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

Alcohol Ethoxylates

Version 2.0

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This version is based on the Version 1 (May 2007) where the equation 4.2 (page 24) has been corrected

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1. Executive summary

General

Alcohol ethoxylates (AE) are a major class of non-ionic surfactants which are widely used in laundry detergents and to a lesser extent in household cleaners, institutional and industrial cleaners, cosmetics, agriculture, and in textile, paper, oil and other process industries.

Human health

The presence of AE in household detergents gives rise to a variety of possible consumer contact scenarios including direct and indirect skin contact from its use in laundry detergents, inhalation through the use of spray cleaners and oral ingestion derived from residues deposited on dishes. The aggregate consumer exposure to AE has been conservatively estimated to be at maximum 6.48 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. No adverse reproductive or developmental effects were observed. The majority of available toxicity studies revealed NOAELs in excess of 100 mg/kg bw/d but the lowest NOAEL for an individual AE was established to be 50 mg/kg bw/day. This value was subsequently considered as a conservative, representative value in the risk assessment of AE. The effects were restricted to changes in organ weights with no histopathological organ changes with the exception of liver hypertrophy (indicative of an adaptive response to metabolism rather than a toxic effect). It is noteworthy that there was practically no difference in the NOAEL in oral studies of 90-day or 2 years of duration in rats. A comparison of the aggregate consumer exposure and the systemic NOAEL (taking into account an oral absorption value of 75%) results in a Margin of Exposure of 5,800. Taking into account the conservatism in the exposure assessment and the assigned systemic NOAEL, this margin of exposure is considered more than adequate to account for the inherent uncertainty and variability of the hazard database and inter and intra-species extrapolations.

AEs are not contact sensitizers. Neat AE are irritating to eyes and skin. The irritation potential of aqueous solutions of AEs depends on concentrations. Local dermal effects due to direct or indirect skin contact in certain use scenarios where the products are diluted are not of concern as AEs are not expected to be irritating to the skin at in-use concentrations. Potential irritation of the respiratory tract is not a concern given the very low levels of airborne AE generated as a consequence of spray cleaner aerosols or laundry powder detergent dust.

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

Environment

The environmental risk assessment uses the “sum of toxic units” approach, in which the ratio of the predicted environmental concentration (PEC) of each individual AE homologue to the predicted no effect concentration (PNEC) of that AE homologue is

first calculated, and then the sum of these ratios, or "toxic units", is calculated for all AE homologues used in laundry cleaners and household cleaning products. Thus 230 different AE homologues, with hydrocarbon chain lengths from 8 to 18 and with ethylene oxide chain lengths from 0 to 22, are considered in the AE environmental risk assessment.

The environmental concentrations in river water are determined from measured effluent concentrations from European sewage treatment facilities, using recently developed analytical methods able to measure environmental concentrations of individual AE homologues with hydrocarbon chain lengths from 12 to 18 and ethylene oxide chain lengths from 0 to 18. These homologues cover more than 80% of the tonnage used in laundry cleaners and household cleaning products. Conservative estimates have been made for the concentrations of the other AE homologues, and these are included in the risk assessment. The total local AE concentration in river water receiving sewage effluent ($PEClocal_{dissolved}$) is 1.01µg/l.

The equilibrium partitioning method has been used to predict the concentrations of the individual AE homologues in river sediment from the river water concentrations. Maximum values for soil concentrations have been estimated from measured concentrations of several representative European sewage sludges. The local sediment, soil, and sewage treatment plant concentrations have been determined as 1.01mg/kg wet sediment, 0.24 mg/kg wet soil, and 9.8 µg/l respectively, with the sewage treatment plant concentration determined from the measured AE effluent concentrations.

Two complementary methods, both based on high quality chronic effects data, are used to determine the toxicity of the AE homologues in river water. The deterministic method uses a recently published QSAR for the species with the best high quality chronic information, *Daphnia magna*, with an application factor of 10. The probabilistic method uses a chronic QSAR, recently developed using data from 17 different species and an application factor of 1, to predict the NOEC values for each AE homologue. Equilibrium partitioning is then used to determine the toxicity of AE homologues in sediment and soil, with the soil values being supported by acute and chronic single homologue data for some AE homologues.

Two risk assessments, one using the deterministic (D) method and one using the probabilistic method (P), have been carried out for the environmental concentrations of AE used in laundry cleaners and household cleaning products. The resulting risk assessment ratio (PEC/PNEC value) for all the AE homologues in surface water is 0.041 with the deterministic method (D), and 0.024 with the probabilistic method (P). Risk assessment ratios are 0.316(D) and 0.181(P) in sediment, 0.103(D) and 0.068(P) in soil, and 0.007 in the sewage treatment plant, where a simple method assuming the acute data for the most toxic AE mixture applies to all AE has been used. As all the risk assessment ratios are below 1, there is no cause for concern in any of the environmental compartments.

In summary, AE usage in laundry cleaners and household cleaning products is not a cause for concern in the EU environment, as shown by consideration of surface water, sediment, sewage treatment facilities, and soil.

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3. Substance characterisation

Alcohol ethoxylates (AE) are a very widely used class of non-ionic surfactants. Significant quantities of AE are converted to alcohol ethoxysulphates (AES) with the remaining AE used primarily in household laundry detergents. AE have many desirable characteristics such as rapid biodegradation, low to moderate foaming ability, superior cleaning of man-made fibres and tolerance of water hardness. AE are also used in lesser quantities in household cleaners, institutional and industrial cleaners, cosmetics, agriculture, and in textile, paper, oil and other process industries. Uses in household cleaning products, relevant to the HERA program of risk assessments, include laundry detergents, hand dishwashing liquids, and various hard surface cleaners. Chapter 5.1.1 details the household cleaning applications and typical finished product concentration ranges of all AEs used in household products.

3.1 CAS number and grouping information

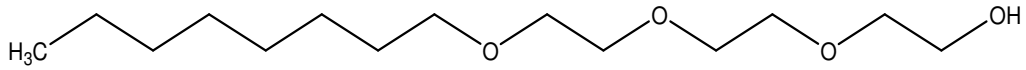
There are numerous CAS numbers describing AE. A comprehensive list is presented in Annex I of this document. Although clearly important from a regulatory perspective, the AEs covered in this assessment report are not characterized by CAS numbers, but by a definition of their chemical structure in terms of the respective carbon chain length and ethoxylation degree.

3.2 Chemical structure and composition

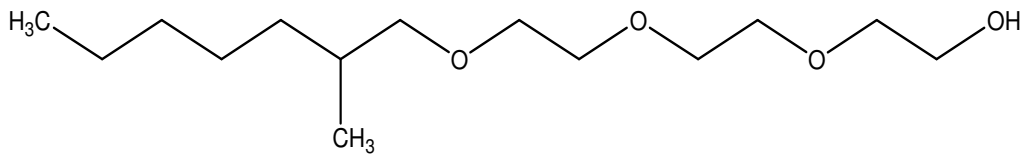
3.2.1. Chemical Structure

The AE family is defined for HERA purposes to be of the basic structure $C_{x-y}AE_n$. The subscript following the 'C' indicates the range of carbon chain units. AEs with carbon unit range between C_8 to C_{18} are most commonly used in household detergent products. Further, AEs contain an ethylene oxide (E) chain attached to the alcohol. The degree of ethylene oxide polymerization is indicated by a subscript which indicates the average number of ethylene oxide units. In household products the average ethylene oxide chain length commonly ranges between 3 and 12 units.

Two principle structures of AEs present in household cleaning products are presented below.



Linear AE (C_8EO_3)



Essential linear, methyl branched AE (C_8EO_3)

Further details on the structures included in the AE family are given in Section 3.3., with information concerning homologue distributions given in section 3.4.

3.2.2 Physicochemical Properties

The family of AE are composed of compounds that differ in chain length with respect to the number of carbon units and the number of ethylene oxide units. The physico-chemical properties of AE therefore span a broad range. Although very little specific information is available concerning several of the physicochemical properties of specific AE homologues, an extensive data set is available for the alcohols (the EO=0 homologues). In many cases it is possible to use the alcohol information to set upper or lower limits for the specific physicochemical property for the other AE homologues, and thus evaluate their chemical and physical chemical behaviour.

In general, information about several physicochemical properties of AE homologues is necessary in order to carry out an environmental risk assessment according to the principles of the EU TGD (2003), especially if the EUSES program is used to carry out the assessment. The most important of these physicochemical properties are the water solubility, the vapour pressure, the octanol/water partition coefficient or the associated K_{oc}[1] and K_d[2] values used to quantify adsorption onto various environmental solids, and the Henry's law constant, which quantifies the air/water partitioning behaviour. However, melting point and boiling point information are also useful for the environmental risk assessment. The experimentally available data are discussed below. It is also possible to calculate these physical chemical properties for AEs, using programs such as EPIWIN (US EPA 2000). Appendix III gives the physical chemical properties calculated by EPIWIN for any AE isomers for which the calculated value has been used in the HERA environmental AE assessment.

3.2.2.1. Solubility in water

The EU risk assessment protocol described in the TGD requires that information on the solubility of a chemical be available, in order to ensure that aqueous concentrations determined as part of the environmental risk assessment do not exceed the solubility of the chemical in water, and to ensure that the chemical concentrations used in eco-toxicity testing are below the limit of aqueous solubility. Although no direct information is available for the other AE homologues, aqueous solubilities for the alcohols (EO=0 homologues) have been collected as part of the long chain alcohol

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[1] K_{oc} quantifies the adsorption onto organic carbon. It is useful for expressing adsorption onto environmental solids whose organic carbon content can be quantified, and is used extensively in the EU TGD (2003) to describe adsorption to activated sludge, suspended matter in surface waters, and solid particles in sediment and soil.

[2] K_d quantifies the adsorption onto a specific solid type, such as sediment obtained from a specific site. It incorporates the effects of hydrophilic and ionic or other sorption mechanisms.

SIAR (SIAR 2006) and are given in Table 3.1.These data have been given Klimisch[3] (See Klimisch et al, 1997) scores of 2 by the authors of the long chain alcohol SIAR (2006). As the addition of the ethylene oxide group makes the AE homologue more soluble in aqueous media, the various AE homologues will be expected to be more soluble than the alcohol solubility values given in Table 3.1. Thus even the C18 AE homologues are expected to have an aqueous solubility of at least 1 µg/l.

Additional information on water solubility can be obtained from information on the measured critical micelle concentration (cmc) of several AE homologues, as the homologue must be soluble, as monomer, at aqueous concentrations up to the cmc to allow the cmc measurements to be made. The cmc information provided by Nikkol (2006) is shown in table 3.1, in bold, and is given a Klimisch score of 2. For the three EO8 homologues shown, this solubility is more than 10 times the solubility of the corresponding alcohol, with the excess solubility over alcohol increasing with the hydrocarbon chainlength of the AE homologue. The trend in the available information suggests that longer chainlength AE homologues could have an aqueous solubility in excess of 25 times the water solubility of the corresponding alcohol at EO=8, with further small increases in solubility expected for higher EO chain numbers.

Further cmc information for commercial AE mixtures has been provided by Sasol (personal communication, 2006). This information is shown in italics in table 3.1, and is presently given a Klimisch score of 4, as the communication is a secondary reference. The EO values for these commercial materials are average values for the commercial product, and thus some longer chain EO material will be present in these samples. However, the data demonstrate that these AE mixtures are water soluble in the milligram per litre range and above, with solubility increasing with the number of EO groups.

It is also possible to calculate water solubility for AEs, using programs such as EPIWIN (US EPA 2000). Appendix III gives the water solubility calculated by EPIWIN for any AE isomers for which the calculated value has been used in the HERA environmental AE assessment.

[3] Klimisch Scores are 1 = valid without restriction; 2 = valid with restriction; 3 = not reliable; 4 = not assignable. Further information and guidance on the selection of data for HERA reports is given in Appendix C of the HERA Methodology Document (HERA 2005).

Table 3.1. Aqueous solubilities of long chain alcohols, from the alcohol SIAR (2006), and also cmc data[1] for several AE isomers.

EO / C	C8	C10	C12	C13	C14	C15	C16	C18
0	551 mg/l at 25C	39.5 mg/l	1.93 mg/l at 20C	0.38 mg/l at 20C	0.191mg/l at 25C	0.102 mg/l at 25C	0.013mg/l at 25C	0.0011mg/l at 25C
2			*Linear C12-14 75 mg/l*		*Linear C12-14 75 mg/l*			
3			*Linear C12-14 11 mg/l*	*Branched C13 3 and 19 mg/l*[2]	*Linear C12-14 11 mg/l*	*Essentially linear C14-15 1 mg/l*		
5			**26.4mg/l**	*Branched C13 26mg/l*		*Essentially linear C14-15 2 mg/l*		
6			**30.6mg/l** *See also C14*	*Branched C13 32and 40mg/l*[2]	*Linear C12-14 12 mg/l*			
7			**34.1mg/l** *See also C14*	*Branched C13 34mg/l*	*Linear C12-14 15 mg/l*	*Essentially linear C14-15 2 mg/l*		
8		**510mg/l**	**38.2mg/l**	*Branched C13 57 and70 mg/l*[2]	**5.1mg/l**			
9			*Linear C12-14 18mg/l*	*Branched C13 67and 80 mg/l*[2]	*Linear C12-14 18mg/l*	*Essentially linear C14-15 3 mg/l*		
10				*Branched C13 74 mg/l*				
12				*Branched C13 110 mg/l*				
20				*Branched C13 250mg/l*				
40				*Branched C13 1000mg/l*				

[1]Cmc data in for single homologues in bold. Cmc data for commercial mixtures in italics. [2]Two different methods have been used to measure the cmc for these AEs.

3.2.2.2. Melting point

Melting point information is used in the EU risk assessments (EU TGD 2003) mainly to determine whether the substance of interest is solid or liquid at room temperature. There is some melting point information available for AE homologues, with the information on the alcohol melting points from the long chain alcohol SIAR (SIAR 2006) and the data on other specific homologues given by the manufacturer, Nikkol (2006), who prepared several specific AE homologues for research purposes, and by Tolls and Sijm (2000), who quote the original synthesizer of the pure chain AE homologues. The Nikkol data is given a Klimisch (Klimisch et al, 1997) score of 2, and the alcohol SIAR data is rated 4 by the authors of the SIAR, except for the C8, C10, C14, C16, and C18 (unsaturated at the 9 position) data, which has been rated 2. The Tolls and Sijm data is given a Klimisch score of 4, as it has been obtained from a reference work. The available data is given in Table 3.2. AE homologues with no data have been omitted from the table. Here the higher melting temperatures seen for the alcohols compared to several of the lower EO homologues reflects the greater ease

Table 3.2. Melting temperatures (in degrees centigrade) for several AE homologues

C / EO	C8	C10	C12	C13	C14	C15	C16	C18	C18 (=9)[4]
0	-15.5 to -17	[1]6.4	[1]22.6 - 24	[1]30.6 or 32-33	[1]39-40	[1]44 or 45-46	[1]50	[1]58, 59.5	[1]13-19
2			[2]18.0-18.2		[2]28.0-29.0		[2]36.8-37.2 [3]31.7		
3					[2]25.7-27.0		[2]33.8-34.2		
4					[2]28.5-29.5		[2]36.7-37.0		
5			[2]23.6-24.0		[2]30.0-31.7		[2]37.6-38.0		
6		[3]16.7	[2]25.0-25.4 [3]25.7		[2]32.5-33.0 [3]35.0		[2]38.4-38.9 [3]36.4		
7		[2]20.0-20.1			[2]33.5-34.5		[2]39.4-39.9		
8		[2]25.8-26.0	[2]30.0-31.0		[2]37.0-38.0		[2]43.0-43.5		
9							[3]43.0		
12							[3]45.5		
15							[3]47.0		

[1]Data from Long chain alcohol SIAR. [2]Data provided by Nikkol. [3]Data from Tolls and Sijm (2000). [4] Unsaturated at the C=9 position.

in disassociating the crystal structure of the lower EO homologues. The energy involved in this structure disassociation is also expected to influence the water solubility of the various AE homologues (see section 3.2.2.1).

It is also possible to calculate melting points for AEs, using programs such as EPIWIN (US EPA 2000). Appendix III gives the melting points calculated by EPIWIN for any AE isomers for which the calculated value has been used in the HERA environmental AE assessment.

3.2.2.3. Boiling Point

Boiling point information is available for several of the long chain alcohols and other AE homologues, and is given in Table 3.3. AE homologues with no available data have been omitted from this table. The entries for C8, C10, C14, and C16 are given a

Table 3.3. Boiling point data for AE homologues

C / EO	C8	C10	C12	C13	C14	C16	C18	C18(=9)
0	[3]194 to 195C (ambient)	[3]229C (ambient)	[3]255 to 269C (ambient)	[3]276C (ambient)	[3]289C (ambient)	[3]334-344C (ambient)	[3]210C (15 mm Hg)	[3]333C (ambient)
2		[1]100C (0.4mm Hg)	[1]175-180C (3.0mm Hg)		[1]174-176C (1.5mm Hg)	[1]172-178C (0.5-10.6 mm Hg)		
3		[1]145C (0.48mm Hg)	[1]204-212C (6.0 mm Hg)		[1]181-184C (0.5mm Hg)	[1]203-206C (0.35mm Hg)		
4		[1]173C (0.2mm Hg)	[1]235-245C (3.0-4.0 mmHg) [2]152C at 0.01 mmHg		[1]204-206C (0.55mm Hg)	[1]215-220C (0.3mm Hg)		
5		[1]183C (0.15mm Hg)	[1]202-216C (0.5 mm Hg)		[1]227-229C (0.5mm Hg)	[1]247-253C (0.5 mmHg)		
6		[1]230C (0.5mm Hg) [2]200C at 0.02 mmHg	[2]205C at 12 mmHg		[2]206C at 0.02 mmHg	[2]234C at 0.05 mmHg		
8			[2]232C at 0.01 mmHg					
12			[2]281C at 0.01 mmHg					

[1]AE homologue data from Nikkol (2006).
[2]AE data from Boethling and Mackay (2000).
[3]Alcohol data from long chain alcohol SIAR (2006).

