications (HERA Tonnage)

To determine the total AES tonnage used in products falling within the scope of HERA (i.e., household detergents and cleaning products), a survey was conducted among detergent formulator companies (data from members of AISE) and companies manufacturing AES (via the CESIO Statistics Group). From the data received an estimated distribution between carbon chain lengths has also been determined. This is shown in Table 1.

Table 1. Estimated tonnage and Chain length distribution of AES within the scope of HERA

Chain length	CESIO : Total AES Tonnage		CESIO : Estimate of Volume used in Household Cleaning Products		AISE : Estimate of Volume used in Household Cleaning Products	
	Percent	*Tonnes*	*Percent*	*Tonnes*	*Percent*	*Tonnes*
C10	0.2	568	0.2	229		
Cl1	-	-	0.1	115	0.4	285
C12	62.1	176 511	57.4	65 786	46	32 770
C13	8.5	24 160	15	17 191	31.9	22 725
C14	24.6	69 922	21.5	24641	18.1	12 894
C15	1.6	4 548	2.7	3 094	3.6	2 565
C16	2.1	5 969	2.1	2 407	-	-
C17	-	-				
C18	0.8	2 274	0.9	1031		
ΣC_{10-18}		284 236		114 609		71239

Of the estimated total European AES production volume (305 000) and estimated total AES volume used in household cleaning products (138 000) - the distribution between carbon chain lengths has been determined for 284 236 tonnes and 114 609 tonnes, respectively. These chain length data are considered a reasonable representation of the distribution applicable for the total tonnages.

Alcohol ethoxysulphates are also used in a number of applications outside of the HERA scope. CESIO estimates that 47% (123,000 tonnes) of the captive use volume is used in other applications. Second to use in household detergents and cleaning products, Personal Care applications consume the next largest volume of AES, followed by use in Industrial and Institutional cleaners and the Industrial sector (e.g. emulsion polymerisation). These applications are not considered in this assessment.

4. Human Health Assessment

4.1 Consumer Exposure

4.1 .1. Product Types

In line with the objectives of the HERA initiative, this human health assessment will focus on the use of alcohol ethoxysulphates, AES, in household cleaning products. AES are used in many household detergents including laundry powders (typical concentration range: 0.1 – 0.8%), laundry liquids (typical concentration range: 1.5 – 18%), laundry additives (typical concentration range: 1 – 2%), dishwashing liquids (typical concentration range: 3 – 27%) and hard surface (typical concentration range: 0.3 – 3.1%) and toilet cleaners (typical concentration range: 3.5 – 6%).

4.1.2. Consumer Contact Scenarios

Based on the product types, the following consumer exposure routes were identified and assessed:

Direct skin contact with neat (laundry pre-treatment) or diluted consumer product (hand-washed laundry, hand dishwashing, hard surface cleaning)

Indirect skin contact via release from clothes fibers to skin

Inhalation of detergent dust or aerosols generated by spray cleaners

Oral ingestion of residues deposited on dishes

Oral ingestion of residues in drinking water

Accidental or intentional overexposure

4.1.3. 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/cup, tasks/week, 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 (H&P) of pre-treatment of clothes, additional H&P information for a targeted exposure assessment was directly provided by the member companies of AISE.

4.1.3.1. Direct skin contact from hand-washed laundry

Hand-washed laundry is a common consumer habit. During this procedure, the AES-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 AES 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	20% (0.2) [AISE Internal data]
C	product concentration in mg/ml:	*10 mg/ml* [AISE/HERA Table of H&P, 2002]
KP	dermal penetration coefficient	*1.62.x 10^{-4} cm/h** [Black et al. 1979]
t	duration of exposure or contact	*IO min (0.167h)* [AISE/HERA Table of H&P, 2002]
S_{der}	surface area of exposed skin	*1980cm²* [TGD, 1996]
n	product use frequency (tasks per day)	3 [AISE/HERA Table of H&P, 2002]
BW	body weight	*60 kg*

* the dermal penetration coefficient was calculated from the dermal flux (0.39 µg/cm²) which was determined in an in vivo dermal penetration experiment conducted by Black and Howes according to the following algorithm: Kp = dermal flux/exposure time x concentration of test solution; Kp = 0.00039 mg/cm²/24h x 10 mg/cm³ = 1.62 x 10^{-4} cm/h

$$\mathbf{Exp_{sys}} = [0.2 \times (10\ \mathrm{mg/ml}) \times (1.62 \times 10^{-4}\ \mathrm{cm/h}) \times (0.167\mathrm{h}) \times 3 \times (1980\ \mathrm{cm}^*)] / 60\ \mathrm{kg} = \mathbf{5.4\ \mu g/kg\ bw/day}$$

4.1.3.2. Direct skin contact from laundry tablets

Filling laundry tablets into the dispenser of the washing machine involves only a very short direct skin contact with the neat material. Due to the short contact time and the very small skin contact area, the dermal exposure to AES from this use is considered insignificant.

4.1.3.3. Direct skin contact from pre-treatment of clothes

Consumers typically spot-treat clothing stains by hand with the help of either a detergent paste (i.e. water/laundry powder = 1:1) or a laundry liquid which is applied directly on the garment. In this exposure scenario, only the skin surface of the hand (- 840 cm²) is exposed and the treatment time is typically less than 10 minutes(‘).

The exposure calculation is conducted by using the algorithm described in chapter 5.1.3.1. The AISE/HERA table [AISE Internal data] does not provide sufficient detail on the actual habits & practices of consumers with regard to laundry pre-treatment. The following assumptions are considered to represent a realistic reflection of this scenario:

F_1	percentage weight fraction of substance in product	20% (laundry liquid; 0.2) [AISE Internal data]

C	product concentration in mg/ml:	*1000 mg/ml* [AISE/HERA Table of H&P, 2002]
Kp	dermal penetration coefficient	1.62 *x 1 0^{-4} cm/h* [Black et al. 1979]
t	duration of exposure or contact	*5 min (0.083h)* [AISE/HERA Table of H&P, 2002]
S_{der}	surface area of exposed skin	*840cm^2* [TGD, 1996]
n	product use frequency (tasks per day)	0.5
BW	body weight	*60 kg*

Exp_{sys} = [0.2 x (1000 mg/ml) x (1.62 x 10^{-4} cm/h) x (0.083h) x (840 cm²) x OS]/ 60 kg= 18.8 µg/kg bw/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 which is not reflected in this exposure estimate. It should also be considered that only a fraction of the two hands' surface skin will actually be exposed. The assumption that both hands will be fully immersed leads to a likely overestimate of the true exposure.

4.1.3.4 Direct skin contact from hand dishwashing

The determination of AES exposure from hand dishwashing is conducted in a manner very similar to that of hand-washed laundry. Thus, the algorithm discussed in chapter 5.1.3.1 is used to calculate the dermal exposure to AES from hand dishwashing. The following assumptions have been made to address a reasonable worst case scenario:

F_1	percentage weight fraction of substance in product	*28% (0.28)* [AISE Internal data]
C	product concentration in mg/ml:	*1 mg/ml* [AISE/HERA Table of H&P, 2002]
Kp	dermal penetration coefficient	*1.62 x 10^{-4} cm/h* [Black et al. 1979]
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]
n	product use frequency (tasks per day)	3 [AISE/HERA Table of H&P, 2002]
BW	body weight	*60 kg*

Exp_{sys} = [0.28 x (1 mg/ml) x (1.62 x 10^{-4} cm/h) x (0.75h) x (1980 cm²) x 3] / 60 kg= 3.4 µg/kg bw/day

4.1.3.5. Direct skin contact from hard surface cleaning

During this procedure, the AES-containing hard surface cleaning solution comes in direct contact with the skin of the hands. A hard surface cleaning task takes at maximum 20 minutes [AISE/HERA Table of H&P, 2002]. The exposure to AES 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	*2.5%* (0.025) [AISE Internal data]
C	product concentration in mg/ml:	*12 mg/ml* [AISE/HERA Table of H&P, 2002]
Kp	dermal penetration coefficient	*1.62×10^{-4} cm/h** [Black et al. 1979]
t	duration of exposure or contact	*20 min (0.334h)* [AISE/HERA Table of H&P, 2002]
S_{der}	surface area of exposed skin	*840cm²* [TGD (1996)]
n	product use frequency (tasks per day)	*1* [AISE/HERA Table of H&P, 2002]
BW	body weight	*60kg*

* the dermal penetration coefficient was calculated from the dermal flux (0.39 $\mu g/cm^2$) which was determined in an in vivo dermal penetration experiment conducted by Black and Howes according to the following algorithm: Kp = dermal flux/exposure time x concentration of test solution; Kp = 0.00039 $mg/cm^2/24h$ x 10 mg/cm^3 = 1.62 x 10^{-4} cm/h

$$Exp_{sys} = [0.025 \times (12\ mg/ml) \times (1.62 \times 10^{-4}\ cm/h) \times (0.334h) \times 1 \times (840\ cm')] / 60\ kg = 0.2\ \mu g/kg\ bw/day$$

4.1.3.6. Indirect skin contact from 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 AES is deposited on the fabric following a wash process. This value has, however, been determined for linear alkylbenzene sulphonate (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 LAS, 2.5 g LAS resided per kilogram wash on the fabric. LAS is present in laundry detergents at about the same level (18% LAS versus 20% AES). Given the

similar physico-chemical nature of these two surfactants, it is assumed that AES remains to the same degree on the fabric as LAS [Rodriguez et al., 1994].

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 AES 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 (AES) 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	I% (0.01) [Vermeire et al., 1993]
F_3	percent weight fraction remaining on skin	*100%* (worst case)
F_4	percent weight fraction absorbed via skin	***1%*** (0.01) [Schaefer et al., 1996]
BW	body weight	***60 kg***

* C' was determined by multiplying the experimental value of the amount of LAS 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 [Procter & Gamble, 1996a]).

** For reasons of simplification, not the dermal penetration constant, but an estimated absorbed fraction was used to calculate the exposure. Schaefer and Redelmeier reported that the dermal penetration of ionic substances is very low [Schaefer et al., 1996].

$$\mathbf{Exp_{sys\ (indirect\ skin\ contact)}} = [(2.5 \times 10^{-2}\ mg/cm^2) \times (17{,}600\ cm^2) \times 0.01 \times 1 \times 0.011\ /\ 60kg = 0.73\ \mu g\ /kg\ bw\ day$$

4.1.3.7. Inhalation of detergent dust during washing processes

Studies by van de Plassche et al., 1998 determined an average release of about 0.27 µg dust per cup of product (i.e. laundry powder) used for machine laundering. AES is present in laundry powder detergents at a maximum level of 1% (or 2.7 x 10^{-3} µg AES/use). Taking the worst case assumption that all released dust is inhaled and washing of laundry occurs 3 times daily, the exposure of an adult with an average body weight of 60kg to AES is estimated to be,

$$\mathbf{Exp_{sys\ (inhalation\ of\ detergent\ dust)}} = [(2.7 \times 10^{-3}\ \mu g) \times 3]\ /\ 60\ kg = \mathbf{1.35 \times 10^{-4}\ \mu g/kg\ bw/day}$$

4.1.3.8. Inhalation of aerosols from cleaning sprays

AES is also present in surface cleaning sprays at a typical concentration range of 0.3 – 3.1% and at maximum 6%. The HERA guidance document specifies the algorithm to be used for calculation of consumers' worst case exposure to AES-containing aerosols generated by the spray cleaner:

$$Exp_{sys} = F_1 \times C' \times Q_{inh} \times t \times n \times F_7 \times F_8 / BW$$

F_1	percentage weight fraction of substance in product	**6% (0.06;** worst case) [AISE Internal data]
C'	product concentration in air:	0.35 ***mg/m³*** *[Procter & Gamble, 1996a]
Q_{inh}	ventilation rate	0.8 ***m³/h***
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%***
F_8	weight fraction absorbed or bioavailable	**75%; 075**
BW	body weight	**60** ***kg***

* 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

$$Exp_{sys\ (inhalation\ of\ aerosols)} = [0.06 \times (0.35\ mg/m^3) \times (0.8\ m^3/h) \times (0.17\ h) \times 0.751 / 60\ kg = 0.036\ \mu g/kg\ bw/day$$

4.1.3.9. Oral Exposures to AES

Oral exposure to AES can originate from residues on eating utensils and dishes washed in hand dish washing detergents and from AES residues taken up via drinking water. With regard to the uptake of AES from the drinking water, the Environmental Risk Assessment of AES discussed in chapter 4 has estimated a worst case regional predicted environmental concentration of AES in surface water of 0.055 mg/l.

For the estimation of human exposure to AES via the drinking water, one can assume in worst case assumption that an adult person drinks about 21 water per day [TGD, 1996]. Further, assuming 100% bioavailability of AES and 60kg body weight, the daily human exposure can be estimated as:

$$Exp_{sys\ (oral\ via\ drinking\ water)} = [(0.055\ mg/l) \times (2l)] / 60\ kg = 1.8\ \mu g/kg\ bw/day$$

In reality, this exposure estimate must be regarded as overly conservative. The vast majority of AES (estimated to be > 99%) will be removed during drinking water treatment process using e.g. sand or activated carbon filtration techniques.

The daily exposure to AES 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 HER4 guidance document:

$$Exp_{eve} = F1 \times C' \times Ta' \times Sa / BW$$

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	*28% (0.28);* [AISE Internal data]
C'	concentration of product in dish wash solution:	*1 mg/cm³* [AISE/HERA Table of H&P, 2002]
$T_{a'}$	amount of water left on dishes after rinsing	*5.5 x 10⁻⁵ ml/cm²* [Schmitz, 1973]
S_a	area of dishes in daily contact with food	*5400cm²* (Official publication French legislation, 1990)
BW	body weight, in kg	*60*

$$Exp_{sys\ (oral\ dish\ deposition)} = [0.28 \times (1\ mg/cm^3) \times (5.5 \times 10^{-5}\ ml/cm^2) \times (5400\ cm^2)] / 60\ kg = 1.4\ \mu g/kg\ bw/day$$

4.1.3.10. Accidental or intentional overexposure

Accidental or intentional overexposure to AES may occur via household detergent products, which may contain up to 28 % of AES.

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

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

4.2 Hazard Assessment

4.2.1. Summary of the available toxicological data

4.2.1.1. Acute Toxicity

4.2.1.1 .1. Acute Oral Toxicity

The acute oral toxicity of alcohol ethoxysulphates (AES) was evaluated with rats in several acute oral toxicity studies [Hüls AG, 1997a; Hüls AG, 1986a; Shell Research Ltd. 1975a; Shell Research Ltd., 1978a; Shell Research Ltd., 1978b; Brown, V. et al., 1968; Shell Research Ltd., 1975b; Shell Research Ltd., 1978c; Shell Research Ltd., 1975c; Shell Research Ltd., 1972; Brown, V. et al., 1970; Shell Chemical Co., 1967; Arthur D. Little, 1991]. The test materials were typically AES solutions containing 25 – 70% active material. The dilutions were administered at doses ranging from 2.5 – 10 ml/kg bodyweight. Most of the studies pre-date Good Laboratory Practice (GLP) regulations and in only one of these [Vermeire et al., 1993], the study design included at least 5 animals of each sex per dose group, thus meeting the critical aspect of current testing standards as defined in OECD methodologies. In these studies, the LD50 was estimated to be > 1.3 g active material per kg bodyweight. In a review for the Soap and Detergent Industry Association, Arthur D. Little reported rat oral LD50 values ranging from 1.7 - > 5 g/kg bodyweight [Arthur D. Little, 1991]. The most reliable studies will be discussed in the following paragraph in more detail.

A recent study [Hiils AG, 1997a] which was rated as reliable without restrictions according to the Klimisch criteria [Klimisch et al. (1997)], followed the guidelines of OECD method 401 and was compliant with GLP, a group of ten rats, five of each sex, was given a single oral dose of the triisopranolammonium salt of C12-14AE2S (90% active material) at a dose level of 2000 mg/kg bodyweight. The undiluted liquid was administered by gavage with an application volume of 2 ml/kg bodyweight. The rats were observed daily for any mortalities and clinical symptoms following treatment. Individual body weights were recorded on days 0 (prior to dosing), 7 and 14. At the end of the 14-day observation period, the animals were sacrificed and macroscopically examined. There were no deaths following a single oral application of the tested AES. The animals showed mild clinical symptoms such as increased activity and piloerection as a reaction to the treatment for approximately four hours after dosing. The macroscopic examination on day 14 showed no significant lesions. In conclusion, the acute lethal oral dose to male and female rats of the tested AES was found to be > 2 g/kg.

In a further study, rated as reliable with restrictions according to the Klimisch criteria, was also conducted according to the guidelines of OECD method 401, but not following GLP standards, a 70% solution of NaC12-14AE2S was administered by oral gavage at a dose level of 2.5 g/kg. No mortalities occurred under the dosing conditions. The rats achieved acceptable bodyweight gains throughout the study and showed mild clinical signs (unkempt fur, abdominal position, diarrhoea) as a reaction to the treatment for approximately 2 hours after dosing. The macroscopic examination on day 14 showed no significant lesions.

Conclusion

Alcohol ethoxysulphates are considered to have a low order of acute oral toxicity in the rat. In two recent and guideline compliant acute oral toxicity studies with marketed AES substances,

the LD50 was greater than 2000 mg/kg bodyweight. The clinical findings such as increased activity and piloerection following oral exposure are indicative of gastrointestinal stress and could be explained by the irritant nature of the test solutions under the conditions of oral gavage.

4.2.1.1.2. Acute Inhalation Toxicity

There are no test data available to evaluate the acute inhalation toxicity of AES. Only one study was identified in the review conducted by Arthur D. Little. In this study, rats (group size not specified) survived a 1 hour exposure to 60 mg/l of 59% active material solution of NH_4 C12-14AE3S. No additional details are available.

Conclusion

Given the lack of information on the study protocol and study results, this study is not suitable to assess the acute inhalation toxicity hazard of AES-type surfactants.

4.2.1.1.3. Acute Dermal Toxicity

The acute dermal toxicity of AES has been evaluated in several rat studies [Hüls AG, 1997b; Shell Research Ltd. 1975a; Shell Research Ltd., 1978a; Shell Research Ltd., 1978b; Shell Research Ltd., 1975b; Shell Research Ltd., 1978c; Shell Research Ltd., 1975c; Shell Research Ltd., 1972; Shell Chemical Co., 1967; Arthur D. Little, 1991] and in one rabbit study [Shell Chemical Co., 1967]. Most of the studies did not follow OECD guidelines (e.g. use of small group sizes) and did not comply with GLP regulations. However, despite some protocol deficiencies, the studies were reported in sufficient detail to allow a reasonable assessment of the potential dermal toxicity of AES in laboratory animals. The investigations included mortality and clinical observations. No mortality was observed in the rat studies at the dose level tested and subsequently LD50 values were expressed to be above the highest investigated dose levels, *i.e.*, >0.65 g/kg [Shell Research Ltd., 1978a], >1.12 g/kg [Shell Research Ltd., 1978b], >2.4 g/kg [Shell Research Ltd. 1975a], >1.25 g/kg [Shell Research Ltd., 1972], >1.08 g/kg [Shell Research Ltd., 1975b], >0.54 g/kg [Shell Research Ltd., 1978c], >1.8 g/kg [Shell Research Ltd., 1975c] and 4.6 g/kg [Shell Chemical Co., 1967]. Arthur D. Little, 1991 reported dermal LD50 values for AES on both intact and abraded rabbit skin ranging from 4 – 12 g/kg bodyweight. At highest dosage levels, various degrees of skin irritation (moderate to severe erythema and oedema) were reported and signs of intoxication included sporadic signs of haemorrhage around the eyes and nose, piloerection, and diarrhoea.

An acute dermal toxicity study (limit test) following OECD method 402 and complying with GLP guidelines was performed to assess the acute dermal toxicity of triisopranolammonium salt of C12-14AE2S (90% active material) in the rat. A group of ten rats, five of each sex, was given a single dermal application of the test substance at a dose level of 2 g/kg bodyweight. There were no deaths and no signs of systemic reaction to the treatment. Following removal of the dressing, moderate to severe dermal irritations indicated by inflammation of the epidermis and eschar formation were observed at the treatment site. The effects cleared over time. Some minor residual skin lesions were observed in 1 animal at the end of the 14-day observation period. No abnormalities were recorded at the macroscopic examination on day 14. The acute lethal dermal dose to male and female rats of NH_4C12-14AE2S was determined to be > 2 g/kg bodyweight.

Conclusion

Alcohol ethoxysulphates are considered to be of low acute dermal toxicity to rats. This was demonstrated in a recent, OECD guideline and GLP compliant acute dermal toxicity limit test in rats. This study has been judged to provide reliable information on the dermal toxicity of AES.

This assessment is supported by a substantial number of further acute dermal toxicity studies in rats and rabbits with a lower reliability score, which also demonstrated low acute dermal toxicity of AES-type surfactants.

4.2.1.1.4. Skin Irritation

Several skin irritation studies were conducted on rabbits considering different concentrations (0.1%, 1%, 10%, neat material), exposure duration (4h, 24h, 36 h) and exposure conditions (open application, semi-occlusion, full occlusion) [Hüls AG, 1997c; Hiils AG, 1986b; Shell Research Ltd., 1978d; Shell Research, Ltd., 1978e; Shell Oil Co., 1989; Shell Research Ltd. 1975a; Shell Research Ltd., 1978a; Shell Research Ltd., 1978b; Shell Research Ltd., 1968; Shell Research Ltd., 1978c; Shell Research Ltd., 1975c; Brown et al., 1970, Shell Chemical Co., 1967; Arthur D. Little, 1991, Hüls AG, 1997b.

The triisopranolammonium salt of C12-14AE2S (90% active material) was tested in an EC standard (4h) skin irritation study on rabbits [Hüls AG, 1997b]. The study followed OECD method 404 and was in compliance with GLP regulations. In this study, the undiluted liquid test substance was applied in a single dose for 4 hours to the shorn intact skin of three animals. The administration of the test substance led to well-defined erythema 24 hours after application, and was associated with distinct oedema in two animals and severe oedema in the 3rd animal. Forty–eight (48) hours after application, these signs of irritation were still well-defined and without change in 2 out of 3 animals. The 3rd animal presented with moderately severe erythema, associated with severe oedema, dry skin and scaling, 48 hours after application. Seventy-two (72) hours after application, 2 animals exhibited localized skin irritation in the form of well-defined or moderately severe erythema and oedema, and 1 rabbit had slight subcutaneous haemotrhages. On the 14th day after administration of the test substance, the skin of all the animals was free from signs of irritation. For all 3 animals, an erythema/eschar mean score of 2.33 and an oedema mean score of 2.78 was determined. This score indicates moderate skin irritation properties of the undiluted test substance.

In two further studies [NOTOX, 1994, Hiils AG, 1986b], NaC 12-14AE2 (70% active material) was tested in the EC standard irritation test. Both studies were conducted in compliance with OECD method 404, but only 1 complied with GLP regulations [NOTOX, 1994]. As in the case of the study discussed before, exposure to the test substance for 4 hours resulted in moderate to severe erythema and oedema. After 72 hours, reduced flexibility, fissuring of the skin and severe erythema and oedema were apparent. One study [Hüls AG, 1986b] terminated the observations at the 14th observation day and clinical signs of irritation were still apparent at this time. In the other study [NOTOX, 1994], animals were observed for 21 days and irritation had completely resolved within 21 days after exposure, but patches of bold skin persisted at termination.

As indicated before, further studies were conducted to investigate the skin irritation of effects of various dilutions of AES at different exposure durations and conditions. These studies were investigative in nature and neither was in compliance with OECD guidelines, nor with GLP regulations. However, these studies provide useful information on AES exposure conditions that are of particular relevance in consumer product applications. In 4hr or 24hr skin irritation studies on rabbits, a 0.1% AES solution did not show any signs of irritation, a 1% AES solution showed slight irritation, and solutions containing AES of 10 – 30% were mildly to moderately irritating under the patch conditions of the animal test.

Conclusion

The irritation potential of AES is concentration dependent. Materials with concentrations higher than 70% are moderately to severely irritating to rabbit skin under the conditions of the EC irritation test, and therefore classified as irritating to skin according to EU criteria as laid

down in the Dangerous Substance Directive (67/548/EEC). At concentrations between 10 and 30%, the AES solutions exhibit mild to moderate irritancy under the conditions of an occluded patch test. AES concentrations below 1% are virtually non-irritating under the conditions of the acute skin irritation testing protocol.

4.2.1.2. Eye Irritation

The potential of AES to cause eye irritation under accidental exposure conditions has been evaluated in several rabbit eye irritation studies [Hüls AG, 1997d; Hüls AG, 1986c, Shell Research Ltd. 1975a, Shell Research Ltd., 1978b, Shell Research Ltd., 1975b, Shell Research Ltd., 1978c, Shell Research Ltd., 1972, Brown et al., 1970, Arthur D. Little, 1991]. Most of the studies with undiluted or concentrated AES solutions (e.g. 32.6% C9-11AE2.5S, 70% C12-13AE2S, 28% C12-13AE2S) resulted in extensive cornea1 damage, inflammation of the iris and maximal conjunctival irritation with no significant improvement seen over a 7-day recovery period after product administration [Shell Research Ltd. 1975a' Shell Research Ltd., 1975b, Brown et al., 1970]. In the same studies, which were neither conducted according to OECD guidelines (e.g., protocol deviations such as application volume and observation period), nor followed the principles of GLP, the authors also investigated the same materials at concentrations of 10%, 1% and 0.1%. Generally, solutions containing 10% AES were observed to cause moderately irritating effects while 1% and 0.1% dilutions were virtually non-irritating. The most reliable studies will be discussed in the following paragraph in more detail.

The triisopranolammonium salt of C12-14AE2S (90% active material) was tested in an acute eye irritation study ("Drake test") according to OECD method 405 and following the principles of GLP. In this study, O.lml of the liquid test substance was administered into the conjunctival sac of one eye of each of the 3 rabbits. After an exposure time of 24 hours, the eyes were flushed with warm physiological saline. Twenty-four hours after exposure, the animals were observed to have reactions of the conjunctivae in the form of diffuse crimson red discoloration (individual blood vessels not easily discernible), together with distinct swelling and partial eversion of the eyelids. The cornea was slightly opaque over the entire surface, and the iris of one animal showed severe hyperaemia. Up 'to 72 hours after administration, these signs of irritation were largely unchanged and after 6 days, all signs of irritation began to diminish. After day 17, 2 animals were free from signs of irritation of the eye and mucosa. The 3rd animal was cleared after 24 days.

In another study, 28% active C12-14AE2S was also tested in the Draize test, following the guidelines specified in the OECD method 405. GLP compliance was not mentioned. Again, in this study the tested AES material caused cornea1 opacity, iritis and conjunctivitis in all test animals. While the conjunctivitis appeared to improve in all 3 test animals approximately 8-10 days after exposure to the test material, cornea1 opacity and the circumcomeal injection in the iris were still present in 2 animals after 21 days.

Further investigative studies were conducted to determine the effect of rinsing and AES alkyl chain length on the eye irritation potential in rabbits [Procter & Gamble, 1996b]. It was found that rinsing after instillation greatly reduced the severity of eye effects and that AES in the C 12- 16 range produced more severe effects than AES with longer or shorter chains. This was primarily manifested by longer clearing times (> 7 days versus l-7 days).

Conclusion

In two independent OECD and GLP compliant acute eye irritation studies, the triisopranolammonium salt of C12-14E2S (90% active material) and NaC12-14E2S (28% active material) were shown to be moderately to severely irritating to rabbit eyes. Due to its persistent effects, these materials were to be classified as severely irritating, according to the EU criteria as laid down in the Dangerous Substance Directive (67/548/EEC).

In studies with a lower reliability score it was shown that solutions containing less than 1-10% AES are slightly to moderately irritating to eyes and below 1%, AES solutions are virtually non-irritating.

4.2.1.3. Skin Sensitization

The skin sensitization potential of AES was evaluated in the guinea pig maximization test according the Magnusson-Kligman protocol [Hüls AG, 1989; Henkel KGaA, 1977a; Henkel KGaA, 1985; Henkel KGaA, 1977b; Shell Research Ltd., 1975d; Shell Research Ltd., 1980a; Shell Research Ltd., 1983a, Shell Research Ltd. 1975a, Shell Research Ltd., 1978a, Shell Research Ltd., 1978b, Shell Research Ltd., 1975b, Shell Research Ltd., 1978c, Shell Research Ltd., 1978d, Shell Research, Ltd., 1978e] and in the non-adjuvant Buehler protocol in guinea pigs [Hüls AG, 1997e, Shell Research Ltd., 1975b, Shell Research Ltd., 1972, Brown et al., 1970, Arthur D. Little, 1991]. Further results of skin sensitization studies are listed in a review conducted for the US soap and detergent industry [Arthur D. Little, 1991].

In summary, of 15 studies conducted on different AES batches and materials according to the Magnusson-Kligman protocol, 14 studies revealed no evidence for skin sensitization potential of AES and only 1 study resulted in a positive result, indicating weak sensitization potential of a tested AES batch. Of the available 8 Buehler studies, 6 studies did not indicate any skin sensitization potential of the tested AES batches and 2 studies resulted in a weak positive response. It must be noted that the majority of the available studies were not conducted according to the OECD guideline protocols, nor according GLP standards. Nevertheless, based on the limited information available, these studies appear to be scientifically well conducted and the results should be included in the overall evaluation. The studies reported in most detail will be discussed in the following paragraphs.

NaC12-14AE2S (28% active material) was evaluated in the Magnusson-Kligman guinea pig maximization test [Hüls AG, 1989] according to OECD method 406. In the induction phase, the treatment group was injected on day zero 3 pairs of 0.1ml volume (injection 1: a 1:1 mixture Freunds' complete adjuvant (FCA) and water; injection 2: 0.1% test substance in water; injection 3: 0.1% test substance in a 1: 1 mixture FCA) in the shoulder region of female guinea pigs. A week later, a patch containing 30% solution of the test substance was placed over the injection area for 48 hours in the treatment group. The control groups were treated in the same manner, but without the test substance (i.e., 3 injections on day 0 and patch application on day 7). Two weeks after the induction phase, the flanks of the treated and the control animals were cleared of hair and an occlusive 'challenge' patch containing 10% of the test substance (or water in case of the control group) was applied to one flank of the animals for 24 hours. Approximately 48 and 72 hours from the start of the challenge application, the skin reaction was observed and recorded according to the Magnusson-Kligman grading scale. Under the test conditions, NaC12-14AE2S did not cause skin sensitization in guinea pigs.

Further AES materials such as NaC12-14AE2S (27% active material) and a mixture of sodium laureth sulphate, sodium laureth-8 sulphate and sodium oleth sulphate (5-10EO, 29% active matter) were evaluated according the same protocol and were found to not cause skin sensitization in guinea pigs [Henkel KGaA, 1977a, Henkel KGaA, 1977b]. However, one batch of NaC12-15E3S caused a weak skin sensitization response [Henkel KGaA, 1985]. In this study, 20 animals were induced intradermally with a 0.25% aqueous solution of the test item and complete Freund' adjuvant. One week after, a an occluded patch containing 50% solution of the test substance was placed over the injection area for 48 hours. After a 14 day rest period, the test animals were challenged with an occluded patch containing a 20% solution of the test substance. 24 and 48 hours after removal of the challenge patch, dermal reactions (score 1) were seen in seven animals. A rechallenge was performed seven days later

by applying a 10% aqueous solution of the test substance on the flanks opposite to the treatment area. Two out of twenty animals displayed weak skin effects (score 1).

In a more recent study, the triisopranolammonium salt of C12-14AE2S was tested according the Buehler method in guinea pigs following OECD guidelines 406 and in compliance with GLP standards [Hüls AG, 1997e]. To determine the potential sensitizing effect of this test substance, 20 test animals and 10 control animals were tested with the highest readily tolerated concentration of the test substance, which led to slight to well-defined signs of irritation. A 50% strength formulation was used for treatment during induction phases I, II, and III and a 25% strength formulation of the test substance was administered as the highest non-irritant concentration during challenge. The challenge treatment did not cause any cutaneous reactions in the form of erythema or oedema on the posterior right flank of any treated animal in the test and control groups 30 and 54 hours after administration. Based on these results, the test material NH4C12-14E2S showed no sensitizing effect on guinea pigs under the described test conditions.

In 1966, skin sensitization associated with exposure to ethoxysulphates was reported in Norway. Walker et al., 1973 conducted a series of investigations to determine the source of this response and identified a contaminant in one particular AES batch shown to be the responsible sensitizing agent. Connor et al., 1975 identified the contaminant in AES to be 1-dodecene-1,3-sultone, 1 -tetradecene-1,3 sultone, 2-chloro-1,3 dodecene sultone and 2-chloro-1,3-tetradecene sultone. Connor et al. demonstrated that these sultones could be formed only under very specific, extreme AES manufacturing conditions. It became evident that the unsaturated and the chloro-sultones which are considered to be potent skin sensitizers were the result of conditions not normally present and readily avoidable in AES manufacture. The formation of sultones in the AES production is to date not an issue anymore. Presently, residual levels of unsaturated and chloro-sultones and their precursors are monitored in AES batches on a routine basis.

Conclusion

Taking a weight of evidence approach and considering quality criteria (i.e., compliance with OECD methods, GLP) in evaluating reliability of individual studies, AES are not considered to be a skin sensitizers. The vast majority of available guinea pig studies in which AES was tested for skin sensitization properties demonstrated the absence of skin sensitizing potential of AES. Only a few studies indicated a weak sensitization potential of AES, but it should be taken into consideration that observed reactions may have been confounded with irritation reactions.

4.2.2. Repeated Dose Toxicity

4.2.2.1. Oral route

NaC12-15AE3S was tested at doses of 0%, 0.023%, 0.047%, 0.094%, 0.188%, 0.375%, 0.75%, 1% and 1.5% in a 3-week dietary rat feeding study [Unilever, 1979a]. Three (3) animals per sex per dose and 6 animals of each sex in the control group were used. In summary, the organ most affected by the feeding of NaC12-15AE3S was the liver. No effects were observed in rats fed at 0.188% dietary level (254 mg/kg/body weight per day) and less. The lowest observed effect level, based on hepatocytic hypertrophy was 0.375% which is equivalent to 487 mg/kg body weight per day. Significantly increased organ weights (liver, kidney, brain) were observed in

males and females at doses equal (females) or higher (males and females) than the LOEL established for hepatocytic hypertrophy.

NH4C12-15E3S was tested at doses of 0%, 0.023%, 0.047%, 0.094%, 0.188%, 0.375%, 0.75%, 1% and 1.5% in a 3-week dietary rat feeding study [Unilever, 1979b]. Three (3) animals per sex per dose and 6 animals of each sex in the control group were used. In summary, the only organ affected by the feeding of NH4C12-15E3S was the liver. No effects were observed in rats fed at 0.188% dietary level (232 mg/kg/body weight per day) and less. The lowest observed effect level, based on significant increases in plasma alkaline phosphatase activity, was 0.375% which is equivalent to 465 mg/kg body weight per day. Significantly increased liver weight was observed in males and females at doses higher than the LOEL established for the change in some plasma enzyme levels.

NaC 12-15E3S containing 21.1% ethanol and 1.15% methanol (note: after mixing with the diet and storage for 3-4 days methanol was no longer detectable and more than 98% of remaining ethanol was evaporated) was tested at doses of 0%, 0.023%, 0.047%, 0.094%, 0.188%, 0.375%, 0.75%, 1% and 1.5% in a 3-week dietary rat feeding study [Unilever, 1980a]. Three (3) animals per sex per dose and 6 animals of each sex in the control group were used. In summary, the organ mostly affected by the feeding of NaC12-15E3S was the liver. No effects were observed in rats fed at 0.094% dietary level (108 mg/kg/body weight per day) and less. The lowest observed effect level, based on significant increases in plasma alkaline phosphatase activity, was 0.188% which is equivalent to 217 mg/kg body weight per day. Significantly increased liver weight was observed in males and females at doses equal (females) or higher (males and females) than the LOEL established for the change in some plasma enzyme levels.

NH4C13-15E3S was tested at doses of 0%, 0.023%, 0.047%, 0.094%, 0.188%, 0.375%, 0.75%, 1% and 1.5% in a 3-week dietary rat feeding study [Unilever, 1979c]. Three (3) animals per sex per dose and 6 animals of each sex in the control group were used. In summary, the organ mostly affected by the feeding of NH4C12-15E3S was the liver. No effects were observed in rats fed at 0.375% dietary level (461 mg/kg/body weight per day) and less. The lowest observed effect level, based on hepatocyte hypertrophy, was 0.75% which is equivalent to 857 mg/kg body weight per day. Significantly increased organ weights (liver, brain, testes) were observed in males and females at doses higher than the LOEL established for hepatocytic hypertrophy.