Klimisch (Klimisch et al, 1997) rating of 2 by the authors of the long chain alcohol SIAR (SIAR 2006), and the other alcohol entries are given a Klimisch rating of 4. The boiling point data for the other AE homologues is either supplied by the manufacturer, Nikkol, and given a Klimisch score of 2, or taken from a reference work (Tolls and Sijm, 2000), and given a Klimisch score of 4. Unfortunately, the C18 alcohol data, and all of the boiling point data for the other AE homologues supplied by Nikkol and by Tolls and Sijm were obtained at reduced pressure.

As conversions of boiling point data from reduced pressure to ambient pressure is not straightforward or reliable, it has not been attempted here. The boiling point data is useful in a qualitative way, however, as it indicates that the other AE homologues have relatively high boiling points, and thus might be expected to have relatively low vapour pressure at ambient temperature.

It is also possible to calculate boiling points for AEs, using programs such as EPIWIN (US EPA 2000). Appendix III gives the boiling points calculated by EPIWIN for any AE isomers for which the calculated value has been used in the HERA environmental AE assessment.

3.2.2.4. Vapour pressure data and Henry's Law constant

Vapour pressure data at 20C or 25C is available for long chain alcohols. The data available in the long chain alcohol SIAR (2006) is shown in table 3.4. All of the data have been given a Klimisch score of 2 by the authors of the SIAR.

Table 3.4. Vapour pressure data for long chain alcohols (SIAR 2006)

C8	C9	C10	C11	C12	C13	C14	C15	C16	C18
0.10 hPa at 25°C	0.03 hPa at 25°C	0.0113 hPa at 25°C	0.0039 hPa at 25°C	0.0011 hPa at 25°C;	0.00057 hPa at 25°C	0.00014 hPa at 25 °C	5.12×10^{-5} hPa at 25 °C	1.4×10^{-5} hPa at 25 °C	3.3×10^{-6} hPa at25°C

The vapour pressures are low, especially for the higher chain alcohols. As noted in section 3.2.2.3, the indications of higher boiling points for the non-alcohol AE homologues suggests that the vapour pressure for these will be lower than those given for the alcohols of the same chain length in table 3.4. Tolls and Sijm (2000) note in their review of non-ionic surfactants that "Vapour pressure data are not available for…. non-ionic surfactants. Some alcohol ethoxylates have been analysed by high temperature gas chromatography, but the fact that the elution temperatures of the higher ethoxylated AEs are above 520K on a SE 30 boiling column …. indicates that the vapour pressure of these compounds is comparatively low. This is consistent with the high boiling points of these compounds. In addition, since surfactants are rather water soluble, their Henry's law constants can be expected to be very low……as a result, evaporation of surfactants can be expected to be negligible." These authors also note that no Henry's Law constants have been directly measured for nonionic surfactants.

It is also possible to calculate vapour pressure at 25C for AEs, using programs such as EPIWIN (US EPA 2000). Appendix III gives the vapour pressures calculated by EPIWIN for any AE isomers for which the calculated value has been used in the HERA environmental AE assessment.

3.2.2.5. Log K_{ow}, K_{oc}, and K_d information for AE homologues

The octanol-water partition coefficient, or K_{ow}, is a very useful parameter for environmental risk assessment, and is used extensively in EU risk assessment (EU TGD 2003). Quantitative Structure/Activity Relationships, or QSARs, exist which enable sorption onto several different types of solid to be calculated from log K_{ow} values, by assuming that the sorption mechanism involves substance solubilisation in the organic carbon portion of the solid substance. Bioaccumulation and eco-toxicity relationships may also be calculated from a log K_{ow} value. However, logKow is difficult to measure for surfactants, as surfactants will be located preferentially at the interface(s) in an oil/water system (ECETOC, 2003). This must be remembered whenever logKow data are used for surfactants.

Table 3.5. Measured log Kow values for long chain alcohols

C8	C9	C10	C11	C12	C13	C14	C16	C18
3.15	3.77	4.57	4.72	5.36	5.51	6.03	6.65	7.19

Measured log K_{ow} information is available for the long chain alcohols, but not for the other AE homologues. The data, shown in table 3.5, have all been given a Klimisch reliability score of 2 by the authors of the long chain alcohol SIAR (2006), except for the C11 data, which has been given a Klimisch score of 4. It would be expected that the log K_{ow} values for the other AE homologues would be somewhat lower than the values for alcohols of the same chain length, as the addition of EO groups makes the AE homologues of the same chain length more water soluble. Thus use of the alcohol log K_{ow} values could be a reasonable conservative assumption for many uses of log K_{ow} values (e.g. bioaccumulation) in an AE risk assessment, if more appropriate methods were not usually available.

Kow values for AE homologues can be calculated using the methods of Leo and Hansch (1979), with appropriate Kow contributions for the ethylene oxide group being calculated by the method of Roberts (1991). This has been done in the CSARA AE Workbook (ERASM 2005b)[4] for AE homologues covering the hydrocarbon range from C9 to C18, and the ethylene oxide range from 0 to 20. In table 3.6, the logKow values for C8 and EO 21 and 22 have been calculated by the same method, and added to the information in the CSARA AE Workbook. Agreement with the measured logKow values for the alcohols can be seen by comparison with the alcohol data in Table 3.5. Use of these logKow values is recommended for eco-toxicity QSAR development, and for other areas if more appropriate information is not available.

[4] The CSARA AE Workbook (ERASM 2005b,c) is an EXCEL workbook which has been developed by the CSARA taskforce, sponsored by ERASM. It contains the basic data and calculation methods available to calculate the results of several ecotoxicity and sorption QSARs for each AE homologue from C9 -18 and EO 0 to 20. If environmental concentrations can be provided, the Workbook can enable the full risk assessment process to be carried out, with several choices of method available to the user. This enables more efficient use of QSARS including those developed by CSARA, in the AE risk assessment process.

Table 3.6. Log Kow information calculated by the methods of Leo and Hansch (1979), and of Roberts (1991), using the method in the CSARA AE Workbook (ERASM 2005b)

C/EO	8	9	10	11	12	13	14	15	16	18
0	3.03	3.57	4.11	4.65	5.19	5.73	6.27	6.81	7.35	8.43
1	3.07	3.61	4.15	4.69	5.23	5.77	6.31	6.85	7.39	8.47
2	2.97	3.51	4.05	4.59	5.13	5.67	6.21	6.75	7.29	8.37
3	2.87	3.41	3.95	4.49	5.03	5.57	6.11	6.65	7.19	8.27
4	2.77	3.31	3.85	4.39	4.93	5.47	6.01	6.55	7.09	8.17
5	2.67	3.21	3.75	4.29	4.83	5.37	5.91	6.45	6.99	8.07
6	2.57	3.11	3.65	4.19	4.73	5.27	5.81	6.35	6.89	7.97
7	2.47	3.01	3.55	4.09	4.63	5.17	5.71	6.25	6.79	7.87
8	2.37	2.91	3.45	3.99	4.53	5.07	5.61	6.15	6.69	7.77
9	2.27	2.81	3.35	3.89	4.43	4.97	5.51	6.05	6.59	7.67
10	2.17	2.71	3.25	3.79	4.33	4.87	5.41	5.95	6.49	7.57
11	2.07	2.61	3.15	3.69	4.23	4.77	5.31	5.85	6.39	7.47
12	1.97	2.51	3.05	3.59	4.13	4.67	5.21	5.75	6.29	7.37
13	1.87	2.41	2.95	3.49	4.03	4.57	5.11	5.65	6.19	7.27
14	1.77	2.31	2.85	3.39	3.93	4.47	5.01	5.55	6.09	7.17
15	1.67	2.21	2.75	3.29	3.83	4.37	4.91	5.45	5.99	7.07
16	1.57	2.11	2.65	3.19	3.73	4.27	4.81	5.35	5.89	6.97
17	1.47	2.01	2.55	3.09	3.63	4.17	4.71	5.25	5.79	6.87
18	1.37	1.91	2.45	2.99	3.53	4.07	4.61	5.15	5.69	6.77
19	1.27	1.81	2.35	2.89	3.43	3.97	4.51	5.05	5.59	6.67
20	1.17	1.71	2.25	2.79	3.33	3.87	4.41	4.95	5.49	6.57
21	1.07	1.61	2.15	2.69	3.23	3.77	4.31	4.85	5.39	6.47
22	0.97	1.51	2.05	2.59	3.13	3.67	4.21	4.75	5.29	6.37

However, for sorption onto activated sludge and river water solids, van Compernolle et al (2006) have developed two sorption QSARs for AEs, one predicting $\log K_{oc}$ and the other predicting log K_d, which are both a function of carbon number and EO number. The development and use of these sorption QSARs, which are appropriate for sorption prediction onto these solids and have been chosen for use in this risk assessment, is described in section 4.1.1.1.1.

3.3 Manufacturing route and production volume

AE are most commonly derived from linear or branched primary alcohols and to a lesser extent from linear random secondary alcohols. The alcohols used in the manufacture of AE typically contain an alkyl chain with 8 to 18 carbon atoms while the ethoxylate chain typically averages from 3 to 12 ethylene oxide units (Talmage, 1994).

Primary AE are produced by ethoxylation of primary alcohols with ethylene oxide (EO) using base catalysed reaction with potassium or sodium hydroxide followed by neutralisation with an acid such as acetic or phosphoric acid. Most commercial products are produced and shipped as solid form, paste or solution. Typically, commercial AE contain the active material but also some reaction by-products such as un-reacted alcohol, typically present at about 5% but with variations between different commercial products. Trace levels of certain other chemicals such as ethylene oxide or 1,4-dioxane might also be present, however, at levels that do not pose any safety concerns. The issues of both contaminants are addressed in chapter 5.3.2.1. The HERA AE family is ultimately derived from linear and essentially linear primary alcohols in the C_8 to C_{18} range. These 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 mixtures are substantially interchangeable as precursor substances for AE used in the major applications falling within the scope of HERA. As marketed, such AEs usually contain a distribution of alkyl chain lengths as well as ethoxy unit chain lengths.

The linear alcohols used in the manufacture of linear AEs which are used in household cleaning products are mainly primary -non-branched- aliphatic alcohols containing an even number of carbon atoms, and may be derived from oleochemical or petrochemical feedstocks (SIAR 2006). These alcohols are produced in single carbon fractionations, or more usually as wider fractionations selected from within the range C_6 through C_{22}. Some alcohols derived from oleochemical sources may also contain unsaturated primary –non-branched- aliphatic alcohols (SIAR 2006).

Essentially linear alcohols, also known as oxo-alcohols, are also used to manufacture AEs used within the scope of HERA. These are mixtures of saturated, primary linear aliphatic alcohols and their saturated, mono branched primary alcohol isomers of corresponding carbon chain length (SIAR 2006). The alcohols are derived from olefins via the so-called oxo-chemistry, in which the precursor olefins, typically derived from ethylene or normal paraffin, are used to manufacture aliphatic alcohols. The alcohols of this sub-group may fall in the range C7 – C17 and contain even and odd numbered carbon chains. The proportion of linear alcohols ranges from 90 to around 50%. The mono-branched isomers have a linear backbone (SIAR 2006). This sub-category also contains a closely related mixture of saturated C12-C13 primary alcohols derived from Fischer-Tropsch olefins consisting of approximately 50% linear, 30% mono-methyl branched and 20% other unintended components. This product is referred to as C10-16 alcohols Type B [CAS 67762-41-8] (SIAR 2006).
A small amount (less than 5%) of the alcohol ethoxylates used in household applications have a greater degree of branching, but are readily biodegradable and have similar ecotox properties to the linear and essentially linear AEs. These AEs which are produced from primary alcohols derived from branched butylene oligomers

have also been included in the HERA Assessment. Of the AE used in consumer cleaning applications in Europe, a preliminary estimate gives 30% derived from even carbon numbered linear alcohols, with the remainder derived from odd and even carbon numbered essentially linear alcohols.

The HERA Guidance Document Methodology (HERA 2005, Section 1.4.5) states that complementary tonnage information may be obtained both from the producers of the HERA substance, and from the formulators who use the substance in the fabrication of products used in household detergent and cleaning applications. Both producer (Cesio) and formulator (AISE) associations have provided complementary AE tonnage information for this HERA AE assessment.

A survey conducted among detergent formulator companies covering at least 80% of the market established that, in 2002, almost 220 000 tonnes of AE were found to be used in household cleaning products (AISE/HERA 2003). Thus the expected AE use in 2002 was approximately 275 000 tonnes, if the tonnage reported by the formulators is increased to account for 80% market coverage. In 1999, a survey of AE producers (Cesio, 1999) reported that approximately 290 000 tonnes of AE were thought to be sold for household cleaning products. In addition, approximately 80 000 additional tonnes of AE were thought to be sold for uses outside the scope of HERA, which may be discharged to sewer. However, as information on the hydrocarbon chainlengths and EO chainlengths is not available for this "non-HERA" AE, it is not considered further in this HERA assessment. A tonnage of 290 000 tpa, with the hydrocarbon and EO chainlength distributions established from the formulator's survey (AISE/HERA 2003) and shown in Tables 3.7 and 3.9 below, may be used to derive environmental concentrations in applicable sections of the HERA environmental risk assessment, in areas where higher tier information (for example, environmental concentrations obtained from monitoring data) is not available.

3.4 Homologue distribution in HERA applications

To determine the total AE tonnage that has been used in products falling within the scope of HERA (*i.e.*, household cleaning products), a survey has been conducted among detergent formulator companies (data from members of AISE) and companies manufacturing AE. In the HERA-relevant range of C_8-C_{18}, the distribution between carbon chain lengths has been estimated (Table 3.7) and has been calculated based on more detailed tonnage and product information submitted by the formulator companies. As mentioned in section 3.2.1, AE carbon chains are a mixture of linear alcohols and essentially linear alcohols. In addition, in some cases branched hydrocarbon chains may also be present, but in all cases these will be readily biodegradable substances, which do not contain quaternary carbon atoms.

Table 3.7: Estimated carbon chain distribution of AEs in household cleaning products

Carbon chain length	C_8	C_9	C_{10}	C_{11}	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}	C_{17}	C_{18}
Distribution in household products	<1%	3%	7%	5%	31%	22%	15%	11%	2.5%	0.5%	3%

The survey data showed that a high proportion of the tonnage of the AEs used in HERA applications have nominal or average EO values described by the manufacturer as being in the E7-E8 range (Table 3.8). The data in Table 3.8 refer to the nominal or average ethoxylation degree as indicated by the formulator description for the various market products. However, a wide distribution of EO chainlengths is actually present in each commercial AE product. This leads to a broad overall distribution of EO chainlengths in AE products, as shown in Table 3.9.

Table 3.8: Estimated nominal average EO distribution of AE in household cleaning products

Nominal mean EO chain length	E_3	E_4	E_5	E_6	E_7	E_8	E_9	E_{10}	E_{11}	E_{20}	E_{25}
Distribution in household products	3%	5%	11%	6%	38%	34%	-	1%	-	1%	1%

The reaction of the basis alcohols with ethylene oxides yields mixtures of homologues which are alcohol polyethylene glycol ethers. The content of the individual homologue depends on the degree of polymerization, the catalyst and its concentration. AE's produced by alkaline catalysis have usually a broad homologue distribution with a significant content of un-reacted alcohol (Falbe, 1987).
A more refined EO number distribution which addresses the de-facto broad distribution was derived from analytical data for products having nominal mean EO values in the EO3 to E5 range, and also for products having nominal mean EO values in the E6 to E8 range.. A cross-check of those data among two suppliers confirmed their validity. This refined EO distribution is shown in Table 3.9.
Table 3.9 illustrates that the estimated EO distribution of AEs in household detergents is fairly even up to an EO-degree of about E12, and then becomes more flat and expires at approx. E20, with a significant amount of un-reacted alcohol. These EO distributions cover the known range of commercial products. The percentage of each EO homologue in a specific commercial blend may differ from the overall percentages given in the table.

Table 3.9: Estimated EO distribution of AE in household cleaning products

EO chain length	E_0[1]	E_1	E_2	E_3	E_4	E_5	E_6	E_7	E_8	E_9	E_{10}	E_{11}
Distribution in household products	6%	4%	5%	6%	7%	7%	7%	7%	7%	7%	7%	6%
EO chain length	E_{12}	E_{13}	E_{14}	E_{15}	E_{16}	E_{17}	E_{18}	E_{19}	E_{20}	E_{21}	E_{22}	
Distribution in household products	5%	4%	3%	3%	2%	2%	1%	1%	1%	1%	1%	

[1] Unreacted alcohol

The estimated hydrocarbon chain distribution in Table 3.7 and the estimated EO distributions for the individual AE homologues given in Table 3.9 have been used, together with the maximum estimated AE production tonnage of 290 000 tonnes per annum, to derive environmental concentrations in applicable sections of the HERA AE environmental risk assessment, in areas where higher tier information (for example, environmental concentrations obtained from monitoring data) is not available.