NaC12-14E3S was tested at doses of 0%, 0.023%, 0.047%, 0.094%, 0.188%, 0.375%, 0.75%, 1% and 1.5% in a 3-week dietary rat feeding study [Unilever, 1979d]. Three animals per sex per dose and six animals of each sex in the control group were used. In summary, the only organ affected by the feeding of NH4C12-15E3S was the liver. No effects were observed in rats fed at 0.094% dietary level (120 mg/kg/body weight per day) and less. The lowest observed effect level, based on increase in plasma levels of glutamic-pyruvic transaminase and alkaline phosphatase, was 0.188% which is equivalent to 236 mg/kg body weight per day. Significant changes in organ weights (liver, kidney, heart, adrenals) were observed in males and females at doses higher than the LOEL established for changes in plasma enzyme levels.

NaC16-18E4S was tested at doses of 0%, 0.023%, 0.047%, 0.094%, 0.188%, 0.375%, 0.75%, 1% and 1.5% in a 3-week dietary feeding study [Unilever, 1980b]. Three (3) animals per sex per dose and 6 animals of each sex in the control group were used. In summary, the organ mostly affected by the feeding of NH4C12-15E3S was the liver. No effects were observed in rats fed at 0.375% dietary level (468 mg/kg/body weight per day) and less. The lowest observed effect level, based on hepatocyte hypertrophy and increases in plasma levels of glutamic-pyruvic transaminase, was 0.75% which is equivalent to 969 mg/kg body weight per day.

Significant changes in organ weights (liver, kidney, heart) were observed in males and females at doses higher than the LOEL established for changes in plasma enzyme levels.

NaC12-15E3S was tested at doses of 0%, 0.023%, 0.047%, 0.094%, 0.188%, 0.375%, 0.75%, 1% and 1.5% in a 3-week dietary rat feeding study [Unilever, 1979e]. Three (3) animals per sex per dose and 6 animals of each sex in the control group were used. In summary, the organ mostly affected by the feeding of NH4C12-15E3S was the liver. No effects were observed in rats fed at 0.375% dietary level (441 mg/kg/body weight per day) and less. The lowest observed effect level, based on hepatocyte hypertrophy, was 0.75% which is equivalent to 872 mg/kg body weight per day. Significant changes in organ weights (liver, brain, heart, spleen) were observed in males and females at doses higher than the LOEL established for hepatocyte hypertrophy.

The Unilever studies summarized above were not conducted according to OECD and GLP guidelines. However, the methodology used was similar in many respects to OECD Guideline No. 407.

In a 28-day oral gavage rat study, a blend of alkyl (C14-18) sulphate and C12-13E6.5S was tested at 30, 100,300, and 1000 mg/kg/day [Shell Oil, 1992]. This blend caused irritation to the forestomach of the test animals, evidenced as hyperplasia and hyperkeratosis. Histologically, the hyperplasia appeared as a thickening of the non-glandular stomach epithelium at 100, 300, and 1000 mg/kg/day, but not at 30 mg/kg/day. Similar to the 90-day oral gavage study discussed above, the effects observed in forestomach are considered to be local treatment-related and concentration dependent irritant effects. Since there is no human equivalent to the rat forestomach, these effects are not considered to be relevant to human health assessment. No further information is available on this study and thus, a NOEL or NOAEL for systemic toxicity could not be established.

Synthetic NaC12-15AE3S and natural NaC12AE3S were tested in a 90-day rat diet study at dose levels of 0, 40 200, 1000 and 5000ppm active material [Walker, 1967]. Health, behaviour, body weight, food intake, haematological and urinary parameters remained within normal limits at all doses. Total serum protein was increased in males in the 5000ppm dose group of NaC12-15AE3S. Differences in absolute organ weights were observed at 5000ppm only. Both ethoxysulphates increased kidney weight in males. Liver weight was increased at 5000ppm in both sexes by NaC 12- 15AE3 S. Females receiving NaC 12AE3S showed increased liver, kidney and heart weights. A large variation was reported in male heart weights in rats receiving 1000ppm of NaC12-15AE3S, but the increase was not considered to be treatment related. No increase in heart weight was reported for males receiving 5000ppm. Similarly to the study by Butter-worth [Shell Research Ltd., 1982a], a NOEL or NOAEL was not established by the authors, but based on the available information and taking a conservative approach, the NOAEL could be established at the dose level of 1000ppm. The study was conducted prior to the development of GLP and OECD guidelines. However, the principles and the procedures were similar in various respects to the OECD test guidelines.

NaC12-15E3S was fed to rats at dietary concentrations of active ingredient of 0, 40, 200, 500, 1000 and 5000ppm in a 90-day oral feeding study [Shell Research Ltd., 1982a]. During the study, observations were made on the general health and behaviour, body weight and food intake of each rat. At necropsy, major organs were weighed and specified tissues examined histologically. Terminal blood samples were taken for haematological and clinical chemical examinations. All animals survived until their scheduled necropsy date. The general health and behaviour of control and treated rats were similar throughout the study. No significant change

was found in female body weights. Male body weights were significantly higher than controls at 500ppm from week 10 onwards and at 200ppm at weeks 11 and 13. At higher concentrations, there was no difference in body weights from the control values. Male and female liver weights significantly increased at 5000ppm. Absolute testes weights were increased at 5000ppm. However, no differences were observed when adjusted for terminal body weight. These increases were not accompanied by histological, clinical chemical or haematological changes and were therefore considered to be adaptive in nature and not a toxic effect of the compound. A NOEL or NOAEL was not indicated by the authors, but based on the available information and taking a conservative approach, the NOAEL is considered to be 1000ppm. It was not indicated in the report whether the study followed the principles of the OECD method 407 and was GLP compliant.

NaC12-14AE2S was tested for systemic toxicity at repeated doses by oral gavage of 0 (group 1), 25 (group 2), 75 (group 3), and 225 (group 4) mg/kg bodyweight [Henkel KGaA, 1994a]. The compound was administered by gavage over a period of 90 days. Ten (10) male and female rats were used for each dose. Five (5) male and female animals of groups 1, 3, and 4 were observed to determine the reversibility of possible compound-related alterations for 28-days after treatment. Four (4) animals died during the treatment period. The mortality of the animals was, however, considered to be incidental. Three (3) animals died due to experimental procedures such as anesthesia for blood sampling and the fourth animal was sacrificed due to a traumatic fracture of the mandibula. No systemic treatment-related effects were observed in any test group. The mean food and water consumption was not affected and the total body weight gain showed no deviations in all male and female test groups. Local treatment effects were only seen in the forestomach. The forestomach of the animals of group 4 showed some lesions such as a hyperplasia, submucosal oedema and chronic ulceration. In groups 2 and 3, 3 out of 10 animals showed small eosinophilic foci in the stratified epithelium of the forestomach. In conclusion, according to the study described, a daily administration of NaC 12- 14AE2S revealed no systemic toxicity but local treatment-related concentration dependant irritation to differing degrees in the forestomach in all main test groups 2 - 4. Thus, a NOEL-value was not determined. Since there is no human equivalent to the rat forestomach, these effects are not considered to be relevant to human health assessment. Looking at systemic toxicity, behavioural and clinical abnormalities and other general or specific toxic effects, a no adverse effect level (NOAEL) of 225 mg/kg could be established. The study followed the OECD guideline method 408. GLP compliance was not indicated in the study report.

No unusual fmdings regarding systemic toxicity were noted in a 2-year chronic feeding study in rats in which Cl2 AE3S was given at 0, 0.1 or 0.5% in the diet for 2 years. An occasional tumour (type and incidence unspecified) was found in various groups. The tumours were characterized as "typical" of those commonly found in aged rats and did not appear to be associated with the ingestion of AES [Tusing et al., 1962 quoted in Arthur D. Little, 199 1]. The results of this study suggests that the NOEL for C12AE3S in this 2-year chronic feeding study in rats was greater than 250 mg/kg bw/day. However, the information available is only very limited and thus only a low study reliability score can be assigned.

In a 2-year study, rats (20/sex/group) were administered C12AE3S in the drinking water at a concentration of 0.1% [Arthur D. Little, 1991]. At termination, survival, growth, food consumption, body weights, clinical laboratory findings, hematology and urinalyses were all comparable in control and treated animals. The only unusual finding was slight, but consistently higher water consumption by all rats receiving the test compound in their drinking water and a significant difference in the empty cecum to body weight ratio of females. Absolute organ weights were all comparable to controls and no consistent gross or histopathology was found.

Generally, pathological findings for controls and treated rats after 2 years were varied and consisted predominantly of incidental findings attributable to advanced age. Various types of benign and malignant tumours were found in both groups. The incidence and types of tumours observed in the treated group was similar to that of control animals. A NOEL greater than 75 mg/kg bw/day (equals a dose of 0.1% in drinking water) can be estimated on the basis of the available information.

A few more repeated oral toxicity studies on AES or AES containing formulations are published elsewhere [Arthur D. Little, 1991]. Detailed study descriptions for these studies were not available, but taking the summaries into account these studies appear to confirm the data and information presented in this chapter.

4.2.2.2. Inhalation

Long-term inhalation studies on AES are not available.

4.2.2.3. Dermal route

Subchronic percutaneous toxicity studies were conducted on 2 liquid dishwashing detergents containing anionic surfactant C12-14AES (detergent A: 23%; detergent B: 27%), C12-14 alkyl sulphate (detergent A: 5%; detergent B: 0%), C12-14 alkylamine oxide (detergent A: 3%; detergent B: 5%), ethanol (detergent A: 5%; detergent B: 7%) and water (balance). The detergents were administered dermally to the shaved backs of rabbits (10 animals per group; 5 of each sex) at concentrations of 0, 0.5, 1 .O, and 2.5% in distilled water for 6 hr/day, 5 days/week for a total of 65 treatments (91 days). The dose selection was based on the local irritation effects observed in a 14-day pilot study conducted with each detergent. No adverse systemic effects were observed by assessment of haematological parameters or by gross or microscopic tissue examination. Transient slight to moderate dermal irritation at the detergent application site was observed with detergent A. Slight to moderate dermal irritation confined to the detergent application site was noted in the detergent B study [Petersen, 1988].

No further studies investigating the toxicity of AES, other than irritation, after repeated exposure via the dermal route were available.

Table 1 - Summary table of the repeated dose toxicity tests with AES

Animal	Route	Duration	Test Material	Estimated NOEL*	Doses	Reference
Rat	Drinking water	2 years	C12AE3S	>75 mg/kg/d** (0.1%)	0.1%	Arthur D. Little, 1991
Rat	Oral feeding	2 years	C12AE3S	250 mg/kg/d*** (0.5%)	0, 0.1, 0.5%	Arthur D. Little, 1991
Rat	Oral gavage	90 days	NaC12-14AE2S	225 mg/kg/day for systemic toxicity; (local effects in forestomach at all doses)	25, 75, 225 mg/kg/day	Henkel KGaA, 1994a

Rat	Oral feeding	90 days	NaC12-15E3S	50 mg/kg/d*** (1000ppm)	40, 200, 500, 1000, 5000ppm	Shell Research Ltd., 1982a
Rat	Oral feeding	90 days	C12-15E3S C12E3S	50 mg/kg/d*** (1000ppm)	40, 200, 500, 1000, 5000 ppm	Walker, 1967
Rabbits	Dermal	90 days	2 hand dish detergents containing AES at levels of 23 and 27%	> 12.5 mg/kg/d	0, 0.5%, 1%, 2.5%	Petersen, 1988
Rat	Oral gavage	28 days	Blend of C14-18S and C12-13E6.5S		30, 100,300, 1000 mg/kg bwld	Shell Oil, 1992
Rat	Oral feeding	21 days	NaC12-15E3S	254 mg/kg bw/d (0.188%)	0.023%, 0.047%, 0.094%, 0.188%, 0.375%, 0.75%, 1%, 1.5%	Unilever, 1979a
Rat	Oral feeding	21 days	NH4C12-15E3S	232 mg/kg bw/d (0.188%)	0.023%, 0.047%, 0.094%, 0.188%, 0.375%, 0.75%, 1%, 1.5%	Unilever, 1979b
Rat	Oral feeding	21 days	NaC12-15E3S cont. alcohol	108 mg/kg bw/d (0.094%)	0.023%, 0.047%, 0.094%, 0.188%, 0.375%, 0.75%, 1%, 1.5%	Unilever, 1980a
Rat	Oral feeding	21 days	NH4C13-15E3S	461 mg/kg bwld (0.375%)	0.023%, 0.047%, 0.094%, 0.188%, 0.375%, 0.75%, 1%, 1.5%	Unilever, 1979c
Rat	Oral feeding	21 days	NaC12-14E3S	120 mg/kg bwld (0.094%)	0.023%, 0.047%, 0.094%,	Unilever, 1979d

					0.188%, 0.375%, 0.75%, 1%, 1.5%	
Rat	Oral feeding	21 days	NH4C16-18E4S	468 mg/kg bw/d (0.375%)	0.023%, 0.047%, 0.094%, 0.188%, 0.375%, 0.75%, 1%, 1.5%	Unilever, 1980b
Rat	Oral feeding	21 days	NaC12-15E3S	441 mg/kg bwld (0.375%)	0.023%, 0.047%, 0.094%, 0.188%, 0.375%, 0.75%, 1%, 1.5%	Unilever, 1979e

* NOELs were not expressed in the original study reports, but estimated. based on the available information

** estimated based on the assumption of a mean adult rat body weight of 0.4kg and a water consumption of 30ml/day [US Environmental Protection Agency, 1978]

*** estimated based on the assumption of a mean adult rat body weight of 0.4kg and a food consumption of 20g per day (lppm in food equals 0.05 mg/kg/day) [US Environmental Protection Agency, 1978]

Conclusion

The available oral repeated dose toxicity studies provide a coherent picture on the subacute, subchronic and chronic oral toxicity of AES. In 2 chronic toxicity studies investigating carcinogenicity of AES and four subchronic toxicity studies (3 oral studies with AES, 1 dermal study with AES containing dishwashing liquids), no adverse effects, behavioral or clinical abnormalities of AES were observed up to a dose level of 250 mg/kg body weight per day.

In the subchronic oral gavage study, local treatment related effects were observed in the forestomach of the test animals. These effects can be explained by the irritating nature of the test solutions on the epithelium of the forestomach after repeated administration under the conditions of oral gavage. This is considered to be a response secondary to the irritant properties of AES and specific to the administration procedure. A similar response was not observed when the test material was administered via the diet. Administration via oral gavage is not considered to be relevant for humans because this exposure route is an unlikely scenario for human exposure. Also, there is no equivalent in man to the rat forestomach.

In the subchronic oral feeding studies with AES, general health, body weight and food intake remained within normal limits up to the highest tested dose of 250 mg/kg bw/day, but increased organ weights (liver, kidney) were determined in the highest dose group (250 mg/kg bw/day) of the 2 subchronic oral feeding studies. These increases were unaccompanied by

histological changes and are considered to be of an adaptive nature rather than a toxic effect of the test article. The dose level of 250 mg/kg/day is considered to represent a NOAEL.

In a series of 21-day oral feeding studies various AES were evaluated for their repeated dose toxicity. The no observed effect levels derived from these toxicity studies ranged from 108 – 460 mg/kg body weight per day. The organ mostly affected in these studies was the liver, expressed by increased liver weight at high doses, hepatic hypertrophy and occasionally changes in biochemical parameters such as increase of enzyme levels in plasma, generally at levels higher than 250 mg/kg bw/day. Significant increases in weight were also observed in other organs (e.g. kidney, heart, brain) in some of these studies, but only at doses higher than LOELs established for above mentioned liver parameters. With regard to these information, it must be noted that care should be taken in the interpretation due to the low number of animals in the dose groups and the limited information available on the studies. It was considered that, in particular, the observations at dose levels below 250 mg/kg bw/day were not adverse in nature. This evaluation takes into account that at approximately the same dose levels, no adverse effects were seen in the above mentioned subchronic and chronic toxicity studies.

From the available repeated toxicity studies, only the 90-day oral gavage study with NaC12-14AE2S and the 90-day oral feeding study were indicated to be in compliance with the OECD method 407 and GLP regulations and should be considered as most reliable [Henkel KGaA, 1994a, Shell Research Ltd., 1982a]. Although none of the other studies fully complied with the principles of OECD method 407 or indicated compliance with GLP regulations, their results were consistent with the most reliable studies. In particular, the chronic rat drinking water study and the 2nd rat oral feeding study were conducted following principles and procedures similar to those of OECD method 407 and thus, should be regarded as suitable for inclusion in a weight of evidence approach to evaluating the toxicity of AES.

4.2.3. Genetic Toxicity

4.2.3.1. In Vitro

Bacterial tests

Several alcohol ethoxysulphates were assessed for their potential to induce reverse mutations in the presence and absence of a metabolic activation system in an *in vitro* bacterial system, the so-called Ames test [Hüls AG, 1996; Hüls AG, 1994 ; Henkel KGaA, 1988; Shell Research, 1980b].

Representing the whole range of studies, a recent OECD method 471 and GLP compliant study [Hüls AG, 1996] should be mentioned at this place: In this study, *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA 1537 were treated with the triisopranolammonium salt of C 12-14AE2S in the Ames test plate incorporation assay as well as the preincubation method. Dose levels covering the range of 1 to 5000 @plate, in triplicate both with and without the addition of a metabolizing system (Aroclor 1254 induced rat liver S9 mix) were employed. All 4 bacterial strains exhibited mutagenic responses to the appropriate positive control substances. Solvent controls were also tested with each strain and the mean numbers of spontaneous revertants were in an acceptable range. Mutagenic activity of the test compound to any of the tester strains was not observed with and without metabolic activation. It was therefore concluded that under the chosen test conditions, the triisopranolammonium salt of Cl 2-14AE2S is not a bacterial mutagen.

The majority of the studies evaluated the mutagenicity of AES in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA 1537 and TA 1538. One study [Shell Research, 1980b], however, evaluated the mutagenicity of NaC 12- 15E3S in presence and absence of a metabolic activation system in the *Escherichia coli* strains WP2 and WP2uvrA, in addition to the *Salmonella typhimurium* strains. Also, in these E. coli strains, the tested AES compounds were not mutagenic under the test conditions.

In all tested systems, AES were not found to be mutagenic to bacterial systems.

Non bacterial tests

The mutagenic activity of NaC12-15AE3S was further evaluated in a Saccharomyces gene conversion assay [Shell Research, 1980b]. In this study, it was concluded that the addition of NaC12-15AE3S to liquid suspension cultures of *Saccharomyces cerevisiae* JD1 with or without metabolic activation did not induce a consistent increase in mitotic gene conversion at either gene locus in two replicate experiments.

AES was examined for mutagenic activity by assaying for the induction of trifluorothymidine resistant mutants in L5 178Y TK+/- mouse lymphoma cells after *in vitro* treatment in the absence and presence of S9 metabolic activation [Research Toxicology Centre S.p.A., 1995]. Under the reported experimental conditions, it was concluded that in the presence and absence of metabolic activation, the test material NaC12-14AE2S did not induce gene mutations in L5 178Y TK+/- mouse lymphoma cells. This study was conducted in compliance with OECD method 476 and GLP regulations.

The ability of NaC 12-15E3S to induce chromatid and chromosome aberrations was studied in rat liver cells [Shell Research, 1980b]. In slide cultures of rat liver cells exposed to culture medium containing NaC12-15E3S at concentrations of 25, 50 and 100 µg/ml the frequency of chromatid and chromosome aberrations did not differ significantly from that of the controls cultures.

No morphological cell transformations were observed in Syrian golden hamster embryo cells exposed in culture to concentrations up to 50 mg/ml C12-13E2.5S [Inoue et al., 1980].

In an *in vitro* transformation study with NaC12-15E3S [Shell Research Ltd., 1983], the transforming activities of NaC12-15E3S and 1,4-dioxane were determined using cultured C3H 10T1/2 mouse embryo fibroblasts as the target cell population. Monolayer cell cultures were incubated for 24 hours in growth medium containing NaC12-15E3S or 1 A-dioxane. Transformation frequencies were assessed by counting the number of actively dividing, darkly stained cell foci per dish, 3 or 4 weeks after test compound treatment. In conclusion, there was no evidence to suggest that either NaC 12-15E3S or 1,4-dioxane increased the frequency of 1 0T1/2 mouse embryo fibroblasts under the experimental conditions described.

4.2.3.2. In Vivo

NaC12-15E3S has been evaluated in an alkaline elution assay [Shell Research Ltd., 1982b]. In this screen which aims to measure DNA single-strand breaks induced in DNA by reaction with electrophiles, NaC12-15E3S did not cause measurable DNA-strand damage when administered to Wistar rats as a single oral dose of 2.5 ml/kg (equals about half of the LD50 of NaC12-15E3S) for an exposure period of 6 hours. Based on this result it was concluded

that neither NaC12-15E3S nor its *in situ* generated metabolites have any effect upon the integrity of rat liver DNA in *vivo* under the conditions of the test.

In a series of studies with a 55% AES:45% LAS mixture, no significant differences from control values were noted in a dominant lethal study or in *vivo* or *in vitro* cytogenicity studies [Arthur D. Little, 1991]. In the dominant lethal assay, male mice were orally administered either 100, 150, or 200 mg/kg subacutely or 500, 750, or 1000 mg/kg acutely of the surfactant mixture. No significant differences from water-dosed controls were observed in the mutagenic index. Similarly, no significant differences in chromosomal anomalies were found in bone marrow cells of male rats given 40, 500, or 1000 mg/kg of the surfactant mixture orally, then killed 18, 24 or 48 hours post-dosing. Likewise, human leukocytes incubated for 18, 24, or 48 hours with 4, 40 or 200 µg/l of the surfactant mixture exhibited no increased incidence of chromosomal anomalies above the water control group.

Another published *in vivo* study indicated that AES is not clastogenic. Hope [Hope, 1977] reported that the incorporation of C 12- 1 5AES into the diet of rats at a maximum tolerated dose (1.13% active ingredient) for 90 days had no effect on the chromosome of rat bone marrow cells.

Conclusion

A structure activity analysis did not reveal any functional groups in the chemical structure of AES that were associated with mutagenic or genotoxic properties. In all available *in vitro* and *in vivo* genotoxicity assays, there is no indication of genetic toxicity of AES. Only 2 studies, an Ames test [Hüls AG, 1997f] and a mouse lymphoma assay [Research Toxicology Centre S.p.A., 1995] were conducted according to OECD guideline methodologies and GLP regulations. However, all the other available *in vitro* and *in vivo* studies appear to be well documented and conducted. Some of these studies were published in peer-reviewed journals. Based on the presented data, it is therefore concluded that there is no evidence that AES are either mutagenic or genotoxic.

4.2.4. Carcinogenicity

In a 2-year study, rats (20/sex/group) were administered C12AE3S in the drinking water at a concentration of 0.1%. At termination, survival, growth, food consumption, body weights, clinical laboratory findings, haematology and urinalyses were all comparable in control and treated animals. The only unusual findings were slight, but consistently higher water consumption by all rats receiving the test compound in their drinking water and a significant difference in the empty cecum to body weight ratio of females. Absolute organ weights were all comparable to controls and no consistent gross or histopathology was found. Generally, pathological findings for controls and treated rats after two years on test were varied and consisted predominantly of incidental findings attributable to advanced age. Various types of benign and malignant tumors were found in both groups. The frequency of tumours in the treated group was not significantly different from that of control animals [Arthur D. Little, 1991].

No indications of an increased incidence in tumours were noted in a 2-year chronic feeding study in rats in which Cl2 AE3S was given at 0, 0.1 or 0.5% in the diet for 2 years. An occasional tumour (type and incidence unspecified) was found in various groups. The tumours were characterized as "typical" of those commonly found in aged rats and did not appear to be associated with the ingestion of AES [Tusing et al., 1962 quoted in Arthur D. Little, 1991].

An 5% aqueous solution of C12E3S (O.lml) was applied twice weekly on the skin of 30 female Swiss mice [Tusing et al., 1962 quoted in Arthur D. Little, 1991]. No papillomas or other tumours were found under these exposure conditions.

In its report to the Soap and Detergent industry [Arthur D. Little, 1991], Arthur D. Little reported on a study in which an aqueous solution of 18.5% C16-18AES and 15.6% LAS was applied 3 times a week on the skin of Swiss ICR mice for 18 months. Under these conditions, the test solutions did not induce any carcinogenic response either on the skin or systemically.

Conclusion

The available oral and dermal long term toxicity/carcinogenicity studies, even if not performed according to accepted guidelines for carcinogenicity bioassays, appear to be conducted and documented in an acceptable manner. It is therefore concluded that there is sufficient evidence that AES is not carcinogenic in the tested species under the conditions described.

4.2.5. Reproductive toxicity

As part of a chronic feeding study, 10 rats/sex/group fed diets containing 0.1% of C12AES were mated after 14 weeks on the test [Arthur D. Little, 1991]. The F1 generation was maintained on the parental diet and mated at 100 days of age. The F2 generation was fed the same diet for 5 weeks, and then killed. No adverse effects on fertility, lactation, litter size or survival and growth of the offspring were seen. Haematological, biochemical and histopathological findings were comparable to controls. From this study it can be concluded that the NOEL for reproductive toxicity is estimated to be greater than 50 mg/kg bw/day. This estimation was based on the assumption of a mean adult rat body weight of 0.4kg and a water consumptions of 30 ml/day [US Environmental Protection Agency, 1978].

No adverse parental toxicity or significant differences in either litter parameters or viabilityof offspring were noted in two generations of rats fed diets containing either 0.1% C12AES [Tusing et al., 1962] or 1% (reported to equal an exposure of 800 mg/kg/day) of a detergent formulation containing 55%TE3S and 45% LAS [Nolen, et al., 1975].

In available subchronic [Henkel KGaA, 1994a, Shell Research Ltd., 1982a, Walker, 1967] and chronic toxicity studies [Arthur D. Little, 1991, Hiils AG, 1997b] on various AES (NaC12-14AE2S, CaC123-15AE3S, C12AE3S), the primary sex organs of the males and females did not show evidence for treatment-related adverse effects as indicated by organ weight differences, gross examination, and microscopic histology examination at the highest tested exposure levels of 250 mg/kg bw/day.

Further information can be deduced from a two-generation reproduction study with NaC12-14AE2S [Henkel 1999]. This GLP-study followed the OECD guideline method 416. Four groups of thirty male and thirty female Sprague Dawley rats (strain Crl:CD(SD)BR) (FO generation) were dosed via the drinking water. Concentrations used were 0 (control), 0.03, 0. 1 and 0.3 %, which corresponded to daily doses of ca. 0, 30, 100 and 300 mg/kg/day.

There were some changes indicative of parental toxicity in the group treated with 0.3 % of the test substance, which were characterised by reduced straight line velocity of the sperm. The observed reduced triglyceride levels (female) and increased percentage neutrophil counts (males) were slight and within the range of the historical control data. There was evidence of

toxicity on pup development at this dose level that was characterised by an increase in the time taken for sexual development of the male (not significant) and female (significant) offspring. No other developmental parameters were affected.

There were some changes seen in reduced straight line velocity of the sperm, reduced tigylceride levels (female) and increased percentage neutrophil counts (males) in the group treated at 0.1 %. All the changes were either not statistically significant or within the range of the historical control data. There was no evidence of toxicity on pup development.

There was no evidence of toxicity on pup development in the group treated with 0.03 %.

Decreased liver weights of the FO and F1 male dose groups were observed which was not confirmed in the F2 generation dose group.

The male FO generation showed a small but significant reduction in bodyweight-liver weight ratios, but the corresponding brain related liver weights and the absolute liver weights developed not in a dose dependant way. For the F1 generation where similar results were reported, no dose-response relationship was detected either. No influence on liver weight development was seen in the F2 generation. None of the groups revealed any histopathological or clinical-chemical findings, which could be attributed to hepatotoxicity. This led to the conclusion that this untypical liver weight reduction was of no toxicological relevance, additionally underlined by the absence of such effects in the studies for subchronic toxicity mentioned above.

In summary, there was no effect of treatment at any dose level on reproduction of the parents or offspring (NOAEL > 3 %; > 300 mg/kg/day)

Based on this study an overall NOAEL for systemic effects of 0.1 % (86.6 mg/kg bw) for the FO generation and a NOAEL of 0.1 % (149.5 mg/kg bw) for the F1 generation can be deduced.

Conclusion

Alcohol ethoxysulphates were evaluated for reproductive effects in rats. The key study (Henkel, 1999) fulfilled OECD guideline protocols and was conducted according to GLP standards. No information on the guidelines and GLP was available for another reproduction study that was cited in the scientific literature [Arthur D. Little, 1991]. AES did not adversely affect reproduction in the rat and the NOAEL for reproductive effects was > 300 mg/kg; slight systemic effects were observed in the parental and F1 generation with a NOAEL of 86 and 149 mg/kg, respectively.

4.2.6. Developmental Toxicity / Teratogenicity

4.2.6.1. Oral route

NaC12-14AE2S was tested in a segment II embryotoxicity study [Henkel KGaA, 1994b]. The purpose of the study was to assess the effects of orally administered NaC12-14AE2S on embryonic and foetal development in pregnant CD-rats. The study followed the guidelines of OECD method 414 "Teratogenicity" and complied with the OECD principles of GLP. In this study, NaC12-14AE2S was administered orally by gavage at dose levels of 0, 100, 300, and 1000 mg/kg body weight once daily from day 6 to day 15 of gestation. Each group consisted

of at least 24 female rats. A standard dose volume of 10 ml/kg body weight was used and the control animals were dosed with the vehicle alone over the period described. Clinical condition and reaction to treatment were recorded at least once daily. Body weights were reported for days 0, 6, 16 and 20 of gestation. All surviving females were sacrificed on day 20 of gestation and the foetuses were removed by caesarean section. At necropsy the females were examined macroscopically and live foetuses were weighed, sexed and examined for visceral and skeletal abnormalities. In summary, the results of the study showed that repeated oral administration (day 6 – day 15 post coitum) of NaC12-14AE2S to pregnant rats did not cause symptoms of cumulative toxicity up to a dose level of 1000 mg/kg/day. No compound-related symptoms were observed and no treatment-related abnormalities were found at necropsy of the females. All females had viable foetuses. Pre-implantation loss, post-implementation loss, mean number of resorptions, embryonic deaths, total foetuses, mean foetal placental and uterus weights were not affected by the treatment. Foetal sex ratio was comparable in all groups. There were no treatment-related foetal abnormalities at necropsy and no treatment-related effects in the reproduction data. In conclusion, in the described embryotoxicity study, NaC 12-14AE2S was not cumulatively toxic to pregnant rats and did not reveal any teratogenic potential at the tested dose levels. Thus, based on the available information, the NOAEL for teratogenicity and developmental toxicity are assessed to be greater than 1000 mg/kg bw/day.

NaC12-15AE3S was administered orally by gavage to pregnant Colworth-Wistar rats at dose levels of 0, 375 and 750 mg/kg/day once daily from day 6 to 15 of gestation [Unilever, 1980c]. Two different samples of the test material were tested. Fifteen (15) animals were used per dose group, 10 for dissection and 5 for natural parturition. Throughout the study, the females were monitored for signs of toxicity. Upon necropsy, fetal toxicity was determined by evaluating pre-implantation and post-implantation fetal loss and fetal weight. Fetuses were evaluated for externally visible malformations, as well as malformations of the internal organs and skeleton. In the post-partum phase pup mortalities, body weights and litter size as well as incidence of external and gross visceral and skeletal defects were monitored until weaning day 21. The resulting data were compared to the control group. In summary, NaC12-15AE3S induced maternal toxicity, indicated by body weight changes and other clinical and behavioural observations, when administered by gavage to pregnant rats at doses of 750 mg/kg. The authors were unable to detect any specific abnormality which would indicate a developmental toxicity or teratogenic response related to the treatment. This study was not conducted according to any recognized guideline. However, the study was conducted according to GLP, is well-documented and judged to be scientifically acceptable. Based on the available information the NOAEL for maternal toxicity was estimated to be 375 mg/kg bw/day and the NOAEL for teratogenic effects or developmental toxicity is greater than 750 mg/kg bw/day.

NH4C13-15AE3S was administered orally by gavage to pregnant Colworth-Wistar rats at dose levels of 0, 63, 125, 250 and 500 mg/kg/day once daily from day 6 to 15 of gestation [Unilever, 1986a]. Fifteen (15) animals were used per dose group, 10 for dissection and 5 for natural parturition. No detailed information was available on the study design. Some slight maternal toxicity indicated by body weight changes and other clinical observations (e.g. diarrhoea, respiratory wheeziness) was seen in rats with exposure to 250 and 500 mg/kg bw/day, but given the limited information available, there is some uncertainty regarding the severity of these effects. No evidence of developmental toxicity or a teratogenic response to the treatment were reported at any dose level. This study was not conducted according to GLP

or according to any recognized guideline. Given the lack of information and the uncertainty mentioned before, a NOAEL could not be reliably determined.

NaC12-14AE3S was administered orally by gavage to pregnant Colworth-Wistar rats at dose levels of 0, 93, 187, 375 and 750 mg/kg/day once daily from day 6 to 15 of gestation [Unilever, 1986b]. Fifteen (15) animals were used per dose group, 10 for dissection and 5 for natural parturition. Maternal and foetus effects were evaluated as described previously (i.e study with NaC12-15AE3S). The treatment of pregnant rats with NaC12-14AE3S during days 6-15 of gestation did induce some maternal toxicity at the dose level of 750 mg/kg bw/day. No evidence of treatment-related teratogenic effects or developmental toxicity was reported. This study was not conducted according to GLP or according to any recognized guideline. However, the study appeared well-conducted, was well-documented and judged to be scientifically acceptable. Based on the available information the NOAEL for maternal toxicity was determined to be 375 mg/kg bw/day and the NOAEL for teratogenic or developmental effects is estimated to be greater than 750 mg/kg bw/day.

NaC16-18AE4S was administered orally by gavage to pregnant Colworth-Wistar rats at dose levels of 0, 63, 125, 250 and 500 mg/kg/day once daily from day 6 to 15 of gestation [Unilever, 1986c]. Twenty (20) animals were used per dose group, 15 for dissection and 5 for natural parturition. Forty (40) animals were used for the negative control. Maternal, foetus and post-partum effects were evaluated as described previously (i.e study with NaC12-15AE3S). In summary, there was no evidence of teratogenic potential or developmental toxicity. This study was not conducted according to any recognized guideline. The study was conducted according to GLP, is well-documented and judged to be scientifically acceptable. Based on the available information, the NOAEL for both maternal toxicity, teratogenic and developmental effects appeared to be greater than 500 mg/kg bw/day.

In a last study of this series, NaC12-15E3S was administered orally by gavage to pregnant Colworth-Wistar rats at dose levels of 0, 125,250, 500 and 1000 mg/kg/day once daily from day 6 to 15 of gestation [Unilever, 1979f]. Fifteen (15) animals were used per dose group, 10 for dissection and 5 for natural parturition. Maternal, foetus and post-partum effects were evaluated as described previously. The authors of the study concluded that a degree of maternal toxicity indicated by a significant reduction in body weight gain of NaC12-15E3S was observed at the highest dose level of 1000 mg/kg. However, no evidence of treatment-related developmental toxicity or teratogenic effects was detected. This study was not conducted in compliance with GLP or according to any recognized guideline. The study appeared well-conducted, was well-documented and judged to be scientifically acceptable.

Pregnant rats were administered 50, 100, and 500 mg/kg/day of C12-13AES by oral gavage on days 6-l 5 of gestation. Effects observed were a decrease in maternal body weight gain and food consumption [Arthur D. Little, 1991]. There were no treatment-related maternal effects noted at necropsy or following a uterine examination on day 13 of gestation. The incidence of foetal malformations in AES-treated groups was not different from the control group.

Several investigators have studied the effects of administering a commercial liquid detergent formulation containing both AES and LAS to pregnant mice, rats and rabbits [Iseki, 1972; Nolen, et al., 1975; Palmer, et al., 1975]. Except at dosage levels which were toxic to the dams, no significant differences in the litter parameters of laboratory animals compared to control values were noted in these studies. Levels up to 300 mg/kg of a mixture containing 55% TE3S and 45% LAS given orally to rabbits on days 2-16 of gestation up to 800 mg/kg given to rats on days 6-15 of gestation gave no indications of any embryotoxic or teratogenic

effects attributable to AES [Nolen, et al., 1975]. In these exploratory investigations, there were no indications that detergent formulations containing AES at doses which are several orders of magnitude above possible human exposure levels posed any teratogenic hazard to laboratory animals.

4.2.6.2. Dermal route

There are no studies available that examined the teratogenicity and developmental toxicity of AES after dermal exposure.