4. Environmental assessment

This HERA environmental assessment makes use of the advances in analytical methodology which have enabled the detection and quantification of environmental levels of AE homologues containing hydrocarbon chainlengths from C12 to C18, including those with low numbers (0-2) of ethoxylate units (Dunphy et al 2001). The risk assessment methodology compares the PEC for each homologue with the corresponding PNEC information, which in most cases has been obtained from commercial AE mixtures with different homologue distributions. The QSAR-based approach used to determine the appropriate homologue-specific PNEC values and the use of monitoring data which provides the basis for the PEC determination are both higher tier risk assessment tools. Thus the aquatic component of this HERA environmental risk assessment does not make extensive use of the EUSES program, as is normal for a screening level HERA risk assessment. However, the principles of the EU TGD (2003) are adhered to throughout.

4.1. Environmental Exposure Assessment

4.1.1. Environmental Fate

AE used in HERA applications in Europe is generally released, after use, as an aqueous solution to sewer or to private treatment or dispersal facilities. In the sewer or during sewage treatment processes some of the AE will be adsorbed to solids, and may then undergo anaerobic biodegradation in a digester before the resulting sludge is released to agricultural land, for use as fertiliser. AE remaining in aqueous solution is subject to aerobic biodegradation processes during sewage treatment, which results in substantial AE removal before the effluent is released to surface water. In surface water, sediment, and soil further aerobic and anaerobic biodegradation will occur. In addition, AE may be taken up by plants or animals living in the surface water or soil.
This section discusses these basic adsorption and degradation processes as well as bioconcentration potential before focussing on removal during sewage treatment. The removal section is largely based on measured effluent concentrations obtained from environmental monitoring data. However, measured concentrations in sewage sludge have also been used as part of the terrestrial assessment.

4.1.1.1. Partitioning between environmental compartments

AE can potentially be transferred from the aqueous phase to suspended solids, activated sludge, or soil solids by adsorption, and from the aqueous or solid phases to the atmosphere by volatilisation. These processes are discussed in sections 4.1.1.1.1 and 4.1.1.1.2.

4.1.1.1.1. Adsorption to soil, sediment, and activated sludge

AE adsorption to soil, sediment, and to a lesser extent to activated sludge depends upon both the properties of the individual AE homologue and the properties of the material to which it is adsorbed. The EU TGD (2003) recognises that adsorption may be influenced by factors other than the organic carbon content of the sorbate, but in the absence of other information bases the QSARs used to predict adsorption on the Koc of the adsorbing substance and the organic carbon content of the sorbate. As real

sorbates, especially sediments and soils, will have different amounts of material, for example clay, which can also be a sorption site for AE homologues (van Compernolle et al 2006), the uncertainty in the determination of homologue-specific Kd values intended to cover all sediments and soils will be large. However, the use of a QSAR developed from measured sorption data should give more realistic results than those determined from the default TGD (2003) methodology. Thus this HERA AE assessment uses the AE-specific QSARs developed from measured sorption data by van Compernolle et al (2006) to characterise the adsorption of the various AE homologues to both sewage sludge and suspended solids in river water.
The recent work by van Compernolle et al (2006) has investigated the adsorption of several radio-labelled specific AE homologues onto activated sludge and river water solids, and has used these results, together with results from validated published and unpublished work, to derive both Kd and Koc based sorption QSARs. The experimental data used to develop these QSARs covers the EO range from 0 to 10, with good coverage for both the alcohols (C12, 14, 16, and 18) and EO=9 (C10, 12, 13, 14, 15, and 16) and substantial coverage of intermediate homologues. The complete matrix of the AE homologues used to develop these sorption QSARs is shown in table 4.1.

Table 4.1 AE homologues used to develop sorption QSARs by van Compernolle et al (2006). Values given are logK_d values.

		Carbon Number							
		10	12	13	13.5	14	15	16	18
EO Number	0		3.48			3.93		4.38	4.90
	1					***3.63***			
	2			2.79					
	3	1.61	***2.87****	***2.48***		***3.78***		***3.98***	
	4			2.88					
	5	1.68	2.86			3.54		3.68	
	6		***3.07***	***3.09***		***3.80***		***4.34***	
	7								
	8	2.1	3.09	3.13		3.55		3.79	
	9	2.22	2.87	2.98	3.34	***3.65***	***3.41***	4.18	
	10		3.45						

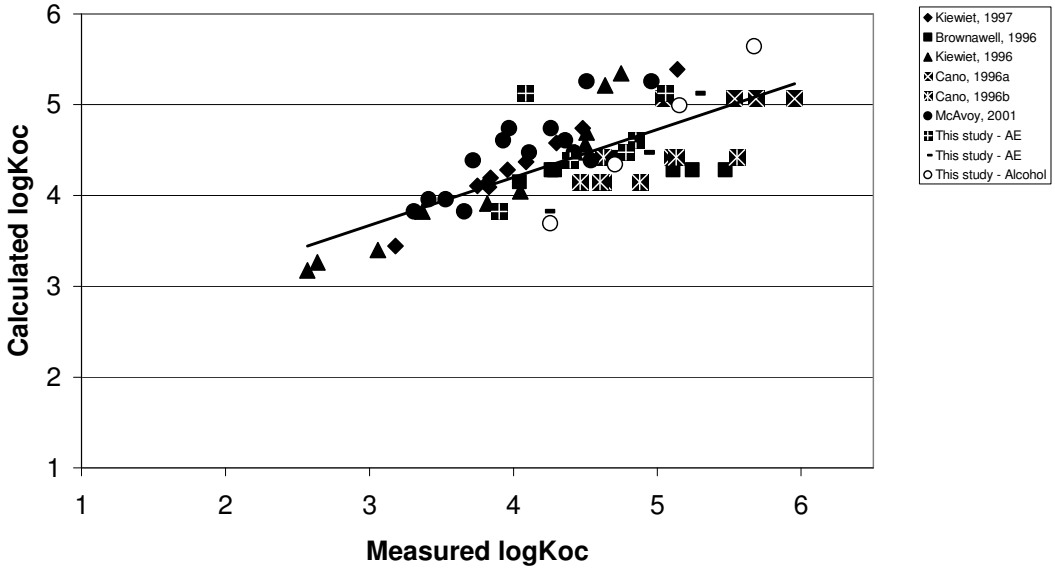
Values in **Bold** are the average of two or more values

For those AE homologues expected to be the most sorptive, except for the C18 chainlength, the range of applicability of the QSARs is excellent. For C18, the homologue expected to have the highest sorptivity has experimental data, and the sorption of other C18 AE homologues is expected to be lower. The errors in using the QSARS developed from this (quite extensive) dataset to predict sorption for the

remaining C18 homologues and for AE homologues with EO>10 are considered to be small with respect to the other errors, such as those involving the inorganic-based sorption capacity of the sorbents involved in QSAR application.

The experimental work carried out by van Compernolle et al (2006) on the alcohols and the AE homologues C12, C14, and C16 EO3s, C14 EO1, and C14 EO6 is of an excellent standard, and should be given a Klimisch rating of 1. The methodology used to evaluate and use the literature data, comprising the linearalisation of that data reported as Freundlich isotherms and the interconversion between data reported as log Kd and as log Koc, is also scientifically very sound, and should be given a Klimisch rating of 2. Thus all of the data used in QSAR development is good data. In addition, the QSAR development approach is very sound, and entirely appropriate. Thus it has been decided to use the QSARs developed by van Compernolle et al (2006) in the HERA assessment, as they are more applicable to AE homologues than the Koc based default QSARs used, in default of further information, by the EU TGD (2003).

Figure 4.1 Measured vs. predicted logK_{oc} based on equation 4.2. From van Compernolle et al (2006).



Van Compernolle et al (2006) used the hydrocarbon chainlength C and ethylene oxide number EO for the AE homologues and the respective log Kd information from their own work and from the literature to generate a QSAR predicting log Kd for specific AE homologues, using the regression feature within the Excel spreadsheet. The resulting equation

$$\log K_d = 0.334C - 0.0114EO - 1.137 \quad (R^2 = 0.64) \qquad \text{(Equation 4.1)}$$

has 95% confidence intervals for the coefficients of (0.272 to 0.395) for the C coefficient, (-0.0445 to 0.0218) for the EO coefficient, and (-2.011 to –0.263) for the

Table 4.2. Koc values calculated according to equation 4.2

C/ EO	8	9	10	11	12	13	14	15	16	18
0	240	504	1057	2218	4656	9772	20512	43053	90365	398107
1	267	561	1178	2472	5188	10889	22856	47973	100693	443609
2	298	625	1312	2754	5781	12134	25468	53456	112202	494311
3	332	697	1462	3069	6442	13521	28379	59566	125026	550808
4	370	776	1629	3420	7178	15066	31623	66374	139316	613762
5	412	865	1816	3811	7998	16788	35237	73961	155239	683912
6	459	964	2023	4246	8913	18707	39264	82414	172982	762079
7	512	1074	2254	4732	9931	20845	43752	91833	192752	849180
8	570	1197	2512	5272	11066	23227	48753	102329	214783	946237
9	635	1334	2799	5875	12331	25882	54325	114025	239332	1054387
10	708	1486	3119	6546	13740	28840	60534	127057	266686	1174898
11	789	1656	3475	7295	15311	32137	67453	141579	297167	1309182
12	879	1845	3873	8128	17061	35810	75162	157761	331131	1458814
13	979	2056	4315	9057	19011	39902	83753	175792	368978	1625549
14	1091	2291	4808	10093	21184	44463	93325	195884	411150	1811340
15	1216	2553	5358	11246	23605	49545	103992	218273	458142	2018366
16	1355	2844	5970	12531	26303	55208	115878	243220	510505	2249055
17	1510	3170	6653	13964	29309	61518	129122	271019	568853	2506109
18	1683	3532	7413	15560	32659	68549	143880	301995	633870	2792544
19	1875	3936	8260	17338	36392	76384	160325	336512	706318	3111716
20	2089	4385	9204	19320	40551	85114	178649	374973	787046	3467369
21	2328	4887	10257	21528	45186	94842	199067	417830	877001	3863670
22	2594	5445	11429	23988	50350	105682	221820	465586	977237	4305266

intercept. It is often useful to have a Koc rather than a Kd based equation for sorption, for example when using sorption predictions in conjunction with standard EU TGD (2003) defaults. For this reason, extensive efforts were made by van Compernolle et al (2006) to find the Koc values of the sediments used in the literature work, and to convert the sorption coefficients reported as Kd values to a Koc basis. This resulted in the following QSAR:

$$\log K_{oc} = 0.322C + 0.047EO - 0.196 \quad (R^2 = 0.53) \qquad \text{(Equation 4.2)}$$

with 95% confidence intervals of (0.249 to 0.401) for the C coefficient, (0.0036 to 0.0854) for the EO coefficient, and (-1.28 to 0.870) for the intercept. The greater uncertainty in the Koc based equation is due in part to the fact that Koc does not reflect all of the sorption mechanisms for AEs, and also due to the necessity to convert more of the original data from a Kd to a Koc basis than to undertake the

reverse Koc to Kd conversion. A graphic idea of the uncertainty can be seen in figure 4.1, taken from van Compernolle et al (2006), in which the original measured (or converted) log Koc values are plotted against the predicted Koc value resulting from equation 4.2.

The Koc based QSAR, equation 4.2, is used to predict AE adsorption in this HERA risk assessment, as the main sorbents, activated sludge, sediment solids, and soil are described only in terms of the (default) organic carbon content. Table 4.2 gives the Koc values, calculated with the CSARA AE Workbook (ERASM 2005b) using equation 4.2, which have been used in this HERA AE environmental assessment. The results of using equation 4.2 are much more applicable to the AE homologues than would be the result of using the TGD (2003) default QSAR methodology, and the use of QSARs determined for the specific substance from measured data is entirely in accord with the EU TGD (2003) methodology. The results of using this QSAR approach will be discussed in the PEC sections for the respective environmental compartments (i.e. surface water, sediment, and soil).

4.1.1.1.2. Volatilisation

As discussed in section 3.2.2.4, the vapour pressures for AE homologues will range from about 10^{-3} hPa to less than 10^{-5} hPa. This range is based upon measured values for the long chain alcohols, and the expectation that the higher molecular weight homologues with EO >0 will have lower vapour pressures than the alcohols of the corresponding hydrocarbon chain length. Calculated values for AE homologues shown in appendix III give vapour pressures lower than 10^{-20} for many AE homologues. Tolls and Sijm (2000) note in their review of non-ionic surfactants that, since surfactants including AEs are rather water soluble and the vapour pressures of AEs are relatively low, the Henry's law constants of AEs can be expected to be very low. As a result, volatilisation of surfactants can be expected to be negligible. Thus volatilisation of AEs will not be considered further in this HERA assessment.

4.1.1.2. Biotic and abiotic degradability

This section discusses the various degradation processes by which AEs may be removed during sewage treatment and while present in surface water, sediment, or soil. As a class, alcohol ethoxylates undergo rapid primary and ultimate biodegradation under both laboratory and field conditions (Talmage, 1992, Danish EPA 2001). The biodegradability of the different AE homologues used in HERA applications is relatively unaffected by the alkyl carbon chain length and the number of EO units. Linear AE are normally easily degraded under aerobic conditions, with only small differences in the time needed for ultimate degradation of linear AE with different alkyl chain lengths. Further information on abiotic degradation, ultimate aerobic biodegradation under stringent screening level ready test conditions, primary aerobic biodegradation in surface water and during sewage treatment, and anaerobic biodegradation for the AEs used in household detergent and cleaning products is given in the sections below.

4.1.1.2.1. Abiotic degradation in water, soil, sediment, and air

Alcohol ethoxylates are not expected to undergo hydrolysis under normal environmental conditions (pH range 4 to 9). Photolysis in the atmosphere, in water, or when adsorbed to solid surfaces such as soil and sediment surfaces is also not expected to occur, due to the chemical structure of the AE homologues. Hydrolysis has also been discounted for the alcohols (EO=0 homologues) in the SIAR for long chain alcohols (SIAR 2006). Thus abiotic degradation processes are not further considered for AEs in this HERA environmental risk assessment.

4.1.1.2.2. Ready tests for ultimate aerobic biodegradability

The OECD ready tests (OECD 1992) have been developed as the first tier of a more complex testing scheme, to provide preliminary screening of organic chemicals, using relatively simple tests of ultimate biodegradability, in order to identify those chemicals for which more detailed, and hence more costly, studies are needed. Ready tests are stringent screening tests, conducted under aerobic conditions, in which a high concentration of the test substance (in the range of 2 to 100 mg/L) is used and biodegradation is measured by non-specific parameters like Dissolved Organic Carbon (DOC), Biochemical Oxygen Demand (BOD) and CO2 production. Domestic sewage, activated sludge or secondary effluent is the typical source of microorganisms (inoculum) in tests for ready biodegradability (OECD 2006).

A "pass" in a ready test can be used, according to the EU TGD (2003), to indicate a conservative level of substance removal, due to biodegradation, from environmental compartments such as soil, sediment, and surface water. Although the HERA risk assessment for the local aqueous compartment is mainly based on measured sewage treatment effluent AE concentrations for homologues with hydrocarbon chainlengths from 12 to 18, establishing the ready biodegradability of the various AE homologues is necessary to support the treatment of biodegradation in surface water, sediment, and soil in the regional risk assessment.

AE with a typical alkyl chain (e.g., C_{12} to C_{15}) will normally reach more than 60% ultimate degradation[5] in standardized tests for ready biodegradability (Danish EPA 2001). For AE containing more than 20 EO units, a reduced rate of biodegradation has been observed (Danish EPA 2001, referencing Scharer *et al.* 1979, and Holt *et al.* 1992). However, natural adaptation of environmental degrading organisms may make some of the older biodegradation test data obsolete. Currently available data (see table 4.3) shows that C16 and C18 homologues with up to 30 EO units should pass the current ready test (OECD 2006), which does not now contain the 10-day window requirement for substances which are technical mixtures.

The 10-day window criterion in a ready test formerly applied to all substances but does not now apply to technical mixtures such as commercial surfactants (OECD

[5] Ultimate degradation involves complete mineralisation, or conversion of all carbon and hydrogen to CO_2 and H_2O

2006). It requires that, after initial evidence of biodegradation has been demonstrated by 10% substance removal, further biodegradation leading to the pass level[6] must be completed within 10 days. This procedure was introduced to increase the stringency of the ready test procedures, and is usually successfully applied to standard testing on individual substances. However, the CSTEE has decided that the 10-day window criterion is not a requirement for surfactants (CSTEE 1999). The CSTEE give several conceptual and technical reasons that the application of the 10-day window does not improve the stringency of ready tests on surfactant materials. The main reason given is that, as surfactant degradation is generally characterised by multiphase kinetics that may be inevitable with a mixed microflora and possibly a multi-component substrate, the 10-day window is not appropriate, as it might interfere with the aim of the ultimate biodegradability test, which is to assess the capability (of a percentage) of a product to be fully degraded in simple compounds during a 28-day period. The OECD has now taken the same position, stating that the 10-day window is not appropriate for technical mixtures containing several components such as surfactants (OECD 2006). Thus, in general, the 10-day window criterion is not considered in establishing the ultimate ready biodegradability of the AEs covered in this HERA risk assessment.