Conclusion

Alcohol ethoxysulphates were evaluated for teratogenic or embryotoxic effects mainly in rats, but in a few investigations also in mice and rabbits. Although the majority of these studies did not fulfill all requirements of existing guideline protocols and were not conducted according to GLP standards, the studies appeared to be well conducted and documented. The following sentence doesn't make sense. Noteworthy is the segment II embryotoxicity study [Henkel KGaA, 1994b] which followed OECD guidelines and complied with the OECD principles of GLP. In this study which which was rated to be reliable without limitations according to the Klimisch criteria [Klimisch et al., 1997], AES showed no cumulative toxicity in pregnant rats and did not reveal any embryotoxic or teratogenic potential at the highest tested dose levels of 1000 mg/kg body weight.

The absence of a teratogenic potential and developmental toxicity of AES was confirmed in a series of teratology screening studies [Unilever, 1979f]. Although there were limitations in the design of the study, in particular with regard to the size of the dose groups and the absence of some clinical/biochemical parameters, the overall quality of these studies is judged to be appropriate and scientifically valid.

Based on the presented information, it is concluded that there is sufficient evidence that AES is not teratogenic or a developmental toxicant under the conditions described. A NOAEL greater than 1000 mg/kg bw/day can be estimated for teratogenicity and embryotoxicity on the basis of the segment II embryotoxicity study which is judged to be of highest reliability. The NOAEL for developmental toxicity appears to be greater than 750 mg/kg bw/day.

4.2.7. Biokinetics

McDermott et al. (1975) studied the absorption of C 16AE3S and C 16AE9S, labelled with ^{14}C in the l-position of the alkyl chain, after oral exposure in man and rats. Seventy-two hours after administration of C16AE3S, radioactive material was mainly excreted via urine (man: 80%; rat: 50%) and to a lesser extent via faeces (man: 9%; rat: 26%) and air (man: 7%; rat: 12%). For C16AE9S however, the radioactivity was mainly excreted via faeces (man: 75%; rat: 82%) and to a lesser extend via urine (man: 4%; rat: 0.6%) and air (man: 6%; rat: 4%). The length of the ethoxylate portion of an AES molecule appears to determine the metabolic fate of the compound following oral administration in both man and rat. There was no evidence of hydrolysis of the sulphate group or of metabolism of the ethoxylate portion of the molecule. The major metabolite found in urine had the following structure: $-OOCCH_2(OCH_2CH_2)_xOSO_3^-$ where x equals either 3 or 9, respectively [McDermott et al., 1975].

In a similar investigation, Taylor et al. (1978) studied the metabolic fate of orally, intraperitoneally or intravenously administered ^{14}C-C1 1AE3S and ^{14}C-C12AE3S in the rat. The

authors observed that both compounds were extensively metabolized (ω, β oxidation) with the proportion of radioactivity appearing in urine and respired air generally independent of the route of administration. Some sex differences in the proportions of radioactivity excreted in urine and respired air was seen, but total recoveries for both compounds were comparable. By the oral route, 67% of the administered radioactivity with Cl 1AE3S appeared in the urine of male rats compared to 45% in females; expired air contained 19% and 35% of administered radioactivity respectively; 4-5% was present in faeces for both sexes. The major urinary metabolite of C 12AE3S was identified as 2-(triethoxy sulphate) acetic acid, with C 1 1AE3S, the major urinary metabolite was tentatively identified as 3-(triethoxysulfate) propionic acid.

Taylor et al. (1978) measured the percutaneous absorption of ^{14}C-labelled NaC12AE3S. The NaC12AE3S was applied to rats as 150 µl of a 1% v/v solution. The ^{14}C-levels were measured in urine collected over 48 hours. Penetration of NaC12AE3S was 0.39 +/- 0.12 µg/cm^2. In experiments in which application was continued for up to 20 minutes, skin penetration was proportional to the duration of the contact. It was also proportional to the number of applications.

Conclusion

Following oral exposure, AES is readily absorbed in the gastrointestinal tract in man and rat and excreted principally via the urine. The length of the ethoxylate portion in an AES molecule seem to have an important impact on the biokinetics of AES in humans and in the rat. Alcohol ethoxysulphates with longer ethoxylate chains (>7-9 EO units) are excreted at a higher proportion in the faeces. Once absorbed, AES is extensively metabolized by beta- or omega oxidation.

The dermal absorption of AES is relatively poor as can be expected from an ionic molecule. The percutaneous absorption of C12AE3S was measured in a rat *in vivo* study. The study determined a dermal flux of the tested compound of 0.0163 µg/cm^2/h.

4.2.8. Experience from human exposure

Allergic contact sensitisation:

Over the years very many formulations containing a variety of AES concentrations are reported to have been tested in Human Repeat Insult Patch tests (HRIPT) failing to show evidence of contact sensitisation (see, e.g., [Nusair TL et al., 19881). Available detailed examples include two HRIPTs reported as follows:

In one test [Procter & Gamble, 1998], 102 volunteers were treated with patches of a 0.05% (w/v) aqueous solution of a detergent formulation containing 37% AES (Na AE1.4S, CAS# 68585-34-2). The patches were applied on the upper arms, under fully occlusive conditions. Test material was applied for 24 hours, 3 times a week, for 3 weeks during the induction period. After a 14-17-day rest, a 24-hour challenge patch was applied on the original and alternate arm sites. There was no evidence of skin sensitisation in any of the 102 subjects who completed the test.

In another test [Procter & Gamble, 1994], 87 volunteers were treated with patches of a 0.2% (w/v) aqueous solution of a formulation containing 6% AES (Na AE3S, CAS# 68585-34-2). The patches were applied on the upper arms, under fully occlusive conditions. Test material was applied for 24 hours, 3 times a week at the same skin site, for 3 weeks during the induction

period. After a 14-17-day rest, a 24-hour challenge patch was applied on the original and alternate arm sites. There was no evidence of skin sensitisation in any of the 87 subjects who completed the test.

Skin irritation

The cumulative skin irritation effects of formulations containing AES have been investigated in six separate "24-hour Repeat Application Patch Test" studies [Procter & Gamble, 2000a]; [Procter & Gamble, 20013; [Procter & Gamble, 2000b]; [Procter & Gamble, 2000c] [Procter & Gamble, 2000d], [Procter & Gamble, 2000e]. In each study 12 volunteers were treated with patches of a 0.1% (w/v) aqueous solution of detergent formulations containing AES (Na AES CAS# 68585-34-2). The patches were applied on the upper arms, under fully occlusive conditions. Test material was applied for 24 hours, 3 times a week at the same skin site, for a total of one week. After the end of each 24 hour application period, the skin was graded for irritation according to a 0 – 4 scoring scale. A total of 12 different detergent formulations were tested with the following AES concentrations (% w/v): 11, 13, 16, 18, 19, 20. A total of 72 volunteers were tested. All the formulations tested resulted in cumulative average skin irritation scores lower than 0.8 (they ranged between 0.05 and 0.79), which corresponds to a very mild effect.

In a separate, similar study the cumulative irritancy potential of a detergent formulation containing 11.4% (w/v) AES (Na AES CAS# 68891-38-3) was investigated under open (non-occlusive) conditions [Procter & Gamble, 2001]. A total of 12 volunteers were treated with 0.3 ml of undiluted, 30% (w/v), and 10% (w/v) aqueous dilutions of the detergent formulation, which were applied on an open application patch on the upper arms. Test materials were applied for 24 hours, 3 times a week at the same skin site, for a total of one week. After the end of each 24 hour application period, the skin was graded for irritation according to a 0 – 4 scoring scale. The cumulative average scores for the undiluted, 30%, and 10% detergent formulation were 0.26, 0.03, and 0.03, respectively. These score are all indicative of a very mild effect.

Conclusion

The human experience data supports the lack of allergic contact sensitisation potential of formulations containing AES. The skin irritation potential of aqueous solutions of detergent product formulations under conditions simulating relevant consumer use can be expected to be mild after repeated contact with human skin.

4.2.9. Identification of critical endpoints

4.2.9.1. Overview on hazard identification

Alcohol 'ethoxysulphates are considered to be of low toxicity after acute oral and dermal exposure. The estimated LD50 is higher than 2000 mg/kg body weight. Reliable data on acute inhalation are not available, but given the irritant nature of AES, it is expected that a high AES aerosol concentration may be irritating to the respiratory tract. However, inhalation is not viewed as a significant route of exposure. AES is mainly used in liquid media and due to its very low vapour pressure, exposure is unlikely to occur. The only possible exposure could be due to the use of powdered formulations or the use of AES in spray cleaner formulations **(see chapter 5.1 – Consumer Exposure).**

The skin and eye irritation potential is concentration dependent. AES concentrations higher than 70% are moderately to severely irritating to rabbit skin under the conditions of 4-hour semi-occluded patch tests and moderately to severely irritating to rabbit eyes. Formulations containing more than 20% AES are classified as skin and eye irritants unless data are available that show absence of irritation potential as defined by the EC criteria. At concentrations below 1%, AES are considered as virtually non-irritating.

AES are not considered to be skin sensitizers. A substantial amount of skin sensitization studies in guinea pigs following either the Magnusson-Kligman maximization or the Buehler testing protocol demonstrate the absence of skin sensitization potential and only very few studies indicated a weak sensitization potential of individual AES. Human experience further supports the assessment that AES are not sensitizing.

The available oral and derrnal repeated dose toxicity studies provide a coherent picture on the subacute, subchronic and chronic toxicity of AES. In 2 chronic and four subchronic toxicity studies (3 oral studies with AES, 1 dermal study with AES containing dishwashing liquids), no systemic adverse effects of AES were observed up to the highest tested dose levels of 250 mg/kg bw/day. In 2 subchronic oral feeding studies a slight, but significant increase in organ weights (liver in males and females in both studies, male kidney in one study) was observed at the dose of 250 mg/kg bw/day, but these increases were not accompanied by histological changes and were therefore considered to be adaptive in nature and not a toxic effect of the AES. In two out of seven 21-day oral feeding studies, hepatic hypertrophy and slight increases in plasma enzyme levels were observed at doses of about 120 mg/kg/d. However, in the other 5 21 -day oral feeding studies the estimated NOELs ranged from 232 – 468 mg/kg/d. Only little information was available on these 21-days studies, but similarly to above mentioned subchronic and chronic oral toxicity studies, the effects seen in the liver are not considered to be of adverse nature.

AES are not considered to be mutagenic, genotoxic or carcinogenic. Although most studies addressing these endpoints were not performed according to accepted guidelines, the picture is very coherent. In all the *in vitro* and *in vivo* assays there was no indication of genetic toxicity of AES. Long-term carcinogenicity studies did not indicate any potential of AES to induce tumours.

Substantial information is available on teratogenicity, embryotoxicity and toxicity to reproduction of AES. Taken all together, it can be concluded that AES is not cumulatively toxic to pregnant rats and did not reveal teratogenic, developmental reproductive effects at the highest tested dose levels of >300 mg/kg body weight per day.

4.2.9.2 Rationale for identification of critical endpoints

Dermal exposure is the main exposure route for consumers and subsequently, dermal effects such as skin irritation and sensitization as well as long-term dermal toxicity have to be considered with regard to the human risk assessment. A substantial amount of data is available addressing the skin irritation and skin sensitization potential of AES solutions and AES containing consumer product formulations. Dermal penetration studies in rats have shown that AES has the potential to penetrate the skin and become systemically available. There are only a few dermal studies available, but by using bridging assumptions, systemic effects after dermal exposure can also be assessed using the results of oral repeated dose toxicity studies in experimental animals.

4.2.9.3 Adverse effects related to accidental exposure

The acute oral and dermal LD50 of solutions containing AES at concentrations up to 70% is greater than 2000 mg/kg. This level of toxicity is generally considered as low. AES is present in detergent formulations at 28% as a maximum. Generally, accidental oral exposure to a surfactant containing formulation such as detergents poses a minor risk of aspiration.

The available information suggest that concentrated solutions containing AES at concentrations above 20-30% may be moderately to severely irritating to eyes and slightly to moderately irritating to skin. Thus, eye and prolonged skin contact with neat products should be avoided. Other surfactants present in the formulation could contribute to these effects. It has, however, been observed that the overall irritation profile of AES containing detergent and cleaning formulations is not necessarily additive and is less than expected based on the individual components. Nevertheless, in case of accidental eye contact, immediate rinsing with plenty of water is recommended. This immediate action has been shown in animal experiments to minimize irritation effects.

4.2.10. Determination of NOAEL or quantitative evaluation of data

As discussed before, the available oral and dermal repeated dose toxicity studies provide a coherent picture and demonstrate low toxicity of AES.

In the available chronic and subchronic toxicity studies, no effects were seen at levels up to 75 mg/kg bw/day and no adverse effects of AES were observed up to the highest tested dose levels of 250 mg/kg bw/day. In 2 subchronic oral feeding studies a slight, but significant increase of organ weights (e.g. liver) was observed at the dose of 250 mg/kg bw/day. These increases were not accompanied by histological changes and were therefore considered to be an adaptation to the test material and not a toxic effect of the AES. In a subchronic oral gavage study in rats, local treatment effects were observed in the test animals. These effects can be explained by the irritating nature of the test solutions on the epithelium of the forestomach under the test conditions. These types of effects are not considered to be relevant for humans because they are a concentration-dependent response to a direct irritation and also the fact that the exposure scenario reflected in the oral gavage study is not of relevance to human exposure scenarios occurring in real life. There is also no equivalent to the rat forestomach in man. Following this rationale, a NOAEL of 250 mg/kg bw/day could be established. With regard to teratogenicity of AES, a NOAEL greater than 1000 mg/kg bw/day is suggested. At this exposure level, no evidence for teratogenicity was found in a reliable segment II embryotoxicity study. In a series of teratology screening studies which monitored pup development up to weaning day 21 no developmental effects were observed for AES at the highest exposure level of 750 mg/kg/day.

However, it is recognized that there might be a different view with regard to the interpretation of the data and the establishment of a NOEL (or NOAEL) for systemic toxicity of AES. Alternatively to the discussion above, there might be the conservative view that the increase in the liver weight accompanied by the increase of certain enzymes in the plasma in one of the subchronic oral feeding studies is indicative of an (adverse) effect.

For assessing the risk associated with human exposure to AES in context of its use in laundry and cleaning products, it is therefore suggested to take a conservative approach by using a no observed effect level (NOEL) of 75 mg/kg bw/day. This value was derived from the results of a 2-year drinking water study in rats.

4.3. Risk Assessment

4.3.1. Margin of Exposure Calculation

The margin of exposure (MOE) is the ratio of the No Observed Adverse Effect Level (NOAEL) or an appropriate substitute (e.g. NOEL) to the estimated or actual level of human exposure to a substance. For alcohol ethoxysulphates (AES), a NOEL of 75 mg/kg bw/day has been established on the basis of a chronic drinking water study (see chapter 5.2.3 and 5.2.10) [McDermott et al., 1975].

4.3.1.1. Exposure scenario: direct skin contact from hand washed laundry

For calculation of the MOE, the NOEL of 75 mg/kg bw/day was divided by the daily systemic dose of 5.4 µg/kg bw/day which was estimated for the dermal exposure to AES from hand-washed laundry.

$$MOE_{\text{direct skin hand-washed laundry}} = 75000/5.4\ [\mu g/kg\ bw/day] = 13888$$

4.3.1.2. Exposure scenario: direct skin contact from pre-treatment of clothes

The MOE was calculated by dividing the NOEL of 75 mg/kg bw/day by the estimated exposure from pre-treatment of clothes of 18.8 µg/kg bw/day.

$$MOE_{\text{direct skin pre-treatement}} = 75000/18.8\ [\mu g/kg\ bw/day] = 3989$$

4.3.1.3. Exposure scenario: direct skin contact from hand dishwashing

The MOE was calculated by dividing the NOEL of 75 mg/kg bw/day by the estimated exposure from hand dishwashing of 3.4 µg/kg bw/day.

$$MOE_{\text{direct skin hand dishwashing}} = 75000/3.4\ [\mu g/kg\ bw/day] = 22058$$

4.3.1.4. Exposure scenario: direct skin contact from hard surface cleaning

Based on the calculations presented in chapter 5.1.3.5, the systemic dose from skin contact during hard surface cleaning was estimated to be 0.2 µg/kg bw/day. This exposure results in a very large MOE (>100000) and thus does not significantly add to the overall exposure. It will therefore not be considered in the risk assessment.

4.3.1.5. Exposure scenario: indirect skin exposure from wearing clothes

The systemic dose from indirect skin exposure to AES residues on washed fabric was estimated to be 0.74 µg/kg bw/day. This exposure subsequently results in a very large MOE (>100000) and thus does not significantly add to the overall exposure. It will therefore not be considered in the risk assessment.

4.3.1.6. Exposure scenario: inhalation of dust during washing process

The systemic dose of AES via inhalation via detergent dust during the washing process was estimated to amount 1.35 x 10^{-4} µg/kg bw/day. The MOE that could be calculated from this low exposure is much greater than 100000. The described exposure does not significantly add to the overall AES exposure and will therefore not be considered in the risk assessment.

4.3.1.7. Exposure scenario: inhalation of aerosols from cleaning sprays

For calculation of the MOE, the NOEL of 75 mg/kg bw/day was divided by the daily systemic dose of 0.036 µg/kg which was estimated for the inhalation of AES-containing aerosols in spray cleaning applications. This exposure results in a very large MOE (>> 100000) and does not significantly add to the overall exposure. It will therefore not be considered in the risk assessment

4.3.1.6. Exposure scenario: oral route from drinking water containing AES

For calculation of the MOE, the NOEL of 75 mg/kg bw/day was divided by the daily systemic dose of 1.8 µg/kg estimated for the uptake of AES from drinking water. This calculation was, however, based on the estimated worst case regional predicted environmental concentration of AES in surface water. In reality, this exposure must be regarded as unreasonable conservative. The vast majority of AES (estimated to be >99%) will be removed during the drinking water treatment process and thus, consumer exposure to AES via drinking water should be regarded as negligible.

4.3.1.9. Exposure scenario: oral route from residues left on dinnerware

The MOE was calculated by dividing the NOEL of 75 mg/kg bw/day by the estimated oral exposure from AES residues left on eating utensils and dinnerware of 1.4 µg/kg bw/day.

$$MOE_{oral\ route} = 75000/1.4\ [\mu g/kg\ bw/day] = 53571$$

4.3.1.10. Exposure scenario: oral route from accidental ingestion and accidental eye contact

Accidental ingestion of a few milligrams of AES 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 toxicity profile of laundry and cleaning products in general, and AES 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 AES.

Accidental eye contact with undiluted laundry or cleaning products containing AES as a major surfactant block at a concentration between 20 – 28% are expected to cause mild to moderate irritation which is fully reversible shortly after the accidental exposure. Nevertheless, in the case of accidental eye contact, immediate rinsing with plenty of water is recommended. This immediate action has been shown in animal experiments to minimize irritation effects.

Eye contact with AES containing solutions under usage conditions (e.g., in hand-washed laundry or hand dishwashing) is not expected to cause more than a very mild irritation.

4.3.1 .1 1. Total Consumer Exposure

In a worst case scenario, the consumer exposure from direct and indirect skin contact of neat or diluted AES containing product, inhalation of AES containing aerosols from spray cleaner applications and from the oral route via the drinking water or AES residues on eating utensils and dinnerware, results in an estimated systemic AES exposure of 29 µg/kg bw/day. The MOE can be calculated by dividing the NOEL of 75000 µg/kg bw/day by the total exposure:

$$MOE_{total} = 75000/29\ [\mu g/kg\ bw/day] = 2586$$

4.3.2 Risk Characterisation

4.3.2.1. Systemic Toxicity

Consumers are exposed to AES 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 NOEL determined in subchronic and chronic toxicity studies. The MOE for the systemic dose resulting from the total consumer exposure is 2586. This MOE calculation reflects the total of all possible exposure scenarios using mostly worst case assumptions, an exposure situation which is very unlikely to occur in real life.

The determined MOE is certainly large enough to account for the inherent uncertainty and variability of the hazard data on which it is based on. The MOE is based on worst case exposure assumptions and a very conservative, systemic NOEL. The true consumer exposure is with a very high likelihood significantly lower than presented here and there are very good scientific reasons to assume that the NOAEL of AES is about three times higher than the NOEL used in the MOE calculations.

The available toxicological information indicates that AES is not mutagenic, genotoxic or carcinogenic, nor was there any evidence for reproductive toxicity, developmental or teratogenic effects in animals at the highest AES doses tested. The only effects observed in 2 subchronic toxicity studies were related to a slight, but significant increase of the liver (in both studies in males and females) and kidney (only in males of one study) weights accompanied in one study with a slight increase of plasma enzyme levels. In both studies these effects were not accompanied by histological changes and should not therefore be considered a toxic effect of AES.

Some concerns were raised due to the presence of traces of 1,4-dioxane in some AES batches. 1,4-dioxane is a chemical classified as possibly carcinogenic (2B) by IARC (IARC, 1999).

This issue was thoroughly evaluated in context of consumer products (Appel, 1988, European Chemicals Bureau, 2002). It was concluded that given the very low levels of 1,4-dioxane in AES formulated consumer products, the presence of 1,4-dioxane does not pose a health risk to the consumer.

A large proportion of the total systemic AES exposure results from the percutaneous absorption of AES in applications involving skin contact. The percutaneous absorption of AES was calculated by using a dermal penetration constant which was determined experimentally in an *in vivo* rat study. Generally, rat skin is considered to be more permeable than human skin [Schaefer et al., 1996]. While the exact relationship between rat and human skin has not been established and differs depending on the physico-chemical characteristics of the chemical substance, the additional level of conservatism needs to be considered in the overall assessment.

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

4.3.2.2. Local Toxicity

AES is not a contact sensitizer and its irritation potential is concentration dependent. Under normal use conditions with direct skin contact (e.g., in hand laundering or in hand dishwashing) the consumer is exposed to detergent solutions containing 0.02 – 0.2% AES. At these concentration levels, AES is virtually non-irritating to skin. This has been demonstrated in clinical situations as well as in animal studies. Short-term 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.

AES is present in laundry and cleaning products at concentrations between 0.1 and 28%. Accidental eye contact with undiluted detergent product may cause mild to moderate irritation which is fully reversible shortly after exposure. This assessment is supported by poison control center data demonstrating that accidental eye contact with AES containing products will at worst result in a transient irritation which heals after 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 AES 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 AES and AES 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.

4.3.3. Summary and Conclusion

Consumers are exposed to AES through its presence in laundry and cleaning products mainly via the dermal route, but to some extend also via the oral and the inhalator-y route. Skin exposure occurs mainly in hand-washed laundry, laundry pre-treatment and hand dishwashing and to a very minor extent also through AES residues in the fabric after the washing cycle and skin contact during hard surface cleaning. Consumers are orally exposed to AES through residues deposited on eating utensils and dishes after hand dishwashing. Since AES is also used in spray cleaners, the consumer can also be exposed to AES containing aerosols

generated by the sprayer. The consumer aggregate exposure to AES has been estimated to be at maximum 29 µg/kg bw/day.

A substantial amount of toxicological data and information *in vivo* and *in vitro* demonstrates that there is no evidence for AES being genotoxic, mutagenic or carcinogenic. There wasn't also any evidence of reproductive toxicity, teratogenic, or developmental effects in animals at the highest doses tested. The long-term toxicity of AES was evaluated in several subacute, subchronic and chronic toxicity studies. In the available chronic and subchronic oral toxicity studies, no adverse effects of AES were observed at the highest tested dose level of 250 mg/kg/day. In 2 subchronic oral feeding studies a slight, but significant increase of organ weights (e.g. liver) was observed at the dose of 250 mg/kg bw/day. These increases were not accompanied by histological changes and should therefore not be considered a toxic effect of AES. In a subchronic oral gavage study in rats, local treatment and concentration-dependent irritant effects were observed in the forestomach of the rats. These effects are considered to be a direct irritation response under the test conditions and thus not relevant for humans in general and in particular, the AES consumer exposure scenarios considered in this assessment.

Recognizing the fact that there might be a different view with regard to the interpretation of the subchronic toxicity data (i.e. slight increase in organ weight is considered to be an effect), a conservative approach was taken to assess the risk associated with human exposure to AES in context of its use in laundry and cleaning products by using a NOAEL of 75 mg/kg bw/day.

The comparison of the aggregate exposure and the systemic NOEL results in a MOE of 2586. This is a very large margin of exposure, large enough to account for the inherent uncertainty and variability of the hazard database and inter and intra-species extrapolations, which are usually considered by a factor of 100.

Neat AES is an irritant to eyes and skin. The irritation potential of aqueous solutions of AES depends on concentration. Local dermal effects due to direct or indirect skin contact with AES containing solutions in hand-washed laundry or hand dishwashing are not of concern because AES is not a contact sensitizer and AES is not expected to be irritating to the skin at in-use concentrations.

In summary, the human health risk assessment has demonstrated that the use of AES in household laundry and cleaning detergents is safe and does not cause concern with regard to consumer use.

6. Contributors to this Risk Assessment

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

Cognis, Henkel, Huntsman, Procter & Gamble, Sasol, Shell Chemicals Ltd. (lead), Stepan Europe, Unilever, and The Weinberg Group (consultant).

Additional input was given by the HERA Human Health Task Force:

Carlos Rodriguez (Chairman):	Procter & Gamble Eurocor
Klemeris Berthold:	Bayer AG
Frieda Bielen:	Procter & Gamble Eurocor
Juanell Boyd:	Colgate-Palmolive
Philip Carthew:	Unilever

Norbert Fedtke :	Henkel
Otto Grundler:	BASF
Sylvia Jacobi :	Degussa-Hüls
Sheila Kirkwood :	McBride
Marcus Kleber :	Cognis
Reinhard Kreiling :	Clariant
Garrett Moran :	Unilever
James Plautz :	Ciba
Thomas Roth :	Clariant
Gauke Veenstra :	Shell

References

AISE (Association Intemationale de la Savonnerie, de la Detergence et des Produits d' Entretien): Table of Habits and Practices for Consumer Products in Western Europe. Developed within the HERA project in 2002.

AISE: Internal data – Unpublished data.

Appel, K.E, (1988) Health Evaluation of Dioxane, Bundesgesundheitsblatt 31, No. 2, 37-46.

Arthur D. Little, Inc. (1991) Environmental and human safety of major surfactants. Volume 1. Anionic surfactants. Part 2. Alcohol ethoxy sulfates. Final report to : The Soap and Detergent Association.

Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinarmedizin (BgVV), Artzliche Mitteilungen bei Vergiftungen 1999, ISBN 3-93 1675-59-9.

Black, J.G. and Howes, D. (1979) Skin penetration of chemically related detergents. J. Soc. Cosmet. Chem. 30, 157-165.

Brown, V.K.H. and Muir, C.M.C. (1970) The toxicities of some coconut alcohol and Dobanol 23 derived surfactants. Tenside 7, 137-l 39.

Connor, D.S., et al. (1975) Identification of certian sultones as the sensitizers in an alkyl ethoxy sulfate. Fette und Selfen Anstrichmittel77, 182- 185.

European Chemicals Bureau, European Risk Assessment Report, 1,4-Dioxane, 2002.

Henkel KGaA, Unpublished data, Test of skin sensitising properties of Texapon N1 1, 1977a.

Henkel KGaA, Unpublished data, Test of skin sensitising properties of Texapon N40, Texapon ASV and Texapon T42, 1977b.

Henkel KGaA, Unpublished data, Test of skin sensitising properties of CJL 394,1985.

Henkel KGaA, Unpublished data, Determination of the mutagenicity of Texapon N25 in the Ames mutagenicity test, 1988.

Henkel KGaA, Unpublished data, Texapon N70: 90 day oral (gavage) subchronic toxicity study in the rat, 1994a.

Henkel KGaA, Unpublished data, Texapon N70: Embryotoxicity study (including teratogenicity) in the rat (segment II), 1994b.

Henkel KGaA, Unpublished data, report no. R 9900771. 1999

HERA AES manufacturers – personal communication (May 2002)

Hope, J. (1977) Absence of chromosome damage in the bone marrow of rats fed detergent actives for 90 days. Mutat. Res. 56, 47-50.

Hiils AG, Unpublished data, Acute oral toxicity of Marlinat 24/70 in rats, 1986a.

Hiils AG, Unpublished data, Test of the acute irritation of the skin by Marlinat 24/70, 1986b.

Hüls AG, Unpublished data, Test of the acute irritant effects on eyes and mucosa of Marlinate 24/28, 1986c.

Hüls AG, Unpublished data, Determination of the mutagenicity of Marlinat 242/70 in the Ames mutagenicity test, 1994.

Hüls AG, Unpublished data, S. typhimurium reverse mutation assay (Ames test) with Marlinat 242/90T, 1996.

Hüls AG, Unpublished data, Test of the skin sensitizing effect of Marlinat 242/90T, 1989.

Hiils AG, Unpublished data, Marlinat 242/90T: Acute oral toxicity to the rat, 1997a.

Hüls AG, Unpublished data, Marlinat 242/90T: Acute dermal toxicity to the rat, 1997b.

Hüls AG, Unpublished data, Test of the acute skin irritation of the skin by Marlinat 242/90T on rabbits, 1997c.

Hüls AG; Unpublished data, Test of the acute irritant effect on eyes and mucosa of Marlinat 242/90T in rabbits, 1997d.

Hüls AG, Unpublished data, Test of the skin sensitizing effect of Marlinat 242/90T in guinea pigs (Buehler method), 1997e.

International Agency for Research on Cancer, IARC, (1999) Vol 17, 589-601.

Inoue, K.T., et al. (1980) Studies of in vitro cell transformation and mutagenicity by surfactants and other compounds. Fd. Cosmet. Toxicol. 18, 289-296.

Iseki, H. (1972) On teratogenicity of some detergents. Mie Kaibo Gyosekishu 20, 119.

Klimisch et al. (1997) A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regul. Toxicol. Pharmacol. 25, l-5.

McDermott, J.A., et al. Alkyl ethoxy sulfates: Absorption, distribution, excretion and metabolite identification studies in rats and man. Presented at the Society of Toxicology Meetings, March 14, 1975.

Nolen, C.A. (1975) Teratology studies of a mixture of tallow alkyl ethoxylate and linear alkylbenzene sulfonate in rats and rabbits. Toxicol. 4, 23 l-243.

NOTOX, Unpublished data, 1994.

Nusair TL, Danneman PJ, Stotte J, Bay PHS (1988) Consumer Products: risk assessment process for contact sensitisation. Toxicologist 8,258.

Official publication of the French legislation ("Journal Officiel de la Republique Francaise") concerning substances used in dish care products which may come in contact with foods, 1990

Palmer, A.K., et al. (1975) Assessment of the teratogenic potential of surfactants. Part I-LAS, ARS and CLD. Toxicol. 3, 91-106.

Petersen, D.W. (1988) Subchronic percutaneous toxicity testing of two liquid hand dishwashing detergents. Fd. Chem. Toxic. Vol. 26, No.9, 803-806.

Procter & Gamble Company, Unpublished data, 1994.

Procter & Gamble Company, Unpublished data, 1996a.

Procter & Gamble Company, Unpublished data, 1996b.

Procter & Gamble Company, Unpublished data, 1998.

Procter & Gamble Company, Unpublished data, 2000a.

Procter & Gamble Company, Unpublished data, 2000b.

Procter & Gamble Company, Unpublished data, 2000c.

Procter & Gamble Company, Unpublished data, 2000d.

Procter & Gamble Company, Unpublished data, 2000e.

Procter & Gamble Company, Unpublished data, 200 1.

Research Toxicology Centre S.p.A., Unpublished data, 1995.

Rodriguez, C., et al. (1994) Skin effects associated with wearing fabrics washed with commercial laundry detergents. J. Toxicol.-Cutaneous & Ocular Toxicol.13, 39-45.

Schaefer, H. and Redelmeier, T.E. (1996) Skin barrier: Principles of percutaneous absorption. S. Karger AG, P.O. Box, CH-4009 Base1 (Switzerland), ISBN 3-8055-6326-4

Schmitz, J. (1973) Tenside Detergents, 10, 1 l-l 3

Shell Chemical Co., Unpublished data, Acute toxicity studies on Neodol 25-3S, 1967.

Shell Oil Co., Unpublished data, Primary skin irritation in rabbits of Neodol 25-3S, 1989.

Shell Oil Co., Unpublished data. Letter to US EPA, 1992.

Shell Research Ltd., Unpublished data, The comparative toxicity of some Dobanol derived detergent materials and their Laurex NC matches. Part I: The skin irritancy and acute oral toxicity of the mono and triethanolamine sulphates and the ethoxysulphate, 1968.

Shell Research Ltd., Unpublished data, The toxicity of the two Stanlow derived surfactants, Dobanol 25-9 and Dobanol 25-3S, 1972.

Shell Research Ltd, Unpublished data, The toxicity of detergents: Acute toxicity, skin and eye irritancy and skin sensitization potential of the detergent Dobanol 25-3S/60, 1975a.

Shell Research Ltd., Unpublished data, The toxicity of detergents: Acute toxicity, skin and eye irritancy and skin sensitization potential of Dobanol 25-3S/27, 1975b.

Shell Research Ltd., Unpublished data, The toxicity of detergents: Acute toxicity, skin and eye irritancy and skin sensitization potential of Dobanol 25-3A/60, 1975c.

Shell Research Ltd., Unpublished data, The toxicity of detergents: Skin sensitization potential of Dobanol 23-2S/28, 1975d.

Shell Research Ltd., Unpublished data, Toxicology of detergents: Acute toxicity, skin and eye irritancy and skin sensitizing potential of Teepol PB, 1978a.

Shell Research Ltd., Unpublished data, Toxicology of detergents: Acute toxicity, skin and eye irritancy and skin sensitizing potential of Dobanol 23-2S/28, 1978b.

Shell Research Ltd., Unpublished data, Toxicology of detergents: Acute toxicity, skin and eye irritancy and skin sensitizing potential of Dobanol 23- 3S/27 (ex Z and S Italy), 1978c.

Shell Research Ltd., Unpublished data, Toxicology of detergents: Skin irritancy and skin sensitizing potential of Dobanol 25-3S/27 (bleached and unbleached), 1978d.

Shell Research Ltd., Unpublished data, Toxicology of detergents: Skin irritancy and skin sensitizing potential of Dobanol 25-3A/60 (bleached and unbleached), 1978e.

Shell Research Ltd., Unpublished data, Toxicology of detergents: Skin sensitizing potential of 12 batches of Dobanol 25-3S/27, 1980a.

Shell Research Ltd., Unpublished data, 1980b.

Shell Research Ltd., Unpublished data, 1982a.

Shell Research Ltd., Unpublished data, 1982b.

Shell Research Ltd., Unpublished data, The skin sensitizing potential of Dobanol 25-3S/27, 1983a.

Shell Research Ltd., Unpublished data, 1983b.

Taylor, A.J., et al. (1978) Metdolism of the surfactants sodium undecyltriethoxy sulphate and sodium dodecyltriethoxy sulphate in the rat. Biochem. J. 174, 405-412.

TGD, EEC 1996, Technical Guidance Document in support of Commission Directive 93/67/EEC on risk assessment for new notified substances and Commission Regulation (EC) No. 1488/94 on risk assessment for existing substances, EU Commission, Luxembourg, 1996

Tusing, T.W., et al. (1962) Toxicologic studies on sodium lauryl glyceryl ether sulfonate and sodium lauryl trioxyethylene sulfate. Toxicol. Appl. Pharmacol. 4, 402-409.

Unilever, Unpublished data, Ingestion toxicity of ether sulphates. Part 2. 3-Week Feeding Trial with Dobanol25 3E0 sulphate, sodium salt, 1979a.

Unilever, Unpublished data, Ingestion toxicity of ether sulphates. Part 3. 3-Week Feeding Trial with Dobanol25 3E0 sulphate, ammonium salt, 1979b.

Unilever, Unpublished data, Ingestion toxicity of ether sulphates. Part 4. 3-Week Feeding Trial with Acropol 13- 15 3E0 sulphate, ammonium salt, 1979c.

Unilever, Unpublished data, Ingestion toxicity of ether sulphates. Part 4. 3-Week Feeding Trial with Alfol 12-14 3E0 sulphate, sodium salt, 1979d.

Unilever, Unpublished data, Ingestion toxicity of ether sulphates. Part 5. 3-Week Feeding Trial with Lial 25L 3E0 sulphate, sodium salt, 1979e.

Unilever, Unpublished data, Ingestion toxicity of ether sulphates. Part 5. 3-Week Feeding Trial with Lial 25L 3E0 sulphate, sodium salt, 1979f.

Unilever, Unpublished data, Ingestion toxicity of ether sulphates. Part 7. 3-Week Feeding Trial with Dobanol25 3E0 sulphate, sodium salt, containing alcohol, 1980a.