It will be assumed, in this HERA assessment, that ready biodegradability for a commercial mixture results from most of the homologues contained in the mixture being readily biodegradable, especially if the pass level in the ready test is significantly in excess of the required pass level. The available ready test data from company reports and published literature, including information collected by the Danish EPA (Danish EPA 2001) is shown in Table 4.2.

The information in Table 4.3 shows that AE homologues with hydrocarbon chainlengths ranging from 8 to 18 and with from 2 to more than 20 ethylene oxide units are readily biodegradable. In addition, the SIAR for Long Chain alcohols (SIAR 2006) establishes the ready biodegradability of alcohols containing 6-18 ethylene oxide units. Information from several company reports shows that C16 and C18 homologues with up to 30 EO units also pass the ready test.

As well as ready test information for AEs with linear hydrocarbon chains, Table 4.3 also contains information concerning essentially linear AEs. Information showing the ready biodegradability of the AE homologues included in this HERA assessment which are derived from branched butylene oligomers is also included in Table 4.3. The biodegradation of 2-branched AE is similar to the biodegradation of linear AE. Swisher (1987 – referred to in Danish EPA 2001) found that a branch of one single methyl group had no effect on biodegradation compared to entirely linear AE. Marcomini et al (2000a,b) found that, for a series of AE with an average of five EO groups and between 11 and 15 carbon atoms in the hydrocarbon chain, short 2-alkyl substituents (i.e., methyl and ethyl groups) allow the central cleavage mechanism to occur, which leads to the formation of polyethylene glycols, whereas AE with longer alkyl substituents, such as 2Bu-C8AE, biodegrade through hydrolytic oxidation of the alkyl and polyethoxylic chains, leading to formation of AE metabolites with

[6]60% or 70% removal depending on the measurement technique chosen for the test

carboxylic groups on both the hydrophobic and hydrophilic moieties. However, 60% biodegradation was reached under ready test conditions for 2Bu-C8AE. Thus the 2-branched AE with predominantly methyl branching used in HERA applications would be expected to be readily biodegradable, predominantly via the central cleavage mechanism.

Table 4.3 Ready biodegradation test data for alcohol ethoxylates

Hydrocarbon Chainlength description	**EO Chainlength description**	**Test Method**	**Biodegradation Extent**	**Reference (Reliability)**
C8	EO4 (mean)	OECD 301-D (Closed Bottle)	74% ThOD	Cognis Deutchland GmbH, 2003 (2)
C_{9-11}	EO8	Closed bottle test, 28 d	80% ThOD	Danish EPA 2001 (4) citing Madsen *et al.* 1994
C10-C12	6 EO (mean)	OECD301-B	83% $ThCO_2$ evolution (mean of 2 replicates)	Sasol Germany GmbH, 2001a (1)
Oxo-C_{11} 10% branching	EO7-8	Die away screening test, 28 d	100% DOC	Kaluza and Taeger 1996 (4)
C12	4 EO (mean)	OECD301-B	85% $ThCO_2$ evolution	Sasol Germany GmbH, 2001b (1)
C12-C14	6 EO (mean)	OECD301-F (Manometric respirometer)	60% of ThOD obtained	Sasol Germany GmbH, 1995f (1)
C12-C14	EO7-8	Die away screening test, 28 d	100% DOC	Kaluza and Taeger 1996 (2)
C_{12-15}	EO7	BOD, 30 d	92% ThOD	Danish EPA 2001 (4) citing Kravetz *et al*. 1991

Table 4.3 (continued)				
Hydrocarbon Chainlength description	**EO Chainlength description**	**Test Method**	**Biodegradation Extent**	**Reference (Reliability)**
C_{12-15}	EO9	CO_2 evolution test, 28 d	64-79% $ThCO_2$	Danish EPA 2001 (4) citing Kravetz *et al.* 1991
C_{12-18}	EO10-14	Closed bottle test, 28 d	69-86% ThOD	Danish EPA 2001 (4) citing Schöberl *et al.* 1988
C13, mixture of different isomers	3 EO (mean)	OECD 301-B	75% of $ThCO_2$ in 28 days	Sasol Germany GmbH, 1999a (1)
C 13, branched	3 EO (mean)	OECD 301-B	Range 70-80% $ThCO_2$ in 28 days	BASF 2005a (2)
C13, mixture of different isomers	5 EO (mean)	OECD 301-B	74% of $ThCO_2$ in 28 days	Sasol Germany GmbH, 1999b (1)
C 13, branched	5 EO (mean)	OECD 301-B	Range 60-70% of $ThCO_2$ in 28 days	BASF 1995 (2)
C13, mixture of different isomers	12EO Broad range	OECD 301-B	95.4% of $ThCO_2$ in 28 days	Sasol Germany GmbH, 2005c (1)
C13, mixture of different isomers	6EO mean Broad range	OECD 301-B	65% of $ThCO_2$ in 28 days	Sasol Germany GmbH 1999c (1)
C13	EO7-8	Die away screening test, 28 d	100% DOC	Kaluza and Taeger 1996 (2)
C 13, branched	8 EO (mean)	OECD 301-B	Over 90% $ThCO_2$ evolved in 28 days	BASF 1999 (2)
C13, mixture of different isomers	9 EO mean	OECD 301-B	70% of $ThCO_2$ in 28 days	Sasol Germany GmbH 1999d (1)

Table 4.3 (continued)				
Hydrocarbon Chainlength description	**EO Chainlength description**	**Test Method**	**Biodegradation Extent**	**Reference (Reliability)**
C13, mixture of different isomers	9 EO mean	OECD 301-E	80% Primary[1] biodegradation in 18 days	Sasol Germany GmbH 2000 (1)
C 13, branched	12 EO (mean)	OECD 301-B	Range 70-80% $ThCO_2$ in 28 days	BASF 2005b (2)
C_{13-15}	EO7-8	Die away screening test, 28 d	100% DOC	Kaluza and Taeger 1996 (2)
Oxo-C_{13-15} 10% branching	EO7-8	Die away screening test, 28 d	100% DOC	Kaluza and Taeger 1996 (2)
Oxo-C_{13-15}	EO3-12	Modified OECD screening test, 28 d	75% DOC	Danish EPA 2001 (4) citing Schöberl *et al*. 1988
C_{14-15}	EO7	BOD, 30 d	83% ThOD	Danish EPA 2001 (4) citing Kravetz *et al*. 1991
C_{14-15}	10 EO (mean)	Modified Sturm	78% CO_2 formation in 28 days	Talmage 1994 (4) citing Birch 1991a
Oxo-C_{14-15}	EO9-20	Die away screening test, 28 d	65-75% DOC	Danish EPA 2001 (4) citing Schöberl *et al*. 1988
C_{14-15}	20 EO (mean)	Modified Sturm	65% CO_2 formation in 28 days	Talmage 1994 (4) citing Birch 1991a

Table 4.3 (continued)				
Hydrocarbon Chainlength description	**EO Chainlength description**	**Test Method**	**Biodegradation Extent**	**Reference (Reliability)**
C16	EO2	Sapromat	87% ThOD in 28 days	Cognis/Henkel, 1997g (1)
C_{16-18}	EO5	Closed bottle test, 28 d	65-75% ThOD	Danish EPA 2001 (4) quoting Schöberl *et al*. 1988
C16-C18 mixture	11 EO mean (broad range)	OECD301-B	85.3% $ThCO_2$ evolution (mean of 2 replicates)	Sasol Germany GmbH, 2005a (1)
Commercial mixture C16-18	12 EO (mean)	Closed bottle (OECD 301-D)	>92% ThOD in 30 days	Cognis/Henkel 1999b (1)
C16-18 mixture	>20 EO (mean)	OECD301-B	85.3% $ThCO_2$ evolution (mean of 3 replicates)	Sasol Germany GmbH, 2005b (1)
Commercial mixture C16-18	30 EO (mean)	Closed bottle (OECD 301-D)	>86% ThOD in 30 days	Cognis Deutchland GmbH, 2004 (1)

The data in Table 4.3 confirm that AE homologues with linear hydrocarbon chainlengths from C8 to C15 and mean values ranging from 3-20 EO units are readily biodegradable. AE homologues with C16 or C18 hydrocarbon chain lengths and mean values between 2 tand more than 20 ethylene oxide units are also readily biodegradable. Good quality data (Klimisch score 1) is also available for a 2-butyl substituted oxo-C12EO5 AE homologue, showing more than 60% $ThCO_2$ removal in a 28-day CO_2 evolution test (Marcomini et al, 2000b), which indicates that the presence of one short alkyl chain in the 2 position, especially one of 4 carbons or less, will not reduce the ability of the homologue to pass a ready biodegradability test.
In conclusion, the available data confirm that the AE homologues used in detergent and household cleaning applications and included in this HERA assessment are readily biodegradable.

4.1.1.2.3. Primary biodegradability in river water

The ready biodegradability data discussed in section 4.1.1.2.2 shows that the AEs used in household detergent products will be ultimately degraded (to the inorganic components carbon dioxide and water) in the environment. A conservative estimation of the rate at which this degradation occurs in river water can be estimated, according to the EU TGD (2003), for compounds which pass the ready test. However, for risk assessment it is important to consider the rate of primary biodegradation of the AE homologues, as much of the eco-toxicity is lost in the first step in the biodegradation process.

Table 4.4. AE river water die-away study results, 10 °C (JSDA 2006)

AE homologue*	Time after start					blank	detection limit	*unit*
	0 hour	3 hours	6 hours	9 hours	24 hours			
AE, C12 ,n=2	0.05	N.D.**	N.D.**	N.D.**	N.D.**	N.D.**	0.02	*µg/L*
AE, C12 ,n=3	0.09	0.04	N.D.**	N.D.**	N.D.**	N.D.**	0.02	*µg/L*
AE, C12 ,n=4	0.12	0.07	0.03	N.D.**	N.D.**	N.D.**	0.02	*µg/L*
AE, C12 ,n=5	0.16	0.08	0.05	0.03	N.D.**	N.D.**	0.02	*µg/L*
AE, C12 ,n=6	0.19	0.13	0.09	0.06	N.D.**	N.D.**	0.02	*µg/L*
AE, C12 ,n=7	0.23	0.15	0.12	0.08	N.D.**	N.D.**	0.02	*µg/L*
AE, C12 ,n=8	0.26	0.19	0.15	0.11	0.04	N.D.**	0.02	*µg/L*
AE, C12 ,n=9	0.27	0.21	0.17	0.12	0.05	N.D.**	0.02	*µg/L*
AE, C12 ,n=10	0.28	0.22	0.17	0.13	0.06	N.D.**	0.02	*µg/L*
AE, C12 ,n=11	0.30	0.23	0.19	0.14	0.07	N.D.**	0.02	*µg/L*
AE, C12 ,n=12	0.28	0.22	0.18	0.14	0.07	N.D.**	0.02	*µg/L*
AE, C12 ,n=13	0.26	0.21	0.18	0.14	0.07	N.D.**	0.02	*µg/L*
AE, C12 ,n=14	0.23	0.18	0.16	0.13	0.07	N.D.**	0.02	*µg/L*
AE, C12 ,n=15	0.18	0.16	0.14	0.11	0.06	N.D.**	0.02	*µg/L*
AE, C12 ,n=16	0.15	0.14	0.12	0.10	0.05	N.D.**	0.02	*µg/L*
AE, C12 ,n=17	0.12	0.11	0.10	0.08	0.05	N.D.**	0.02	*µg/L*
AE, C12 ,n=18	0.10	0.08	0.08	0.07	0.04	N.D.**	0.02	*µg/L*
AE, C12 ,n=19	0.08	0.07	0.06	0.05	0.03	N.D.**	0.02	*µg/L*
AE, C12 ,n=20	0.06	0.05	0.05	0.04	0.03	N.D.**	0.02	*µg/L*
AE, C13 ,n=2	0.04	N.D.**	N.D.**	N.D.**	N.D.**	N.D.**	0.02	*µg/L*
AE, C13 ,n=3	0.07	0.02	N.D.**	N.D.**	N.D.**	N.D.**	0.02	*µg/L*
AE, C13 ,n=4	0.11	0.05	N.D.**	N.D.**	N.D.**	N.D.**	0.02	*µg/L*
AE, C13 ,n=5	0.15	0.07	0.03	N.D.**	N.D.**	N.D.**	0.02	*µg/L*
AE, C13 ,n=6	0.19	0.11	0.07	0.04	N.D.**	N.D.**	0.02	*µg/L*
AE, C13 ,n=7	0.22	0.13	0.10	0.06	N.D.**	N.D.**	0.02	*µg/L*
AE, C13 ,n=8	0.26	0.18	0.13	0.07	N.D.**	N.D.**	0.02	*µg/L*

Table 4.4 (continued)

AE homologue*	Time after start					blank	detection limit	*unit*
	0 hour	3 hours	6 hours	9 hours	24 hours			
AE, C13 ,n=9	0.28	0.20	0.15	0.10	0.03	N.D.**	0.02	*µg/L*
AE, C13 ,n=10	0.28	0.22	0.16	0.11	0.03	N.D.**	0.02	*µg/L*
AE, C13 ,n=11	0.29	0.23	0.18	0.12	0.04	N.D.**	0.02	*µg/L*
AE, C13 ,n=12	0.28	0.22	0.18	0.12	0.04	N.D.**	0.02	*µg/L*
AE, C13 ,n=13	0.25	0.20	0.17	0.11	0.05	N.D.**	0.02	*µg/L*
AE, C13 ,n=14	0.21	0.17	0.14	0.10	0.05	N.D.**	0.02	*µg/L*
AE, C13 ,n=15	0.18	0.16	0.13	0.09	0.04	N.D.**	0.02	*µg/L*
AE, C13 ,n=16	0.15	0.13	0.11	0.08	0.04	N.D.**	0.02	*µg/L*
AE, C13 ,n=17	0.12	0.10	0.09	0.07	0.04	N.D.**	0.02	*µg/L*
AE, C13 ,n=18	0.09	0.08	0.07	0.05	0.03	N.D.**	0.02	*µg/L*
AE, C13 ,n=19	0.07	0.06	0.05	0.04	0.02	N.D.**	0.02	*µg/L*
AE, C13 ,n=20	0.05	0.05	0.04	0.04	N.D.**	N.D.**	0.02	*µg/L*
AE, C14 ,n=2	0.02	N.D.**	N.D.**	N.D.**	N.D.**	N.D.**	0.02	*µg/L*
AE, C14 ,n=3	0.04	N.D.**	N.D.**	N.D.**	N.D.**	N.D.**	0.02	*µg/L*
AE, C14 ,n=4	0.07	0.02	N.D.**	N.D.**	N.D.**	N.D.**	0.02	*µg/L*
AE, C14 ,n=5	0.10	0.04	N.D.**	N.D.**	N.D.**	N.D.**	0.02	*µg/L*
AE, C14 ,n=6	0.13	0.08	0.04	N.D.**	N.D.**	N.D.**	0.02	*µg/L*
AE, C14 ,n=7	0.16	0.11	0.06	0.03	N.D.**	N.D.**	0.02	*µg/L*
AE, C14 ,n=8	0.18	0.13	0.08	0.04	N.D.**	N.D.**	0.02	*µg/L*
AE, C14 ,n=9	0.20	0.15	0.10	0.06	N.D.**	N.D.**	0.02	*µg/L*
AE, C14 ,n=10	0.21	0.16	0.11	0.06	0.02	N.D.**	0.02	*µg/L*
AE, C14 ,n=11	0.22	0.17	0.12	0.07	0.03	N.D.**	0.02	*µg/L*
AE, C14 ,n=12	0.20	0.15	0.12	0.07	0.03	N.D.**	0.02	*µg/L*
AE, C14 ,n=13	0.18	0.15	0.11	0.07	0.03	N.D.**	0.02	*µg/L*
AE, C14 ,n=14	0.16	0.12	0.10	0.07	0.03	N.D.**	0.02	*µg/L*
AE, C14 ,n=15	0.14	0.11	0.09	0.06	0.03	N.D.**	0.02	*µg/L*
AE, C14 ,n=16	0.12	0.10	0.08	0.05	0.02	N.D.**	0.02	*µg/L*
AE, C14 ,n=17	0.09	0.07	0.06	0.05	N.D.**	N.D.**	0.02	*µg/L*
AE, C14 ,n=18	0.07	0.05	0.05	0.03	N.D.**	N.D.**	0.02	*µg/L*
AE, C14 ,n=19	0.05	0.04	0.04	0.03	N.D.**	N.D.**	0.02	*µg/L*
AE, C14 ,n=20	0.04	0.03	0.03	0.02	N.D.**	N.D.**	0.02	*µg/L*
AE, C12 total	3.4	2.5	2.0	1.5	0.69	N.D.**		*µg/L*
AE, C13 total	3.3	2.4	1.8	1.2	0.41	N.D.**		*µg/L*

Table 4.4 (continued)

AE homologue*	Time after start					blank	detection limit	*unit*
	0 hour	3 hours	6 hours	9 hours	24 hours			
AE, C14 total	2.4	1.7	1.2	0.71	0.19	N.D.**		*µg/L*
AE, C12-14 total	9.1	6.6	5.0	3.4	1.3	N.D.**		*µg/L*

**AE is alkyl ethoxylate.C is the number of carbon atoms in the alkyl chain, and n is the number of ethylene oxide units.*

***N.D. is not detected.*

Primary biodegradation of AE homologues in river water has been addressed in an experiment sponsored by the Japanese SDA (JSDA 2006), in which river water collected immediately downstream of the effluent outlet of a well-operated activated sludge treatment facility has been collected, incubated at the river temperature of 10C, and used in laboratory experiments to determine the rate of disappearance of C12, C13, and C14 AE homologues with EO chainlengths ranging from 2 to 20. The AE analytical method used (Electrospray LC/MS- see Evans, KA et al, 1997) was not capable of resolving the E0=0 and EO=1 homologues. After an environmentally realistic initial total AE concentration of 10µg/l had been added, samples were taken for analysis at 0, 4, 8, 12, and 24 hours. The results are shown in Table 4.4.

Table 4.5 Proposed half-lives, in hours, for AE homologues in river water at approximately 12degrees C

C / EO	8	9	10	11	12	13	14	15	16	18
0	***4***	4	4	4	4	4	4	4	4	4
1	4	4	4	4	4	4	4	4	4	4
2	4	4	4	4	**4**	**4**	**4**	4	4	4
3	4	4	4	4	**4**	**4**	**4**	4	4	4
4	4	4	4	4	**4**	**4**	**4**	4	4	4
5	4	4	4	4	**4**	**4**	**4**	4	4	4
6	8	8	8	8	**8**	**8**	**8**	8	8	8
7	12	12	12	12	**12**	**8**	**8**	8	8	8
8	12	12	12	12	**12**	**8**	**8**	8	8	8
9	12	12	12	12	**12**	**8**	**8**	8	8	8
10	12	12	12	12	**12**	**8**	**8**	8	8	8
11	12	12	12	12	**12**	**12**	**8**	12	12	12
12	12	12	12	12	**12**	**12**	**8**	12	12	12
13	12	12	12	12	**12**	**12**	**8**	12	12	12
14	12	12	12	12	**12**	**12**	**8**	12	12	12
15	12	12	12	12	**12**	**12**	**8**	12	12	12
16	12	12	12	12	**12**	**12**	**8**	12	12	12
17	24	24	24	24	**24**	**24**	**12**	24	24	24
18	24	24	24	24	**24**	**24**	**12**	24	24	24
19	24	24	24	24	**24**	**24**	**12**	24	24	24
20	24	24	24	24	**24**	**24**	**12**	24	24	24

The initial homologue concentrations ranged from twice to about 15 times the limit of detection (0.02µg/l). The subsequent concentrations for each AE homologue were scrutinised to see if at least half of the AE present at the start of any 4 hour period had been removed by the end of that period, and if so that AE homologue was assigned a maximum half-life of 4 hours. For those AE homologues with less removal, a similar process was followed to assign maximum half-lives of 8, 12, and 24 hours, as appropriate. These maximum half-lives have been entered, in bold text, in Table 4.5. It can be seen that the half-lives increase with increasing EO number, and may decrease somewhat with increasing C number, in the C12 to C14 region. The data, obtained at 10 degrees C, are considered conservative for the standard European conditions of 12 degrees C (TGD 2003).

Supporting information giving rate constants for primary biodegradation under river water conditions is given in Tolls and Sijm (2000). Although test details including the concentration of test substances and the temperatures of the tests are not given, rate constants giving half–lives between 3.8 and 5 hours are given for C12EO8, C12EO9, C14EO8, and C14EO3. These rates are similar to the more extensively documented data available in the JSDA (2006) study. Further supporting evidence is available from an ultimate biodegradation study, in which Larson and Games (1981), studied the ultimate biodegradation of two AE homologues, C12EO9 and C16EO3, in Ohio River water collected below the discharge from a municipal wastewater treatment facility treating mainly domestic waste. Laboratory studies at 3, 14, 25, and 34°C established that degradation was first order for concentrations between 1µg/l and 100µg/l. At 25 °C, ultimate biodegradation half-lives of 1 to 2.5 days were observed, which is broadly consistent with the primary biodegradation half-lives observed in the JSDA (2006) study.

Supporting information is also available for some of the long chain alcohols. Primary biodegradation half-lives for C6, C7, and C8 primary alcohols of 8.7, 5.6, and 1.9 hours respectively have been observed at 25 °C (Yoshitaka Yonezawa and Yoshikuni Urushigawa, 1979). Although the several experimental details, including the activated sludge concentrations used, are not reported in the paper, the information may still provide useful supporting information. By assuming that the degradation rates will continue to increase for the C9 and longer alcohols, and that reaction rates approximately double for each 10C° rise in temperature, estimated half-lives of about 4 hours for the C8 alcohol and less than 4 hours for the higher alcohols at approximately 12°C can be predicted. These predictions, consistent with both the lower alcohol data and the measured AE data for the higher AE homologues, have been entered in ordinary text in Table 4.5. The C8 alcohol data has been entered in bold italic text.

The measured AE homologue data in Table 4.5 has been extrapolated from the C12, C13, and C14 data to cover the C8-11 and C15-C18 regions. These extrapolations are shown in normal text in the table. In order to be conservative, the C12 half-lives have been taken to be appropriate for the lower hydrocarbon chainlengths, while the C13 half-lives rather than the shorter C14 half-lives have been used for C15 to C18 chainlengths. Considering the conservative nature of the JSDA (2006) data (10^{o}C data, and maximum half-lives used), the extrapolations are considered to be reasonable or conservative estimates of primary biodegradation half lives for the AE homologues of interest. These half-lives in river water are used in the HERA AE

environmental assessment as part of the determination of the AE Regional background concentration, in **section 4.1.2.1.7**

4.1.1.2.4. Primary biodegradation in sewage treatment

Primary AE degradation in sewage treatment involves higher concentrations, by several orders of magnitude, of both the AE to be degraded and the degrading organisms, compared with river water. Thus the kinetics of degradation are quite different from those found in river water, with Monod kinetics rather than first order kinetics being often employed (HERA 2005, section 2.2.2.3). For this reason experiments carried out under sewage treatment plant conditions are needed to establish the kinetics and rate of removal of AE during sewage treatment.

Federle and Itrich (2006) studied the degradation of C12, C14, and C16 alcohols radio-labelled in the ^{1}C position, in an activated sludge die away test with activated sludge at typical sewage treatment concentrations. C13EO8 and C16EO8, radio-labelled in the ^{1}C alkyl chain position, were also studied. The experiments, which took place at 20 $^{\circ}C$, were evaluated assuming that primary biodegradation proceeded by a double first order mechanism, rather than by Monod kinetics. The faster degradation time was attributed to dissolved parent AE, while the slower degradation was attributed to adsorbed AE. However, the half-life for the faster decay process, which, except for hexadecanol, accounted for most of the overall decay in the model, was 1 minute or less for all the compounds studied.

In a previous paper, Itrich and Federle (2004) carried out similar experiments for other C12, C14, and C16 AE homologues. In these experiments all homologues except C14EO3 had most of the decay attributed to the faster decay process. The available data, which are shown in Table 4.6, establish that, at 20 $^{\circ}C$, all the AE homologues except EO-labelled C16EO6 decayed with half-lives of less than a minute. Increasing the half-lives by 50% to account for the 5C° drop in temperature to the 15 $^{\circ}C$ standard European conditions still produces half-lives of less than one minute for all the AE homologues studied, except for C16EO6 labelled on the second carbon of the EO group closest to the alkyl chain. Note that the small differences seen in decay rate may be due to several factors, such as different positions of the ^{14}C radiolabel in the AE homologue, or uncontrolled differences between experimental runs. One would expect similar half-lives, of less than a minute under standard European sewage treatment plant conditions, to be applicable to the C9 to C11 AE homologues, although somewhat longer half-lives might be required for C18AE homologues. In addition, Federle and Itrich (2006) show that the percentages of removal calculated using the rate constants given in their paper and standard sewage treatment residence times (HRT=6 hours, SRT = 10 days) were greater than 99.7%, consistent with monitoring data. Thus half-lives of 1 minute or less for removal of AE homologues under sewage treatment conditions is reasonable, if first order kinetics is assumed for the removal process.

Table 4.6. Half-lives of AE homologues under sewage treatment conditions, at 20C

AE Homologue	Position of ^{14}C radiolabel	k_1, hr^{-1}	*Half-life, minutes*
*C12OH	$CH3(CH2)_{10}{}^{14}COH$	113	*0.37*
C12E6	$CH3(CH2)_{11}OCH_2{}^{14}CH_2(OC_2CH_2)_5OH$	69	*0.6*
*C13E8	$CH3(CH2)_{11}{}^{14}CH_2O(OC_2CH_2)_8OH$	146	*0.28*
*C14OH	$CH3(CH2)_{12}{}^{14}COH$	87	*0.48*
C14E1	$CH3(CH2)_{13}OCH_2{}^{14}CH_2OH$	62	*0.68*
C14E3	$CH3(CH2)_{13}O(C_2CH_2O)_2CH_2{}^{14}CH_2OH$	71	*0.59*
C14E6	$CH3(CH2)_{13}OCH_2{}^{14}CH_2(OC_2CH_2)_5OH$	70	*0.59*
C14E6	$CH3(CH2)_{13}OCH_2{}^{14}CH_2(OC_2CH_2)_5OH$	61	*0.68*
C14E9	$CH3(CH2)_{13}OCH_2{}^{14}CH_2(OC_2CH_2)_8OH$	78	*0.53*
*C16OH	$CH3(CH2)_{14}{}^{14}COH$	103	*0.40*
C16E6	$CH3(CH2)_{15}OCH_2{}^{14}CH_2(OC_2CH_2)_5OH$	18	*2.32*
**C16E8*	*$CH3(CH2)_{14}{}^{14}CH_2O(OC_2CH_2)_8OH$*	*106*	*0.39*

*Data from Federle and Itrich (2006), evaluated as Klimisch score 2. All other data is from Itrich and Federle (2004), again evaluated as Klimisch score 2.

The information in this section, leading to a primary biodegradation half-life in sewage treatment of 1 minute or less for AE homologues has been used in the EUSES calculations described in **section 4.1.2.1.7,** as part of the determination of an appropriate Regional background concentration for AE homologues in surface waters.

4.1.1.2.5. Anaerobic biodegradability

Alcohol ethoxylates are anaerobically biodegradable. The Danish EPA (2001) note that most studies of the anaerobic biodegradability of AE have been performed with linear AE. Anaerobic biodegradation tests have been performed using both anaerobically digested sludge and anaerobic sediment as inocula. The initial laboratory work (Steber and Wierich 1987), carried out with radio-labelled C18 EO7 in a laboratory-scale anaerobic digester running at 35°C for up to 4 weeks, showed that more than 80% of radioactivity from both the ethoxylate and the 1 position of the hydrocarbon chain (i. e, the last chain carbon to be digested by ω-oxidation followed by β-elimination) was removed as $^{14}CO_2$ and $^{14}CH_4$. The distribution of the remaining radioactivity is shown in Table 4.7.

Table 4.7 Fate of ^{14}C-labelled stearyl alcohol ethoxylate after biodegradation in a model sludge digester. From Steber and Wierich (1987).

	^{14} C Distribution (% of initial radioactivity				
Test compound **Radio labelled C18EO7**	Residual surfactants	Metabolites	14C-gas	L4C-biomass	Total degraded
Uniformly labeled EO carbons	6.9	1.2	83.6	8.3	[1]91.9
Labeled on the 1 carbon of the hydrocarbon chain	3.8	0.5	87.3	7.0	[1]94.8

[1]Klimisch reliability score 2

In these experiments the AE concentrations were 300-500 µg of surfactant per g of dry sludge, which Steber and Wierich (1987) say compares well with nonionic surfactant concentrations (50-500 ppm) determined in raw and digested sludges of municipal sewage treatment plants (Hellmann, 1981, quoted in Steber and Wierich 1987).

The Danish EPA (2001) have tabulated results of anaerobic biodegration of AEs in digested sludge and in anoxic sediment systems. They find that the mineralization observed in experiments with ^{14}C-labelled surfactants suggests that almost complete degradation of linear AE may be expected in anaerobic digesters and that the lower mineralization observed in some screening tests, characterized by measurement of gas production, (e.g. Madsen et al. 1996b) was caused by inhibition. Valid results obtained for AEs used in household detergent and cleaning products are shown in

Table 4.8
Ultimate anaerobic biodegradability of AE in digested sludge. (from Danish EPA (2001)

Compound	**Type of test and duration**	**Result**	**Reference***	**Klimisch[7] Score**
$C_{9\text{-}11}$ EO8	Measurement of gas production, 35° C, 40-50 d	60-83% $ThCH_4$	Salanitro and Diaz 1995	4
$C_{9\text{-}11}$ EO8	Measurement of gas production, 35° C, 56 d	79% ThGP	Madsen *et al.* 1996a	4
C_{18} EO7	Measurement of $^{14}CH_4$ and $^{14}CO_2$ evolution, 35° C, 28 d	84% $ThCH_4$ + $ThCO_2$	Steber and Wierich 1987	2

*References may be found in Danish EPA (2001)

[7] Klimisch Scores are 1 = valid without restriction; 2 = valid with restriction; 3 = not reliable; 4 = not assignable. Further information and guidance on the selection of data for HERA reports is given in Appendix C of the HERA Methodology Document (HERA 2005).

Table 4.9
Ultimate anaerobic biodegradability of AE in sediments. (from Danish EPA (2001)

Compound	Type of test and duration	Result	Reference[1]
C_{9-11} EO8	Measurement of gas production in freshwater swamp material, 35° C, 56 d	77% ThGP	Madsen *et al.* 1996a
C_{9-11} EO8	Measurement of gas production in marine sediment, 35° C, 56 d	66% ThGP	Madsen *et al.* 1996a
C_{10-12} EO7.5	Measurement of CH_4-production in polluted creek mud, 28° C, 37 d	70% $ThCH_4$	Wagener and Schink 1987
C_{12} EO8-9	Measurement of $^{14}CH_4$ and $^{14}CO_2$ evolution in wastewater pond sediment, 22° C, 87 d	24-40% $ThCH_4$ + $ThCO_2$	Federle and Schwab 1992
C_{12} EO8-9	Measurement of $^{14}CH_4$ and $^{14}CO_2$ evolution in pond sediment, 22° C, 87 d	13% $ThCH_4$ + $ThCO_2$	Federle and Schwab 1992
C_{12} EO23	Measurements of CH_4-production in polluted creek mud, 28° C, 37 d	80% $ThCH_4$	Wagener and Schink 1987

[1]All references have Klimisch score of 4, as are from a secondary reference. References may be found in Danish EPA, 2001

Table 4.8. Linear AE were also degraded in anoxic sediments, where a lower mineralization was observed at 22° C compared to the mineralization at higher temperatures (Table 4.9**).** The data in both tables supports the conclusion that alcohol ethoxylates are anaerobically biodegradable.

In summary, alcohol ethoxylates have the potential to biodegrade anaerobically in sediments and during sewage treatment. At least 80% removal of AE should be expected during anaerobic digestion used as part of the sewage treatment process.

4.1.1.3. Removal in sewage treatment

Removal of surfactants during sewage treatment generally refers to the removal of the parent compound, with primary biodegradation and adsorption to sludge being the main removal mechanisms. A considerable amount of information is available which establishes that overall AE removals exceeding 99% are generally observed in sewage treatment. For example, in a laboratory study using continuous activated sludge plants, Wind et al (2006) found AE removals ranging from 99.70% for C18 homologues to more than 99.98% for C12 to C16 homologues. In a recent study of AE removal in the US and Canada, Morrall et al (2006) found total AE removal percentages ranging from 99.4 to 99.9% for two trickling filter plants, two activated sludge plants, two plants utilising oxidation ditches, and one rotating biological contactor. The two lagoons studied by Morrall et al (2006) had removal percentages

of 99.4 and 97.2%, with the lower percentage removal of 97.2% which was calculated for one lagoon being due to a low influent concentration, rather than a higher effluent concentration, thus giving a smaller amount of AE removed. The lagoon effluent concentrations in Morrall et al (2006) were similar to those obtained from the other types of treatment facility studied.

Although the concept of percentage removal is easy to understand and apply in simple calculations, it has been shown that the percentage removal concept is not appropriate for substances, such as AE and indeed many of the higher volume HPV substances, which are present in sewage treatment plant influents at concentrations high enough to support growth of the biological treatment organisms. Berg and Nyholm (1996) specifically state that biodegradation of high volume household chemicals during sewage treatment will not proceed via first order kinetics, for which the percentage removal concept is appropriate. Primary biodegradation of these high volume household chemicals follows Monod kinetics, which Birch (1991b) has shown leads to a constant effluent concentration, whose level is generally dependent upon specific sewage treatment plant characteristics, mainly the sludge retention time. The use of Monod kinetics is described in the TGD (2003) as appropriate for higher tier risk assessment, and the HERA Methodology Guidance Document (2005, section 2.2.2.3) specifies its suitability for substances present at influent concentrations of 100 ppb or higher (Berg and Nyholm 1996, Nyholm et al 1996). This applies to AEs, with average influent concentration levels of a few mg/L (Wind, et al, 2006).

In this HERA assessment, rather than using percentage removals, the measured concentration of AE homologues in sewage treatment plant effluent, which results mainly from biodegradation occurring according to Monod kinetics, will be used to determine the effluent concentrations of AE homologues from which $PEC_{aquatic}$ will be derived. This data has been obtained from the recent effluent monitoring data obtained by Eadsforth et al (2006), which has been obtained from 12 representative activated sludge sewage treatment plants from five European countries. The description of this data and its use in the development of $PEC_{aquatic}$ is given in section 4.1.2.1.