Unilever, Unpublished data, Ingestion toxicity of ether sulphates. Part 6. 3-Week Feeding Trial with Alfol 16-l 8 4E0 sulphate, sodium salt, 1980b.

Unilever, Unpublished data, Mutagenicity and teratology of alternative surfactants. Part 25. Teratology of Dobanol25 E3 sulphate. Oral administration in the rat, 198Oc.

Unilever, Unpublished data, Mutagenicity and teratology of alternative surfactants. Part 22. Teratology of Acropol 13-l 5 E3 sulphate. Oral administration in the rat, 1986a.

Unilever, Unpublished data, Mutagenicity and teratology of alternative surfactants. Part 13. Teratology of Alfol 12-14 E3 sulphate. Oral administration in the rat, 1986b.

Unilever, Unpublished data, Mutagenicity and teratology of alternative surfactants. Part 31. Teratology of Alfol 16- 18 E4 sulphate. Oral administration in the rat, 1986c.

US Environmental Protection Agency (1986) Reference values for risk assessment. Final Draft ECAO-CIN-477.

van de Plassche et al. (1998) Moret Ernst & Young Management Consultants (Second Draft), Rep. No. 601503 013, pgs.l-64.

Vermeire, T.G., et al. (1993) Estimation of consumer exposure to chemicals: Application of simple models. Science Total Environ. 136: 155- 176.

Walker, A.I.T., et al. (1967) Toxicity of sodium lauryl sulphate , sodium lauryl ethoxysulphate and corresponding surfactants derived from synthetic alcohols. Fd. Cos. Tox. 5, 763-769.

Walker, A.P., et al. (1973) Some characteristics of the sensitizer in alkyl ethoxy sulphate. Acta. Dermatovener 53, 141-144.

Appendix 1. Literature Search

A search of:

- BIOSIS Previews (1969-Present)
- CA SEARCH. Chemical Abstracts (1967-Present)
- TOXLINE
- Registry of Toxic Effects of Chemical Substances

was performed. The search combined each of the following CAS Numbers/chemical name descriptors with the search terms:

REPRODUC*
DEVELOPMENT*
ACUTE CHRONIC SUBCHRONIC with TOXIC*
TERATOGEN*
CARCINOGEN*
MUTAGEN*
GENOTOXIC*
IRRIT*
DERMATITIS
EYE*
SKIN
DERMIS
DERMAL
OCULAR
RAT*
MICE
MOUSE
DOG*
RABBIT*
MONKEY*
HAMSTER*
GUINEA PIG*
HUMAN*
MAMMAL*
OCCUPATIONAL CONSUMER HOUSEHOLD with EXPOSURE*

CAS Number	CAS Description
27028-82-6	Ethanol, 2,2',2"-nitrilotris-, compd. with a-sulfo-w-(dodecyloxy)poly(oxy-1,2-ethanediyl) (1: 1)
54116-08-4	Poly(oxy-1,2-ethanediyl), a-sulfo-w-tridecyloxy)-, sodium salt
67762-19-O	Poly(oxy-1,2-ethanediyl), a-sulfo-w-hydroxy-, C 1 O-l 6-alkyl ethers, ammonium salts
68037-05-g	Poly(oxy-1,2-ethanediyl), a-sulfo-w-hydroxy-, C6-10-alkyl ethers, ammonium salts
68037-06-9	Poly(oxy-1,2-ethanediyl), a-sulfo-w-hydroxy-, C6-10-alkyl ethers
68540-47-6	Ethanol, 2,2',2"-nitrilotris-, compd. with a-sulfo-w-(tetradecyloxy)poly(oxy-1,2-ethanediyl) (1: 1)
68585-34-2	Poly(oxy-1 ,2-ethanediyl), a-sulfo-w-hydroxy-, C 1 O-l 6-alkyl ethers, sodium salts
68585-40-o	Poly(oxy- 1,2-ethanediyl), a-sulfo-w-hydroxy-, C 16- 18-alkyl ethers, sodium salts
68891-38-3	Poly(oxy-1,2-ethanediyl), a-sulfo-w-hydroxy-, C12-14-alkyl ethers, sodium salts
96130-61-9	Poly(oxy-1,2-ethanediyl), a-sulfo-w-hydroxy-, C9-11-alkyl ethers, sodium salts
105859-96-9	Ethanol, 2,2',2"-nitrilotris-, compds. with polyethylene glycol hydrogen sulfate C 1 1- 15-sec-alkyl ether ammonium salts
125301-92-o	Poly(oxy-1,2-ethanediyl), a-sulfo-w-hydroxy-, C 12-15-alkyl ethers, sodium salts
125304-06-5	Ethanol, 2,2',2"-nitrilotris-, compds. with polyethylene glycol hydrogen sulfate C 16-l 8-alkyl ether
129783-23-9	Ethanol, 2,2'-iminobis-, compds. with polyethylene glycol hydrogen sulfate C 12- 15-alkyl ethers
157627-92-4	Alcohols, C 10- 16, etboxylated, sulfates, mono(hydroxyethyl)ammonium salts (>1 <2.5 mol EO)
157707-82-9	Alcohols, C 14-16, ethoxylated, sulfates, sodium salts (> 1 <2.5 mol EO)

162201-45-8	Ethanol, 2-amino-, compds. with polyethylene glycol hydrogen sulfate C 12-15-alkyl ethers
174450-50-l	Alcohol, C12-14, ethoxylated, sulfates, triisopropanolamine salts
102783-14-2	Poly(oxy-1,2-ethanediyl), a-sulfo-w-hydroxy-, C10-18-alkyl ethers, sodium salts
9004-82-4	Sodium lauryl ether sulfate
2523 l-22-5	Poly(oxy-1,2-ethanediyl), .alpha.-[(tridecyloxy)sulfonyl]-.omega.-hydroxy-, sodium salt
3443 l-25-9	Polyethylene glycol octyl ether sulfate, sodium salt
52286-19-8	Polyethylene glycol decyl ether sulfate, ammonium salt
67762-2 l-4	Poly(oxy-1,2-ethanediyl), .alpha.-sulfo-.omega.-hydroxy-, Cl 0-16-alkyl ethers, magnesium salts
68081-91-4	Poly(oxy-1,2-ethanediyl), .alpha.-sulfo-.omega.-hydroxy-, Cl 2-l 8-alkyl ethers, sodium salts
68 184-04-3	2-Aminoethanol compd. with .alpha.-sulfo-.omega.-(dodecyloxy)poly(oxy-1,2-ethanediyl) (1: 1)
68610-22-o	Poly(oxy-1,2-ethanediyl), .alpha.-sulfo-.omega.-hydroxy-, C 12-18-alkyl ethers, ammonium salts
6889 l-29-2	Poly(oxy-1,2-ethanediyl), .alpha.-sulfo-.omega.-hydroxy-, C8-10-alkyl ethers, ammonium salts
6889 l-30-5	Poly(oxy-1,2-ethanediyl), .alpha.-sulfo-.omega.-hydroxy-, Cl l-l 5-branched alkyl ethers, ammonium salts
73665-22-2	Poly(oxy- 1,2-ethanediyl), .alpha.-sulfo-.omega.-hydroxy-, C6-10-alkyl ethers, sodium salts
157627-95-7	Poly(1,2-ethanediyl), .alpha.-sulfo-.omega.-hydroxy-C 16-18 and C 18 unsaturated alkyl ethers, sodium salts
160104-51-8	Poly(1,2-ethanediyl), .alpha.-sulfo-.omega.-hydroxy-C12-14 alkyl ethers, magnesium salts
160 104-52-9	Poly(1,2-ethanediyl), .alpha.-sulfo-.omega.-hydroxy-C16-18 and Cl8 unsaturated alkyl ethers, magnesium salts
67762-l 9-O	Poly(oxy-1,2-ethanediyl), .alpha.-sulfo-.omega.-hydroxy-, C 1 O-l 6-alkyl ethers, ammonium salts

13150-00-0	Ethanol, 2-[2-[2-(dodecyloxy)ethoxy]ethoxy]-, hydrogen sulfate, sodium salt
32612-48-9	Poly(oxy-1,2-ethanediyl), .alpha.-sulfo-.omega.-(dodecyloxy)-, ammonium salt

In addition, a call-in was made for data from AISE/CESIO companies.

201-16316

RECEIVED

2006 JUL 18 AM 7:37

HERA

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

Alcohol Ethoxysulphates (AES) Environmental Risk Assessment

AN rights reserved. No part of this publication may be used, reproduced, copied, stored or transmitted in any form of by any means, electronic, mechanical, photocopying, recording or otherwise without the prior written permission of the HERA Substance Team or the involved company.

The content of this document has been prepared and reviewed by experts on behalf of HERA with all possible care and from the available scientific information. It is providedfor information only. HERA cannot accept any responsibility or liability and does not provide a warranty for any use or interpretation of the material contained in this publication.

1 Contents

1 Contents 2

2 Executive Summary 5

3 Substance Characterisation 6

3.1 CAS No and Grouping information 6

3.2 Chemical structure and composition 6

3.3 Manufacturing Route and Production/Volume Statistics 6

3.4 Homologue distribution in HERA applications 7

4 Data Search Strategy.. 9

5 Exposure 9

5.1 Tonnage.. 9

5.2 Derivation of PEC.. 9

5.2.1 Tonnage Scenarios 10

5.2.2 Physico-Chemical Properties 10

5.2.3 Removal.. 11

5.2.4 PEC Calculations 14

6 Effects 18

6.1 Aquatic toxicity.. 18

6.1.1 Acute data 18

6.1.2 Chronic data 18

6.1.3 Mesocosm data 21

6.2 $PNEC_{aquatic}$ derivation 21

6.2.1 Justification for PNEC based on chronic data 21

6.2.2 Trends in Toxicity/QSAR 22

6.2.3 $PNEC_{aquatic}$. 24

6.3 Other Compartments Toxicity 25

6.3.1 Microbial toxicity 25

6.3.2 Soil and Sediment Toxicity Data.. 25

6.3.3 $PNEC_{sediment}$ and $PNEC_{soil}$ 25

7 Risk Characterisation 26

7.1 Aquatic Compartment 26

7.2 Microbial toxicity 26

7.3 Sediment Compartment 26
7.4 Soil Compartment 26
8 CONCLUSIONS.. ..... .27
9 CONTRIBUTORS TO THIS RISK ASSESSMENT.. 27
10 REFERENCES 27
11 ANNEXES 32

Tables

Table 1 Estimated tonnage and Chain length distribution of AES 8
Table 2 Estimated tonnage and EO distribution of AES 8
Table 3 Tonnages used in PEC assessments.. ..... '10
Table 4 Fate of AES with E02.7 in STP (fractional distribution). 12
Table 5 Primary degradation of AES in higher tier tests.. 12
Table 6 Fate of AES with E02.7 based on 97.5% degradation.. 12
Table 7 Simpletreat PEC estimates (Scenario I). 14
Table 8 Simulation test PEC estimates (Scenario II) 15
Table 9 AES with EO=2.7 uptake by Humans – as calculated with EUSES* 16
Table 10 Comparison of modelled PEC and field monitored concentrations 16
Table 11 Chronic toxicity data 18
Table 12 Mesocosm data 21
Table 13 QSAR estimates of toxicity 22
Table 14 *Brachionus calyciflorus* toxicity data.. 23
Table, 15 $PNEC_{aquatic}$ (mg/l). 24
Table 16 $PNEC_{soil}$ (mg/kg) 26
Table 17 Aquatic risk quotients 26
Table 18 Soil risk quotients 27

Annex

Annex 1 CAS # covered in family 32
Annex 2 Physchem Properties 33

Annex 3 RCR based on Total Tonnage 35

2 Executive Summary

Alcohol ethoxysuiphates (AES) are a widely used class of anionic surfactants. They are used in household cleaning products, personal care products, institutional cleaners and industrial cleaning processes, and as industrial process aids in emulsion polymerisation and as additives during plastics and paint production. Uses in household cleaning products, the scope of HERA, include laundry detergents, hand dishwashing liquids, and various hard surface cleaners.

The total volume of AES surfactants used in Europe is estimated to be 276,000 tonnes/year on an active matter basis of which 108,000 tonnes/year is used in household detergents and cleaning products (CESIO, 2000).

A large environmental data set is available for AES. On the environmental fate side, this includes standard biodegradation studies, advanced simulation studies of removal in treatment systems, and field monitoring data. On the environmental effects side, acute as well as chronic single-species data are available, as well as advanced studies in micro- and mesocosm systems.

To determine the Predicted Environmental Concentration (PEC), chemical removal in wastewater treatment plants was determined from advanced simulation test data. Monitoring studies on sewage treatment plant effluents indicate that the exposure estimates in this assessment are likely to be conservative.

The Predicted No-Effect Concentration (PNEC) was based on chronic ecotoxicity data. Mesocosm studies'suggest that the effects assessment based on laboratory studies is also conservative.

By means of these higher tier exposure and effects data, it could be shown that the use of AES in HERA applications (household detergents and cleaning products) results in risk characterization ratios less than one, indicating no concern, for all environmental compartments.

An additional exposure scenario was included in this risk assessment, by assuming the entire AES tonnage used in Europe is disposed of down the drain. Using the same exposure and effects assessment approach, the absence of environmental concerns can also be demonstrated for this total tonnage.

3 Substance Characterisation

Alcohol ethoxysulphates (AES) are a widely used class of anionic surfactants. They are used in household cleaning products, personal care products including toothpaste and shampoos, hand and other personal cleaning products, institutional cleaners and industrial cleaning processes, and as industrial process aids in emulsion polymerisation and as additives during plastics and paint production. Uses in household cleaning products, relevant to the HERA program of risk assessments, include laundry detergents, hand dishwashing liquids, and various hard surface cleaners.

3.1 CAS No and Grouping information

There are several CAS Numbers describing AES. A comprehensive list is presented in Annex 1 of this document. Although clearly important from a Regulatory perspective, this assessment is not based on CAS Nos., but on the environmental fate and effects of the components of the products.

3.2 Chemical structure and composition

The alcohol ethoxysulphate family is defined for HERA purposes to encompass commercial grades of linear-type primary alcohol ethoxysulphates containing AES components of basic structure C_nH_{2n} $(C_2H_4O)_mSO_4X$,) where n=12- 18 and m = O-8 and X = sodium, ammonium or triethanolamine (TEA). Sodium salts of AES are by far the most commonly used grades. Further detail on the structures included in the AES family are given in Section 3.3.

3.3 Manufacturing Route and Production/Volume Statistics

Alcohol ethoxysulphates are produced by sulphation of the ethoxylates of primary alcohols using sulphur trioxide or chlorosulphonic acid followed by immediate neutralisation with base to produce typically a sodium salt, less commonly an ammonium salt. Minor volumes are neutralised with alkanolamines, usually triethanolamine (TEA). Most commercial alcohol ethoxysulphates are produced as low or high aqueous active solutions e.g. 2530% or 68-70%. Many grades of AES are produced commercially differing in the parent detergent alcohol, the ethoxylate (number of moles of EO), the concentration of AES active matter in water, whether shipped as a solution, a paste or in solid form. Commercial sodium AES typically contain, approximately 2-4% of unsulphated alcohol ethoxylate, 1-2% unreacted alcohol and 15-45% alcohol sulphate, and optionally trace amounts of inorganic pH buffering agents, depending on the active matter content and the degree of ethoxylation. The molecules included in the HERA AES family are ultimately derived from linear-type primary alcohols in the C_{12} to C_{18} range. As marketed, such alcohols usually contain a distribution of alkyl chain lengths.

The linear-type alcohols include those which are mixtures of entirely linear alkyl chains, and those which are mixtures of linear and mono-branched alkyl chains, though still with a linear backbone. Such alcohols and their blends are substantially interchangeable as feedstocks for AES used in the major applications falling within the scope of HERA.

The entirely-linear alcohol feedstocks include those derived from vegetable or animal sources via oleochemical processes and those derived from ethylene via Ziegler chemistry. Such alcohols contain even numbered alkyl chains only, and are produced in single carbon cuts or more usually wider cuts from C6 through C22+. C 12 through C 18 grades are feedstocks for HERA AES.

The essentially-linear alcohol feedstocks, also known as linear oxo-alcohols, are derived from linear higher olefins via oxo-chemistry. The feedstock linear olefins are typically derived from ethylene or normal paraffins. Such alcohols contain mixtures of even/odd or odd numbered alkyl chains depending on the feedstock olefin, and are produced in grades ranging from C7 through C 15. Typically 90-40% of the carbon chains are linear, the remainder being mono-branched 2-alkyl isomers, predominantly 2-methyl. The mono-branched isomers thus have a linear backbone. Cl 2 through C 15 grades are feedstocks for HERA AES.

The principle structures present in HERA C_{12} AES for example are:

$$CH_3(CH_2)_{11}O(EO)_nSO_3Na$$

$$CH_3(CH_2)_{8-m}\underset{\underset{(CH_2)_mCH_3}{|}}{C}HCH_2O(EO)_nSO_3Na$$

where n varies from O-8 and m varies from O-4, but is primarily 0. The average value of n is 2.7 for AES sold into household use and 2.4 for the total AES produced.

Of the AES used in consumer cleaning applications in Europe, approximately 71% is derived from even carbon numbered linear alcohols (C 12- 14 and C 16- 1 S), with the remaining 29% derived from odd and even carbon numbered essentially-linear oxo alcohols.

Excluded from the HERA AES family are alcohol ethoxysulphates derived from alcohols shorter than C_{12}. The tonnages of these products are very small (<1000 tonnes/year) and their toxicity is less than that of longer chainlengths. Also excluded from the family are AES with other alkyl chain structures such as multi-branched alcohols, for example commercial *iso*-tridecanols. These grades of AES are not typically used in household cleaning products. Their uses are small and specialised and they are not considered further in this assessment.

The European (EU, CH and NO) production volume of AES surfactants on an active matter basis is estimated to be 320,000 tonnes/y (CESIO statistics for 2000; CESIO = European Committee for Surfactants and their Organic Intermediates, a sector group of the European Chemical Industry Council, CEFIC). About 276,000 tonnes/y are estimated to remain in Europe, the remainder (44000 tonnes/yr) is exported. The imported volume is thought to be negligible. CESIO estimates that 39% (108,000 tonnes) of the captive use volume is used in HERA applications.

3.4 Homologue distribution in HERA applications

To determine the carbon-number distribution of products falling within the scope of HERA (i.e., household detergents and cleaning products), a survey was conducted among detergent formulator companies (data from members of AISE) and companies manufacturing AES (via the CESIO Statistics Group). From the data received,

estimated distributions between carbon chain lengths have been determined. In the HERA-relevant range of C12-C18, the distribution between carbon chain lengths has been determined for 303,388 tonnes of the estimated total European AES production volume (320 000) and for 102,480 tonnes of the estimated total AES volume used in household cleaning products (108 000) (Table 1). These chainlength data are considered a reasonable representation of the distribution applicable for the marketed tonnages.

Table 1 Estimated tonnage and Chain length distribution of AES

Chain length	CESIO : Total AES Tonnage		CESIO : Estimate of Volume used in Household Cleaning Products		AISE : Estimate of Volume used in Household Cleaning Products	
	Percent	*Tonnes*	*Percent*	*Tonnes*	*Percent*	*Tonnes*
C12	60.9	184 847	57.6	59 045	46.2	32 770
C13	8.9	26 981	15.1	15 447	32.0	22 725
C14	24.8	75 315	21.6	22 145	18.2	12 894
C15	2.4	7 170	2.7	2 730	3.6	2 565
C16	2.2	6 787	2.1	2 200	-	-
C17	-	-	-	-	-	-
C18	0.8	2 288	0.9	913	-	-
ΣC_{12-18}		303 388*		102 480**		70 954**

* Compared to EU Production Tonnage of 320 000 (of which 44 000 t/a are exported)
** Compared to 108 000 t/a used in HERA applications.

CESIO estimates that 61% (168,000 tonnes) of the captive use volume is used in applications outside of the HERA scope. Second to use in household detergents and cleaning products, Personal Care applications consume the next largest volume of AES, followed by use in Industrial and Institutional cleaners and the Industrial sector (e.g. emulsion polymerisation). These applications are not considered in the body of this assessment, although an environmental assessment based on the total EU-captive tonnage is included in Annex 3.

A separate survey was performed to determine the average EO number of products used in HERA applications. The total tonnages from this survey are very similar to those from the distribution by carbon number survey. The information extracted from this EO-distribution survey is the average EO number, hence the slight difference in total tonnage will have little effect (Table 2).

Table 2 Estimated tonnage and EO distribution of AES

Commercial Product	CESIO	CESIO

AVERAGE EO	Household volume (tonnes)	AES Total tonnage (tonnes)
1	1,492	1,492
2	18,693	161,577
2.5	37,000	89,250
3	43,850	47,703
	1,750	4,500
Total tonnage	102,785	304,522
Average EO	2.7	2.4

4 Data Search Strategy

Chemical names were extracted from the STN database, Registry file. Chemical names and CAS numbers were searched in STN database, CAPlus file and the Dialog databases BIOSIS file, Enviroline tile and Pollution Abstracts file. Additional searches were made of ECOTOX (U.S. EPA) and TOMES databases.

In addition, a call-in was made for data from AISE/CESIO companies with a request for information on toxicity, fate and tonnage marketed.

5 Exposure

5.1 Tonnage

The European (EU, CH and N) production volume of AES surfactants on an active matter basis is estimated to be 320,000 tonnes/y (CESIO statistics for 2000). About 276 000 tonnes/y are estimated to remain in Europe, the remainder is exported. The imported volume is thought to be negligible. An estimated 108,000 tonnes/y is used in formulations for household use. Assessments are made based on both 108,000 for HER4 applications (Section 5.2) and 276,000 tonnes for the total captive tonnage (Annex 3).

Estimates of the distribution of carbon chainlengths and EO distribution within this tonnage are shown in Table 1 and Table 2 respectively. In the following assessment it is assumed that the carbon chainlength distribution of the tonnage for which data were available is representative of the total tonnage.

5.2 Derivation of PEC

The PEC is derived on the basis of individual C# with an average EO of 2.7 for HERA applications (2.4 for total tonnage assessment, Annex 3). The only way to estimate the physchem properties of E02.7 or E02.4 is by interpolation of values for E02 and E03. Values are shown in Annex 2.

The use of individual C# is needed because there is some evidence to suggest that toxicity may show a parabolic relationship with carbon chainlength (Section 6.2.2.2). However, there appears to be an essentially linear relationship of EO and toxicity and therefore use of an average EO is justified.

52.1 Tonnage Scenarios

The AISE and CESIO data for tonnage in household applications differ both quantitatively and qualitatively (Table 1). The qualitatively greatest difference is that AISE attributes greater percentage tonnage to Cl3 and less to C12. Interpretation of this variability to estimate PEC values based on household use has been managed by scaling the highest percentage estimate to the total tonnage. For example, for C13, the AISE estimate is the highest at 32%. The sum of highest percentages for all chainlengths is 117.9%. For each carbon chainlength, the CESIO total tonnage in household use, 108,000 t/a, has been scaled by the highest percentage for that particular carbon chainlength divided by the sum percentage of the carbon chainlengths. For Cl3 this is 108,000 t * 32.0%/1 17.9% to give 29312 t/a.

The tonnages used to estimate PEC's arising from the total AES marketed in EU are derived from CESIO estimates of the C# distribution of the Total Tonnage applied to the EU captive tonnage.

Adopting this approach, the tonnages used in the PEC assessments were those shown in Table 3.

Table 3 Tonnages used in PEC assessments

	Household use		Total AES production	
	Highest estimated %	Total tonnage x Highest estimated %	CESIO estimate %	Total tonnage[1] x CESIO estimate %
C12	57.6%	52763	60.9	168160
C13	32.0%	29312	8.9	24545
C14	21.6%	19786	24.8	68516
C15	3.6%	3298	2.4	6523
C16	2.1%	1924	2.2	6174
C18	0.9%	824	0.8	2081
TOTAL	117.9%	108000	100	276000

[1]= Production tonnage minus export tonnage

5.2.2 Physico-C hemical Properties

The most important phys/chem properties for an environmental risk assessment are aqueous solubility, vapour pressure, and the octanol/water partition coefficient or other partition coefficients, for example, those between water and environmental matrices such as soil, sediment, or sewage sludge. Details of physchem properties used in modelling the PEC are shown in Annex 2.

For Alkyl Ethoxy Sulphate, all groups of homologues have sufficiently low volatility that the sensitivity of the risk assessment to the values of this parameter will be negligible.

It should be noted that for surfactants a physically meaningful log Kow cannot be measured but can be modelled from molecular structure. Therefore, all assessments based on partitioning coefficients that are not established experimentally but calculated from log Kow-values should be considered only as a first and conservative estimate

5.2.3 Removal

5.2.3.1 Biodegradation pathways

The risk assessment of a parent compound should be restricted to that compound unless the metabolites are persistent and/or more ecotoxic than the parent. There are 3 starting routes of AES degradation which all seem to occur: i) o-/P-oxidation of the alkyl chain, ii) enzymatic cleavage of the sulphate substituent leaving an alcohol ethoxylate, iii) cleavage of an ether bond in the AES molecule producing either the alcohol (central cleavage) or an alcohol ethoxylate and an oligo(ethylene glycol) sulphate (Swisher 1987, Steber and Berger 1995). The subsequent degradation of the resulting intermediates encompasses oxidation of the alcohol to the corresponding fatty acid (itself then degraded via &oxidation) or degradation of the alcohol ethoxylate (via central cleavage or degradation from either end of the molecule) or degradation of the oligo(ethylene glycol) sulphate. The ultimate biodegradability of alcohol ethoxylates is well established (Swisher 1987, Holt et al. 1992) and glycol ether sulphates have also been shown to be fully degradable by mixed cultures forming inorganic sulphate and carbon dioxide (Griffith et al 1986, White and Russell 1988). The conclusion that AES degradation will not produce any recalcitrant metabolite is in line with the experimental findings on AES in the "Test for detecting recalcitant metabolites" (Gerike and Jasiak 1986). In addition, Yoshimura et al (1982) reported test data showing that the (fish) toxicity of AES decreases in the course of AES degradation. Consequently, there is no indication for the formation of persistent or markedly toxic metabolites from AES, and so primary AES removal data obtained with methods such as MBAS, LCMS and ^{14}C-radiolabelled studies are suitable for use in this assessment.

5.2.3.2 Aerobic Degradation & WWTP fate

5.2.3.2. I Ready Biodegrability Data

Several reviews highlight that AES are readily biodegradable, with alkyl-chain length having little effect (Madsen et al 2000, BKH 1994, Painter 1992, ADL 1991).

X2.3.2.2 Scenario I - SimpleTreat calculation

EUSES calculates degradation in a 9-box STP model ranging from 75% for C 18EO2.7S to 87% for C 12EO2.7S (Table 4). These calculations are based on AES being readily biodegradable.

Table 4 Fate of AES with E02.7 in STP (fractional distribution)

C#	12	13	14	15	16	18
air	<1.0E-10	<1.0E-10	<1.0E-10	<1.0E-10	<1.0E-10	<1.0E-10
water	0.13	0.13	0.13	0.13	0.12	0.11
sludge	7.0E-4	1.8E-3	4.4E-3	1.1E-2	2.6E-2	0.14
degraded	0.87	0.87	0.87	0.86	0.85	0.75

A PEC scenario (Scenario 1) using these data is developed in Section 5.2.4.1.

5.2.3.2.3 Simulation Test Data

Information from higher tier tests was collected from producers and reported in BKH's 1994 review. Primary removal in higher tier tests is shown in Table 5.

Table 5 Primary degradation of AES in higher tier tests

C	EO	Removal %	Method	Source quoted in BKH 1994
12	2	97.2	CONF	Henke183
12	8	95.6	CONF	Henke184
12	12	95.4	CONF	Henkel 85
13.3	3.19	100	SCAS	Vista 33
12-14	2	98	CONF	Huls 111
14-15	2	98	CONF	Henkel 88
14-15	3	97.9	CONF	Henkel89
16-18	7.8	98.6	CONF	Henke186
16-18	10.3	97	CONF	Henke187

CONF: CECD CAS test (confirmatory test)

The primary removal data listed above suggest no consistent removal trend with alkyl chainlength or degree of ethoxylation. Consequently, a geometric mean of the data (97.5% removal) has been used in subsequent analyses (Scenario II). Scaling the SimpleTreat distributions assuming 97.5% removal is shown in Table 6. These data were used to develop Scenario II shown below.

Table 6 Fate of AES with E02.7 based on 97.5% degradation

C#	12	13	14	15	16	18
air	<1.0E-10	<1.0E-10	<1.0E-10	<1.0E-10	<1.0E-10	<1.0E-10
water	2.5E-02	2.5E-02	2.5E-02	2.5E-02	2.5E-02	2.5E-02
sludge	7.8E-04	2E-03	4.9E-03	1.2E-02	2.9E-02	0.15
degraded	0.97	0.97	0.97	0.96	0.95	0.82

It is clear from these studies that greater removal should be expected from a STP than is modelled by the default values attributed to readily biodegradable substances in the TGD by SimpleTreat.

5.2.3.3 Anaerobic Degradation

Based on the chemical structure of AES and the proven easy anaerobic biodegradability of the structurally related alcohol ethoxylates and alkyl sulphates, good anaerobic biodegradability of AES is likely (Steber and Berger, 1995). This is supported by the result from testing C12-14EO2S in a stringent anaerobic biodegradability screening test (ECETOC test) which showed a gas (CO_2 + methane) production of 75 % within the 41 -day incubation period (Steber 1991). In addition, Nuck & Federle (1996) tested AES in a lab digester that simulated the situation in practice except that the system was static while real digesters are mainly run semi-continuously. Within the 17-day incubation period 88% ultimate biodegradation (based on ^{14}C-gas formation) was found for C14[^{14}C]EO3S.

Taking these mineralisation data into account it is expected that the removal of the parent AES compound under digester conditions is at least 90%. However, the organic moiety of the sewage sludge (about 50% of the sludge dry matter) is also reduced during the digestion process, typically by about 50%, suggesting a reduction in sludge volume of 25%. Scaling the reduction of AES concentration to take account of sludge volume reduces the reduction in AES concentration by a factor of 1.3 (100/75%) and consequently, AES anaerobic removal is estimated as 87 % rather than the 90% calculated when the reduction in the organic content of sludge is not taken into account.

The EUSES program does not include anaerobic degradation during sludge digestion. Instead, this process has been included in the HERA risk assessment by manual modification (i.e. reduction by 87%) of the concentrations in agricultural soil calculated by EUSES.

5.2.3.4 Degradation in other media

Federle et al (1997) compare rate constants for 9 chems including C14-15EO2.25S in different tests. The publication doesn't give individual rates but Federle (pers. comm.) provided the following mineralization rates (l/day):

	Sturm	Activated Sludge	River	Soil
Mineralization rate (day-')	0.18	1.79	0.48	0.29
Equivalent ?&life (days)	3.9	0.39	1.4	2.4

These data suggest that degradation will be considerably faster than assumed by the surface-water and soil rate constants used for readily biodegradable substances according to the EU-TGD (k= 0.047, t1/2 = 15 d for surface water and k=0.023, t1/2 = 30 d for soil for a substance with logKow ≤ 4.4).

Schröder (1995) investigated the half-life of AES in River water and showed a half-life of about 1 hour in a sample from the Rur river. This would be equivalent to a rate constant of 16.6 (d^{-1}).

Based on the ready biodegradability of all chainlengths of AES, it is assumed that the rate constant for degradation in bulk surface water for C14-15EO2.25S determined by Federle et al (1997) is applicable to other chainlengths. Therefore, a surface-water degradation rate of 0.48 d-l has been applied to all chainlengths in the calculation of PEC values. The value of 0.48 d-l indicates more rapid degradation than the default rate constant proposed in the TGD for readily biodegradable substances (0.047 d-l), but is far more conservative compared to the rate of 0.7 h-l determined by Schröder (1995).

Lee et al (1997) report on mineralization in a stream mescosm exposed to different surfactants including C45EO2.17S and show that temperature (13-25 oC) has no effect on degradation rate.

For degradation in soil, the biodegradation kinetic obtained from the work by Federle et al (k= 0.29 d^{-1}, t1/2 = 2.4 d) was used to determine.the PEC calculations instead of using the TGD default value (k = 0.023 d^{-1}, t1/2 = 30 d). Federle's figure is considered conservative because it is based on the mineralisation rate, i.e. the removal of the parent surfactant will have been much higher. Further support for the use of this figure is provided by comparing the assumed AES half life (2.4 d) with the corresponding figure for LAS which, in a field study run under realistic conditions was in the range 3-7 days (Küchler et al.,1997).

5.2.4 PEC Calculations

5.2.4.1 Local $PEC_{aquatic}$

EUSES was used to calculate local PEC based on household use tonnage which includes a contribution from the regional PEC. HERA default values were used: 7% of the continental tonnage is applied to the region and the average discharge to WWTP is increased by a factor of 1.5 to take account of local variability (HERA, 2002). The Federle et al degradation rate constants for surface water and soil were used to override the default values. The resulting PEC values are shown in Table 7.

Table 7 Simpletreat PEC estimates (Scenario I

Carbon #	12	13	14	15	16	18
Local PEC surface water (mg/l)	5.0E-2	2.8E-2	1.9E-2	3.1E-3	1.8E-3	6.9E-4
Local PEC sediment (mg/kg wwt)	4.7E-2	3.3E-2	3.4E-2	1.0E-2	1.3E-2	2.8E-2
Local PEC agric 30 d (mg/kg wwt)	1.1E-3	1.6E-3	2.6E-2	1.1E-3	1.6E-2	3.5E-3
Local PEC agric 30 d with 87% anaerobic degradation (mg/kg wwt)	1.4 E-4	2.1E-4	3.4E-4	1.4E-4	2.1E-4	4.6E-4
PECstp microorgs (mg/l)	0.48	0.27	0.18	3.0E-2	1.7E-2	6.6E-3

Regional PEC surface water total (mg/l)	2.2E-3	1.2E-3	8.2E-4	1.4E-4	7.9E-5	3.3E-5

Simulation test degradation showed no consistent trend across carbon chainlength and an average of 97.5% degradation (Section 5.2.3.2.3). Overriding EUSES defaults to reflect the STP distribution shown in Table 6, results in the PEC values shown in Table 8.

Table 8 Simulation test PEC estimates (Scenario II)

Karbon #	12	13	14	15	16	18
Local PEC surface water (mg/l)	1.1E-02	6.3E-03	4.2E-03	7.0E-04	4.1E-04	1.8E-.04
Local PEC sediment (mg/kg wwt)	1.1E-02	7.4E-03	7.6E-03	2.3E-03	2.9E-03	7.1E-03
Local PEC agric 30 d (mg/kg wwt)	1.3E-3	1.7E-3	3.0E-3	1.2E-3	1.7E-3	3.9E-3
Local PEC agric 30 d with 87% anaerobic degradation (mg/kg wwt)	1.6E-4	2.3E-4	3.8E-4	1.6E-4	2.3E-4	5.0E-4
PECstp microorgs (mg/l)	9.5E-02	5.3E-02	3.6E-02	5.9E-03	3.5E-03	1.5E-03
Regional PEC surface water total (mg/l)	1.8E-03	9.9E-04	6.7E-04	1.1E-04	6.5E-05	2.8E-05

5.2.4.2 Indirect Exposure to Humans

As a starting point for the calculation of indirect human exposure via drinking water, the EUSES calculations for indirect uptake via regional exposure can be used (taking into account that drinking water will not be sourced immediately downstream of wastewater emissions). These are shown in Table 9 below, with the calculated uptake from a local source given for comparison. The total human uptake calculated by EUSES is also shown in the table, although known inadequacies with the current model for plant uptake mean that these calculated values will considerably overestimate the uptake from food. Thus these total regional uptake values may not be considered to be acceptably realistic for the HERA Human Health Assessment.

Table 9 AES with EO=2.7 uptake by Humans – as calculated with EUSES*

AS Fraction	Regional (mg/kg/day)		Local (mg/kg/day)	
	Drinking Water	Total Food + Water Uptake	Drinking Water	Total Food + Water Uptake
C12	5.1E-5	5.9E-5	3.2E-4	3.7E-4
C13	2.8E-5	3.8E-5	1.8E-4	2.4E-4
C14	1.9E-5	3.2E-5	1.2E-4	2.0E-4
C15	3.2E-6	7.9E-6	2.0E-5	5.0E-5
C16	1.9E-6	8.5E-6	1.2E-5	5.4E-5
C18	7.9E-7	2.0E-5	5.0E-6	1.3E-4

*EUSES defaults modified according to the HERA Detergent Scenario and taking account of 97.5% removal in STP, 87% anaerobic degradation in sludge and degradation rates in surface water and soil based on measured data.

5.2.4.3 Validation of modelling using monitoring data

STP Effluent Monitoring

Data on STP monitoring can be used to validate modelling data based on laboratory confirmatory studies and/or default values applied to laboratory screening data. Literature reports of AES monitoring generally do not distinguish between carbon chainlengths. In addition, field monitoring for AES has used analytical methods that cover C12-15 only. Therefore monitoring data have been compared with the sum of PEC values for C 12-l 5 only. Additionally, monitoring analytical methods cannot distinguish between AS from AES and from AS itself, and therefore the sum of AES + AS will overestimate the AES PEC. Considering the tonnage of AS marketed relative to that originating from AES the error due to inclusion of AS from sources other than AES may be quite large. In addition, AES monitoring data will combine AES from detergents with that from other sources.

Comparison of monitoring and modelling data is shown in Table 10. The highest values from STP influent monitoring data are similar to EUSES estimates. EUSES estimates of STP effluent concentrations based on simulation test data are greater by more than one order of magnitude compared to the concentrations monitored in activated sludge plants. This emphasises that the aquatic PEC must be considered as a very conservative estimate. Consequently, the monitoring data suggest that a more accurate, less conservative modelling of fate in STP would lead to lower PEC values.

Table 10 Comparison of modelled PEC and field monitored concentrations

	Value	Reference	Notes	Homologues covered
C_{infl} (mg/l)	[illegible]	[illegible]	[illegible]	[illegible]

	0.57	Popenoe et al 1994	RBC plant	C12-15 EO0-8
	0.016 & 1.0	McAvoy et al 1998	2 act. slu. plants. 0.016 value may be particularly low due to long residence time in equalization basin before the STP	C12-15 EO0-6
	0.74	Wind pers. comm., 2002	Median value, Sum of AS+AES (approx. 90 samples) at 9 STP	C12/14 EO1-5 C12-18 AS
	3.8	Matthijs et al 1997	Average value, sum AS+AES at 7 STP	C12-15 EO0-8
	0.23 & 0.74	Schröder (1995)	Median value, Sum of AS+AES at 2 STP	C12/14 EO1-5 C12-18 AS
	0.4 - 5.1	Schröder et al 1999	Sum of AS+AES determined in 2-h composite samples (1 STP) over a 24h period	C12/14 EO1-5 C12-18 AS
C_{eff} (mg/l)	[redacted]	[redacted]	[redacted]	[redacted]
	0.004 & 0.018	McAvoy et al 1998	2 AS plants. Also 0.032-0.164 from 4 TF plants	C12-15 EO0-6
	0.012	Matthijs et al 1997	Avg, sum of AS+AES @ 7 plants	C12-15EO0-8
	<0.001	Schröder et al 1999	Sum of AS+AES determined in 2-h composite samples (1 STP) over a 24h period	C12/14 EO1-5 C12-18 AS
	0.003 & 0.008	Schröder 1995	Median value, Sum of AS+AES at 2 plants	C12/14 EO1-5 C12-18 AS
	0.002	Wind pers. comm. (2002)	Median value, Sum of AS+AES (approx. 90 samples) at 9 STP	C12/14 EO1-5 C 12-18 AS
Regional PEC surface water (mg/l)	[redacted]	[redacted]	[redacted]	[redacted]
	0.0103	Popenoe et al 1994	Upstream of STP	C12-15 EOO-8
	0.001	Schröder 1995	Upstream of STP	

5.2.4.4 PEC for other compartments

There are no measured concentrations of AES in sediment or soil, not even bulk AES without characterisation by C#. Local $PEC_{sediment}$ and PEC_{soil} are calculated by EUSES, although the PEC_{soil} is modified to take account of anaerobic biodegradation, and the results are included in Section 5.2.4.1.

6 Effects

6.1 Aqua tic toxicity

6.1 .1 Acute data

Acute toxicity data are available in several review articles (ADL 1991; BKH 1994; Madsen 2000). As a large chronic data base exists (Section 6.1.2) the acute data have not been further considered for the HERA risk assessment.

6.1.2 Chronic data

The following chronic toxicity data are available in reviews or have been identified during this HER4 assessment project.

Table 11 Chronic toxicity data

Fish and other aquatic vertebrates

C#		EO#		Linearity	Species	Endpoint	Exposure	Value	Ref
Avg	Distn	Avg	Distn					(mg/l)	
12		0		?	Saccobranchus fossilis	60 d	Semi-static	>2.24	Dalela et al, 1981
?	12-13	1	?	?	P. promelas	30 d NOEC	?	0.88	BKH 1994
?	12-14	2	?	?	0. mykiss	28 d growth	flow-through	0.1	Scholz 1997
?	12-15	3	?	?	0. mykiss	28 d NOEC	flow-through Measured	0.12	BUA 1997
13.7	?	2.25	?	?	P. promelas	365 d NOEC	Measured	0.1	Maki 1979
?	14-15	2.25	?	?	P. promelas (juvenile)	45 d LC50	? (flow-through)	0.44	ADL 1991
?	14-15	2.25	?	?	P. promelas (fry)	45 d LC50	? (flow-through)	0.63	ADL 1991
?	14-15	2.25	?	?	P. promelas	45 d LC50	? (flow-through)	0.94	ADL 1991

?	14-16	2.25	?	?	P. promelas	45 d LC50	?	0.1	BKH 1994
17.3	16-18	0			Brachydanio rerio	OECD 204, NOEC		1.7	Steber et al 1988
17	?	3	?	?	P. promelas	365 d NOEC	?	0.13	BKH 1994

Invertebrates

C#		EO#		Linearity	Species	Endpoint	Exposure	Value	Ref
Avg	Dist'n	Avg	Distn						
12	99%	0	-	-	C. dubia	7 d NOEC	Flow-through	0.88	Dyer et al 1997
12	>95% Pure	1	>95% Pure	?	C. dubia	7 d NOEC	Flow-through	0.34	Dyer et al 2000
12	>95% Pure	2	>95% Pure	?	C. dubia	7 d NOEC	Flow-through	6.3	Dyer et al 2000
12	100% Pure	2	100% Pure	?	Brachionus calyciflorus	2 d EC20	Measured	0.97-1.1	Versteeg et al, 1997
12	>95% Pure	4	>95% Pure	?	C. dubia	7 d NOEC	Flow-through	2.7	Dyer et al 2000
12	99% pure	4	99% pure	?	B. calyciflorus	2 d EC20	Measured	2.3	Versteeg et al 1997
12	>90% Pure	8	>90% Pure	?	C. dubia	7 d NOEC	Flow-through	1.2	Dyer et al 2000
?	12-14	2	?	?	D. magna	21 d repro	Semi-static Nominal	0.72	Scholz 1997
?	12-14	>2	?	?	D. magna	21 d NOEC	Semi-static	0.7	BKH 1994
?	12-15	3	?	?	D. magna	21 d repro	Semi-static Measured	0.34	BUA 1997
13	>95% Pure	2	>95% Pure	?	C. dubia	7 d NOEC	Flow-through	0.28	Dyer et al 2000
13	100% pure	2	100% pure	?	B. calyciflorus	2 d EC20	Measured	0.49	Versteeg et al 1997
13.67	13-15	2.25	?	?	D. magna	21 d NOEC	Measured	0.27	Maki 1979
14	>95%	0	-	-	C. dubia	7 d NOEC	Flow-through	0.<0.062	Dyer et al 1997
14	>95% Pure	1	>95% Purc	?	C. dubia	7 d NOEC	Flow-through	0.34	Dyer et al 2000
14	>95% Pure	2	>95% Pure	?	C. dubia	7 d NOEC	Flow-through	0.31	Dyer et al 2000
14	100% pure	2	100% pure	?	B. calyciflorus	2 d EC20	Measured	0.13	Versteeg et al 1997
14	>95% Pure	4	>95% Pure	?	C. dubia	7 d NOEC	Flow-through	1.1	Dyer et al 2000

14	98% pure	4	98% pure	?	B. calyciflorus	2 d EC20	Measured	0.37	Versteeg et al 1997
?	14-15	0			C. dubia	7 d NOEC	Flow-through	0.081	Dyer et al 1997
?	14-15	2.25	?	?	D. magna	21 d NOEC	Nominal	0.18	BKH 1994
?	14-16	2.25	?	?	D. magna	21 d NOEC	?	0.27	BKH 1994
15	>95%	0	-	-	C. dubia	7 d NOEC	Flow-through	0.23	Dyer et al 1997
15	>95% Pure	1	>95% Pure	?	C. dubia	7 d NOEC	Flow-through	0.08	Dyer et al 2000
15	>95% Pure	2	>95% Pure	?	C. dubia	7 d NOEC	Flow-through	0.06	Dyer et al 2000
15	>95% Pure	4	>95% Pure	?	C. dubia	7 d NOEC	Flow-through	0.15	Dyer et al 2000
15	99% pure	4	99% pure	?	B. calyciflorus	2 d EC20	Measured	0.22	Versteeg et al 1997
15	>90% Pure	8	>90% Pure	?	C. dubia	7 d NOEC	Flow-through	5.8	Dyer et al 2000
16	>95% pure	0		-	C. dubia	7 d NOEC	Flow-through	0.20	Dyer et al 1997
17.3	16-18	0			D. magna	21 d NOEC		16.5	Steber et al 1988
18	>95% pure	0			C. dubia	7 d NOEC	Flow-through	0.60	Dyer et al 2000

Algae

C#		EC#		Linearity	Species	Endpoint	Exposure	Value	Ref
Avg	Dist'n	Avg	Distn						
12		0			S. capricomutum	96 h NOEC Growth inhibtion		12	Nyholm & Damgaard, 1990
12		?			River water 'community'	Chlorophyll a NOEC	3 weeks	70 mg/l (enhancement at 5 mg/l)	Drewa 1989
?	12-13	?	?	?	Selenastrum capricomutum	?	5 d NOEC	50.5	BKH 1994
?	12-14	2	?	?	Scenedesmus subspicatus	72 h NOEC AUGC	Static Nominal	0.72	Scholz 1997
?	12-14	2	?	?	Scenedesmus subspicatus	96 h NOEC	Static Nominal	0.35	BKH 1994
?	12-15	3	?	?	Scenedesmu	72h	Static	0.9	BUA

					s subspicatus	NOEC	Measured		1997
?	14-15	?	?	?	Selenastrum capricornutum	NOEC Test duration unknown	?	21	BKH 1994
17.3	16-18	0			Scenedesmus subspicatus	72 h NOEC	Static	17	Henkel 1996

6.1.3 Mesocosm data

Several mesocosm/microcosm studies have been performed with AES.

Table 12 Mesocosm data

C#		EO#		Linearity	Species	Endpoint	Exposure	Value	Ref
Avg	Distn	Avg	Distn						
14.5	14-15	2.17	?	?	Corbicula fluminea (Asian clam)	8 weeks NOEC	Flow-through	0.075 mg/l	Belanger et al 1995a
14.5	14-15	2.17	?	?	Goniobasis spp (a snail)	8 weeks LOEC	Flow-through	>0.73 mg/l	Belanger et al 1995a
14.5	14-15	2.17	?	?	Periphyton	4 weeks NOEC	Flow-through	0.61 mg/l	Belanger et al 1996
14.5	14-15	2.17	?	?	46 invertebrate spp	8 weeks NOEC species density	Flow-through	0.25 mg/l	Belanger et al 1995b
13.2	12-15	3		80%	Fish, invertebrate and algal taxa	30d NOEC	Flow-through	>2 mg/l	Lizotte et al 2002
?	16-18	0		?	Algae, protozoa, rotifer, bacteria spp	21 d NOEC		0.55	Steber et al 1989

6.2 PNEC$_{aquatic}$ derivation

6.2.1 Justification for PNEC based on chronic data

The abundance of chronic toxicity data is such that it is justified to base the PNEC on chronic toxicity data.

6.2.2 Trends in Toxicity/QSAR

6.2.2.1 Relative spp sensitivities,

Understanding the relative sensitivity of different taxa is important because the PNEC should be based on the most sensitive taxonomic level.

Inspection of the chronic toxicity data listed above indicates no consistent difference in the sensitivity of invertebrates and fish. For C12-14EO2S fish appear more sensitive than *D.* magna or algae, but a flow-through test was used for the fish, a semi-static for the *D. magna* and a static design for the algae. For Cl 3.7E02.25 fish appear 2.7 times more sensitive than D. magna based on measured concentrations, but C14-15EO2.25 appears more toxic to invertebrates, although the actual exposure concentrations were not confirmed. BKH (1994, Table 6) concluded fish were more sensitive than invertebrates to AES, but they did not take account of C#/EO#.

Lizotte et al (2002), mesocosm data suggest fish are more sensitive than periphyton/macrophytes and invertebrates. Belanger et al (1995b), mesocosm data cannot be used to determine relative sensitivity of fish compared to invertebrates and algae, because fish were not included in the experiment.

Van de Plassche et al (1999) normalised all chronic NOECs to a $C_{12.5}EO_{3.4}SO_4$ structure and showed that *B. calyciflorus* (invertebrte) is more sensitive than *P. promelas* (fish).

On the basis of this analysis, PNEC could be derived based on either fish or invertebrate data. Since the invertebrate database is more extensive than that for fish, the PNEC will be based on invertebrate data.

6.2.2.2 Justification for PNEC based on averages

Different AES homologues are expected to differ in their toxicity. In theory, a PNEC could be derived for each homologue, related to the PEC for each homologue and the resulting quotients summed to determine the risk of the AES family (a toxic units approach). However, the complexity of this approach is not warranted if the toxicity of a single structure is the same as that of a homologue distribution with an average structure equivalent to the single homologue. Choosing an average structure approach, a toxic units approach or some combination (eg consideration of individual carbon chain lengths but with average EO #) requires consideration of toxicity QSAR.

Dyer et al (2000) have developed QSAR for chronic toxicity to *Cerioduphniu* using data on single AES homologues, including EO=0, ie AS. The chronic toxicity QSAR was based on C 12-1 5, EOO-8 plus C 16EO0 and C 18EO0, but R^2 was approximately 0.7 and solubility difficulties were noted for some homologues. The QSAR developed was:

$$\log NOEC\ (mol/l) = 0.128C^2 - 3.767C + 0.152EO + 21.182$$

The QSAR estimates of toxicity are shown in Table 13.

Table 13 QSAR estimates of toxicity

EO#

		0	1	2	3	4	5	6	7	8
	10	53	88	140	230	360	570	880	1400	2100
	11	4.7	7.7	12	20	31	49	75	120	180
	12	0.74	1.2	2.0	3.1	4.8	7.5	12	18	27
C #	13	0.21	0.34	0.55	0.86	1.4	2.1	3.2	4.9	7.5
	14	0.11	0.17	0.28	0.44	0.68	1.1	1.6	2.5	3.7
	15	0.10	0.16	0.25	0.4	0.62	0.95	1.5	2.2	3.4
	16	0.16	0.26	0.41	0.65	1.0	1.6	2.4	3.6	5.5
	17	0.49	0.78	1.2	1.9	3.0	4.5	6.9	11	16
	18	2.6	4.2	6.5	10	15	24	37	56	84

Values interpolated within the training set are in bold.

The chronic QSAR estimates a parabolic relationship between carbon number and toxicity with toxicity increasing from C 12 to Cl 5 and then decreasing. However, with the exception of EOO (ie AS), the QSAR is based on an extrapolation for carbon chainlengths longer than C 15. Furthermore, solubility difficulties were observed in some of the tests (C14EO1S, C15EO0S, C15EO1S, C16S and C18S). Since the Dyer et al QSAR is based on MBAS determined in samples of water from the test vessels, it may not represent the truly dissolved concentrations (bioavailable fraction) and consequently, the 'real' concentration causing effects may have been less than that reported suggesting that the QSAR is underestimating toxicity. Alternatively, the dissolution diffkulties may have caused physical fouling rather than chemical toxicity. Therefore it is unclear whether the parabolic nature of the QSAR is an artifact of solubility problems (ie longer carbon chainlengths are really more toxic than predicted, the error being caused by measured concentrations overestimating the bioavailable fraction), or whether the QSAR overestimates the toxicity of the longer chainlengths due to physical fouling. Fouling would explain toxic effects, even for those carbon chainlengths for which the concentration causing effects is greater than the water solubility.

Comparison of toxicity as predicted by the chronic NOEC QSAR developed for C. ***dubia*** (7 d NOEC) with the observed toxicity (2 d EC20) to ***B. calyciflorus*** shows agreement within a factor of 3 (average 1.9) with ***B. calyciflorus*** being slightly more sensitive than C. ***dubia.*** Nevertheless ***the*** use of ***C. dubia*** data is favoured since ***B. calyciflorus*** is not a traditional test species.

Table 14 ***Bruchionus calyciflorus*** **toxicity data**

Carbon chain	EO#	2 d EC20		
		Observed[1]	Predicted (QSAR)[2]	Observed / Expected
12	2	0.97-1.1	2.0	0.48-0.55
12	4	2.3	4.8	0.48
13	2	0.49	0.55	0.89
14	2	0.13	0.28	0.46

14	4	0.37	0.68	0.54
15	4	0.22	0.62	0.33

[1] Observed toxicity to B. *calyciflorus* (Versteeg et al, 1997)

[2] Toxicity predicted by QSAR for C. *dubia* 21 d NOEC (Dyer et al 2000)

The C. dubia chronic toxicity QSAR (Dyer et al 2000) suggests that the best fit to the data is parabolic with respect to alkyl chain length. Consequently, for chronic toxicity it is not justified to use an average structure for alkyl chain length. The effect of increasing the number of EO units is to reduce the toxicity. The effect of EO on logNOEC is essentially linear and therefore, for a single alkyl-chain length, a single homologue of EO=x will have approximately the same toxicity as a distribution of EO homologues with an average of EO=x. Consequently, a pragmatic option for development of PNEC(s) is to develop a single PNEC for each alkyl chain length, each estimated on the basis of average EO#.

Notwithstanding the parabolic nature of the chronic toxicity QSAR, there are data on the toxicity of complex structures (range of C# and EO# that can be compared with the toxicity of a single homologue as predicted by the Dyer et al (2000) QSAR. Maki (1979) published a *D. magna* 2 1 d NOEC for Cl 3.67EO2.25S (average structure, C-range 13- 15, EO range not known) of 0.27 mg/l while Dyer et al's QSAR would suggest a chronic NOEC for this structure of 0.34 mg/l. Belanger et al (1995b) report a mesocosm study on C14.5EO2.17S (alkyl range 14-15, EO range not known) that gave a NOEC of 0.25 mg/l. Dyer's chronic toxicity QSAR would suggest an identical NOEC for this structure (0.25 mg/l). Lizotte et al (2002) report a mesocosm study on C13.5EO2.8S (alkyl range C12-15, EO 0-10-t) that gave a lowest NOEC invertebrates of 4.3 mg/l. Dyer et al's QSAR would suggest a NOEC for this structure of 0.49 mg/l.

The congruence of these data with the toxicity predicted by the Dyer et al chronic toxicity QSAR suggests that using a single PNEC for an average AES structure is justified. Nevertheless, since none of these tests used an AES that spanned the whole range of C# included in the AES family, and since the Dyer et al data suggest a parabolic relationship between toxicity and C#, separate PNEC will be determined for each C# based on the average EO# marketed.

6.2.3 $PNEC_{aquatic}$

The chronic toxicity QSAR (Dyer et al, 2000) has been used to derive PNEC values, using an application factor of 10. The application factor of 10 is justified by the taxonomic diversity of the overall dataset (Section 6.1.2). The resulting PNEC are shown in Table 15.

Table 15 $PNEC_{aquatic}$ (mg/l)

Carbon #	12	13	14	15	16	18
$PNEC_{aquatic}$ (mg/l)	0.27	0.076	0.038	0.035	0.057	0.89

6.3 Other Compartments Toxicity

6.3.1 Microbial toxicity

Goodnow & Harrison (1972) report the toxicity of AES ($C_{12}EO_3S$) to 45 isolated strains of bacteria growing in peptone medium. Growth inhibition greater than 50% was shown in 5 of the 42 strains tested at 10 mg/l but in 3 of these the AES was >90% degraded in 72 h. Only one strain tested at 100 mg/l showed complete inhibition. Lundahl et al (1973) showed a LOEC of 2 g/l for the growth of *Escherichia coli* on agar plates. Urano et al (1985) report degradation at different concentrations of C12EO5S. Degradation rate is lower at higher concentrations, but even at 100 mg/l degradation occurs. Verge et al (1996) report an OECD 209 respiration inhibition test with C12-14EO2.35 in which the 3h EC50>1600 mg/l. This last test is considered most appropriate as a basis for estimating a PNEC and consequently the microbial PNEC is set at 16 mg/l in accordance with the TGD.

6.3.2 Soil and Sediment Toxicity Data

There are no measured sediment toxicity data. Stora (1972) describes toxicity tests with a sediment dwelling polychaete, *Scololepis fuliginosa* but the exposure was in a water-only system and therefore is uninformative as to sediment toxicity.

In soil, Painter (1992) reports that 100-l 000 mg AES/l gave increased germination rates and yields of soybean, pea, onion and dwarf Coleus *salicifolius. The* original reference for this work is not available, but the units of effect suggest that the exposure used a water-only system again and therefore is uninformative as to soil toxicity.

Some information is available on AS (See HERA AS assessment) and this indicates low soil toxicity. For example, the 48 h EC50 root growth inhibition of C12EO0S to *Cicer arietinum* is 361 mg/kg (Schmidt 1988) and C16-18 (avg C17.3) EOOS NOEC to 'several spp' is >1000 mg/kg (BUA 1996). It is unclear how concentrations of AES causing toxic effects would compare to AS concentrations causing effects since the more hydrophilic AES is expected to be more bioavailable but also less toxic.

Consequently it is concluded that there are no useful sediment or soil toxicity data for AES.

6.3.3 $PNEC_{sediment}$ and $PNEC_{soil}$

Since there are no measured sediment exposure data (Section 5.2.4.4) nor any sediment toxicity data, and since the logKow of none of the AES homologues exceeds logKow 5, the TGD states that the RCR for the aquatic compartment should be used for the sediment compartment. Consequently $PNEC_{sediment}$ is not calculated.

To estimate $PNEC_{soil}$ by equilibrium partitioning, the sorption behaviour of AES homologues is needed. The only sorption value found for AES was measured for $C_{12}EO_5S$ in river sediments and gave K_{oc}=1. 1 (Urano et al, 1984). This compares to a K_{oc} of 2.3 calculated using the QSAR for 'Predominantly hydrophobics' from Sablijic & Gusten (1995) referenced in the TGD (logKoc=0.8 1 logKow + 0.1). The applicability of this QSAR to surfactants is questionable, but in the absence of other measured K_{oc} values, $PNEC_{soil}$ have been derived using this QSAR, TGD defaults for soil properties and the PNECaquatic values derived above (Table 16).

Table 16 $PNEC_{soil}$ (mg/kg)

Carbon #	12	13	14	15	16	18
$PNEC_{soil}$	3.6E-02	1.1E-02	5.6E-03	5.3E-03	9.2E-03	0.16

7 Risk Characterisation

7.1 Aqua tic Compartment

RCR have been calculated using the PEC estimations, based on the household use tonnage (Table 7 and Table 8), and the PNEC derived using the C. *dubia* chronic toxicity QSAR (Table 15). The results using SimpleTreat default estimates of STP degradation (Scenario I) or primary degradation from OECD CAS or SCAS tests (Scenario II), are shown in Table 17.

Table 17 Aquatic risk quotients

PEC/PNEC (AF=10)

Carbon #	12	13	14	15	16	18	Total RCR
Scenario I	0.19	0.37	0.5	8.9E-02	3.2E-02	7.7E-04	1.2
Scenario II	4.1E-02	8.3E-02	0.11	2.0E-02	7.2E-03	2.0E-04	0.26

As discussed in Section 5.2.4.3, Scenario II implies a very conservative exposure estimate while Scenario I is considered to be unrealistically worst case. Consequently, the RCR based on Scenario I can be neglected.

7.2 Microbial toxicity

EUSES estimates of C_{effl} can be used as the $PEC_{micro\text{-}organisms}$. The sum of C12-18 is 0.98 mg/l. The microbial toxicity reported in Section 6.3.1 demonstrated no effect at substantially higher concentrations. Consequently, the RCR for WWTP microorganisms is <1.

7.3 Sediment Compartment

In the absence of measured data, the RCR for the sediment compartment is the same as that for the aquatic compartment.

7.4 Soil Compartment

The RCR for the soil compartment are estimated from:

- EUSES estimates of soil concentrations derived using simulation data to estimate degradation in WWTP, and 87% anaerobic degradation.

- Soil toxicity based on equilibrium partitioning.

Table 18 Soil risk quotients

Carbon #	12	13	14	15	16	18	Total RCR
EO=2.7	4.5E-03	2.1 E-02	6.8E-02	3.0E-02	2.5E-02	3.2E-03	[illegible]

8 CONCLUSIONS

This assessment shows that the use of AES in HERA applications results in risk characterization ratios (Σ(PEC/PNEC)) less than one. To demonstrate this, higher tier exposure and effects data were needed. PEC values were estimated based on simulation test data for removal in wastewater treatment plants and receiving waters and PNEC values were based on chronic effects data.

9 CONTRIBUTORS TO THIS RISK ASSESSMENT

This risk assessment was developed by experts from the following companies: Cognis, Henkel, Procter&Gamble, and Shell Chemicals (Lead). Additional input was given by the HERA Environmental Task Force.

10 REFERENCES

ADL (1991)
Environmental and human safety of major surfactants. Volume 1. Anionic surfactants. Part 2. Alcohol ethoxy sulfates.
Final report to the Soap and Detergent Association

Belanger SE, Rupe KL & Bausch RG (1995a)
Responses of invertebrates and fish to alkyl sulfate and alkyl ethoxylate sulfate anionic surfactants during chronic exposure.
Bull. Environ. Contam. Toxicol. 55: 75 l-758

Belanger SE, Meiers EM, Bausch RG (1995b)
Direct and indirect ecotoxicological effects of alkyl sulfate and alkyl ethoxysulfate on macroinvertebrates in stream mesocosms
Aquatic Toxicology 33: 65-87.

Belanger SE, Rupe, KL, Lowe RL, Johnson D & Pan Y (1996)
A flow-through laboratory microcosm suitable for assessing effects of surfactants on natural periphyton.
Enviromnental Toxicology and Water Quality: An International Journal 11: 65-76.

BKH (1994)
Environmental data review of Alkyl Ether Sulphates (AES).
Final report to Nederlandse Vereniging van Zeepfaloikanten (NVZ)

BUA (1996)
Fatty Alkyl Sulphates.
BUA Report 189, August 1996, S. Hirzel, Stuttgart.

BUA (1997)
Ecotoxicology of selected surfactants for the detergent and cleanser sector.
Report 206

Dalela RC, Tyagi AK, Pal N & Verma SR (198 1)
Water, Air, Soil Pollution 15: 3-9

Drewa G, Zbytniewski Z, Andruszczak D, Kowalska B, Korsak V, Kozica-Raszeja L, Kozlowska H & Palgan, K (1989)
Laboratory studies of the effect of an anionic detergent and fuel oil on the levels of chlorophyll, oxygen and total suspended particulate matter in water of the Brda River.
Pol. Arch. Hydrobiol. 36(1):161-168, 1989

Dyer SD, Lauth JR, Morrall SW, Herzog RR & Cherry DS (1997)
Development of a chronic toxicity structure-activity relationship for alkyl sulfates.
Environ. Toxicol. Water Qual. 12;295-303.

Dyer SD, Stanton DT, Lauth JR & Cherry DS (2000)
Structure-activity relationships for acute and chronic toxicity of alcohol ether sulfates.
Environmental Toxicology and Chemistry 19: 608-6 16.

Federle TW, Gasior SD & Nuck BA (1997)
Extrapolating mineralization rates from the ready CO_2 screening test to activated sludge, river water, and soil.
Environ. Toxicol. Chem. 16(2): 127-134

Gerike P & Jasiak W (1986)
How completely are surfactants biodegraded?
Tenside Deterg. 23:300-304

Goodnow RA & Harrison AP (1972)
Bacterial degradation of detergent compunds.
Applied Microbiology 24: 555-560

Griffith E.T., Hales S.G., Russell N.J., Watson G.K. (1986). Metabolite production during the biodegradation of the surfactant sodium dodecyltriethoxy sulphate under mixed-culture die-away conditions. J. Gen. Microbiol. 132, 963-972

Henkel(1996)
Sulfopon T55: Subacute/chronic Toxicity Algae.
P. Wierich 10.06.1996. Henkel KgaA, unpub. data, Reg. Nr. 6796.

HERA (2002)
HERA Guidance Document Methodology – April 2002.
http://www.heraproject.com/files/Guidance_document_22_April_2002.pdf

Holt MS, Mitchell GC, Watkinson RJ (1992).
The environmental chemistry, fate and effects of nonionic surfactants. In: The Handbook of Environmental Chemistry (0. Hutzinger, ed.), Vol. 3 Part F: Anthropogenic Compounds - Detergents. Springer-Verlag, Berlin

Küchler T & Schnaak W. (1997)
Behaviour of LAS in sandy soils with low amounts of organic matter
Chemosphere 35: 153-167

Lee DM, Guckert JB, Belanger SE & Feijtel TCJ (1997)
Seasonal temperature declines do not decrease periphytic surfactant biodegradation or increase algal species sensitivity.
Chemosphere 35: 1143-l 160

Lizotte RE Jr., Dom PB, Steinriede RW Jr, Wong DCL, & Rodgers, JH Jr (2002)
Ecological Effects of an Anionic C 12-15 AE-3S Alkylethoxysulfate Surfactant in Outdoor Stream Mesocosms
Environmental Toxicology & Chemistry, 2 1: 2472-275 1.

Lundahl P, Cabridenc R, Xuereff R (1972)
6th Surf Cong Zurich, Chemie and Anwendungstechnik er Grenzflachenacktiven Stoffe, Carl Hauser, Munich 1973 3:689
In: Painter HA (1992) Anionic surfactants. In de Oude NT (Ed) The Handbook of Environmental Chemistry Vo13 Part F, Anthropogenic Compounds, Detergents, Springer Verlag, pp l-88.

Madsen T, Buchardt Boyd H, Nylen D, Rathmann Pedersen A, Petersen, GI, Simonsen F (2000)
Environmental and health assessment of substanes in household detergents and cosmetic detergent products.
DHI Water & Environment

Maki AW (1979)
Correlations between *Daphnia magna* and fathead minnow (*Pimephales promelas*) chronic toxicity values for several classes of test substances.
Fish Res. Board Can. 36: 411-421

Matthijs E, Holt MS, Kiewiet A & Rijs GBJ (1997)
Fate of surfactants in activated sludge waste water treatment plants
Tenside Surf. Det. 34:238-24 1.

McAvoy DC, Dyer SD, Fendinger NJ, Eckhoff WS, Lawrence DL & Begley WM (1998)
Removal of alcohol ethoxylates, alkyl ethoxylate sulfates and linear alkylbenzene sulfonates in wastewater treatment.
Environmental Toxicology & Chemistry 17: 1705- 17 11

Nuck B.A., Federle T.W. (1996).
A batch test for assessing the mineralisation of 14C-radiolabeled compounds under realistic conditions.
Envir. Science Technol. 30, 3597-3603

Nyholm N & Damgaard BM (1990)
A comparison of the algal growth inhibition toxicity test method with the short-term 14C-assimilation test.
Chemosphere 21: 671-679.

Painter HA (1992)
Anionic surfactants. In de Oude NT (Ed) The Handbook of Environmental Chemistry Vo13 Part F, Anthropogenic Compounds, Detergents, Springer Verlag, pp l-88.

Popenoe DD, Morris SJ, Horn PS & Norwood KT (1994)
Determination of alkyl sulfates and alkyl ethoxysulfates in wastewater treatment plant influents and effluents and in river water using liquid chromatography/ion spray mass spectrometry.
Anal. Chem. 66: 1620- 1629

Sabljic A & GüstenH (1995).
QSARs for soil sorption. In: Overview of structure-activity relationships for environmental endpoints. Hermens JLM (ed), Report prepared within the framework of the project 'QSAR for Prediction of Fate and Effects of Chemicals in the Environment', an international project of the Environmental Technologies RTD Programme (DG XII/D-l) of the European Commission under contract number EV5V-CE92-02 11.

Schmidt JM (1988)
Bestimmung der toxischen Grenzkonzentration und der 50%-Hemmkonzentration von Natriumchlorid, Kupfersulfat, Dodecylhydrogensulfat-Na-Salz und Calciumcyanamid an Lupinus albus und Cicer arictinum
Z.f. Wasser- und Abwasserforschung 2 1: 107-l 09.

Scholz N (1997)
Ecotoxicology of surfactants
Tenside Surf. Det. 34: 229-232

Schröder FR (1995)
Concentrations of anionic surfactants in receiving riverine water
Tenside Surf. Det. 32:492-497

Schröder FR, Schmitt M, Reichensperger U (1999)
Effect of waste water treatment technology on the elimination of anionic surfactants.
Waste Manage. 19:125-131.

Schmidt JM (1988).
Bestimmung der toxischen Grenzkonzentration und der 50%-Hemmkonzentration von Natriumchlorid, Kupfersulfat, Dodecylhydrogensulfat-Na-Salz und Calciumcyanamid an Lupinus albus und Cicer arictinum.
Z.f. Wasser- und Abwasserforschung 21, 107-l 09.

Steber J. (1991).Wie vollständig sind Tenside abbaubar? Textilveredlung 26, 348-354

Steber J & Berger H. (1995).
Biodegradability of anionic surfactants. In: Biodegradability of surfactants (D.R. Karsa and M.R. Potter, eds). Blackie Academic & Professional, London

Steber J, Gode P & Guhl W (1988)
Fettalkoholsulphate – Die okologische Absicherung einer wichtigen Gruppe von Waschmitteltensiden, Sonderdruck aus Fett, Wissenschaft, Technologie, 32-38.

Stora G (1972)
Contributrion a l'étude de la notion de concentration lethale limite moyenne (CL50) appliquée a des invertébrés marins. 1. Etude méthodologique
Tethys 4, 597-644

Swisher RD (1987)
Surfactant Biodegradation (2nd edition, revised and expanded).
Marcel Dekker N.Y.

Urano K, Saito, M & Murata C (1984)
Adsporption of surfactants in sediments.
Chemosphere 13: 293-300.

Urano K & Saito M (1985)
Biodegradability of surfactants and inhibition of surfactants to biodegradation of other pollutants.
Chemosphere 14: 1333-1342

Van de Plassche de Burijn, J & Feijtel T (1999)
Risk assessment of four major surfactant groups in the Netherlands.
Tenside Surf. Det. 34: 242-249

Verge C & Moreno A (1996)
Toxicity of anionic surfactants to the bacterial population of a waste water treatment plant.
Tenside Surf. Det. 33: 323-327

Versteeg D, Stanton, DT, Pence MA & Cowan C (1997)
Effects of surfactants on the rotifer, *Brachionus calyciflorus*, in a chronic toxicity test and in the development of QSARS
Environmental Toxicology and Chemistry 16: 105 l-1058

White G.F., Russell N.J. (1988).
Mechanisms of bacterial biodegradation of alkyl sulphate and alkyl-polyethoxy sulphate surfactants. In: 7th Int. Biodeterioration Symp., Cambridge (D.L. Houghton, R.N. Smith, H.O.W. Eggins, eds.), Elsevier, Barking, UK, pp. 325-332

Wind pers. comm. (2002)
Henkel internal data.