4.1.1.4. Bioconcentration

The AE homologues used for domestic cleaning applications are very likely to have a

Table 4.10 Whole body BCF values of AE in fish, based on AE concentrations determined by chemical analysis (Danish EPA, 2001)

Compound/species	Uptake/ depuration period	BCF	Reference[1]
C_{12} EO8 Fathead minnow *(Pimephales promelas)*	54-72 h/-	12.7	Tolls 1998
C_{13} EO4 Fathead minnow	54-72 h/-	232.5	Tolls 1998

C_{13} EO8 Fathead minnow	54-72 h/-	29.5-55.0	Tolls 1998
C_{14} EO4 Fathead minnow	54-72 h/24 h	237.0	Tolls 1998
C_{14} EO8 Fathead minnow	54-72 h/24 h	56.7-135.2	Tolls 1998
C_{14} EO11 Fathead minnow	54-72 h/24 h	15.8	Tolls 1998
C_{14} EO14 Fathead minnow	54-72 h/24 h	< 5	Tolls 1998
C_{16} EO8 Fathead minnow	54-72 h/24 h	387.5	Tolls 1998

[1]Klimisch score 2 . Same data in Tolls et al 2000.

$\log K_{ow}$ value greater than 3, as can be seen from the measured $\log K_{ow}$ values for the EO=0 homologues in Table 3.5 and calculated $\log K_{ow}$ values for other the AE homologues in Table 3.6 (section 3.2.2.5). Thus bioconcentration and bioaccumulation must be considered for alcohol ethoxylates.

The Danish EPA report on surfactants (Danish EPA, 2001) found that bioaccumulation of alkyl ethoxylates in aquatic organisms had been determined only for fish. The majority of the limited data were based on studies with ^{14}C-labelled compounds that do not allow the distinction between the parent compound and metabolites, or material incorporated into the cells during growth. Because alcohol ethoxylates are metabolized in aquatic organisms (Danish EPA, 2001), the bioconcentration factor for the parent compound may well be overestimated in experiments in which ^{14}C-labelled model surfactants are used. Tolls (1998, and Tolls et al 2000) combined ^{14}C-techniques and chemical analysis to determine the amount of AE actually present as the parent molecule in the fish. This showed that the parent AE (e.g. C_{13} EO8) was rapidly eliminated by transformation into metabolites, which were eliminated at a slower rate. The BCF factors which Tolls obtained using this combined technique for several AE homologues in fathead minnow are shown in Table (4.10).

The Danish EPA concluded that the data in Table 4.10 indicate that the more hydrophobic AE (e.g. C_{13} EO4, C_{14} EO4, and C_{16} EO8) have a moderate bioaccumulation potential (Danish EPA 2001). They also note that in the study by Tolls (1998) the BCF values ranged from < 5 to 387.5, whereas the uptake rates ($k1$) varied from 330 to 1660 (l x kg x d^{-1}) and the elimination rates ($k2$) varied from 3.3 to 59 (d^{-1}). The time to steady state and the BCF for AE increase with decreasing length

of the ethoxylate chain (e.g., t_{95} for C_{13} EO8 = 2.4 h and BCF = 30-55, and t_{95} for C_{13} EO4 = 17.1 h and BCF = 233) (Danish EPA 2001).

Tolls et al (2000) conclude that the high values of the elimination rate constants suggest that fathead minnows efficiently biotransform AE, thereby preventing AE from attaining high concentrations in fish. They note that estimates of the bioconcentration potential of AE mixtures in influents and effluents of several wastewater treatment plants were similar to each other, implying that the bioconcentration potential was not significantly altered by the processes involved in wastewater treatment. These estimates of the bioconcentration potential were conservative and ranged around 140 L/kg.

Further work reported by Environment Canada and Health Canada (2006) has established that the degree of bioaccumulation expected from AE is well below the Canadian bioconcentration criterion of 5000. The sixteen measured BCF values for 15 AE homologues showed the lack of a linear relationship between alkyl or ethoxylate chain length and BCF, with the highest measured BCF value being under 800. Environment Canada (2006) concluded that it is evident that the AE metabolism rates prevent any significant accumulation. The data indicated that there may be an optimal structural combination of ethoxylate and alkyl chain lengths, at or around C14EO7, where BCF is maximized, but even the measured BCF for this chemical is well below the criterion of 5000. Thus Environment Canada (2006) concluded that ethoxylated aliphatic alcohols are not bioaccumulative.

4.1.2. PEC Calculations

The TGD (2003) defines Predicted Environmental Concentrations (PEC) for the local (PEClocal), the regional (PECregional) and the continental (PECcontinental) scale, as well as for the different environmental compartments, e.g. surface waters ($PEC_{aquatic}$) and the terrestrial compartment (PEC_{soil}). The PECs can either be derived from an environmental transport and transformation ("fate") model such as EUSES[8] (ECB 2005), or directly, based on environmental monitoring data. In the later case the 90th percentile of a representative range of analytical data should be used (EU 2003).

The aquatic exposure assessment of the alcohol ethoxylates is based on environmental monitoring data. Recent progress in the analytics of trace amounts of alcohol ethoxylates in environmental matrices (Dunphy et al 2001) has allowed the determination of a set of 114 AE homologues (chain lengths from 12-18 and EO from 0-18) in waste water treatment plant effluents from 12 different European STPs. PEClocal is derived from the monitoring data by taking into account the EU default 1:10 dilution of the STP effluent by the receiving water body (EU 2003), and the addition of the appropriate regional (PECregional) and continental (PECcontinental)

[8]The PC program EUSES is designed to be a decision-support system for the evaluation of the risks of substances to man and the environment. The system is fully described in the EUSES documentation and is based on the EU Technical Guidance Documents for risk assessment of new and existing substances. The documentation and program can be obtained from the European Chemicals Bureau, Ispra, Italy.

background concentrations. Due to the lack of background concentration monitoring data for PECregional and PECcontinental, EUSES (ECB 2005), calibrated using the available monitoring results, is used to determine the background concentration to $PEClocal_{aquatic}$. PECsediment is derived from PEC aquatic, using the equilibrium partitioning method (EU 2003), while a combined method using both EUSES and measured AE concentrations in sewage sludge is used to determine PECsoil.

The scope of the HERA risk assessment includes all AE homologues used in household detergents, i.e. AE C8-18 with EO 0-22. Thus, it is necessary to derive appropriate PEC data for the homologues not covered by the original monitoring data. This is done here by applying conservative upper limit values based on the nearest hydrocarbon chain and EO chain lengths with measured data, to the AE homologues for which monitoring data is not available.

As the overall procedure for PEC calculations is complex, an overview of the different steps to be carried out and a guide to the corresponding sections in this HERA report, where more detailed information will be found, are given in the overview box below.

Overview of PEC derivations from AE monitoring data

1. Aquatic compartment – determination of $PEC_{local_{dissolved}}$ from monitoring data

1.1. Obtain high quality effluent monitoring data from representative European sewage treatment plants. This has been possible for AE homologues in the C_{12}-C_{18}, EO_0 to EO_{18} range. **See section 4.1.2.1.1**

1.2. Remove any alcohol which does not originate from AE from these local effluents by using the Alcohol Cap. **See section 4.1.2.1.2.**

1.3. Determine an overall representative local effluent concentration from 90th percentile of monitoring data, for each AE homologue measured (C_{12}-C_{18}, EO_0 to EO_{18}). **See section 4.1.2.1.3.**

1.4. Dilute this 90th percentile effluent concentration matrix for the AE homologues by 10, to give the total (adsorbed + dissolved) concentration for each AE homologue in river water. **See section 4.1.2.1.4**

1.5. Apply partitioning according to the TGD methods using the Koc QSAR described in section 4.1.1.1.1. to determine the local concentration which is dissolved in the river water (called $Clocal_{dissolved}$) for each AE homologue. **See section 4.1.2.1.5**

1.6. Expand the AE homologue matrix in a conservative manner to include AE homologues with hydrocarbon chainlengths from 8 to 11 and EO chainlengths from 19 to 22. **See section 4.1.2.1.6**

1.7. Incorporate an appropriate background concentration for each dissolved AE homologue present in surface water due to AE use in the continent and region. **See section 4.1.2.1.7**

1.7.1. Carry out EUSES calculations for representative AE homologues using the best available input parameters, to calculate both local and background concentrations for the AE homologues dissolved in surface water, and the resulting $PEClocal_{Dissolved}$ created by adding these quantities.

1.7.2. Calibrate these EUSES-calculated results using the dissolved surface water concentrations $Clocal_{dissolved}$ obtained from the 90 percentile from the effluent monitoring data (Result of step 1.4)

1.7.3. Fill in the representative calibrated conversion matrix by interpolation, generating a conversion matrix based on EUSES calculations which, when multiplied by the $Clocal_{dissolved}$ matrix, will generate $PEClocal_{Dissolved}$.

1.8. Calculate $PEClocal_{Dissolved}$ **See section 4.1.2.1.8**

2. **Sediment compartment:** generate PEC sediment from $PEClocal_{Dissolved}$, following TGD principles (equilibrium partitioning). **See section 4.1.2.2**

3. **Sewage treatment plant:** use PEC effluent (see point 1.3 above) with other AE homologues included (see point 1.6 above) **See section 4.1.2.3**

4. **Terrestrial Compartment:** generate PEC in sludge and soil from monitoring data from digested sewage sludge, following the TGD **See section 4.1.2.4**

4.1.2.1. Determination of PEC values for the aquatic compartment

This section describes the determination of predicted environmental concentrations of AE homologues in the local and regional environments, as described in the EU TGD (2003). As high quality sewage treatment plant effluent data are available for AE homologues with hydrocarbon chainlengths of 12 to 18 these data are used as the basis of the aquatic PEC determination. However, EUSES calculations, calibrated by the measured effluent concentration data, are used to determine an appropriate regional background concentration. The various steps in this process, set out in the Overview Box on the preceding page, are described in the sections below.

4.1.2.1.1. Description of the effluent monitoring data

Before the development of the analytical methodology of Dunphy, et al (2001), which uses 2-fluoro-N-methylpyridinium p-toluenesulphonate derivatisation followed by electrospray LC/MS detection, methods for determining AE concentrations in environmental samples were only able to detect AE homologues with hydrocarbon chainlengths between 12 and 15 and with between 3 and 18 EO units. The Dunphy, et al (2001) method is able to detect all 114 AE homologues in the range $C_{12\text{-}18}$ and $EO_{0\text{-}18}$ at ng/L levels in environmentally relevant aquatic samples. This allows a much more complete environmental profile of AE homologue distribution to be obtained.

Sewage treatment effluents from 9 representative US treatment plants, 8 representative Canadian sewage treatment plants, and 12 representative European sewage treatment plants have been obtained in monitoring exercises, and analysed using the method of Dunphy et al (2001) (Eadsforth et al, 2006; Morrall et al, 2006). In Europe, Eadsforth et al (2006) obtained 24-hour, flow proportional composite

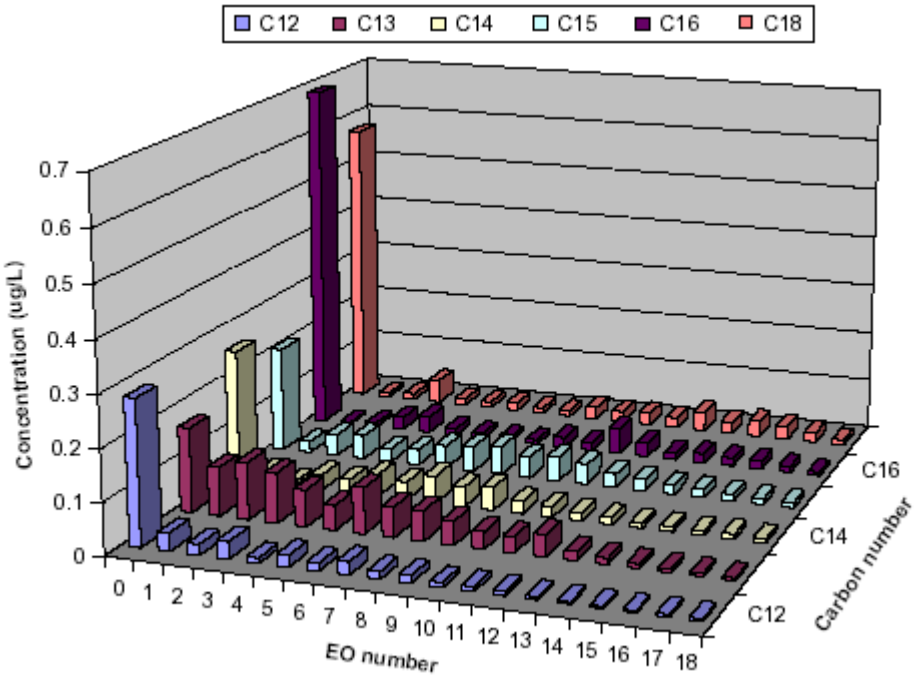


Figure 4.2 Average concentration of alcohol ethoxylate homologues in European effluents from activated sludge wastewater treatment plants. Figure from Eadsforth et al (2006).

samples from 12 representative, acceptably performing activated sludge plants from five European countries (NL, UK, ES, DE, and IT). The plants served populations from 40000 to 1 900 000, and had industrial inputs ranging from 0 to 35% by volume. The total AE effluent concentrations ranged from 1.1µg/l to 16.8 µg/l, with an average value of 4.9 µg/l. For all chainlengths, the alcohol (EO=0) component was the largest component present. The average European EO homologue distribution from the publication of Eadsforth et al (2006) is shown in figure 4.2.

4.1.2.1.2. The alcohol cap

As explained by Wind et al (2006), one finding of recent monitoring studies of municipal effluent concentrations in Europe, Canada, and the USA (Eadsforth et al, 2006; Morrall et al, 2006) was that high mole fractions of long chain alcohols were observed, accounting for up to 4.5 times the ethoxylated material (EO numbers 1-18) present, on a molar basis. As there are potentially many different sources for long chain alcohols in municipal treatment plant effluent, ranging from natural sources to commercial and domestic products as well as alcohols arising from decomposition of both natural and anthropogenic materials, Wind et al (2006) carried out a paired CAS study with synthetic sewage to determine the molar fraction of long chain alcohols which would result from AE degradation. The CAS units were operated with a sludge retention time of 10 days, which is appropriate for European sewage treatment plants. The influent AE, whose total concentration was at the realistic environmental influent level of 4 mg/L, was prepared by combining two commercial AE mixtures. One mixture had a hydrocarbon chain length range from 12 to 15 and an EO range of 0-18 giving an average ethoxylate number of 7, while the other had a C16 to C18 hydrocarbon chainlength and an average ethoxylate number of 11 from an EO range of 0-22). Although this AE mixture has a homologue distribution with a significantly lower C12 and significantly higher C16 and C18 components when compared to the average European AE hydrocarbon chain distribution shown in Table 3.7, the results from degradation allowed a maximum molar percentage of alcohol due to AE degradation to be established, for each hydrocarbon chainlength. The results of this study, given in **Table 4.11,** show, for each hydrocarbon chainlength, the maximum number of moles of alcohol in a sewage treatment plant effluent which can be attributed to AE biodegradation, for each mole of combined EO_{1-18} in the effluent. This maximum amount of alcohol is termed the "alcohol cap" by Wind et al (2006).

Table 4.11 Alcohol resulting from AE degradation, for each hydrocarbon chain length. Data from Wind et al (2006).

Hydrocarbon chain length	**C12**	**C13**	**C14**	**C15**	**C16**	**C18**	***Mean***
Mean: Mole Ratio EO_0 to EO_{1-18}	*1.1*	*2.6*	*0.38*	*0.40*	*0.63*	*0.12*	*0.25*

The alcohol cap appropriate to each hydrocarbon chainlength has been applied to the AE homologue concentration data for each of the twelve 24-hour flow proportional EU effluent samples described in **section 4.1.2.1.1.** (Shell Research Ltd. 2002, 2003). The CSARA AE Workbook (ERASM 2005b) was used for this procedure. This removes any measured alcohol which is not the result of AE degradation processes from the AE homologue matrix, and thus from specific consideration in the AE

environmental risk assessment. The resultant capped AE homologue effluent matrices have been used to determine the 90th percentile concentration matrix for AE in local effluent, as explained below.

4.1.2.1.3. Determination of the local 90% effluent matrix for C12 to C18 AE in European sewage treatment effluent

The EU TGD (2003) allows the use of representative monitoring data for PEC determination. However, in order to generate a reasonable worst case PEC, the 90th percentile of the monitoring data, rather than the mean of the monitoring data, is considered to be appropriate. A 90th percentile homologue matrix has been calculated from the monitoring results (Shell Research Ltd. 2002, 2003), using the following procedure:

1. Each of the 12 sets of 24-hour flow proportional monitoring data, each consisting of 114 AE homologues measured at one of the representative EU activated sludge sewage treatment plants, was subjected to the molar alcohol capping procedure described in Wind, et al (2006), and shown in **Table 4. 11,** to remove all alcohol which is not produced by AE degradation, as described in section 4.1.2.1.2.
2. Each of the 114 homologues in each of the 12 data matrices, now containing only the amount of alcohol which can be attributed to AE use, was considered individually, with the mean and the 90th percentile for each of the 114 homologues being determined from the data for that homologue determined from each of the 12 European effluents.
3. A 90th percentile matrix, consisting of the 90th percentiles of the data obtained for each homologue from each of the 12 effluents, was then constructed. This 90th percentile matrix, consisting of the 90th percentiles of each of the 114 AE homologues, has been termed the $^{90}Clocal_{effluent}$ matrix. This matrix, which represents the reasonable worst case for AE homologue effluent concentrations, is shown in **Table 4.12** below.