Yoshirmura K & Masuda F (1982)
Biodegradation of Sodium Alkyl Poly(oxyalkylene)sulfates
J. Am. Oil Chem. Soc. 59:328-332

11 ANNEXES

Annex 1 CAS # covered in family

CAS Number	CAS Description
27028-82-6	Ethanol, 2,2',2"-nitrilotris-, compd. with a-sulfo-w-(dodecyloxy)poly(oxy-1,2-ethanediyl) (1: 1)
54116-08-4	Poly(oxy-1,2-ethanediyl), a-sulfo-w-tridecyloxy)-, sodium salt
67762-19-o	Poly(oxy-1,2-ethanediyl), a-sulfo-w-hydroxy-, C 10-l 6-alkyl ethers, ammonium salts
68037-05-g	Poly(oxy-1,2-ethanediyl), a-sulfo-w-hydroxy-, C6-1 0-alkyl ethers, ammonium salts
68037-06-9	Poly(oxy-1,2-ethanediyl), a-sulfo-w-hydroxy-, C6-10-alkyl ethers
68540-47-6	Ethanol, 2,2',2"-nitrilotris-, compd. with a-sulfo-w-(tetradecyloxy)poly(oxy- 1,2-ethanediyl) (1: 1)
68585-34-2	Poly(oxy- 1,2-ethanediyl), a-sulfo-w-hydroxy-, C 10- 16-alkyl ethers, sodium salts
68585-40-O	Poly(oxy- 1,2-ethanediyl), a-sulfo-w-hydroxy-, C 16- 18-alkvl ethers- sodium salts
68891-38-3	Poly(oxy-1,2-ethanediyl), a-sulfo-w-hydroxy-, C12-14-alkyl ethers, sodium salts
96130-61-9	Poly(oxy-1,2-ethanediyl), a-sulfo-w-hydroxy-, C9-1 1-alkvl ethers. sodium salts
105859-96-9	Ethanol, 2,2',2"-nitrilotris-, compds. with polyethylene glycol hydrogen sulfate C 1 1 - 15-sec-alkyl ether ammonium salts
125301-92-o	Poly(oxy-1 ,2-ethanediyl), a-sulfo-w-hydroxy-, C 12-15-alkyl ethers, sodium salts
125304-06-5	Ethanol, 2,2',2"-nitrilotris-, compds. with polyethylene glycol hydrogen sulfate C 16- 18-alkyl ether
129783-23-9	Ethanol, 2,2'-iminobis-, compds. with polyethylene glycol hydrogen sulfate C 12- 15-alkyl ethers
157627-92-4	Alcohols, C 10- 16, ethoxylated, sulfates, mono(hydroxyethyl)ammonium salts (>1 <2.5 mol EO)
157707-82-9	Alcohols, C 14-16, ethoxylated, sulfates, sodium salts (>1 <2.5 mol EO)
162201-45-g	Ethanol, 2-amino-, compds. with polyethylene glycol hydrogen sulfate C 12- 15-alkyl ethers

174450-50-l	Alcohol, C12-14, ethoxylated, sulfates, triisopropanolamine salts
102783-14-2	Poly(oxy-1,2-ethanediyl), a-sulfa-w-hydroxy-, C10-18-alkyl ethers, sodium salts
9004-82-4	Sodium lauryl ether sulfate
2523 l-22-5	Poly(oxy-1,2-ethanediyl), .alpha.-[(tridecyloxy)sulfonyl]-.omega.-hydroxy-, sodium salt
3443 l-25-9	Polyethylene glycol octyl ether sulfate, sodium salt
52286-19-8	Polyethylene glycol decyl ether sulfate, ammonium salt
67762-21-4	Poly(oxy-1,2-ethanediyl), .alpha.-sulfo-.omega.-hydroxy-, C 10- 16-alkyl ethers, magnesium salts
6808 l-9 l-4	Poly(oxy-1,2-ethanediyl), .alpha.-sulfo-.omega.-hydroxy-, C12-18-alkyl ethers, sodium salts
68 184-04-3	2-Aminoethanol compd. with .alpha.-sulfo-.omega.-(dodecyloxy)poly(oxy-1,2-ethanediyl) (1: 1)
68610-22-o	Poly(oxy-1,2-ethanediyl), .alpha.-sulfo-.omega.-hydroxy-, C12-18-alkyl ethers, ammonium salts
68891-29-2	Poly(oxy- 1,2-ethanediyl), .alpha.-sulfo-.omega.-hydroxy-, C8-10-alkyl ethers, ammonium salts
68891-30-5	Poly(oxy-1,2-ethanediyl), .alpha.-sulfo-.omega.-hydroxy-, C 1 l-l 5-branched alkyl ethers, ammonium salts
73665-22-2	Poly(oxy-1,2-ethanediyl), .alpha.-sulfo-.omega.-hydroxy-, C6-10-alkyl ethers, sodium salts
157627-95-7	Poly(1,2-ethanediyl), .alpha.-sulfo-.omega.-hydroxy-C16-18 and C 18 unsaturated alkyl ethers, sodium salts
160104-51-8	Poly(1,2-ethanediyl), .alpha.-sulfo-.omega.-hydroxy-C12-14 alkyl ethers, magnesium salts
160 104-52-g	Poly(1,2-ethanediyl), .alpha.-sulfo-.omega.-hydroxy-C16-1 8 and C 18 unsaturated alkyl ethers, magnesium salts
67762-l 9-O	Poly(oxy- 1,2-ethanediyl), .alpha.-sulfo-.omega.-hydroxy-, C 10- 16-alkyl ethers, ammonium salts
13150-00-0	Ethanol, 2-[2-[2-(dodecyloxy)ethoxy]ethoxy]-, hydrogen sulfate, sodium salt
32612-48-9	Poly(oxy-1,2-ethanediyl), .alpha.-sulfo-.omega.-(dodecyloxy)-, ammonium salt

Annex 2 Physchem Properties

All values estimated by interpolation of values for E02 and E03 calculated using SRC sofhvare

EO2.7 – Average for HERA applications

Carbon #	12	13	14	15	16	18
Molecular weight (g mol^{-1})	407	422	436	450	464	492
Melting point (°C)	298	304	309	315	320	331
Boiling point (°C)	684	695	707	719	730	754
Vapour pressure at 25°C (Pa)	1.2E-13	4.9E-14	2.1E-14	8.8E-15	3.8E-15	6.2E-16
Octanol-water partition coefficient (log_{10}) SRC	0.95	1.4	1.9	2.4	2.9	3.9
Water solubility (mg l^{-1})	425	133	41	13	4.0	0.38

EO2.4 – Average for total captive tonnage

Carbon #	12	13	14	15	16	18
Molecular weight (g mol^{-1})	394	409	423	437	451	479
Melting point (°C)	293	299	304	310	315	326
Boiling point (°C)	673	684	696	708	719	743
Vapour pressure at 25°C (Pa)	2.1E-13	8.8E-14	3.8E-14	1.6E-14	6.9E-15	1.1E-15
Octanol-water partition coefficient (log_{10}) SRC	1.0	1.5	2.0	2.5	3.0	4.0
Water solubility (mg l^{-1})	437	136	42	13	4.1	0.39

Annex 3 RCR based on Total Tonnage

PEC values have been calculated for the total EU-captive tonnage using the same assumptions as used for the HERA tonnage. Export tonnages have been omitted in estimating PEC_{local} values.

PEC - Simpletreat estimates

Carbon #	12	13	14	15	16	18
Local PEC surface water (mg/l)	0.16	2.3E-2	6.5E-2	6.1E-3	5.7E-3	1.7E-3
Local PEC sediment (mg/kg wwt)	0.16	2.9E-2	0.13	2.3E-2	4.7E-2	8.0E-2
Local PEC agric 30 d (mg/kg wwt)	4.1E-3	1.5E-3	1.1E-2	2.5E-3	5.8E-3	1.OE-2
Local PEC agric 30 d with 87% anaerobic degradation (mg/kg wwt)	5.3E-4	2.2.0E-4	1.41.4E-	3.33.3E-4	7.5I7.5E-4	1.3E-3
PECstp microorgs (mg/l)	1.5	0.22	0.62	5.9E-2	5.5E-2	1.6E-2
Regional PEC surface water total (mg/l)	6.9E-3	.OE	-3	2.8E-3	2.7E-4	2.5E-4 8.3E-5

PEC - Simulation test degradation estimates

Scaling the STP fate to 97.5% degradation, as was done for the HERA tonnage, reduces the PEC values to:

Carbon #	12	13	14	15	16	18
Local PEC surface water (mg/l)	3.6E-2	5.4E-3	1.5E-2	1.4E-3	1.1E-3	4.4E-4
Local PEC sediment (mg/kg wwt)	3.5E-2	6.6E-3	2.9E-2	5.2E-3	9.1E-3	2.1E-2
Local PEC agric 30 d (mg/kg wwt)	4.6E-3	1.7E-3	1.2E-2	2.8E-3	6.5E-3	1.1E-2
Local PEC agric 30 d with 87% anaerobic degradation (mg/kg wwt)	6.0E-4	2.2E-4	1.6E-3	3.6E-4	8.4E-4	1.4E-3
PECstp microorgs (mg/l)	0.30	4.4E-2	0.12	1.2E-2	9.1E-3	3.7E-3
Regional PEC surface water total (mg/l)	5.7E-3	8.30E-4	2.3E-3	2.2E-4	2.1E-4	7.0E-5

PNEC

PNEC values (mg/l) were derived using the equation in Section 6.2.3:

	Carbon #					
	12	13	14	15	16	18
Aquatic (mg/l)	0.23	0.066	0.033	0.03	0.05	0.78
Soil (mg/kg)	3.1E-02	9.3E-03	4.9E-03	4.7E-03	8.1E-03	0.14

Indirect Exposure

AES with EO=2.4 uptake by Humans – as calculated with EUSES*

AS Fraction	Regional (mg/kg/day)		Local (mg/kg/day)	
	Drinking Water	Total Food + Water Uptake	Drinking Water	Total Food + Water Uptake
C12	1.6E-4	1.9E-4	1 .0E-3	1.2E-3
C13	2.4E-5	3.3E-5	1.5E-4	2.0E-4
C14	6.6E-5	1.2E-4	4.2E-4	7.3E-4
C15	6.3E-6	1.7E-5	4.0E-5	1.1E-4
C16	5.9E-6	3.1E-5	3.2E-5	1.7E-4
C18	2.0E-6	5.8E-5	1.3E-5	3.7E-4

*EUSES defaults modified according to the HERA Detergent Scenario and taking account of 97.5% degradation in STP and 87% anaerobic degradation in sludge

RCR

Carbon #	12	13	14	15	16	18	Total RCR
Aquatic	0.15	8.2E-02	0.45	4.6E-02	2.2E-02	5.6E-04	0.72
Soil	1.9E-2	2.4E-2	0.31	7.7E-2	0.14	1.OE-2	0.55

RECEIVED
OPPT CBIC

2006 SEP 21 AM 9: 58

201-16316

DOSSIER
Alcohol Ethoxysulphates (AES)

Excerpted from:

Human & Environmental Risk Assessments on ingredients of European household cleaning products:

Alcohol Ethoxysulphates (AES): Human Health Risk Assessment

(Draft 2003)

Alcohol Ethoxysulphates (AES): Environmental Risk Assessment

1. Substance Characterisation

Alcohol ethoxysulphates (AES), also known as alkyl ethersulphates, are a widely used class of anionic surfactants. They are used in household cleaning products, personal care products including toothpaste and shampoos, hand and other personal cleaning products, institutional cleaners and industrial cleaning processes, and as industrial process aids in emulsion polymerisation and as additives during plastics and paint production. Uses in household cleaning products, relevant to the HERA program of risk assessments, include laundry detergents, hand dishwashing liquids, and various hard surface cleaners.

1.1. CAS No and Grouping information

There are more than 36 CAS Numbers describing AES. A comprehensive list is presented in Appendix 1 of this document. Although clearly important from a Regulatory perspective, this assessment is not based on CAS Nos., but on a clear definition of the product family's composition.

1.2. Chemical structure and composition

The alcohol ethoxysulphate family is defined for HERA purposes to encompass commercial grades of linear-type primary alcohol ethoxysulphates containing AES components of basic structure $C_nH_{2n+}O(C_2H_4O)_mSO_3X$) where n=10-18 and m = 0-8 and X = sodium, ammonium or triethanolamine (TEA). Sodium salts of AES are by far the commonly used grades.

2.2 Hazard Assessment

2.2.1. Summary of the available toxicological data

2.2.1.1. Acute Toxicity

2.2.1.1.1. Acute Oral Toxicity

The acute oral toxicity of alcohol ethoxysulphates (AES) was evaluated with rats in several acute oral toxicity studies [Hüls AG, 1997a; Hüls AG, 1986a; Shell Research Ltd. 1975a; Shell Research Ltd., 1978a; Shell Research Ltd., 1978b; Brown, V. et al., 1968; Shell Research Ltd., 1975b; Shell Research Ltd., 1978c; Shell Research Ltd., 1975c; Shell Research Ltd., 1972; Brown, V. et al., 1970; Shell Chemical Co., 1967; Arthur D. Little, 1991]. The test materials were typically AES solutions containing 25 – 70% active material. The dilutions were administered at doses ranging from 2.5 – 10 ml/kg bodyweight. Most of the studies pre-date Good Laboratory Practice (GLP) regulations and in only one of these [Vermeire et al., 1993], the study design included at least 5 animals of each sex per dose group, thus meeting the critical aspect of current testing standards as defined in OECD methodologies. In these studies, the LD50 was estimated to be > 1.3 g active material per kg bodyweight. In a review for the Soap and Detergent Industry Association, Arthur D. Little reported rat oral LD50 values ranging from 1.7 - > 5 g/kg bodyweight [Arthur D. Little, 1991]. The most reliable studies will be discussed in the following paragraph in more detail.

A recent study [Hüls AG, 1997a] which was rated as reliable without restrictions according to the Klimisch criteria [Klimisch et al. (1997)], followed the guidelines of OECD method 401 and was compliant with GLP, a group of ten rats, five of each sex, was given a single oral dose of the triisopranolammonium salt of C12-14AE2S (90% active material) at a dose level of 2000 mg/kg bodyweight. The undiluted liquid was administered by gavage with an application volume of 2 ml/kg bodyweight. The rats were observed daily for any mortalities and clinical symptoms following treatment. Individual body weights were recorded on days 0 (prior to dosing), 7 and 14. At the end of the 14-day observation period, the animals were sacrificed and macroscopically examined. There were no deaths following a single oral application of the tested AES. The animals showed mild clinical symptoms such as increased activity and piloerection as a reaction to the treatment for approximately four hours after dosing. The macroscopic examination on day 14 showed no significant lesions. In conclusion, the acute lethal oral dose to male and female rats of the tested AES was found to be > 2 g/kg.

In a further study, rated as reliable with restrictions according to the Klimisch criteria, was also conducted according to the guidelines of OECD method 401, but not following GLP standards, a 70% solution of NaC12-14AE2S was administered by oral gavage at a dose level of 2.5 g/kg. No mortalities occurred under the dosing conditions. The rats achieved acceptable bodyweight gains throughout the study and showed mild clinical signs (unkempt fur, abdominal position, diarrhoea) as a reaction to the treatment for approximately 2 hours after dosing. The macroscopic examination on day 14 showed no significant lesions.

Conclusion

Alcohol ethoxysulphates are considered to have a low order of acute oral toxicity in the rat. In two recent and guideline compliant acute oral toxicity studies with marketed AES substances, the

LD50 was greater than 2000 mg/kg bodyweight. The clinical findings such as increased activity and piloerection following oral exposure are indicative of gastrointestinal stress and could be explained by the irritant nature of the test solutions under the conditions of oral gavage.

2.2.1.1.2. Acute Inhalation Toxicity

There are no test data available to evaluate the acute inhalation toxicity of AES. Only one study was identified in the review conducted by Arthur D. Little. In this study, rats (group size not specified) survived a 1 hour exposure to 60 mg/l of 59% active material solution of NH_4 C12-14AE3S. No additional details are available.

Conclusion

Given the lack of information on the study protocol and study results, this study is not suitable to assess the acute inhalation toxicity hazard of AES-type surfactants.

2.2.1.1.3. Acute Dermal Toxicity

The acute dermal toxicity of AES has been evaluated in several rat studies [Hüls AG, 1997b; Shell Research Ltd. 1975a; Shell Research Ltd., 1978a; Shell Research Ltd., 1978b; Shell Research Ltd., 1975b; Shell Research Ltd., 1978c; Shell Research Ltd., 1975c; Shell Research Ltd., 1972; Shell Chemical Co., 1967; Arthur D. Little, 1991] and in one rabbit study [Shell Chemical Co., 1967]. Most of the studies did not follow OECD guidelines (e.g. use of small group sizes) and did not comply with GLP regulations However, despite some protocol deficiencies, the studies were reported in sufficient detail to allow a reasonable assessment of the potential dermal toxicity of AES in laboratory animals. The investigations included mortality and clinical observations. No mortality was observed in the rat studies at the dose level tested and subsequently LD50 values were expressed to be above the highest investigated dose levels, *i.e.*, >0.65 g/kg [Shell Research Ltd., 1978a], >1.12 g/kg [Shell Research Ltd., 1978b], >2.4 g/kg [Shell Research Ltd. 1975a], >1.25 g/kg [Shell Research Ltd., 1972], >1.08 g/kg [Shell Research Ltd., 1975b], >0.54 g/kg [Shell Research Ltd., 1978c], >1.8 g/kg [Shell Research Ltd., 1975c] and 4.6 g/kg [Shell Chemical Co., 1967]. Arthur D. Little, 1991 reported dermal LD50 values for AES on both intact and abraded rabbit skin ranging from 4 – 12 g/kg bodyweight. At highest dosage levels, various degrees of skin irritation (moderate to severe erythema and oedema) were reported and signs of intoxication included sporadic signs of haemorrhage around the eyes and nose, piloerection, and diarrhoea.

An acute dermal toxicity study (limit test) following OECD method 402 and complying with GLP guidelines was performed to assess the acute dermal toxicity of triisopranolammonium salt of C12-14AE2S (90% active material) in the rat. A group of ten rats, five of each sex, was given a single dermal application of the test substance at a dose level of 2 g/kg bodyweight. There were no deaths and no signs of systemic reaction to the treatment. Following removal of the dressing, moderate to severe dermal irritations indicated by inflammation of the epidermis and eschar formation were observed at the treatment site. The effects cleared over time. Some minor residual skin lesions were observed in 1 animal at the end of the 14-day observation period. No

abnormalities were recorded at the macroscopic examination on day 14. The acute lethal dermal dose to male and female rats of NH_4C12-14AE2S was determined to be > 2 g/kg bodyweight.

Conclusion

Alcohol ethoxysulphates are considered to be of low acute dermal toxicity to rats. This was demonstrated in a recent, OECD guideline and GLP compliant acute dermal toxicity limit test in rats. This study has been judged to provide reliable information on the dermal toxicity of AES.

This assessment is supported by a substantial number of further acute dermal toxicity studies in rats and rabbits with a lower reliability score, which also demonstrated low acute dermal toxicity of AES-type surfactants.

2.2.1.1.4. Skin Irritation

Several skin irritation studies were conducted on rabbits considering different concentrations (0.1%, 1%, 10%, neat material), exposure duration (4h, 24h, 36 h) and exposure conditions (open application, semi-occlusion, full occlusion) [Hüls AG, 1997c; Hüls AG, 1986b; Shell Research Ltd., 1978d; Shell Research, Ltd., 1978e; Shell Oil Co., 1989; Shell Research Ltd. 1975a; Shell Research Ltd., 1978a; Shell Research Ltd., 1978b; Shell Research Ltd., 1968; Shell Research Ltd., 1978c; Shell Research Ltd., 1975c; Brown et al., 1970, Shell Chemical Co., 1967; Arthur D. Little, 1991, Hüls AG, 1997b.

The triisopranolammonium salt of C12-14AE2S (90% active material) was tested in an EC standard (4h) skin irritation study on rabbits [Hüls AG, 1997b]. The study followed OECD method 404 and was in compliance with GLP regulations. In this study, the undiluted liquid test substance was applied in a single dose for 4 hours to the shorn intact skin of three animals. The administration of the test substance led to well-defined erythema 24 hours after application, and was associated with distinct oedema in two animals and severe oedema in the 3rd animal. Forty–eight (48) hours after application, these signs of irritation were still well-defined and without change in 2 out of 3 animals. The 3rd animal presented with moderately severe erythema, associated with severe oedema, dry skin and scaling, 48 hours after application. Seventy-two (72) hours after application, 2 animals exhibited localized skin irritation in the form of well-defined or moderately severe erythema and oedema, and 1 rabbit had slight subcutaneous haemorrhages. On the 14th day after administration of the test substance, the skin of all the animals was free from signs of irritation. For all 3 animals, an erythema/eschar mean score of 2.33 and an oedema mean score of 2.78 was determined. This score indicates moderate skin irritation properties of the undiluted test substance.

In two further studies [NOTOX, 1994, Hüls AG, 1986b], NaC12-14AE2 (70% active material) was tested in the EC standard irritation test. Both studies were conducted in compliance with OECD method 404, but only 1 complied with GLP regulations [NOTOX, 1994]. As in the case of the study discussed before, exposure to the test substance for 4 hours resulted in moderate to severe erythema and oedema. After 72 hours, reduced flexibility, fissuring of the skin and severe erythema and oedema were apparent. One study [Hüls AG, 1986b] terminated the observations at the 14th observation day and clinical signs of irritation were still apparent at this time. In the other study [NOTOX, 1994], animals were observed for 21 days and irritation had completely resolved within 21 days after exposure, but patches of bold skin persisted at termination.

As indicated before, further studies were conducted to investigate the skin irritation of effects of various dilutions of AES at different exposure durations and conditions. These studies were investigative in nature and neither was in compliance with OECD guidelines, nor with GLP regulations. However, these studies provide useful information on AES exposure conditions that are of particular relevance in consumer product applications. In 4hr or 24hr skin irritation studies on rabbits, a 0.1% AES solution did not show any signs of irritation, a 1% AES solution showed slight irritation, and solutions containing AES of 10 – 30% were mildly to moderately irritating under the patch conditions of the animal test.

Conclusion

The irritation potential of AES is concentration dependent. Materials with concentrations higher than 70% are moderately to severely irritating to rabbit skin under the conditions of the EC irritation test, and therefore classified as irritating to skin according to EU criteria as laid down in the Dangerous Substance Directive (67/548/EEC). At concentrations between 10 and 30%, the AES solutions exhibit mild to moderate irritancy under the conditions of an occluded patch test. AES concentrations below 1% are virtually non-irritating under the conditions of the acute skin irritation testing protocol.

2.2.1.2. Eye Irritation

The potential of AES to cause eye irritation under accidental exposure conditions has been evaluated in several rabbit eye irritation studies [Hüls AG, 1997d; Hüls AG, 1986c, Shell Research Ltd. 1975a, Shell Research Ltd., 1978b, Shell Research Ltd., 1975b, Shell Research Ltd., 1978c, Shell Research Ltd., 1972, Brown et al., 1970, Arthur D. Little, 1991]. Most of the studies with undiluted or concentrated AES solutions (e.g. 32.6% C9-11AE2.5S, 70% C12-13AE2S, 28% C12-13AE2S) resulted in extensive corneal damage, inflammation of the iris and maximal conjunctival irritation with no significant improvement seen over a 7-day recovery period after product administration [Shell Research Ltd. 1975a Shell Research Ltd., 1975b, Brown et al., 1970]. In the same studies, which were neither conducted according to OECD guidelines (e.g., protocol deviations such as application volume and observation period), nor followed the principles of GLP, the authors also investigated the same materials at concentrations of 10%, 1% and 0.1%. Generally, solutions containing 10% AES were observed to cause moderately irritating effects while 1% and 0.1% dilutions were virtually non-irritating. The most reliable studies will be discussed in the following paragraph in more detail.

The triisopranolammonium salt of C12-14AE2S (90% active material) was tested in an acute eye irritation study ("Draize test") according to OECD method 405 and following the principles of GLP. In this study, 0.1ml of the liquid test substance was administered into the conjunctival sac of one eye of each of the 3 rabbits. After an exposure time of 24 hours, the eyes were flushed with warm physiological saline. Twenty-four hours after exposure, the animals were observed to have reactions of the conjunctivae in the form of diffuse crimson red discoloration (individual blood vessels not easily discernible), together with distinct swelling and partial eversion of the eyelids. The cornea was slightly opaque over the entire surface, and the iris of one animal showed severe hyperaemia. Up to 72 hours after administration, these signs of irritation were largely unchanged and after 6 days, all signs of irritation began to diminish. After day 17, 2 animals were free from signs of irritation of the eye and mucosa. The 3rd animal was cleared after 24 days.

In another study, 28% active C12-14AE2S was also tested in the Draize test, following the guidelines specified in the OECD method 405. GLP compliance was not mentioned. Again, in this study the tested AES material caused corneal opacity, iritis and conjunctivitis in all test animals. While the conjunctivitis appeared to improve in all 3 test animals approximately 8-10

days after exposure to the test material, corneal opacity and the circumcorneal injection in the iris were still present in 2 animals after 21 days.

Further investigative studies were conducted to determine the effect of rinsing and AES alkyl chain length on the eye irritation potential in rabbits [Procter & Gamble, 1996b]. It was found that rinsing after instillation greatly reduced the severity of eye effects and that AES in the C12-16 range produced more severe effects than AES with longer or shorter chains. This was primarily manifested by longer clearing times (> 7 days versus 1-7 days).

Conclusion

In two independent OECD and GLP compliant acute eye irritation studies, the triisopranolammonium salt of C12-14E2S (90% active material) and NaC12-14E2S (28% active material) were shown to be moderately to severely irritating to rabbit eyes. Due to its persistent effects, these materials were to be classified as severely irritating, according to the EU criteria as laid down in the Dangerous Substance Directive (67/548/EEC).

In studies with a lower reliability score it was shown that solutions containing less than 1-10% AES are slightly to moderately irritating to eyes and below 1%, AES solutions are virtually non-irritating.

2.2.1.3. Skin Sensitization

The skin sensitization potential of AES was evaluated in the guinea pig maximization test according the Magnusson-Kligman protocol [Hüls AG, 1989; Henkel KGaA, 1977a; Henkel KGaA, 1985; Henkel KGaA, 1977b; Shell Research Ltd., 1975d; Shell Research Ltd., 1980a; Shell Research Ltd., 1983a, Shell Research Ltd. 1975a, Shell Research Ltd., 1978a, Shell Research Ltd., 1978b, Shell Research Ltd., 1975b, Shell Research Ltd., 1978c, Shell Research Ltd., 1978d, Shell Research, Ltd., 1978e] and in the non-adjuvant Buehler protocol in guinea pigs [Hüls AG, 1997e, Shell Research Ltd., 1975b, Shell Research Ltd., 1972, Brown et al., 1970, Arthur D. Little, 1991]. Further results of skin sensitization studies are listed in a review conducted for the US soap and detergent industry [Arthur D. Little, 1991].

In summary, of 15 studies conducted on different AES batches and materials according to the Magnusson-Kligman protocol, 14 studies revealed no evidence for skin sensitization potential of AES and only 1 study resulted in a positive result, indicating weak sensitization potential of a tested AES batch. Of the available 8 Buehler studies, 6 studies did not indicate any skin sensitization potential of the tested AES batches and 2 studies resulted in a weak positive response. It must be noted that the majority of the available studies were not conducted according to the OECD guideline protocols, nor according GLP standards. Nevertheless, based on the limited information available, these studies appear to be scientifically well conducted and the results should be included in the overall evaluation. The studies reported in most detail will be discussed in the following paragraphs.

NaC12-14AE2S (28% active material) was evaluated in the Magnusson-Kligman guinea pig maximization test [Hüls AG, 1989] according to OECD method 406. In the induction phase, the treatment group was injected on day zero 3 pairs of 0.1ml volume (injection 1: a 1:1 mixture

Freunds' complete adjuvant (FCA) and water; injection 2: 0.1% test substance in water; injection 3: 0.1% test substance in a 1:1 mixture FCA) in the shoulder region of female guinea pigs. A week later, a patch containing 30% solution of the test substance was placed over the injection area for 48 hours in the treatment group. The control groups were treated in the same manner, but without the test substance (i.e., 3 injections on day 0 and patch application on day 7). Two weeks after the induction phase, the flanks of the treated and the control animals were cleared of hair and an occlusive 'challenge' patch containing 10% of the test substance (or water in case of the control group) was applied to one flank of the animals for 24 hours. Approximately 48 and 72 hours from the start of the challenge application, the skin reaction was observed and recorded according to the Magnusson-Kligman grading scale. Under the test conditions, NaC12-14AE2S did not cause skin sensitization in guinea pigs.

Further AES materials such as NaC12-14AE2S (27% active material) and a mixture of sodium laureth sulphate, sodium laureth-8 sulphate and sodium oleth sulphate (5-10EO, 29% active matter) were evaluated according the same protocol and were found to not cause skin sensitization in guinea pigs [Henkel KGaA, 1977a, Henkel KGaA, 1977b]. However, one batch of NaC12-15E3S caused a weak skin sensitization response [Henkel KGaA, 1985]. In this study, 20 animals were induced intradermally with a 0.25% aqueous solution of the test item and complete Freund' adjuvant. One week after, an occluded patch containing 50% solution of the test substance was placed over the injection area for 48 hours. After a 14 day rest period, the test animals were challenged with an occluded patch containing a 20% solution of the test substance. 24 and 48 hours after removal of the challenge patch, dermal reactions (score 1) were seen in seven animals. A rechallenge was performed seven days later by applying a 10% aqueous solution of the test substance on the flanks opposite to the treatment area. Two out of twenty animals displayed weak skin effects (score 1).

In a more recent study, the triisopranolammonium salt of C12-14AE2S was tested according the Buehler method in guinea pigs following OECD guidelines 406 and in compliance with GLP standards [Hüls AG, 1997e]. To determine the potential sensitizing effect of this test substance, 20 test animals and 10 control animals were tested with the highest readily tolerated concentration of the test substance, which led to slight to well-defined signs of irritation. A 50% strength formulation was used for treatment during induction phases I, II, and III and a 25% strength formulation of the test substance was administered as the highest non-irritant concentration during challenge. The challenge treatment did not cause any cutaneous reactions in the form of erythema or oedema on the posterior right flank of any treated animal in the test and control groups 30 and 54 hours after administration. Based on these results, the test material NH4C12-14E2S showed no sensitizing effect on guinea pigs under the described test conditions.

In 1966, skin sensitization associated with exposure to ethoxysulphates was reported in Norway. Walker et al., 1973 conducted a series of investigations to determine the source of this response and identified a contaminant in one particular AES batch shown to be the responsible sensitizing agent. Connor et al., 1975 identified the contaminant in AES to be 1-dodecene-1,3-sultone, 1-tetradecene-1,3 sultone, 2-chloro-1,3 dodecene sultone and 2-chloro-1,3-tetradecene sultone. Connor et al. demonstrated that these sultones could be formed only under very specific, extreme AES manufacturing conditions. It became evident that the unsaturated and the chloro-sultones which are considered to be potent skin sensitizers were the result of conditions not normally

present and readily avoidable in AES manufacture. The formation of sultones in the AES production is to date not an issue anymore. Presently, residual levels of unsaturated and chloro-sultones and their precursors are monitored in AES batches on a routine basis.

Conclusion

Taking a weight of evidence approach and considering quality criteria (*i.e.,* compliance with OECD methods, GLP) in evaluating reliability of individual studies, AES are not considered to be a skin sensitizers. The vast majority of available guinea pig studies in which AES was tested for skin sensitization properties demonstrated the absence of skin sensitizing potential of AES. Only a few studies indicated a weak sensitization potential of AES, but it should be taken into consideration that observed reactions may have been confounded with irritation reactions.

2.2.2. Repeated Dose Toxicity

2.2.2.1. Oral route

NaC12-15AE3S was tested at doses of 0%, 0.023%, 0.047%, 0.094%, 0.188%, 0.375%, 0.75%, 1% and 1.5% in a 3-week dietary rat feeding study [Unilever, 1979a]. Three (3) animals per sex per dose and 6 animals of each sex in the control group were used. In summary, the organ most affected by the feeding of NaC12-15AE3S was the liver. No effects were observed in rats fed at 0.188% dietary level (254 mg/kg/body weight per day) and less. The lowest observed effect level, based on hepatocytic hypertrophy was 0.375% which is equivalent to 487 mg/kg body weight per day. Significantly increased organ weights (liver, kidney, brain) were observed in males and females at doses equal (females) or higher (males and females) than the LOEL established for hepatocytic hypertrophy.

NH4C12-15E3S was tested at doses of 0%, 0.023%, 0.047%, 0.094%, 0.188%, 0.375%, 0.75%, 1% and 1.5% in a 3-week dietary rat feeding study [Unilever, 1979b]. Three (3) animals per sex per dose and 6 animals of each sex in the control group were used. In summary, the only organ affected by the feeding of NH4C12-15E3S was the liver. No effects were observed in rats fed at 0.188% dietary level (232 mg/kg/body weight per day) and less. The lowest observed effect level, based on significant increases in plasma alkaline phosphatase activity, was 0.375% which is equivalent to 465 mg/kg body weight per day. Significantly increased liver weight was observed in males and females at doses higher than the LOEL established for the change in some plasma enzyme levels.

NaC12-15E3S containing 21.1% ethanol and 1.15% methanol (note: after mixing with the diet and storage for 3-4 days methanol was no longer detectable and more than 98% of remaining ethanol was evaporated) was tested at doses of 0%, 0.023%, 0.047%, 0.094%, 0.188%, 0.375%, 0.75%, 1% and 1.5% in a 3-week dietary rat feeding study [Unilever, 1980a]. Three (3) animals per sex per dose and 6 animals of each sex in the control group were used. In summary, the organ mostly affected by the feeding of NaC12-15E3S was the liver. No effects were observed in rats fed at 0.094% dietary level (108 mg/kg/body weight per day) and less. The lowest observed effect level, based on significant increases in plasma alkaline phosphatase activity, was 0.188% which is equivalent to 217 mg/kg body weight per day. Significantly increased liver weight was

observed in males and females at doses equal (females) or higher (males and females) than the LOEL established for the change in some plasma enzyme levels.

NH4C13-15E3S was tested at doses of 0%, 0.023%, 0.047%, 0.094%, 0.188%, 0.375%, 0.75%, 1% and 1.5% in a 3-week dietary rat feeding study [Unilever, 1979c]. Three (3) animals per sex per dose and 6 animals of each sex in the control group were used. In summary, the organ mostly affected by the feeding of NH4C12-15E3S was the liver. No effects were observed in rats fed at 0.375% dietary level (461 mg/kg/body weight per day) and less. The lowest observed effect level, based on hepatocyte hypertrophy, was 0.75% which is equivalent to 857 mg/kg body weight per day. Significantly increased organ weights (liver, brain, testes) were observed in males and females at doses higher than the LOEL established for hepatocytic hypertrophy.

NaC12-14E3S was tested at doses of 0%, 0.023%, 0.047%, 0.094%, 0.188%, 0.375%, 0.75%, 1% and 1.5% in a 3-week dietary rat feeding study [Unilever, 1979d]. Three animals per sex per dose and six animals of each sex in the control group were used. In summary, the only organ affected by the feeding of NH4C12-15E3S was the liver. No effects were observed in rats fed at 0.094% dietary level (120 mg/kg/body weight per day) and less. The lowest observed effect level, based on increase in plasma levels of glutamic-pyruvic transaminase and alkaline phosphatase, was 0.188% which is equivalent to 236 mg/kg body weight per day. Significant changes in organ weights (liver, kidney, heart, adrenals) were observed in males and females at doses higher than the LOEL established for changes in plasma enzyme levels.

NaC16-18E4S was tested at doses of 0%, 0.023%, 0.047%, 0.094%, 0.188%, 0.375%, 0.75%, 1% and 1.5% in a 3-week dietary feeding study [Unilever, 1980b]. Three (3) animals per sex per dose and 6 animals of each sex in the control group were used. In summary, the organ mostly affected by the feeding of NH4C12-15E3S was the liver. No effects were observed in rats fed at 0.375% dietary level (468 mg/kg/body weight per day) and less. The lowest observed effect level, based on hepatocyte hypertrophy and increases in plasma levels of glutamic-pyruvic transaminase, was 0.75% which is equivalent to 969 mg/kg body weight per day. Significant changes in organ weights (liver, kidney, heart) were observed in males and females at doses higher than the LOEL established for changes in plasma enzyme levels.

NaC12-15E3S was tested at doses of 0%, 0.023%, 0.047%, 0.094%, 0.188%, 0.375%, 0.75%, 1% and 1.5% in a 3-week dietary rat feeding study [Unilever, 1979e]. Three (3) animals per sex per dose and 6 animals of each sex in the control group were used. In summary, the organ mostly affected by the feeding of NH4C12-15E3S was the liver. No effects were observed in rats fed at 0.375% dietary level (441 mg/kg/body weight per day) and less. The lowest observed effect level, based on hepatocyte hypertrophy, was 0.75% which is equivalent to 872 mg/kg body weight per day. Significant changes in organ weights (liver, brain, heart, spleen) were observed in males and females at doses higher than the LOEL established for hepatocyte hypertrophy.

The Unilever studies summarized above were not conducted according to OECD and GLP guidelines. However, the methodology used was similar in many respects to OECD Guideline No. 407.