Entries for each homologue are the 90%iles of the 12 measured sewage treatment plant effluents. Homologues reported in italics suffered from analytical interference in more than one effluent.

The $^{90}Clocal_{effluent}$ matrix has been developed using conservative assumptions for some of the AE homologues, those which have been affected by interference in the analytical procedure. For example, the C18 EO13 homologue was affected by interference in several of the sewage treatment plant effluents, which accounts for its relatively large magnitude in the $^{90}Clocal_{effluent}$ matrix. In all other cases, the magnitude of the peak affected by interference was much smaller. In all cases in which interference was identified (Shell Research Ltd. 2002, 2003), the value used consists of the sum of the signal and the interference, as it is not possible to separate the two. This is conservative on two levels. At the first level, the effluent concentration for any affected homologue is reported to be larger than its true value, thus leading to a conservative PEC/PNEC value in the risk assessment for that homologue. At the second level, the alcohol cap, being dependent on the number of moles of EO_{1-18} present, will be larger than it should be, as the number of moles of EO_{1-18} will be artificially increased by the presence of the interference signal. Thus some alcohol which does not originate from AE will be included in the AE risk

assessment, due to the presence of interference in some of the EO_{1-18} signals. Although the interference may account for a substantial proportion of the signal for a few homologues, notably C18EO13, due to the large number of AE homologues and the generally low level of most homologue concentrations the general effect on the overall AE PEC levels will be small. For all non-detects, half of the limit of quantisation has been used as the signal level for the appropriate homologue in the specific effluent sample.

Table 4.12 $^{90}Clocal_{effluent}$ for AE in Europe, with units in ng/l.

EO / C	C12	C13	C14	C15	C16	C18
0	231.0	308.2	112.6	151.1	153.1	32.5
1	53.6	181.7	50.8	*38.3*	6.7	11.0
2	40.3	138.9	13.9	97.6	17.2	14.9
3	81.7	257.3	77.5	55.9	49.9	66.9
4	20.8	160.0	95.0	45.7	70.2	25.9
5	45.3	102.1	105.3	68.7	31.9	19.8
6	31.7	286.6	77.7	*62.9*	23.6	14.9
7	42.9	169.6	72.3	*91.5*	16.2	26.0
8	25.0	113.6	89.0	182.1	29.8	30.3
9	21.0	*89.2*	75.5	96.5	48.8	27.8
10	*16.9*	*75.0*	45.0	114.2	36.7	25.5
11	15.7	*55.7*	35.5	*93.9*	134.5	27.1
12	13.0	*93.6*	30.5	*62.8*	77.3	23.7
13	*9.0*	*29.5*	23.8	52.2	35.1	*274.2*
14	6.9	*24.7*	16.8	*37.4*	*24.3*	*59.5*
15	*6.0*	*18.0*	18.8	*29.5*	*23.1*	*40.3*
16	*6.9*	*13.8*	13.9	*29.1*	*15.6*	*22.4*
17	*8.8*	*10.0*	18.5	24.5	*12.4*	*12.6*
18	*6.7*	8.9	10.7	17.0	*6.9*	*9.8*
Sum of AE homologues = 6.73 µg/l						

4.1.2.1.4. Determination of the total local surface water concentration

The local concentration in surface water has been determined from $^{90}Clocal_{effluent}$ by the standard TGD (2003) procedure of dilution by a factor of 10 due to the receiving water. This concentration contains both dissolved AE and AE adsorbed to suspended matter, and is referred to as the total local concentration. The AE homologue matrix

for the Total Local surface water concentration, a matrix in which each homologue has 1/10 the value of the value shown for $^{90}C_{effluent}$ in Table 4.12, is shown in Table 4.13.

Table 4.13 Total local surface water concentrations for AE homologues, with units in ng/l

EO / C	C12	C13	C14	C15	C16	C18
0	23.1	30.8	11.3	15.1	15.3	3.3
1	5.4	18.2	5.1	3.8	0.7	1.1
2	4.0	13.9	1.4	9.8	1.7	1.5
3	8.2	25.7	7.8	5.6	5.0	6.7
4	2.1	16.0	9.5	4.6	7.0	2.6
5	4.5	10.2	10.5	6.9	3.2	2.0
6	3.2	28.7	7.8	6.3	2.4	1.5
7	4.3	17.0	7.2	9.2	1.6	2.6
8	2.5	11.4	8.9	18.2	3.0	3.0
9	2.1	8.9	7.6	9.7	4.9	2.8
10	1.7	7.5	4.5	11.4	3.7	2.6
11	1.6	5.6	3.6	9.4	13.5	2.7
12	1.3	9.4	3.1	6.3	7.7	2.4
13	0.9	3.0	2.4	5.2	3.5	27.4
14	0.7	2.5	1.7	3.7	2.4	6.0
15	0.6	1.8	1.9	3.0	2.3	4.0
16	0.7	1.4	1.4	2.9	1.6	2.2
17	0.9	1.0	1.9	2.5	1.2	1.3
18	0.7	0.9	1.1	1.7	0.7	1.0
Sum of AE homologues = 0. 67 µg/l						

4.1.2.1.5. Determination of the dissolved local surface water concentration

The TGD takes adsorption into account when determining the PEC/PNEC ratio for aqueous species, using dissolved rather than total concentrations for aquatic risk assessment. Thus the dissolved portion of the total local aquatic concentration has also been calculated. The calculation of the dissolved portion of the local river water concentration has been carried out using the sorption isotherms developed for AE homologues, including long chain alcohols, by van Compernolle et al (2006), and

described in **section 4.1.1.1.1.** The authors have carried out sorption studies of radio-labelled alcohols and other AE homologues onto activated sludge and river water solid mixtures, and have combined the results of their work with literature data to develop two sorption QSARs. The more robust QSAR given in equation 4.1 (see

Table 4.14 Dissolved local river water concentrations for AE homologues, with units in ng/l

EO / C	C12	C13	C14	C15	C16	C18
0	22.9	30.4	10.9	14.2	13.5	2.0
1	5.3	17.9	4.9	3.6	0.6	0.7
2	4.0	13.6	1.3	9.0	1.5	0.9
3	8.1	25.2	7.4	5.1	4.2	3.7
4	2.1	15.6	9.1	4.2	5.8	1.3
5	4.5	10.0	10.0	6.2	2.6	1.0
6	3.1	27.9	7.3	5.6	1.9	0.7
7	4.2	16.4	6.8	8.0	1.3	1.1
8	2.5	11.0	8.3	15.8	2.3	1.3
9	2.1	8.6	7.0	8.2	3.6	1.1
10	1.7	7.2	4.1	9.6	2.6	0.9
11	1.5	5.3	3.2	7.7	9.3	0.9
12	1.3	8.9	2.7	5.1	5.2	0.7
13	0.9	2.8	2.1	4.1	2.3	8.0
14	0.7	2.3	1.5	2.9	1.5	1.6
15	0.6	1.7	1.6	2.2	1.4	1.0
16	0.7	1.3	1.2	2.1	0.9	0.5
17	0.8	0.9	1.5	1.7	0.7	0.3
18	0.6	0.8	0.9	1.2	0.4	0.2
Sum of AE homologues = 0. 57µg/l						

section 4.1.1.1.1) allows prediction of Kd as a function of hydrocarbon chain length and EO number. The other QSAR, given in equation 4.2, has been determined from the subset of the data for which the organic carbon content of the activated sludge or sediment could be determined, and predicts logKoc as a function of hydrocarbon chain length and EO number.

$$\log K_{oc} = 0.325C + 0.044EO - 0.207 \quad (R^2 = 0.53) \qquad \text{(Equation 4.2)}$$

The QSAR given in equation 4.2 has been used here, as use of Koc is more generally applicable in the standard TGD adsorption methodology, which ascribes adsorption to partitioning into the organic carbon compartment of the various environmental sorbents such as activated sludge, sediment, and soil solids. The standard TGD suspended sediment has been used, which is present at 15mg/l dry weight suspended solids and contains a weight fraction of organic mater of 0.1. The CSARA AE Workbook, (ERASM 2005b) has been used, with log Koc of each AE homologue calculated according to equation 4.2, to calculate the dissolved concentration of AE in the standard local river water. This matrix of dissolved AE homologues is given in Table 4.14.

4.1.2.1.6. Extrapolation to C8-11 and EO 19-22 AE homologues

Although the published analytical methods are not able to detect the C8, C9, C10, and C11 AE homologues or the AE homologues with EO chains from 19 to 22 EO units, these AE homologues are included in the AE category covered by the HERA assessment. The purpose of this section is to give conservative upper limit values for the C8, C9, C10, and C11 AE homologues, and for the AE homologues with EO chains from 19 to 22 EO units, to enable the entire AE category to be covered.
Estimates for the amounts of AE present in household products given in Table 3.7 show that the sum of the C8 to C11 homologues is approximately half of the C12 homologue tonnage. However, as removal in sewage treatment is probably by Monod kinetics, the effluent concentrations will not necessarily be proportional to the input concentrations. Rapid removal is expected for the C8-11 homologues, but, in order to be conservative, it has been assumed that the 90^{th} percentile effluent concentrations measured for the C12 homologues, the nearest AE homologues with experimental effluent concentration data, should also be used to represent the C8, C9, C10, and C11 AE homologue effluent concentrations. For the C8 chainlength, however, the higher EO homologues have been omitted, as no evidence for AE homologues containing EO > 15 has been found. In a similar manner, the experimental data for the EO=18 homologues has been applied to the EO=19, 20, 21, and 22 homologues. The resulting extended matrix of dissolved AE concentrations in river water is shown in Table 4.15. It is stressed that these estimated concentrations for the C8-C11 and EO19-22 range are intended to be very conservative, thus giving an upper limit for the overall AE concentration.

Table 4.15 Dissolved river water concentrations in ng/l, extrapolated to C8-11 and EO19-22

C / EO	C8	C9	C10	C11	C12	C13	C14	C15	C16	C18
0	**22.9**	**22.9**	**22.9**	**22.9**	22.9	30.4	10.9	14.2	13.5	2.0
1	**5.3**	**5.3**	**5.3**	**5.3**	5.3	17.9	4.9	3.6	0.6	0.7
2	**4.0**	**4.0**	**4.0**	**4.0**	4.0	13.6	1.3	9.0	1.5	0.9
3	**8.1**	**8.1**	**8.1**	**8.1**	8.1	25.2	7.4	5.1	4.2	3.7
4	**2.1**	**2.1**	**2.1**	**2.1**	2.1	15.6	9.1	4.2	5.8	1.3
5	**4.5**	**4.5**	**4.5**	**4.5**	4.5	10.0	10.0	6.2	2.6	1.0
6	**3.1**	**3.1**	**3.1**	**3.1**	3.1	27.9	7.3	5.6	1.9	0.7
7	**4.2**	**4.2**	**4.2**	**4.2**	4.2	16.4	6.8	8.0	1.3	1.1
8	**2.5**	**2.5**	**2.5**	**2.5**	2.5	11.0	8.3	15.8	2.3	1.3
9	**2.1**	**2.1**	**2.1**	**2.1**	2.1	8.6	7.0	8.2	3.6	1.1
10	**1.7**	**1.7**	**1.7**	**1.7**	1.7	7.2	4.1	9.6	2.6	0.9
11	**1.5**	**1.5**	**1.5**	**1.5**	1.5	5.3	3.2	7.7	9.3	0.9
12	**1.3**	**1.3**	**1.3**	**1.3**	1.3	8.9	2.7	5.1	5.2	0.7
13	**0.9**	**0.9**	**0.9**	**0.9**	0.9	2.8	2.1	4.1	2.3	8.0
14	**0.7**	**0.7**	**0.7**	**0.7**	0.7	2.3	1.5	2.9	1.5	1.6
15	**0.6**	**0.6**	**0.6**	**0.6**	0.6	1.7	1.6	2.2	1.4	1.0
16		**0.7**	**0.7**	**0.7**	0.7	1.3	1.2	2.1	0.9	0.5
17		**0.8**	**0.8**	**0.8**	0.8	0.9	1.5	1.7	0.7	0.3
18		**0.6**	**0.6**	**0.6**	0.6	0.8	0.9	1.2	0.4	0.2
19		**0.6**	**0.6**	**0.6**	**0.6**	**0.8**	**0.9**	**1.2**	**0.4**	**0.2**
20		**0.6**	**0.6**	**0.6**	**0.6**	**0.8**	**0.9**	**1.2**	**0.4**	**0.2**
21		**0.6**	**0.6**	**0.6**	**0.6**	**0.8**	**0.9**	**1.2**	**0.4**	**0.2**
22		**0.6**	**0.6**	**0.6**	**0.6**	**0.8**	**0.9**	**1.2**	**0.4**	**0.2**
Sum including extrapolated homologues = 0.87µg/l										

Bold text is extrapolated data, while the normal text indicates concentrations derived from the measured effluent data.

4.1.2.1.7. Determination of the regional background contribution to $PEClocal_{dissolved}$ values

The TGD (2003) specifies that regional and continental background concentrations must be added to the local site concentrations derived above, in order to generate the appropriate PEC_{local} concentrations. In principle, these regional and continental concentrations can be calculated using multimedia models such as EUSES (ECB 2005). However, EUSES is intended to be a screening level model, and thus contains many conservative assumptions. In practice, the continental and regional background levels which result from the EUSES calculations for the AE homologues are generally higher than the AE 90 percentile homologue effluent concentrations shown in Table

4.12. This is not realistic for substances which are both readily and anaerobically degradable and are rapidly removed during sewage treatment.

In this HERA assessment, PEClocal has been determined using an approach which involves both the EUSES model, based on the TGD (2003), and the measured 90th percentile effluent concentration, which has been used as described in sections 4.1.2.1.4 and 4.1.2.1.5 to determine $Clocal_{dissolved}$, the dissolved concentration of each AE homologue in the local receiving water. In this approach, it is assumed that the ratio of $PEClocal_{dissolved}$ to $Clocal_{dissolved}$ as calculated by EUSES should be the same as this ratio based on monitoring data, as the TGD (EU 2003) will accept either the EUSES or the monitoring approach for PEC determination. In effect, the monitoring results are used to calibrate the results of the EUSES calculations, which in principle should be carried out for each AE homologue.

The first step begins with the definition of $PEClocal_{dissolved}$ as the sum of the local concentration $Clocal_{dissolved}$ and the regional (or background) concentration, $PECreg_{dissolved}$. Thus:

$$PEClocal_{dissolved} = Clocal_{dissolved} + PECreg_{dissolved} \qquad (4.3)$$

If each term in equation 4.3 is divided by $Clocal_{dissolved,,}$ this equation becomes:

$$PEClocal_{dissolved} / Clocal_{dissolved} = 1 + (PECreg_{dissolved} / Clocal_{dissolved}) \qquad (4.4)$$

On the right hand side of equation 4.4, the terms $PECreg_{dissolved}$ and $Clocal_{dissolved}$ are determined using EUSES calculations, as described below. On the left hand side of the equation, $Clocal_{dissolved}$ is taken as the value determined from monitoring data (see sections 4.1.2.1.4 and 4.1.2.1.5), and $PEClocal_{dissolved}$ then refers to the PEClocal value based on monitoring results. As all the terms in equation 4.4 except for $PEClocal_{dissolved}$ are known or can be determined using EUSES, $PEClocal_{Dissolved}$ can be determined using equation 4.4.

The EUSES calculations used to determine the right hand side of equation 4.4 were carried out for a subset of the possible 230 AE homologues. These EUSES calculations used the MP, BP, VP, and water solubility data calculated for each of these 29 AE homologues using EPIWIN, a program developed by Syracuse Research and sponsored and recommended by the US EPA. Molecular weight, Log Koc, and Log Kow data were obtained from the CSARA AE Workbook (ERASM 2005b), with equation 4.2 being used to calculate Koc from the C and EO number of the AE homologue. Tonnages used for each homologue were obtained from AISE/HERA (2003), based upon the tonnage distributions given in tables 3.7 and 3.9 and the overall tonnage of 290 000 tpa, as described in section 3.4. The tonnage of each AE homologue was treated as being an HPV chemical, as AE distribution and use is widespread, and the widespread distribution and use should also apply to each AE homologue. A primary biodegradation half-life in the sewage treatment plant of 1 minute was used for all AE homologues, as discussed in section 4.1.1.2.4. River water half-lives were extrapolated from the Japanese river water data, as discussed in section 4.1.1.2.3. The Japanese river water data discussed in Section 4.1.1.2.3 has been extrapolated, as shown in Table 4.5, to cover C8-11 and C15-18. The treatment in section 4.1.1.2.3 is conservative, in that the maximum value of the decay time for

each block of data has been used, and the experimental data relate to 10C, not to the 12C standard EU default.

Sediment half-lives were determined from the half-lives in river water, by applying the standard TGD (2003) application factor of 10. A half-life in soil of 5 days was assumed, as AEs are both aerobically and anaerobically biodegradable, and should degrade more quickly as LAS, whose 7 day half-life in soil is given in the HERA LAS environmental risk assessment (HERA 2004). The EUSES input parameters are summarised in Table 4.16.