In a 28-day oral gavage rat study, a blend of alkyl (C14-18) sulphate and C12-13E6.5S was tested at 30, 100, 300, and 1000 mg/kg/day [Shell Oil, 1992]. This blend caused irritation to the forestomach of the test animals, evidenced as hyperplasia and hyperkeratosis. Histologically, the hyperplasia appeared as a thickening of the non-glandular stomach epithelium at 100, 300, and 1000 mg/kg/day, but not at 30 mg/kg/day. Similar to the 90-day oral gavage study discussed above, the effects observed in forestomach are considered to be local treatment-related and concentration dependent irritant effects. Since there is no human equivalent to the rat forestomach, these effects are not considered to be relevant to human health assessment. No further information is available on this study and thus, a NOEL or NOAEL for systemic toxicity could not be established.

Synthetic NaC12-15AE3S and natural NaC12AE3S were tested in a 90-day rat diet study at dose levels of 0, 40 200, 1000 and 5000 ppm active material [Walker, 1967]. Health, behaviour, body weight, food intake, haematological and urinary parameters remained within normal limits at all doses. Total serum protein was increased in males in the 5000ppm dose group of NaC12-15AE3S. Differences in absolute organ weights were observed at 5000ppm only. Both ethoxysulphates increased kidney weight in males. Liver weight was increased at 5000ppm in both sexes by NaC12-15AE3S. Females receiving NaC12AE3S showed increased liver, kidney and heart weights. A large variation was reported in male heart weights in rats receiving 1000ppm of NaC12-15AE3S, but the increase was not considered to be treatment related. No increase in heart weight was reported for males receiving 5000ppm. Similarly to the study by Butterworth [Shell Research Ltd., 1982a], a NOEL or NOAEL was not established by the authors, but based on the available information and taking a conservative approach, the NOAEL could be established at the dose level of 1000ppm. The study was conducted prior to the development of GLP and OECD guidelines. However, the principles and the procedures were similar in various respects to the OECD test guidelines.

NaC12-15E3S was fed to rats at dietary concentrations of active ingredient of 0, 40, 200, 500, 1000 and 5000ppm in a 90-day oral feeding study [Shell Research Ltd., 1982a]. During the study, observations were made on the general health and behaviour, body weight and food intake of each rat. At necropsy, major organs were weighed and specified tissues examined histologically. Terminal blood samples were taken for haematological and clinical chemical examinations. All animals survived until their scheduled necropsy date. The general health and behaviour of control and treated rats were similar throughout the study. No significant change was found in female body weights. Male body weights were significantly higher than controls at 500ppm from week 10 onwards and at 200ppm at weeks 11 and 13. At higher concentrations, there was no difference in body weights from the control values. Male and female liver weights significantly increased at 5000ppm. Absolute testes weights were increased at 5000ppm. However, no differences were observed when adjusted for terminal body weight. These increases were not accompanied by histological, clinical chemical or haematological changes and were therefore considered to be adaptive in nature and not a toxic effect of the compound. A NOEL or NOAEL was not indicated by the authors, but based on the available information and taking a conservative approach, the NOAEL is considered to be 1000ppm. It was not indicated in the report whether the study followed the principles of the OECD method 407 and was GLP compliant.

NaC12-14AE2S was tested for systemic toxicity at repeated doses by oral gavage of 0 (group 1), 25 (group 2), 75 (group 3), and 225 (group 4) mg/kg bodyweight [Henkel KGaA, 1994a]. The compound was administered by gavage over a period of 90 days. Ten (10) male and female rats were used for each dose. Five (5) male and female animals of groups 1, 3, and 4 were observed to determine the reversibility of possible compound-related alterations for 28-days after treatment. Four (4) animals died during the treatment period. The mortality of the animals was, however, considered to be incidental. Three (3) animals died due to experimental procedures such as anesthesia for blood sampling and the fourth animal was sacrificed due to a traumatic fracture of the mandibula. No systemic treatment-related effects were observed in any test group. The mean food and water consumption was not affected and the total body weight gain showed no deviations in all male and female test groups. Local treatment effects were only seen in the forestomach. The forestomach of the animals of group 4 showed some lesions such as a hyperplasia, submucosal oedema and chronic ulceration. In groups 2 and 3, 3 out of 10 animals showed small eosinophilic foci in the stratified epithelium of the forestomach. In conclusion, according to the study described, a daily administration of NaC12-14AE2S revealed no systemic toxicity but local treatment-related concentration dependant irritation to differing degrees in the forestomach in all main test groups 2 – 4. Thus, a NOEL-value was not determined. Since there is no human equivalent to the rat forestomach, these effects are not considered to be relevant to human health assessment. Looking at systemic toxicity, behavioural and clinical abnormalities and other general or specific toxic effects, a no adverse effect level (NOAEL) of 225 mg/kg could be established. The study followed the OECD guideline method 408. GLP compliance was not indicated in the study report.

No unusual findings regarding systemic toxicity were noted in a 2-year chronic feeding study in rats in which C12 AE3S was given at 0, 0.1 or 0.5% in the diet for 2 years. An occasional tumour (type and incidence unspecified) was found in various groups. The tumours were characterized as "typical" of those commonly found in aged rats and did not appear to be associated with the ingestion of AES [Tusing et al., 1962 quoted in Arthur D. Little, 1991]. The results of this study suggest that the NOEL for C12AE3S in this 2-year chronic feeding study in rats was greater than 250 mg/kg bw/day. However, the information available is only very limited and thus only a low study reliability score can be assigned.

In a 2-year study, rats (20/sex/group) were administered C12AE3S in the drinking water at a concentration of 0.1% [Arthur D. Little, 1991]. At termination, survival, growth, food consumption, body weights, clinical laboratory findings, hematology and urinalyses were all comparable in control and treated animals. The only unusual finding was slight, but consistently higher water consumption by all rats receiving the test compound in their drinking water and a significant difference in the empty cecum to body weight ratio of females. Absolute organ weights were all comparable to controls and no consistent gross or histopathology was found.

Generally, pathological findings for controls and treated rats after 2 years were varied and consisted predominantly of incidental findings attributable to advanced age. Various types of benign and malignant tumours were found in both groups. The incidence and types of tumours observed in the treated group was similar to that of control animals. A NOEL greater than 75 mg/kg bw/day (equals a dose of 0.1% in drinking water) can be estimated on the basis of the available information.

A few more repeated oral toxicity studies on AES or AES containing formulations are published elsewhere [Arthur D. Little, 1991]. Detailed study descriptions for these studies were not available, but taking the summaries into account these studies appear to confirm the data and information presented in this chapter.

2.2.2.2. Inhalation

Long-term inhalation studies on AES are not available.

2.2.2.3. Dermal route

Subchronic percutaneous toxicity studies were conducted on 2 liquid dishwashing detergents containing anionic surfactant C12-14AES (detergent A: 23%; detergent B: 27%), C12-14 alkyl sulphate (detergent A: 5%; detergent B: 0%), C12-14 alkylamine oxide (detergent A: 3%; detergent B: 5%), ethanol (detergent A: 5%; detergent B: 7%) and water (balance). The detergents were administered dermally to the shaved backs of rabbits (10 animals per group; 5 of each sex) at concentrations of 0, 0.5, 1.0, and 2.5% in distilled water for 6 hr/day, 5 days/week for a total of 65 treatments (91 days). The dose selection was based on the local irritation effects observed in a 14-day pilot study conducted with each detergent. No adverse systemic effects were observed by assessment of haematological parameters or by gross or microscopic tissue examination. Transient slight to moderate dermal irritation at the detergent application site was observed with detergent A. Slight to moderate dermal irritation confined to the detergent application site was noted in the detergent B study [Petersen, 1988].

No further studies investigating the toxicity of AES, other than irritation, after repeated exposure via the dermal route were available.

Table 1 - Summary table of the repeated dose toxicity tests with AES

Animal	Route	Duration	Test Material	Estimated NOEL*	Doses	Reference
Rat	Drinking water	2 years	C12AE3S	>75 mg/kg/d** (0.1%)	0.1%	Arthur D. Little, 1991
Rat	Oral feeding	2 years	C12AE3S	250 mg/kg/d*** (0.5%)	0, 0.1, 0.5%	Arthur D. Little, 1991
Rat	Oral gavage	90 days	NaC12-14AE2S	225 mg/kg/day for systemic toxicity; (local effects in forestomach at all doses)	25, 75, 225 mg/kg/day	Henkel KGaA, 1994a

Rat	Oral feeding	90 days	NaC12-15E3S	50 mg/kg/d*** (1000ppm)	40, 200, 500, 1000, 5000ppm	Shell Research Ltd., 1982a
Rat	Oral feeding	90 days	C12-15E3S C12E3S	50 mg/kg/d*** (1000ppm)	40, 200, 500, 1000, 5000 ppm	Walker, 1967
Rabbits	Dermal	90 days	2 hand dish detergents containing AES at levels of 23 and 27%	> 12.5 mg/kg/d	0, 0.5%, 1%, 2.5%	Petersen, 1988
Rat	Oral gavage	28 days	Blend of C14-18S and C12-13E6.5S		30, 100, 300, 1000 mg/kg bw/d	Shell Oil, 1992
Rat	Oral feeding	21 days	NaC12-15E3S	254 mg/kg bw/d (0.188%)	0.023%, 0.047%, 0.094%, 0.188%, 0.375%, 0.75%, 1%, 1.5%	Unilever, 1979a
Rat	Oral feeding	21 days	NH4C12-15E3S	232 mg/kg bw/d (0.188%)	0.023%, 0.047%, 0.094%, 0.188%, 0.375%, 0.75%, 1%, 1.5%	Unilever, 1979b

Rat	Oral feeding	21 days	NaC12-15E3S cont. alcohol	108 mg/kg bw/d (0.094%)	0.023%, 0.047%, 0.094%, 0.188%, 0.375%, 0.75%, 1%, 1.5%	Unilever, 1980a
Rat	Oral feeding	21 days	NH4C13-15E3S	461 mg/kg bw/d (0.375%)	0.023%, 0.047%, 0.094%, 0.188%, 0.375%, 0.75%, 1%, 1.5%	Unilever, 1979c
Rat	Oral feeding	21 days	NaC12-14E3S	120 mg/kg bw/d (0.094%)	0.023%, 0.047%, 0.094%, 0.188%, 0.375%, 0.75%, 1% 1.5%	Unilever, 1979d
Rat	Oral feeding	21 days	NH4C16-18E4S	468 mg/kg bw/d (0.375%)	0.023%, 0.047%, 0.094%, 0.188%, 0.375%, 0.75%, 1%, 1.5%	Unilever, 1980b
Rat	Oral feeding	21 days	NaC12-15E3S	441 mg/kg bw/d (0.375%)	0.023%, 0.047%, 0.094%, 0.188%, 0.375%, 0.75%, 1%, 1.5%	Unilever, 1979e

* NOELs were not expressed in the original study reports, but estimated based on the available information

** estimated based on the assumption of a mean adult rat body weight of 0.4kg and a water consumption of 30ml/day [US Environmental Protection Agency, 1978]

*** estimated based on the assumption of a mean adult rat body weight of 0.4kg and a food consumption of 20g per day (1ppm in food equals 0.05 mg/kg/day) [US Environmental Protection Agency, 1978]

Conclusion

The available oral repeated dose toxicity studies provide a coherent picture on the subacute, subchronic and chronic oral toxicity of AES. In 2 chronic toxicity studies investigating carcinogenicity of AES and four subchronic toxicity studies (3 oral studies with AES, 1 dermal study with AES containing dishwashing liquids), no adverse effects, behavioral or clinical abnormalities of AES were observed up to a dose level of 250 mg/kg body weight per day.

In the subchronic oral gavage study, local treatment related effects were observed in the forestomach of the test animals. These effects can be explained by the irritating nature of the test solutions on the epithelium of the forestomach after repeated administration under the conditions of oral gavage. This is considered to be a response secondary to the irritant properties of AES and specific to the administration procedure. A similar response was not observed when the test material was administered via the diet. Administration via oral gavage is not considered to be relevant for humans because this exposure route is an unlikely scenario for human exposure. Also, there is no equivalent in man to the rat forestomach.

In the subchronic oral feeding studies with AES, general health, body weight and food intake remained within normal limits up to the highest tested dose of 250 mg/kg bw/day, but increased organ weights (liver, kidney) were determined in the highest dose group (250 mg/kg bw/day) of the 2 subchronic oral feeding studies. These increases were unaccompanied by histological changes and are considered to be of an adaptive nature rather than a toxic effect of the test article. The dose level of 250 mg/kg/day is considered to represent a NOAEL.

In a series of 21-day oral feeding studies various AES were evaluated for their repeated dose toxicity. The no observed effect levels derived from these toxicity studies ranged from 108 – 460 mg/kg body weight per day. The organ mostly affected in these studies was the liver, expressed by increased liver weight at high doses, hepatic hypertrophy and occasionally changes in biochemical parameters such as increase of enzyme levels in plasma, generally at levels higher than 250 mg/kg bw/day. Significant increases in weight were also observed in other organs (e.g. kidney, heart, brain) in some of these studies, but only at doses higher than LOELs established for above mentioned liver parameters. With regard to this information, it must be noted that care should be taken in the interpretation due to the low number of animals in the dose groups and the limited information available on the studies. It was considered that, in particular, the observations at dose levels below 250 mg/kg bw/day were not adverse in nature. This evaluation takes into account that at approximately the same dose levels, no adverse effects were seen in the above mentioned subchronic and chronic toxicity studies.

From the available repeated toxicity studies, only the 90-day oral gavage study with NaC12-14AE2S and the 90-day oral feeding study were indicated to be in compliance with the OECD method 407 and GLP regulations and should be considered as most reliable [Henkel KGaA, 1994a, Shell Research Ltd., 1982a]. Although none of the other studies fully complied with the

principles of OECD method 407 or indicated compliance with GLP regulations, their results were consistent with the most reliable studies. In particular, the chronic rat drinking water study and the 2nd rat oral feeding study were conducted following principles and procedures similar to those of OECD method 407 and thus, should be regarded as suitable for inclusion in a weight of evidence approach to evaluating the toxicity of AES.

2.2.3. Genetic Toxicity

2.2.3.1. In Vitro

Bacterial tests

Several alcohol ethoxysulphates were assessed for their potential to induce reverse mutations in the presence and absence of a metabolic activation system in an in vitro bacterial system, the so-called Ames test [Hüls AG, 1996; Hüls AG, 1994 ; Henkel KGaA, 1988; Shell Research, 1980b].

Representing the whole range of studies, a recent OECD method 471 and GLP compliant study [Hüls AG, 1996] should be mentioned at this place: In this study, Salmonella typhimurium strains TA98, TA100, TA1535 and TA 1537 were treated with the triisopranolammonium salt of C12-14AE2S in the Ames test plate incorporation assay as well as the preincubation method. Dose levels covering the range of 1 to 5000 μg/plate, in triplicate both with and without the addition of a metabolizing system (Aroclor 1254 induced rat liver S9 mix) were employed. All 4 bacterial strains exhibited mutagenic responses to the appropriate positive control substances. Solvent controls were also tested with each strain and the mean numbers of spontaneous revertants were in an acceptable range. Mutagenic activity of the test compound to any of the tester strains was not observed with and without metabolic activation. It was therefore concluded that under the chosen test conditions, the triisopranolammonium salt of C12-14AE2S is not a bacterial mutagen.

The majority of the studies evaluated the mutagenicity of AES in Salmonella typhimurium strains TA98, TA100, TA1535, TA 1537 and TA 1538. One study [Shell Research, 1980b], however, evaluated the mutagenicity of NaC12-15E3S in presence and absence of a metabolic activation system in the Escherichia coli strains WP2 and WP2uvrA, in addition to the Salmonella typhimurium strains. Also, in these E. coli strains, the tested AES compounds were not mutagenic under the test conditions. In all tested systems, AES were not found to be mutagenic to bacterial systems.

Non bacterial tests

The mutagenic activity of NaC12-15AE3S was further evaluated in a Saccharomyces gene conversion assay [Shell Research, 1980b]. In this study, it was concluded that the addition of NaC12-15AE3S to liquid suspension cultures of Saccharomyces cerevisiae JD1 with or without metabolic activation did not induce a consistent increase in mitotic gene conversion at either gene locus in two replicate experiments.

AES was examined for mutagenic activity by assaying for the induction of trifluorothymidine resistant mutants in L5178Y TK+/- mouse lymphoma cells after in vitro treatment in the absence and presence of S9 metabolic activation [Research Toxicology Centre S.p.A., 1995]. Under the

reported experimental conditions, it was concluded that in the presence and absence of metabolic activation, the test material NaC12-14AE2S did not induce gene mutations in L5178Y TK+/- mouse lymphoma cells. This study was conducted in compliance with OECD method 476 and GLP regulations.

The ability of NaC12-15E3S to induce chromatid and chromosome aberrations was studied in rat liver cells [Shell Research, 1980b]. In slide cultures of rat liver cells exposed to culture medium containing NaC12-15E3S at concentrations of 25, 50 and 100 μg/ml the frequency of chromatid and chromosome aberrations did not differ significantly from that of the controls cultures.

No morphological cell transformations were observed in Syrian golden hamster embryo cells exposed in culture to concentrations up to 50 mg/ml C12-13E2.5S [Inoue et al., 1980].

In an in vitro transformation study with NaC12-15E3S [Shell Research Ltd., 1983b], the transforming activities of NaC12-15E3S and 1,4-dioxane were determined using cultured C3H 10T1/2 mouse embryo fibroblasts as the target cell population. Monolayer cell cultures were incubated for 24 hours in growth medium containing NaC12-15E3S or 1.4-dioxane. Transformation frequencies were assessed by counting the number of actively dividing, darkly stained cell foci per dish, 3 or 4 weeks after test compound treatment. In conclusion, there was no evidence to suggest that either NaC12-15E3S or 1,4-dioxane increased the frequency of 10T1/2 mouse embryo fibroblasts under the experimental conditions described.

2.2.3.2. In Vivo

NaC12-15E3S has been evaluated in an alkaline elution assay [Shell Research Ltd., 1982b]. In this screen which aims to measure DNA single-strand breaks induced in DNA by reaction with electrophiles, NaC12-15E3S did not cause measurable DNA-strand damage when administered to Wistar rats as a single oral dose of 2.5 ml/kg (equals about half of the LD50 of NaC12-15E3S) for an exposure period of 6 hours. Based on this result it was concluded that neither NaC12-15E3S nor its in situ generated metabolites have any effect upon the integrity of rat liver DNA in vivo under the conditions of the test.

In a series of studies with a 55% AES:45% LAS mixture, no significant differences from control values were noted in a dominant lethal study or in vivo or in vitro cytogenicity studies [Arthur D. Little, 1991]. In the dominant lethal assay, male mice were orally administered either 100, 150, or 200 mg/kg subacutely or 500, 750, or 1000 mg/kg acutely of the surfactant mixture. No significant differences from water-dosed controls were observed in the mutagenic index. Similarly, no significant differences in chromosomal anomalies were found in bone marrow cells of male rats given 40, 500, or 1000 mg/kg of the surfactant mixture orally, then killed 18, 24 or 48 hours post-dosing. Likewise, human leukocytes incubated for 18, 24, or 48 hours with 4, 40 or 200 μg/l of the surfactant mixture exhibited no increased incidence of chromosomal anomalies above the water control group.

Another published in vivo study indicated that AES is not clastogenic. Hope [Hope, 1977] reported that the incorporation of C12-15AES into the diet of rats at a maximum tolerated dose (1.13% active ingredient) for 90 days had no effect on the chromosome of rat bone marrow cells.

Conclusion

A structure activity analysis did not reveal any functional groups in the chemical structure of AES that were associated with mutagenic or genotoxic properties. In all available in vitro and in vivo genotoxicity assays, there is no indication of genetic toxicity of AES. Only 2 studies, an Ames test [Hüls AG, 1997f] and a mouse lymphoma assay [Research Toxicology Centre S.p.A., 1995], were conducted according to OECD guideline methodologies and GLP regulations. However, all the other available in vitro and in vivo studies appear to be well documented and conducted. Some of these studies were published in peer-reviewed journals. Based on the presented data, it is therefore concluded that there is no evidence that AES are either mutagenic or genotoxic.

2.2.4. Carcinogenicity

In a 2-year study, rats (20/sex/group) were administered C12AE3S in the drinking water at a concentration of 0.1%. At termination, survival, growth, food consumption, body weights, clinical laboratory findings, haematology and urinalyses were all comparable in control and treated animals. The only unusual findings were slight, but consistently higher water consumption by all rats receiving the test compound in their drinking water and a significant difference in the empty cecum to body weight ratio of females. Absolute organ weights were all comparable to controls and no consistent gross or histopathology was found. Generally, pathological findings for controls and treated rats after two years on test were varied and consisted predominantly of incidental findings attributable to advanced age. Various types of benign and malignant tumors were found in both groups. The frequency of tumours in the treated group was not significantly different from that of control animals [Arthur D. Little, 1991].

No indications of an increased incidence in tumours were noted in a 2-year chronic feeding study in rats in which C12 AE3S was given at 0, 0.1 or 0.5% in the diet for 2 years. An occasional tumour (type and incidence unspecified) was found in various groups. The tumours were characterized as "typical" of those commonly found in aged rats and did not appear to be associated with the ingestion of AES [Tusing et al., 1962 quoted in Arthur D. Little, 1991].

An 5% aqueous solution of C12E3S (0.1ml) was applied twice weekly on the skin of 30 female Swiss mice [Tusing et al., 1962 quoted in Arthur D. Little, 1991]. No papillomas or other tumours were found under these exposure conditions.

In its report to the Soap and Detergent industry [Arthur D. Little, 1991], Arthur D. Little reported on a study in which an aqueous solution of 18.5% C16-18AES and 15.6% LAS was applied 3 times a week on the skin of Swiss ICR mice for 18 months. Under these conditions, the test solutions did not induce any carcinogenic response either on the skin or systemically.

Conclusion

The available oral and dermal long term toxicity/carcinogenicity studies, even if not performed according to accepted guidelines for carcinogenicity bioassays, appear to be conducted and

documented in an acceptable manner. It is therefore concluded that there is sufficient evidence that AES is not carcinogenic in the tested species under the conditions described.

2.2.5. Reproductive toxicity

As part of a chronic feeding study, 10 rats/sex/group fed diets containing 0.1% of C12AES were mated after 14 weeks on the test [Arthur D. Little, 1991]. The F1 generation was maintained on the parental diet and mated at 100 days of age. The F2 generation was fed the same diet for 5 weeks, and then killed. No adverse effects on fertility, lactation, litter size or survival and growth of the offspring were seen. Haematological, biochemical and histopathological findings were comparable to controls. From this study it can be concluded that the NOEL for reproductive toxicity is estimated to be greater than 50 mg/kg bw/day. This estimation was based on the assumption of a mean adult rat body weight of 0.4kg and a water consumption of 30 ml/day [US Environmental Protection Agency, 1978].

No adverse parental toxicity or significant differences in either litter parameters or viability of offspring were noted in two generations of rats fed diets containing either 0.1% C12AES [Tusing et al., 1962] or 1% (reported to equal an exposure of 800 mg/kg/day) of a detergent formulation containing 55%TE3S and 45% LAS [Nolen, et al., 1975].

In available subchronic [Henkel KGaA, 1994a, Shell Research Ltd., 1982a, Walker, 1967] and chronic toxicity studies [Arthur D. Little, 1991, Hüls AG, 1997b] on various AES (NaC12-14AE2S, CaC123-15AE3S, C12AE3S), the primary sex organs of the males and females did not show evidence for treatment-related adverse effects as indicated by organ weight differences, gross examination, and microscopic histology examination at the highest tested exposure levels of 250 mg/kg bw/day.

Further information can be deduced from a two-generation reproduction study with NaC12-14AE2S [Henkel 1999]. This GLP-study followed the OECD guideline method 416. Four groups of thirty male and thirty female Sprague Dawley rats (strain Crl:CD(SD)BR) (F0 generation) were dosed via the drinking water. Concentrations used were 0 (control), 0.03, 0.1 and 0.3 %, which corresponded to daily doses of ca. 0, 30, 100 and 300 mg/kg/day.

There were some changes indicative of parental toxicity in the group treated with 0.3 % of the test substance, which were characterised by reduced straight line velocity of the sperm. The observed reduced triglyceride levels (female) and increased percentage neutrophil counts (males) were slight and within the range of the historical control data. There was evidence of toxicity on pup development at this dose level that was characterised by an increase in the time taken for sexual development of the male (not significant) and female (significant) offspring. No other developmental parameters were affected.

There were some changes seen in reduced straight line velocity of the sperm, reduced trigylceride levels (female) and increased percentage neutrophil counts (males) in the group treated at 0.1 %. All the changes were either not statistically significant or within the range of the historical control data. There was no evidence of toxicity on pup development.

There was no evidence of toxicity on pup development in the group treated with 0.03 %.

Decreased liver weights of the F0 and F1 male dose groups were observed which was not confirmed in the F2 generation dose group.

The male F0 generation showed a small but significant reduction in bodyweight-liver weight ratios, but the corresponding brain related liver weights and the absolute liver weights developed not in a dose dependant way. For the F1 generation where similar results were reported, no dose-response relationship was detected either. No influence on liver weight development was seen in the F2 generation. None of the groups revealed any histopathological or clinical-chemical findings, which could be attributed to hepatotoxicity. This led to the conclusion that this untypical liver weight reduction was of no toxicological relevance, additionally underlined by the absence of such effects in the studies for subchronic toxicity mentioned above.

In summary, there was no effect of treatment at any dose level on reproduction of the parents or offspring (NOAEL > 3 %; > 300 mg/kg/day)

Based on this study an overall NOAEL for systemic effects of 0.1 % (86.6 mg/kg bw) for the F0 generation and a NOAEL of 0.1 % (149.5 mg/kg bw) for the F1 generation can be deduced.

Conclusion

Alcohol ethoxysulphates were evaluated for reproductive effects in rats. The key study (Henkel, 1999) fulfilled OECD guideline protocols and was conducted according to GLP standards. No information on the guidelines and GLP was available for another reproduction study that was cited in the scientific literature [Arthur D. Little, 1991]. AES did not adversely affect reproduction in the rat and the NOAEL for reproductive effects was > 300 mg/kg; slight systemic effects were observed in the parental and F1 generation with a NOAEL of 86 and 149 mg/kg, respectively.

2.2.6. Developmental Toxicity /Teratogenicity

2.2.6.1. Oral route

NaC12-14AE2S was tested in a segment II embryotoxicity study [Henkel KGaA, 1994b]. The purpose of the study was to assess the effects of orally administered NaC12-14AE2S on embryonic and foetal development in pregnant CD-rats. The study followed the guidelines of OECD method 414 "Teratogenicity" and complied with the OECD principles of GLP. In this study, NaC12-14AE2S was administered orally by gavage at dose levels of 0, 100, 300, and 1000 mg/kg body weight once daily from day 6 to day 15 of gestation. Each group consisted of at least 24 female rats. A standard dose volume of 10 ml/kg body weight was used and the control animals were dosed with the vehicle alone over the period described. Clinical condition and reaction to treatment were recorded at least once daily. Body weights were reported for days 0, 6, 16 and 20 of gestation. All surviving females were sacrificed on day 20 of gestation and the foetuses were removed by caesarean section. At necropsy the females were examined macroscopically and live foetuses were weighed, sexed and examined for visceral and skeletal

abnormalities. In summary, the results of the study showed that repeated oral administration (day 6 – day 15 post coitum) of NaC12-14AE2S to pregnant rats did not cause symptoms of cumulative toxicity up to a dose level of 1000 mg/kg/day. No compound-related symptoms were observed and no treatment-related abnormalities were found at necropsy of the females. All females had viable foetuses. Pre-implantation loss, post-implementation loss, mean number of resorptions, embryonic deaths, total foetuses, mean foetal placental and uterus weights were not affected by the treatment. Foetal sex ratio was comparable in all groups. There were no treatment-related foetal abnormalities at necropsy and no treatment-related effects in the reproduction data. In conclusion, in the described embryotoxicity study, NaC12-14AE2S was not cumulatively toxic to pregnant rats and did not reveal any teratogenic potential at the tested dose levels. Thus, based on the available information, the NOAEL for teratogenicity and developmental toxicity are assessed to be greater than 1000 mg/kg bw/day.

NaC12-15AE3S was administered orally by gavage to pregnant Colworth-Wistar rats at dose levels of 0, 375 and 750 mg/kg/day once daily from day 6 to 15 of gestation [Unilever, 1980c]. Two different samples of the test material were tested. Fifteen (15) animals were used per dose group, 10 for dissection and 5 for natural parturition. Throughout the study, the females were monitored for signs of toxicity. Upon necropsy, fetal toxicity was determined by evaluating pre-implantation and post-implantation fetal loss and fetal weight. Fetuses were evaluated for externally visible malformations, as well as malformations of the internal organs and skeleton. In the post-partum phase pup mortalities, body weights and litter size as well as incidence of external and gross visceral and skeletal defects were monitored until weaning day 21. The resulting data were compared to the control group. In summary, NaC12-15AE3S induced maternal toxicity, indicated by body weight changes and other clinical and behavioural observations, when administered by gavage to pregnant rats at doses of 750 mg/kg. The authors were unable to detect any specific abnormality which would indicate a developmental toxicity or teratogenic response related to the treatment. This study was not conducted according to any recognized guideline. However, the study was conducted according to GLP, is well-documented and judged to be scientifically acceptable. Based on the available information the NOAEL for maternal toxicity was estimated to be 375 mg/kg bw/day and the NOAEL for teratogenic effects or developmental toxicity is greater than 750 mg/kg bw/day.

NH4C13-15AE3S was administered orally by gavage to pregnant Colworth-Wistar rats at dose levels of 0, 63, 125, 250 and 500 mg/kg/day once daily from day 6 to 15 of gestation [Unilever, 1986a]. Fifteen (15) animals were used per dose group, 10 for dissection and 5 for natural parturition. No detailed information was available on the study design. Some slight maternal toxicity indicated by body weight changes and other clinical observations (e.g. diarrhoea, respiratory wheeziness) was seen in rats with exposure to 250 and 500 mg/kg bw/day, but given the limited information available, there is some uncertainty regarding the severity of these effects. No evidence of developmental toxicity or a teratogenic response to the treatment were reported at any dose level. This study was not conducted according to GLP or according to any recognized guideline. Given the lack of information and the uncertainty mentioned before, a NOAEL could not be reliably determined.

NaC12-14AE3S was administered orally by gavage to pregnant Colworth-Wistar rats at dose levels of 0, 93, 187, 375 and 750 mg/kg/day once daily from day 6 to 15 of gestation [Unilever,

1986b]. Fifteen (15) animals were used per dose group, 10 for dissection and 5 for natural parturition. Maternal and foetus effects were evaluated as described previously (i.e study with NaC12-15AE3S). The treatment of pregnant rats with NaC12-14AE3S during days 6-15 of gestation did induce some maternal toxicity at the dose level of 750 mg/kg bw/day. No evidence of treatment-related teratogenic effects or developmental toxicity was reported. This study was not conducted according to GLP or according to any recognized guideline. However, the study appeared well-conducted, was well-documented and judged to be scientifically acceptable. Based on the available information the NOAEL for maternal toxicity was determined to be 375 mg/kg bw/day and the NOAEL for teratogenic or developmental effects is estimated to be greater than 750 mg/kg bw/day.

NaC16-18AE4S was administered orally by gavage to pregnant Colworth-Wistar rats at dose levels of 0, 63, 125, 250 and 500 mg/kg/day once daily from day 6 to 15 of gestation [Unilever, 1986c]. Twenty (20) animals were used per dose group, 15 for dissection and 5 for natural parturition. Forty (40) animals were used for the negative control. Maternal, foetus and post-partum effects were evaluated as described previously (i.e study with NaC12-15AE3S). In summary, there was no evidence of teratogenic potential or developmental toxicity. This study was not conducted according to any recognized guideline. The study was conducted according to GLP, is well-documented and judged to be scientifically acceptable. Based on the available information, the NOAEL for both maternal toxicity, teratogenic and developmental effects appeared to be greater than 500 mg/kg bw/day.

In a last study of this series, NaC12-15E3S was administered orally by gavage to pregnant Colworth-Wistar rats at dose levels of 0, 125, 250, 500 and 1000 mg/kg/day once daily from day 6 to 15 of gestation [Unilever, 1979f]. Fifteen (15) animals were used per dose group, 10 for dissection and 5 for natural parturition. Maternal, foetus and post-partum effects were evaluated as described previously. The authors of the study concluded that a degree of maternal toxicity indicated by a significant reduction in body weight gain of NaC12-15E3S was observed at the highest dose level of 1000 mg/kg. However, no evidence of treatment-related developmental toxicity or teratogenic effects was detected. This study was not conducted in compliance with GLP or according to any recognized guideline. The study appeared well-conducted, was well-documented and judged to be scientifically acceptable.

Pregnant rats were administered 50, 100, and 500 mg/kg/day of C12-13AES by oral gavage on days 6-15 of gestation. Effects observed were a decrease in maternal body weight gain and food consumption [Arthur D. Little, 1991]. There were no treatment-related maternal effects noted at necropsy or following a uterine examination on day 13 of gestation. The incidence of foetal malformations in AES-treated groups was not different from the control group.

Several investigators have studied the effects of administering a commercial liquid detergent formulation containing both AES and LAS to pregnant mice, rats and rabbits [Iseki, 1972; Nolen, et al., 1975; Palmer, et al., 1975]. Except at dosage levels which were toxic to the dams, no significant differences in the litter parameters of laboratory animals compared to control values were noted in these studies. Levels up to 300 mg/kg of a mixture containing 55% TE3S and 45% LAS given orally to rabbits on days 2-16 of gestation up to 800 mg/kg given to rats on days 6-15 of gestation gave no indications of any embryotoxic or teratogenic effects attributable

to AES [Nolen, et al., 1975]. In these exploratory investigations, there were no indications that detergent formulations containing AES at doses which are several orders of magnitude above possible human exposure levels posed any teratogenic hazard to laboratory animals.

2.2.6.2. Dermal route

There are no studies available that examined the teratogenicity and developmental toxicity of AES after dermal exposure.

Conclusion

Alcohol ethoxysulphates were evaluated for teratogenic or embryotoxic effects mainly in rats, but in a few investigations also in mice and rabbits. Although the majority of these studies did not fulfill all requirements of existing guideline protocols and were not conducted according to GLP standards, the studies appeared to be well conducted and documented. Noteworthy is the segment II embryotoxicity study [Henkel KGaA, 1994b] which followed OECD guidelines and complied with the OECD principles of GLP. In this study which which was rated to be reliable without limitations according to the Klimisch criteria [Klimisch et al., 1997], AES showed no cumulative toxicity in pregnant rats and did not reveal any embryotoxic or teratogenic potential at the highest tested dose levels of 1000 mg/kg body weight.

The absence of a teratogenic potential and developmental toxicity of AES was confirmed in a series of teratology screening studies [Unilever, 1979f]. Although there were limitations in the design of the study, in particular with regard to the size of the dose groups and the absence of some clinical/biochemical parameters, the overall quality of these studies is judged to be appropriate and scientifically valid.

Based on the presented information, it is concluded that there is sufficient evidence that AES is not teratogenic or a developmental toxicant under the conditions described. A NOAEL greater than 1000 mg/kg bw/day can be estimated for teratogenicity and embryotoxicity on the basis of the segment II embryotoxicity study which is judged to be of highest reliability. The NOAEL for developmental toxicity appears to be greater than 750 mg/kg bw/day.

2.2.7. Biokinetics

McDermott et al. (1975) studied the absorption of C16AE3S and C16AE9S, labelled with ^{14}C in the 1-position of the alkyl chain, after oral exposure in man and rats. Seventy-two hours after administration of C16AE3S, radioactive material was mainly excreted via urine (man: 80%; rat: 50%) and to a lesser extent via faeces (man: 9%; rat: 26%) and air (man: 7%; rat: 12%). For C16AE9S however, the radioactivity was mainly excreted via faeces (man: 75%; rat: 82%) and to a lesser extend via urine (man: 4%; rat: 0.6%) and air (man: 6%; rat: 4%). The length of the ethoxylate portion of an AES molecule appears to determine the metabolic fate of the compound following oral administration in both man and rat. There was no evidence of hydrolysis of the sulphate group or of metabolism of the ethoxylate portion of the molecule. The major metabolite found in urine had the following structure: $-OOCCH_2(OCH_2CH_2)_xOSO_3^-$ where x equals either 3 or 9, respectively [McDermott et al., 1975].

In a similar investigation, Taylor et al. (1978) studied the metabolic fate of orally, intraperitoneally or intravenously administered ^{14}C-C11AE3S and ^{14}C-C12AE3S in the rat. The authors observed that both compounds were extensively metabolized (ω, β oxidation) with the proportion of radioactivity appearing in urine and respired air generally independent of the route of administration. Some sex differences in the proportions of radioactivity excreted in urine and respired air was seen, but total recoveries for both compounds were comparable. By the oral route, 67% of the administered radioactivity with C11AE3S appeared in the urine of male rats compared to 45% in females; expired air contained 19% and 35% of administered radioactivity respectively; 4-5% was present in faeces for both sexes. The major urinary metabolite of C12AE3S was identified as 2-(triethoxy sulphate) acetic acid, with C11AE3S, the major urinary metabolite was tentatively identified as 3-(triethoxysulfate) propionic acid.

Taylor et al. (1978) measured the percutaneous absorption of ^{14}C-labelled NaC12AE3S. The NaC12AE3S was applied to rats as 150 μl of a 1% v/v solution. The ^{14}C-levels were measured in urine collected over 48 hours. Penetration of NaC12AE3S was 0.39 +/- 0.12 $\mu g/cm^2$. In experiments in which application was continued for up to 20 minutes, skin penetration was proportional to the duration of the contact. It was also proportional to the number of applications.

Conclusion

Following oral exposure, AES is readily absorbed in the gastrointestinal tract in man and rat and excreted principally via the urine. The length of the ethoxylate portion in an AES molecule seems to have an important impact on the biokinetics of AES in humans and in the rat. Alcohol ethoxysulphates with longer ethoxylate chains (>7-9 EO units) are excreted at a higher proportion in the faeces. Once absorbed, AES is extensively metabolized by beta- or omega oxidation.

The dermal absorption of AES is relatively poor as can be expected from an ionic molecule. The percutaneous absorption of C12AE3S was measured in a rat in vivo study. The study determined a dermal flux of the tested compound of 0.0163 $\mu g/cm^2/h$.

2.2.8. Experience from human exposure

Allergic contact sensitisation:
Over the years very many formulations containing a variety of AES concentrations are reported to have been tested in Human Repeat Insult Patch tests (HRIPT) failing to show evidence of contact sensitisation (see, e.g., [Nusair TL et al., 1988]). Available detailed examples include two HRIPTs reported as follows:

In one test [Procter & Gamble, 1998], 102 volunteers were treated with patches of a 0.05% (w/v) aqueous solution of a detergent formulation containing 37% AES (Na AE1.4S, CAS# 68585-34-2). The patches were applied on the upper arms, under fully occlusive conditions. Test material was applied for 24 hours, 3 times a week, for 3 weeks during the induction period. After a 14-17-day rest, a 24-hour challenge patch was applied on the original and alternate arm sites. There was no evidence of skin sensitisation in any of the 102 subjects who completed the test.

In another test [Procter & Gamble, 1994], 87 volunteers were treated with patches of a 0.2% (w/v) aqueous solution of a formulation containing 6% AES (Na AE3S, CAS# 68585-34-2). The patches were applied on the upper arms, under fully occlusive conditions. Test material was applied for 24 hours, 3 times a week at the same skin site, for 3 weeks during the induction period. After a 14-17-day rest, a 24-hour challenge patch was applied on the original and alternate arm sites. There was no evidence of skin sensitisation in any of the 87 subjects who completed the test.

Skin irritation

The cumulative skin irritation effects of formulations containing AES have been investigated in six separate "24-hour Repeat Application Patch Test" studies [Procter & Gamble, 2000a]; [Procter & Gamble, 2001]; [Procter & Gamble, 2000b]; [Procter & Gamble, 2000c] [Procter & Gamble, 2000d], [Procter & Gamble, 2000e]. In each study 12 volunteers were treated with patches of a 0.1% (w/v) aqueous solution of detergent formulations containing AES (Na AES CAS# 68585-34-2). The patches were applied on the upper arms, under fully occlusive conditions. Test material was applied for 24 hours, 3 times a week at the same skin site, for a total of one week. After the end of each 24 hour application period, the skin was graded for irritation according to a 0 – 4 scoring scale. A total of 12 different detergent formulations were tested with the following AES concentrations (% w/v): 11, 13, 16, 18, 19, 20. A total of 72 volunteers were tested. All the formulations tested resulted in cumulative average skin irritation scores lower than 0.8 (they ranged between 0.05 and 0.79), which corresponds to a very mild effect.

In a separate, similar study the cumulative irritancy potential of a detergent formulation containing 11.4% (w/v) AES (Na AES CAS# 68891-38-3) was investigated under open (non-occlusive) conditions [Procter & Gamble, 2001]. A total of 12 volunteers were treated with 0.3 ml of undiluted, 30% (w/v), and 10% (w/v) aqueous dilutions of the detergent formulation, which were applied on an open application patch on the upper arms. Test materials were applied for 24 hours, 3 times a week at the same skin site, for a total of one week. After the end of each 24 hour application period, the skin was graded for irritation according to a 0 – 4 scoring scale. The cumulative average scores for the undiluted, 30%, and 10% detergent formulation were 0.26, 0.03, and 0.03, respectively. These score are all indicative of a very mild effect.

Conclusion

The human experience data supports the lack of allergic contact sensitisation potential of formulations containing AES. The skin irritation potential of aqueous solutions of detergent product formulations under conditions simulating relevant consumer use can be expected to be mild after repeated contact with human skin.

2.2.9. Identification of critical endpoints

2.2.9.1. Overview on hazard identification

Alcohol ethoxysulphates are considered to be of low toxicity after acute oral and dermal exposure. The estimated LD50 is higher than 2000 mg/kg body weight. Reliable data on acute inhalation are not available, but given the irritant nature of AES, it is expected that a high AES aerosol concentration may be irritating to the respiratory tract. However, inhalation is not viewed as a significant route of exposure. AES is mainly used in liquid media and due to its very low vapour pressure, exposure is unlikely to occur. The only possible exposure could be due to the use of powdered formulations or the use of AES in spray cleaner formulations.

The skin and eye irritation potential is concentration dependent. AES concentrations higher than 70% are moderately to severely irritating to rabbit skin under the conditions of 4-hour semi-occluded patch tests and moderately to severely irritating to rabbit eyes. Formulations containing more than 20% AES are classified as skin and eye irritants unless data are available that show absence of irritation potential as defined by the EC criteria. At concentrations below 1%, AES are considered as virtually non-irritating.

AES are not considered to be skin sensitizers. A substantial amount of skin sensitization studies in guinea pigs following either the Magnusson-Kligman maximization or the Buehler testing protocol demonstrate the absence of skin sensitization potential and only very few studies indicated a weak sensitization potential of individual AES. Human experience further supports the assessment that AES are not sensitizing.

The available oral and dermal repeated dose toxicity studies provide a coherent picture on the subacute, subchronic and chronic toxicity of AES. In 2 chronic and four subchronic toxicity studies (3 oral studies with AES, 1 dermal study with AES containing dishwashing liquids), no systemic adverse effects of AES were observed up to the highest tested dose levels of 250 mg/kg bw/day. In 2 subchronic oral feeding studies a slight, but significant increase in organ weights (liver in males and females in both studies, male kidney in one study) was observed at the dose of 250 mg/kg bw/day, but these increases were not accompanied by histological changes and were therefore considered to be adaptive in nature and not a toxic effect of the AES. In two out of seven 21-day oral feeding studies, hepatic hypertrophy and slight increases in plasma enzyme levels were observed at doses of about 120 mg/kg/d. However, in the other 5 21-day oral feeding studies the estimated NOELs ranged from 232 – 468 mg/kg/d. Only little information was available on these 21-days studies, but similarly to above mentioned subchronic and chronic oral toxicity studies, the effects seen in the liver are not considered to be of adverse nature.

AES are not considered to be mutagenic, genotoxic or carcinogenic. Although most studies addressing these endpoints were not performed according to accepted guidelines, the picture is very coherent. In all the in vitro and in vivo assays there was no indication of genetic toxicity of AES. Long-term carcinogenicity studies did not indicate any potential of AES to induce tumours.

Substantial information is available on teratogenicity, embryotoxicity and toxicity to reproduction of AES. Taken all together, it can be concluded that AES is not cumulatively toxic to pregnant rats and did not reveal teratogenic, developmental reproductive effects at the highest tested dose levels of >300 mg/kg body weight per day.

2.2.9.2 Rationale for identification of critical endpoints

Dermal exposure is the main exposure route for consumers and subsequently, dermal effects such as skin irritation and sensitization as well as long-term dermal toxicity have to be considered with regard to the human risk assessment. A substantial amount of data is available addressing the skin irritation and skin sensitization potential of AES solutions and AES containing consumer product formulations. Dermal penetration studies in rats have shown that AES has the potential to penetrate the skin and become systemically available. There are only a few dermal studies available, but by using bridging assumptions, systemic effects after dermal exposure can also be assessed using the results of oral repeated dose toxicity studies in experimental animals.

2.2.9.3 Adverse effects related to accidental exposure

The acute oral and dermal LD50 of solutions containing AES at concentrations up to 70% is greater than 2000 mg/kg. This level of toxicity is generally considered as low. AES is present in detergent formulations at 28% as a maximum. Generally, accidental oral exposure to a surfactant containing formulation such as detergents poses a minor risk of aspiration.

The available information suggest that concentrated solutions containing AES at concentrations above 20-30% may be moderately to severely irritating to eyes and slightly to moderately irritating to skin. Thus, eye and prolonged skin contact with neat products should be avoided. Other surfactants present in the formulation could contribute to these effects. It has, however, been observed that the overall irritation profile of AES containing detergent and cleaning formulations is not necessarily additive and is less than expected based on the individual components. Nevertheless, in case of accidental eye contact, immediate rinsing with plenty of water is recommended. This immediate action has been shown in animal experiments to minimize irritation effects.

2.2.10. Determination of NOAEL or quantitative evaluation of data

As discussed before, the available oral and dermal repeated dose toxicity studies provide a coherent picture and demonstrate low toxicity of AES.

In the available chronic and subchronic toxicity studies, no effects were seen at levels up to 75 mg/kg bw/day and no adverse effects of AES were observed up to the highest tested dose levels of 250 mg/kg bw/day. In 2 subchronic oral feeding studies a slight, but significant increase of organ weights (e.g. liver) was observed at the dose of 250 mg/kg bw/day. These increases were not accompanied by histological changes and were therefore considered to be an adaptation to the test material and not a toxic effect of the AES. In a subchronic oral gavage study in rats, local treatment effects were observed in the test animals. These effects can be explained by the irritating nature of the test solutions on the epithelium of the forestomach under the test conditions. These types of effects are not considered to be relevant for humans because they are a concentration-dependent response to a direct irritation and also the fact that the exposure scenario reflected in the oral gavage study is not of relevance to human exposure scenarios occurring in real life. There is also no equivalent to the rat forestomach in man. Following this rationale, a NOAEL of 250 mg/kg bw/day could be established. With regard to teratogenicity of

AES, a NOAEL greater than 1000 mg/kg bw/day is suggested. At this exposure level, no evidence for teratogenicity was found in a reliable segment II embryotoxicity study. In a series of teratology screening studies which monitored pup development up to weaning day 21 no developmental effects were observed for AES at the highest exposure level of 750 mg/kg/day.

However, it is recognized that there might be a different view with regard to the interpretation of the data and the establishment of a NOEL (or NOAEL) for systemic toxicity of AES. Alternatively to the discussion above, there might be the conservative view that the increase in the liver weight accompanied by the increase of certain enzymes in the plasma in one of the subchronic oral feeding studies is indicative of an (adverse) effect.

For assessing the risk associated with human exposure to AES in context of its use in laundry and cleaning products, it is therefore suggested to take a conservative approach by using a no observed effect level (NOEL) of 75 mg/kg bw/day. This value was derived from the results of a 2-year drinking water study in rats.

3 Effects

3.1 Aquatic toxicity

3.1.1 Acute data

Acute toxicity data are available in several review articles (ADL 1991; BKH 1994; Madsen 2000). As a large chronic data base exists (Section 6.1.2) the acute data have not been further considered for the HERA risk assessment.

3.1.2 Chronic data

The following chronic toxicity data are available in reviews or have been identified during this HERA assessment project.

Table 2 Chronic toxicity data

Fish and other aquatic vertebrates

C#		EO#		Linearity	Species	Endpoint	Exposure	Value	Ref
Avg	Distn	Avg	Distn					(mg/l)	
12		0		?	Saccobranchus fossilis	60 d	Semi-static	>2.24	Dalela et al, 1981
?	12-13	1	?	?	P. promelas	30 d NOEC	?	0.88	BKH 1994
?	12-14	2	?	?	O. mykiss	28 d growth	flow-through	0.1	Scholz 1997
?	12-15	3	?	?	O. mykiss	28 d NOEC	flow-through Measured	0.12	BUA 1997
13.7	?	2.25	?	?	P. promelas	365 d NOEC	Measured	0.1	Maki 1979
?	14-15	2.25	?	?	P. promelas (juvenile)	45 d LC50	? (flow-through)	0.44	ADL 1991
?	14-15	2.25	?	?	P. promelas (fry)	45 d LC50	? (flow-through)	0.63	ADL 1991
?	14-15	2.25	?	?	P. promelas	45 d LC50	? (flow-through)	0.94	ADL 1991
?	14-16	2.25	?	?	P. promelas	45 d LC50	?	0.1	BKH 1994
17.3	16-18	0			Brachydanio rerio	OECD 204, NOEC		1.7	Steber et al 1988
17	?	3	?	?	P. promelas	365 d NOEC	?	0.13	BKH 1994

Invertebrates

C#		EO#		Linearity	Species	Endpoint	Exposure	Value (mg/l)	Ref
Avg	Distn	Avg	Distn						
12	99%	0	-	-	C. dubia	7 d NOEC	Flow-through	0.88	Dyer et al 1997
12	>95% Pure	1	>95% Pure	?	C. dubia	7 d NOEC	Flow-through	0.34	Dyer et al 2000
12	>95% Pure	2	>95% Pure	?	C. dubia	7 d NOEC	Flow-through	6.3	Dyer et al 2000
12	100% Pure	2	100% Pure	?	Brachionus calyciflorus	2 d EC20	Measured	0.97-1.1	Versteeg et al, 1997
12	>95% Pure	4	>95% Pure	?	C. dubia	7 d NOEC	Flow-through	2.7	Dyer et al 2000
12	99% pure	4	99% pure	?	B. calyciflorus	2 d EC20	Measured	2.3	Versteeg et al 1997
12	>90% Pure	8	>90% Pure	?	C. dubia	7 d NOEC	Flow-through	1.2	Dyer et al 2000
?	12-14	2	?	?	D. magna	21 d repro	Semi-static Nominal	0.72	Scholz 1997
?	12-14	>2	?	?	D. magna	21 d NOEC	Semi-static	0.7	BKH 1994
?	12-15	3	?	?	D. magna	21 d repro	Semi-static Measured	0.34	BUA 1997
13	>95% Pure	2	>95% Pure	?	C. dubia	7 d NOEC	Flow-through	0.28	Dyer et al 2000
13	100% pure	2	100% pure	?	B. calyciflorus	2 d EC20	Measured	0.49	Versteeg et al 1997
13.67	13-15	2.25	?	?	D. magna	21 d NOEC	Measured	0.27	Maki 1979
14	>95%	0	-	-	C. dubia	7 d NOEC	Flow-through	0.<0.062	Dyer et al 1997
14	>95% Pure	1	>95% Pure	?	C. dubia	7 d NOEC	Flow-through	0.34	Dyer et al 2000
14	>95% Pure	2	>95% Pure	?	C. dubia	7 d NOEC	Flow-through	0.31	Dyer et al 2000
14	100% pure	2	100% pure	?	B. calyciflorus	2 d EC20	Measured	0.13	Versteeg et al 1997
14	>95% Pure	4	>95% Pure	?	C. dubia	7 d NOEC	Flow-through	1.1	Dyer et al 2000
14	98% pure	4	98% pure	?	B. calyciflorus	2 d EC20	Measured	0.37	Versteeg et al 1997

?	14-15	0			C. dubia	7 d NOEC	Flow-through	0.081	Dyer et al 1997
?	14-15	2.25	?	?	D. magna	21 d NOEC	Nominal	0.18	BKH 1994
?	14-16	2.25	?	?	D. magna	21 d NOEC	?	0.27	BKH 1994
15	>95%	0	-	-	C. dubia	7 d NOEC	Flow-through	0.23	Dyer et al 1997
15	>95% Pure	1	>95% Pure	?	C. dubia	7 d NOEC	Flow-through	0.08	Dyer et al 2000
15	>95% Pure	2	>95% Pure	?	C. dubia	7 d NOEC	Flow-through	0.06	Dyer et al 2000
15	>95% Pure	4	>95% Pure	?	C. dubia	7 d NOEC	Flow-through	0.15	Dyer et al 2000
15	99% pure	4	99% pure	?	B. calyciflorus	2 d EC20	Measured	0.22	Versteeg et al 1997
15	>90% Pure	8	>90% Pure	?	C. dubia	7 d NOEC	Flow-through	5.8	Dyer et al 2000
16	>95% pure	0	-	-	C. dubia	7 d NOEC	Flow-through	0.20	Dyer et al 1997
17.3	16-18	0			D. magna	21 d NOEC		16.5	Steber et al 1988
18	>95% pure	0	-	-	C. dubia	7 d NOEC	Flow-through	0.60	Dyer et al 2000

Algae

C#		EO#		Linearity	Species	Endpoint	Exposure	Value	Ref
Avg	Distn	Avg	Distn						
12		0			S. capricornutum	96 h NOEC Growth inhibition		12	Nyholm & Damgaard, 1990
12		?			River water 'community'	Chlorophy 1 a NOEC	3 weeks	70 mg/l (enhancement at 5 mg/l)	Drewa 1989
?	12-13	?	?	?	Selenastrum capricornutum	?	5 d NOEC	50.5	BKH 1994
?	12-14	2	?	?	Scenedesmus subspicatus	72 h NOEC AUGC	Static Nominal	0.72	Scholz 1997
?	12-14	2	?	?	Scenedesmus subspicatus	96 h NOEC	Static Nominal	0.35	BKH 1994
?	12-15	3	?	?	Scenedesmus subspicatus	72 h NOEC	Static Measured	0.9	BUA 1997
?	14-15	?	?	?	Selenastrum capricornutum	NOEC Test duration unknown	?	21	BKH 1994
17.3	16-18	0			Scenedesmus subspicatus	72 h NOEC	Static	17	Henkel 1996

REFERENCES

ADL (1991) Environmental and human safety of major surfactants. Volume 1. Anionic surfactants. Part 2. Alcohol ethoxy sulfates. Final report to the Soap and Detergent Association.

Arthur D. Little, Inc. (1991) Environmental and human safety of major surfactants. Volume 1. Anionic surfactants. Part 2. Alcohol ethoxy sulfates. Final report to : The Soap and Detergent Association.

BKH (1994) Environmental data review of Alkyl Ether Sulphates (AES). Final report to Nederlandse Vereniging van Zeepfakrikanten (NVZ).

Brown, V.K.H. and Muir, C.M.C. (1970) The toxicities of some coconut alcohol and Dobanol 23 derived surfactants. Tenside 7, 137-139.

BUA (1997) Ecotoxicology of selected surfactants for the detergent and cleanser sector. Report 206.

Connor, D.S., et al. (1975) Identification of certian sultones as the sensitizers in an alkyl ethoxy sulfate. Fette und Selfen Anstrichmittel 77, 182-185.

Dalela RC, Tyagi AK, Pal N & Verma SR (1981) Water, Air, Soil Pollution 15: 3-9.

Drewa G, Zbytniewski Z, Andruszczak D, Kowalska B, Korsak V, Kozica-Raszeja L, Kozlowska H & Palgan, K (1989) Laboratory studies of the effect of an anionic detergent and fuel oil on the levels of chlorophyll, oxygen and total suspended particulate matter in water of the Brda River. Pol. Arch. Hydrobiol. 36(1):161-168, 1989.

Dyer SD, Lauth JR, Morrall SW, Herzog RR & Cherry DS (1997) Development of a chronic toxicity structure-activity relationship for alkyl sulfates. Environ. Toxicol. Water Qual. 12;295-303.

Dyer SD, Stanton DT, Lauth JR & Cherry DS (2000) Structure-activity relationships for acute and chronic toxicity of alcohol ether sulfates. Environmental Toxicology and Chemistry 19: 608-616.

Henkel KGaA, Unpublished data, Test of skin sensitising properties of Texapon N11, 1977a.

Henkel KGaA, Unpublished data, Test of skin sensitising properties of Texapon N40, Texapon ASV and Texapon T42, 1977b.

Henkel KGaA, Unpublished data, Test of skin sensitising properties of CJL 394, 1985.

Henkel KGaA, Unpublished data, Determination of the mutagenicity of Texapon N25 in the Ames mutagenicity test, 1988.

Henkel KGaA, Unpublished data, Texapon N70: 90 day oral (gavage) subchronic toxicity study in the rat, 1994a.

Henkel KGaA, Unpublished data, Texapon N70: Embryotoxicity study (including teratogenicity) in the rat (segment II), 1994b.

Henkel (1996) Sulfopon T55: Subacute/chronic Toxicity Algae. P. Wierich 10.06.1996. Henkel KgaA, unpub. data, Reg. Nr. 6796.

Henkel KGaA, Unpublished data, report no. R 9900771. 1999

Hope, J. (1977) Absence of chromosome damage in the bone marrow of rats fed detergent actives for 90 days. Mutat. Res. 56, 47-50.

Hüls AG, Unpublished data, Acute oral toxicity of Marlinat 24/70 in rats, 1986a.

Hüls AG, Unpublished data, Test of the acute irritation of the skin by Marlinat 24/70, 1986b.

Hüls AG, Unpublished data, Test of the acute irritant effects on eyes and mucosa of Marlinate 24/28, 1986c.

Hüls AG, Unpublished data, Determination of the mutagenicity of Marlinat 242/70 in the Ames mutagenicity test, 1994.

Hüls AG, Unpublished data, S. typhimurium reverse mutation assay (Ames test) with Marlinat 242/90T, 1996.

Hüls AG, Unpublished data, Test of the skin sensitizing effect of Marlinat 242/90T, 1989.

Hüls AG, Unpublished data, Marlinat 242/90T: Acute oral toxicity to the rat, 1997a.

Hüls AG, Unpublished data, Marlinat 242/90T: Acute dermal toxicity to the rat, 1997b.

Hüls AG, Unpublished data, Test of the acute skin irritation of the skin by Marlinat 242/90T on rabbits, 1997c.

Hüls AG; Unpublished data, Test of the acute irritant effect on eyes and mucosa of Marlinat 242/90T in rabbits, 1997d.

Hüls AG, Unpublished data, Test of the skin sensitizing effect of Marlinat 242/90T in guinea pigs (Buehler method), 1997e.

Inoue, K.T., et al. (1980) Studies of in vitro cell transformation and mutagenicity by surfactants and other compounds. Fd. Cosmet. Toxicol. 18, 289-296.

Iseki, H. (1972) On teratogenicity of some detergents. Mie Kaibo Gyosekishu 20, 119.

Klimisch et al. (1997) A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regul. Toxicol. Pharmacol. 25, 1-5.

Madsen T, Buchardt Boyd H, Nylen D, Rathmann Pedersen A, Petersen, GI, Simonsen F (2000) Environmental and health assessment of substanes in household detergents and cosmetic detergent products. DHI Water & Environment.

Maki AW (1979) Correlations between Daphnia magna and fathead minnow (Pimephales promelas) chronic toxicity values for several classes of test substances. Fish Res. Board Can. 36: 411-421.

McDermott, J.A., et al. Alkyl ethoxy sulfates: Absorption, distribution, excretion and metabolite identification studies in rats and man. Presented at the Society of Toxicology Meetings, March 14, 1975.

Nolen, C.A. (1975) Teratology studies of a mixture of tallow alkyl ethoxylate and linear alkylbenzene sulfonate in rats and rabbits. Toxicol. 4, 231-243.

NOTOX, Unpublished data, 1994.

Nusair TL, Danneman PJ, Stotte J, Bay PHS (1988) Consumer Products: risk assessment process for contact sensitisation. Toxicologist 8, 258.

Nyholm N & Damgaard BM (1990) A comparison of the algal growth inhibition toxicity test method with the short-term 14C-assimilation test. Chemosphere 21: 671-679.

Palmer, A.K., et al. (1975) Assessment of the teratogenic potential of surfactants. Part I-LAS, ARS and CLD. Toxicol. 3, 91-106.

Petersen, D.W. (1988) Subchronic percutaneous toxicity testing of two liquid hand dishwashing detergents. Fd. Chem. Toxic. Vol. 26, No.9, 803-806.

Procter & Gamble Company, Unpublished data, 1994.

Procter & Gamble Company, Unpublished data, 1996b.

Procter & Gamble Company, Unpublished data, 1998.

Procter & Gamble Company, Unpublished data, 2000a.

Procter & Gamble Company, Unpublished data, 2000b.

Procter & Gamble Company, Unpublished data, 2000c.

Procter & Gamble Company, Unpublished data, 2000d.

Procter & Gamble Company, Unpublished data, 2000e.

Procter & Gamble Company, Unpublished data, 2001.

Research Toxicology Centre S.p.A., Unpublished data, 1995.

Scholz N (1997) Ecotoxicology of surfactants Tenside Surf. Det. 34: 229-232.

Shell Chemical Co., Unpublished data, Acute toxicity studies on Neodol 25-3S, 1967.

Shell Oil Co., Unpublished data, Primary skin irritation in rabbits of Neodol 25-3S, 1989.

Shell Oil Co., Unpublished data. Letter to US EPA, 1992.

Shell Research Ltd., Unpublished data, The comparative toxicity of some Dobanol derived detergent materials and their Laurex NC matches. Part I: The skin irritancy and acute oral toxicity of the mono and triethanolamine sulphates and the ethoxysulphate, 1968.

Shell Research Ltd., Unpublished data, The toxicity of the two Stanlow derived surfactants, Dobanol 25-9 and Dobanol 25-3S, 1972.

Shell Research Ltd, Unpublished data, The toxicity of detergents: Acute toxicity, skin and eye irritancy and skin sensitization potential of the detergent Dobanol 25-3S/60, 1975a.

Shell Research Ltd., Unpublished data, The toxicity of detergents: Acute toxicity, skin and eye irritancy and skin sensitization potential of Dobanol 25-3S/27, 1975b.

Shell Research Ltd., Unpublished data, The toxicity of detergents: Acute toxicity, skin and eye irritancy and skin sensitization potential of Dobanol 25-3A/60, 1975c.

Shell Research Ltd., Unpublished data, The toxicity of detergents: Skin sensitization potential of Dobanol 23-2S/28, 1975d.

Shell Research Ltd., Unpublished data, Toxicology of detergents: Acute toxicity, skin and eye irritancy and skin sensitizing potential of Teepol PB, 1978a.

Shell Research Ltd., Unpublished data, Toxicology of detergents: Acute toxicity, skin and eye irritancy and skin sensitizing potential of Dobanol 23-2S/28, 1978b.

Shell Research Ltd., Unpublished data, Toxicology of detergents: Acute toxicity, skin and eye irritancy and skin sensitizing potential of Dobanol 23- 3S/27 (ex Z and S Italy), 1978c.

Shell Research Ltd., Unpublished data, Toxicology of detergents: Skin irritancy and skin sensitizing potential of Dobanol 25-3S/27 (bleached and unbleached), 1978d.

Shell Research Ltd., Unpublished data, Toxicology of detergents: Skin irritancy and skin sensitizing potential of Dobanol 25-3A/60 (bleached and unbleached), 1978e.

Shell Research Ltd., Unpublished data, Toxicology of detergents: Skin sensitizing potential of 12 batches of Dobanol 25-3S/27, 1980a.

Shell Research Ltd., Unpublished data, 1980b.

Shell Research Ltd., Unpublished data, 1982a.

Shell Research Ltd., Unpublished data, 1982b.

Shell Research Ltd., Unpublished data, The skin sensitizing potential of Dobanol 25-3S/27, 1983a.

Shell Research Ltd., Unpublished data, 1983b.

Steber J, Gode P & Guhl W (1988) Fettalkoholsulphate – Die okologische Absicherung einer wichtigen Gruppe von Waschmitteltensiden, Sonderdruck aus Fett, Wissenschaft, Technologie, 32-38.

Taylor, A.J., et al. (1978) Metbolism of the surfactants sodium undecyltriethoxy sulphate and sodium dodecyltriethoxy sulphate in the rat. Biochem. J. 174, 405-412.

Tusing, T.W., et al. (1962) Toxicologic studies on sodium lauryl glyceryl ether sulfonate and sodium lauryl trioxyethylene sulfate. Toxicol. Appl. Pharmacol. 4, 402-409.

Unilever, Unpublished data, Ingestion toxicity of ether sulphates. Part 2. 3-Week Feeding Trial with Dobanol 25 3EO sulphate, sodium salt, 1979a.

Unilever, Unpublished data, Ingestion toxicity of ether sulphates. Part 3. 3-Week Feeding Trial with Dobanol 25 3EO sulphate, ammonium salt, 1979b.

Unilever, Unpublished data, Ingestion toxicity of ether sulphates. Part 4. 3-Week Feeding Trial with Acropol 13-15 3EO sulphate, ammonium salt, 1979c.

Unilever, Unpublished data, Ingestion toxicity of ether sulphates. Part 4. 3-Week Feeding Trial with Alfol 12-14 3EO sulphate, sodium salt, 1979d.

Unilever, Unpublished data, Ingestion toxicity of ether sulphates. Part 5. 3-Week Feeding Trial with Lial 25L 3EO sulphate, sodium salt, 1979e.

Unilever, Unpublished data, Ingestion toxicity of ether sulphates. Part 5. 3-Week Feeding Trial with Lial 25L 3EO sulphate, sodium salt, 1979f.

Unilever, Unpublished data, Ingestion toxicity of ether sulphates. Part 7. 3-Week Feeding Trial with Dobanol 25 3EO sulphate, sodium salt, containing alcohol, 1980a.

Unilever, Unpublished data, Ingestion toxicity of ether sulphates. Part 6. 3-Week Feeding Trial with Alfol 16-18 4EO sulphate, sodium salt, 1980b.

Unilever, Unpublished data, Mutagenicity and teratology of alternative surfactants. Part 25. Teratology of Dobanol 25 E3 sulphate. Oral administration in the rat, 1980c.

Unilever, Unpublished data, Mutagenicity and teratology of alternative surfactants. Part 22. Teratology of Acropol 13-15 E3 sulphate. Oral administration in the rat, 1986a.

Unilever, Unpublished data, Mutagenicity and teratology of alternative surfactants. Part 13. Teratology of Alfol 12-14 E3 sulphate. Oral administration in the rat, 1986b.

Unilever, Unpublished data, Mutagenicity and teratology of alternative surfactants. Part 31. Teratology of Alfol 16-18 E4 sulphatc. Oral administration in the rat, 1986c.

Vermeire, T.G., et al. (1993) Estimation of consumer exposure to chemicals: Application of simple models. Science Total Environ. 136:155-176.

Versteeg D, Stanton, DT, Pence MA & Cowan C (1997) Effects of surfactants on the rotifer, Brachionus calyciflorus, in a chronic toxicity test and in the development of QSARS Environmental Toxicology and Chemistry 16: 1051-1058.

Walker, A.I.T., et al. (1967) Toxicity of sodium lauryl sulphate, sodium lauryl ethoxysulphate and corresponding surfactants derived from synthetic alcohols. Fd. Cos. Tox. 5, 763-769.

Walker, A.P., et al. (1973) Some characteristics of the sensitizer in alkyl ethoxy sulphate. Acta. Dermatovener 53, 141-144.

Annex 1 CAS # covered in family

CAS Number	CAS Description
27028-82-6	Ethanol, 2,2',2"-nitrilotris-, compd. with a-sulfo-w-(dodecyloxy)poly(oxy-1,2-ethanediyl) (1:1)
54116-08-4	Poly(oxy-1,2-ethanediyl), a-sulfo-w-tridecyloxy)-, sodium salt
67762-19-0	Poly(oxy-1,2-ethanediyl), a-sulfo-w-hydroxy-, C10-16-alkyl ethers, ammonium salts
68037-05-8	Poly(oxy-1,2-ethanediyl), a-sulfo-w-hydroxy-, C6-10-alkyl ethers, ammonium salts
68037-06-9	Poly(oxy-1,2-ethanediyl), a-sulfo-w-hydroxy-, C6-10-alkyl ethers
68540-47-6	Ethanol, 2,2',2"-nitrilotris-, compd. with a-sulfo-w-(tetradecyloxy)poly(oxy-1,2-ethanediyl) (1:1)
68585-34-2	Poly(oxy-1,2-ethanediyl), a-sulfo-w-hydroxy-, C10-16-alkyl ethers, sodium salts
68585-40-0	Poly(oxy-1,2-ethanediyl), a-sulfo-w-hydroxy-, C16-18-alkyl ethers, sodium salts
68891-38-3	Poly(oxy-1,2-ethanediyl), a-sulfo-w-hydroxy-, C12-14-alkyl ethers, sodium salts
96130-61-9	Poly(oxy-1,2-ethanediyl), a-sulfo-w-hydroxy-, C9-11-alkyl ethers, sodium salts
105859-96-9	Ethanol, 2,2',2"-nitrilotris-, compds. with polyethylene glycol hydrogen sulfate C11-15-sec-alkyl ether ammonium salts
125301-92-0	Poly(oxy-1,2-ethanediyl), a-sulfo-w-hydroxy-, C12-15-alkyl ethers, sodium salts
125304-06-5	Ethanol, 2,2',2"-nitrilotris-, compds. with polyethylene glycol hydrogen sulfate C16-18-alkyl ether
129783-23-9	Ethanol, 2,2'-iminobis-, compds. with polyethylene glycol hydrogen sulfate C12-15-alkyl ethers
157627-92-4	Alcohols, C10-16, ethoxylated, sulfates, mono(hydroxyethyl)ammonium salts (>1 <2.5 mol EO)
157707-82-9	Alcohols, C14-16, ethoxylated, sulfates, sodium salts (>1 <2.5 mol EO)
162201-45-8	Ethanol, 2-amino-, compds. with polyethylene glycol hydrogen sulfate C12-15-alkyl ethers

174450-50-1	Alcohol, C12-14, ethoxylated, sulfates, triisopropanolamine salts
102783-14-2	Poly(oxy-1,2-ethanediyl), a-sulfo-w-hydroxy-, C10-18-alkyl ethers, sodium salts
9004-82-4	Sodium lauryl ether sulfate
25231-22-5	Poly(oxy-1,2-ethanediyl), .alpha.-[(tridecyloxy)sulfonyl]-.omega.-hydroxy-, sodium salt
34431-25-9	Polyethylene glycol octyl ether sulfate, sodium salt
52286-19-8	Polyethylene glycol decyl ether sulfate, ammonium salt
67762-21-4	Poly(oxy-1,2-ethanediyl), .alpha.-sulfo-.omega.-hydroxy-, C10-16-alkyl ethers, magnesium salts
68081-91-4	Poly(oxy-1,2-ethanediyl), .alpha.-sulfo-.omega.-hydroxy-, C12-18-alkyl ethers, sodium salts
68184-04-3	2-Aminoethanol compd. with .alpha.-sulfo-.omega.-(dodecyloxy)poly(oxy-1,2-ethanediyl) (1:1)
68610-22-0	Poly(oxy-1,2-ethanediyl), .alpha.-sulfo-.omega.-hydroxy-, C12-18-alkyl ethers, ammonium salts
68891-29-2	Poly(oxy-1,2-ethanediyl), .alpha.-sulfo-.omega.-hydroxy-, C8-10-alkyl ethers, ammonium salts
68891-30-5	Poly(oxy-1,2-ethanediyl), .alpha.-sulfo-.omega.-hydroxy-, C11-15-branched alkyl ethers, ammonium salts
73665-22-2	Poly(oxy-1,2-ethanediyl), .alpha.-sulfo-.omega.-hydroxy-, C6-10-alkyl ethers, sodium salts
157627-95-7	Poly(1,2-ethanediyl), .alpha.-sulfo-.omega.-hydroxy-C16-18 and C18 unsaturated alkyl ethers, sodium salts
160104-51-8	Poly(1,2-ethanediyl), .alpha.-sulfo-.omega.-hydroxy-C12-14 alkyl ethers, magnesium salts
160104-52-9	Poly(1,2-ethanediyl), .alpha.-sulfo-.omega.-hydroxy-C16-18 and C18 unsaturated alkyl ethers, magnesium salts
67762-19-0	Poly(oxy-1,2-ethanediyl), .alpha.-sulfo-.omega.-hydroxy-, C10-16-alkyl ethers, ammonium salts
13150-00-0	Ethanol, 2-[2-[2-(dodecyloxy)ethoxy]ethoxy]-, hydrogen sulfate, sodium salt
32612-48-9	Poly(oxy-1,2-ethanediyl), .alpha.-sulfo-.omega.-(dodecyloxy)-, ammonium salt