Table 4.16 Input data for the representative AE homologue EUSES calculations

Parameter	***Notes***
Tonnage	*Tonnages used for each homologue were obtained from AISE/HERA(2003), based upon the Tonnage distributions given in tables 3.7 and 3.9 and the overall tonnage of 290 000 tpa, as described in section 3.4.*
C8 and C9 tonnages	*As C8 and C9 input tonnages are small, these tonnages were combined with the C10 tonnages for these calculations. The physico-chemical, MW, and removal parameters for C10 were also used for the combined C8-10 tonnage. Thus no specific EUSES calculations were carried out for the C8 and C9 AE homologues.*
Physico-chemical	*Log Kow information has been taken from Table 3.6 (section 3.2.2.5). Koc data has been taken from Table 4.2 (section 4.1.1.1.1). Other physical chemical data has been taken from the EPI program (Appendix A3.1.1).*
Biodegradation during sewage treatment	*First order kinetics has been assumed, with a half-life of 1 minute for all AE homologues. (Section 4.1.1.2.4)*
Biodegradation in river Water	*River water biodegradation has been grouped into blocks with half lives of less than 4 hours, less than 8 hours, less than 12 hours, and less than 24 hours. Within each block, the maximum biodegradation half-life has been used. See section 4.1.1.2.3, Table 4.5.*
Half-life in sediment	*TGD default of 1/10 the half-life in river water*
Half-life in soil	*5 days*

In EUSES, in-stream removal, especially of the 20% of AE assumed in the TGD (2003) not to be treated in a sewage treatment plant, has a major influence on the calculated regional and continental concentrations, with secondary effects due to the Koc of the AE homologue. Thus it is important to consider independently each of the different river-water half-life blocks as shown in Table 4.5 (section 4.1.1.2.3). The river half-life blocks of 4, 8, 12, and 24 hours maximum are indicated in Table 4.16

by different background colours, with e.g. light grey representing a river half-life of up to 4 hours, and dark grey representing a half-life of 12 to 24 hours.

Table 4.17 Values of the right hand side of equation 4.4, calculated using EUSES

EO /C	8 and 9	10	11	12	13	14	15	16	18
0		1.17	1.16	1.15	1.13	1.10	1.07	1.04	1.02
1									
2									
3		1.16							
4									
5									
6		1.32	*est 1.28*	*est 1.25*	est 1.19	*est 1.17*		*est 1.05*	*est 1.03*
7			1.46	1.39	1.19	*est 1.16*			
8				*est 138*		*est 1.15*			
9		1.48		*est 137*		*est 1.13*	1.07		
10				*est 136*		1.11			
11				*est 135*					
12				*est 133*			1.09	1.06	1.03
13				*est 131*					
14		1.46		1.29	1.19				
15									
16									
17									
18		1.84	1.63	1.44	1.28	1.18	1.11	*est 1.08*	1.04
19									
20									
21									
22									

Key: light grey fill=maximum river half-life of 4 hours. No fill = river half-life of 4 to 8 hours. Mid-grey fill = river half-life between 8 and 12 hours. Dark grey fill = river half-life of 12-24 hours.

Table 4.17 contains the values of the right hand side of equation 4.4, as calculated by EUSES, for 29 representative AE homologues. This value is shown in ordinary text in Table 4.17. As discussed above, this EUSES-calculated value, which is equivalent to the $PEClocal_{dissolved}$ to $Clocal_{dissolved}$ ratio as calculated by EUSES, is a good approximation for the $PEClocal_{dissolved}$ to $Clocal_{dissolved}$ ratio based on monitoring data. It is the value given in Table 4.17 which should be multiplied by the $Clocal_{dissolved}$ values derived from monitoring data and given in Table 4.15 to give the $PEClocal_{dissolved}$ values for the AE homologues.

Table 4.17 also includes some entries for interpolations between AE homologue data in the same river half-life block. These ratios are given in the table in italic text. For all other AE homologues the appropriate value for the right hand side of equation 4.4 has been chosen to be the value of the actual or estimated entry given in the table above the homologue in the same hydrocarbon chain column and the same river half-life block. Thus, for example, C10 EO19, C10EO20, C10EO21, and C10EO22 all have the same $PEClocal_{dissolved}$ to local surface water concentration ratio, 1.84, as that calculated for C10EO18. This is conservative, as within each river half-life block the ratio has been found to decrease with increasing Koc value.

In summary, this section has developed a conservative but realistic method for determining the contributions made by background concentrations of AE homologues to the $PEClocal_{dissolved}$ values of these AE homologues in surface waters. The calculation of these $PEClocal_{dissolved}$ values is described in the next section.

4.1.2.1.8. Determination of $PEClocal_{dissolved}$

$PEClocal_{dissolved}$ has been determined by multiplying the dissolved concentrations in local river water, shown in table 4.15, by the factors derived from EUSES and shown in table 4.17 which increase the local river water concentration to account for AE homologues which are present as part of the regional and continental background concentration. The resultant $PEClocal_{dissolved}$ values are given in table 4.18. The sum of these $PEClocal_{dissolved}$ values for the AE homologues is 1.01µg/l.

$$\mathbf{PEClocal_{dissolved} = 1.01 \mu g/l.}$$

Table 4.18 $PEClocal_{dissolved}$ for AE homologues, in ng/l

C/ EO	C8	C9	C10	C11	C12	C13	C14	C15	C16	C18
0	26.8	26.8	26.8	26.6	26.3	34.4	12.0	15.2	14.0	2.0
1	6.2	6.2	6.2	6.1	6.1	20.2	5.4	3.9	0.6	0.7
2	4.7	4.7	4.7	4.6	4.6	15.4	1.4	9.6	1.6	0.9
3	9.4	9.4	9.4	9.4	9.3	28.5	8.1	5.5	4.4	3.8
4	2.4	2.4	2.4	2.4	2.4	17.6	10.0	4.5	6.0	1.3
5	5.2	5.2	5.2	5.2	5.2	11.3	11.0	6.6	2.7	1.0
6	4.1	4.1	4.1	4.0	3.9	33.2	8.5	6.0	2.0	0.7
7	6.2	6.2	6.2	6.1	5.8	19.5	7.9	8.6	1.4	1.1
8	3.7	3.7	3.7	3.7	3.5	13.1	9.5	16.9	2.4	1.3
9	3.1	3.1	3.1	3.1	2.9	10.2	7.9	8.8	3.8	1.1
10	2.5	2.5	2.5	2.5	2.3	8.6	4.6	10.3	2.7	0.9
11	2.2	2.2	2.2	2.2	2.0	6.3	3.6	8.4	9.9	0.9
12	1.9	1.9	1.9	1.9	1.7	10.6	3.0	5.6	5.5	0.7
13	1.3	1.3	1.3	1.3	1.2	3.3	2.3	4.5	2.4	8.2
14	1.0	1.0	1.0	1.0	0.9	2.7	1.7	3.2	1.6	1.6
15	0.0	0.9	0.9	0.9	0.8	2.0	1.8	2.4	1.5	1.0
16	0.0	1.0	1.0	1.0	0.9	1.5	1.3	2.3	1.0	0.5
17	0.0	1.5	1.5	1.3	1.2	1.2	1.8	1.9	0.8	0.3
18	0.0	1.1	1.1	1.0	0.9	1.0	1.1	1.3	0.4	0.2
19	0.0	1.1	1.1	1.0	0.9	1.0	1.1	1.3	0.4	0.2
20	0.0	1.1	1.1	1.0	0.9	1.0	1.1	1.3	0.4	0.2
21	0.0	1.1	1.1	1.0	0.9	1.0	1.1	1.3	0.4	0.2
22	0.0	1.1	1.1	1.0	0.9	1.0	1.1	1.3	0.4	0.2
Sum of AE homologues = $PEClocal_{dissolved}$ = 1.01µg/l.										

In accordance with the TGD (2003), these $PEClocal_{dissolved}$ values are used to determine the PEC/PNEC values for the local surface water environment. They are also used to determine $PEClocal_{sediment}$, as described below.

4.1.2.2 Determination of $PEClocal_{sediment}$

$PEClocal_{sediment}$ has been calculated from the PEC_{local} values given for each AE isomer in Table 4.18, using the equilibrium partitioning method described in the TGD (2003). The TGD (2003) considers the top layer of sediment to consist of settled suspended solids, and thus uses the suspended solids default values for density (RHOsusp) and fraction of organic carbon (Foc) found in sediment. In the TGD method, given by equation TGD 50 shown below, $PEC_{sediment}$ is calculated in mg/kg wet weight and

$PEC_{sediment}$ = (Ksusp-water/RHOsusp) * $PEC_{local\ water}$ * 1000 **TGD equation 50**

PEClocal $_{water}$ is in mg/l, rather than in ng/l as in Table 4.18. Ksusp-water, the unitless suspended solids-water partition coefficient, has been determined from Koc and Foc as shown in TGD equation 24, with substitutions from TGD equation 23, as shown

Ksusp-water = Fwater$_{susp}$ + F solid$_{susp}$ (Foc*Koc)*RHOsolid/1000)

TGD equation 24/23

Table 4.19 PEC$_{local\text{-}Sediment}$ values for AE homologues in mg/kg wet weight sediment, calculated using equilibrium partitioning, according to the TGD.

EO	C8	C9	C10	C11	C12	C13	C14	C15	C16	C18
0	3.82E-05	3.13E-04	6.34E-04	1.30E-03	2.66E-03	7.21E-03	5.19E-03	1.33E-02	2.43E-02	1.10E-02
1	3.2E-05	8.03E-05	1.63E-04	3.33E-04	6.86E-04	4.72E-03	2.59E-03	3.74E-03	1.18E-03	4.13E-03
2	7.11E-05	6.71E-05	1.37E-04	2.80E-04	5.76E-04	3.99E-03	7.62E-04	1.03E-02	3.25E-03	5.65E-03
3	2.04E-05	1.49E-04	3.05E-04	6.30E-04	1.30E-03	8.21E-03	4.81E-03	6.48E-03	9.98E-03	2.47E-02
4	4.86E-05	4.29E-05	8.78E-05	1.82E-04	3.74E-04	5.65E-03	6.57E-03	5.89E-03	1.51E-02	9.20E-03
5	4.22E-05	1.02E-04	2.09E-04	4.33E-04	8.92E-04	4.02E-03	8.00E-03	9.59E-03	7.39E-03	7.47E-03
6	7.12E-05	8.87E-05	1.82E-04	3.66E-04	7.43E-04	1.31E-02	6.88E-03	9.54E-03	5.95E-03	5.56E-03
7	4.71E-05	1.49E-04	3.08E-04	6.30E-04	1.24E-03	8.57E-03	7.03E-03	1.50E-02	4.43E-03	9.18E-03
8	4.39E-05	9.88E-05	2.04E-04	4.17E-04	8.18E-04	6.39E-03	9.42E-03	3.26E-02	8.51E-03	1.14E-02
9	3.95E-05	9.22E-05	1.90E-04	3.90E-04	7.58E-04	5.54E-03	8.63E-03	1.85E-02	1.44E-02	1.00E-02
10	3.87E-05	8.29E-05	1.71E-04	3.51E-04	6.77E-04	5.15E-03	5.48E-03	2.38E-02	1.13E-02	8.56E-03
11	3.73E-05	8.13E-05	1.68E-04	3.45E-04	6.59E-04	4.20E-03	4.72E-03	2.13E-02	4.40E-02	8.89E-03
12	2.87E-05	7.83E-05	1.62E-04	3.32E-04	6.25E-04	7.82E-03	4.40E-03	1.54E-02	2.65E-02	7.16E-03
13	2.48E-05	6.03E-05	1.25E-04	2.56E-04	4.74E-04	2.72E-03	3.77E-03	1.35E-02	1.26E-02	8.45E-02
14	2.37E-05	5.21E-05	1.07E-04	2.21E-04	4.03E-04	2.48E-03	2.96E-03	1.04E-02	8.78E-03	1.74E-02
15		4.97E-05	1.02E-04	2.11E-04	3.83E-04	2.03E-03	3.47E-03	8.56E-03	8.74E-03	1.12E-02
16		6.45E-05	1.32E-04	2.74E-04	4.97E-04	1.71E-03	2.85E-03	8.85E-03	5.99E-03	5.75E-03
17		1.02E-04	2.12E-04	3.88E-04	7.03E-04	1.41E-03	4.16E-03	7.89E-03	5.04E-03	3.57E-03
18		8.50E-05	1.76E-04	3.23E-04	5.84E-04	1.38E-03	2.73E-03	6.01E-03	3.05E-03	2.43E-03
19		9.46E-05	1.96E-04	3.59E-04	6.48E-04	1.52E-03	2.98E-03	6.46E-03	3.22E-03	2.48E-03
20		1.05E-04	2.18E-04	3.99E-04	7.17E-04	1.68E-03	3.25E-03	6.94E-03	3.38E-03	2.52E-03
21		1.17E-04	2.43E-04	4.43E-04	7.94E-04	1.85E-03	3.53E-03	7.42E-03	3.55E-03	2.57E-03
22		1.30E-04	2.70E-04	4.92E-04	8.78E-04	2.03E-03	3.84E-03	7.92E-03	3.72E-03	2.61E-03
Sum of AE homologues = PEClocal$_{sediment}$ = 1.01 mg/kg wet weight sediment										

above. The Koc values for each AE homologue have been determined using the method of van Compernolle et al (2006), using the CSARA AE Workbook[9] (ERASM 2005c). The TGD defaults of 2.5 for RHOsolid, 0.9 for the fraction of water in suspended solids $Fwater_{susp,}$ and 0.1 for the fraction of solids in suspended solids $Fsolid_{susp}$ have been used, as well as 0.1 for the fraction of organic carbon in suspended solids (Foc).

The $PEClocal_{sediment}$ values obtained using the TGD methodology are given for the AE homologues in Table 4.19. As for the $PEClocal_{water}$ values (shown in table 4.18), these values are conservative, in that they are derived from a reasonable worst case effluent concentration. The sum of the $PEClocal_{sediment}$ concentrations in Table 4.18 is just over 1 mg/kg wet weight sediment.

$PEClocal_{sediment}$ = 1.01 mg/kg wet weight sediment

There is very little monitoring data for AE in sediment, with which these calculated $PEClocal_{sediment}$ values can be compared. However, Cavalli et al (2000) have measured AE concentrations in sediments in the Po valley, including samples from sites containing considerable input from highly populated and industrial areas such as Turin and Milan. The measurements took place in the winter, at a time of low river flow. Thus these measurements would be expected to represent a reasonably worst case, comparable to the $PEClocal_{sediment}$ values which have been determined from 90^{th} percentile effluent data. As the analysis method was not capable of distinguishing individual AE homologues, and included any other material in the sediment which would be co-extracted with AE and be detected in the AE region of the HPLC chromatogram, the concentrations reported will over-estimate the AE concentrations actually present in the sediments. These reported AE sediment concentrations range from less than 0.2 mg/kg to just over 1 mg/kg, supporting the $PEClocal_{sediment}$ values determined by equilibrium partitioning.

In the USA, Dyer et al (2006) have measured similar sediment concentrations at three sites, with the highest concentration being downstream of a trickling filter plant. Fatty alcohol accounted for 27% to 60% of the AE measured at the three sites, but as it was not possible to apply an alcohol cap, this alcohol would have had many non-AE sources. Increasing the measured non-alcohol AE by a factor of 3 to account for unmeasured AE homologues and converting the dry weight sediment concentrations to wet weight sediment according to TGD (2003) gives an AE sediment concentration

[9] The CSARA AE Workbook (ERASM 2005b,c) is an EXCEL workbook which has been developed by the CSARA taskforce, sponsored by ERASM. It contains the basic data and calculation methods available to calculate the results of several ecotoxicity and sorption QSARs for each AE homologue from C9 -18 and EO 0 to 20. If environmental concentrations can be provided, the Workbook can enable the full risk assessment process to be carried out, with several choices of method available to the user. This enables more efficient use of QSARS including those developed by CSARA, in the AE risk assessment process.

including non-AE alcohol of approximately 0.7 mg/kg wet weight, for the highest sediment concentration measured in this study.

4.1.2.3. Determination of local concentrations in the sewage treatment plant

The TGD assumes that, for protection of the local sewage treatment plant, the concentration in the activated sludge plant is equal to the effluent concentration (EC 2003, equation 38). In this HERA report, the effluent concentration has been determined as the 90th percentile of the measured AE effluent concentrations, as described in section 4.2.1.2.3. Thus the PEC_{stp} matrix is formed from the $^{90}Clocal_{effluent}$ matrix given in Table 4.12, conservatively extended to cover hydrocarbon chainlengths from 8 to 11 and EO chainlengths up to 22 as described in section 4.1.2.1.6. The resultant PEC_{stp} values for each AE homologue are shown in Table 4.20. The sum of these AE homologue values is 9.8µg/l. This value is used in the risk assessment section to determine the PEC/PNEC ratio for the local sewage treatment plant.

PEC_{stp} = 9.8µg/l.

Table 4.20. PEC_{stp} values, in µg/l, for AE homologues

C/EO	C8	C9	C10	C11	C12	C13	C14	C15	C16	C18
0	**0.2310**	**0.2310**	**0.2310**	**0.2310**	0.2310	0.3082	0.1126	0.1511	0.1531	0.0325
1	**0.0536**	**0.0536**	**0.0536**	**0.0536**	0.0536	0.1817	0.0508	0.0383	0.0067	0.0110
2	**0.0403**	**0.0403**	**0.0403**	**0.0403**	0.0403	0.1389	0.0139	0.0976	0.0172	0.0149
3	**0.0817**	**0.0817**	**0.0817**	**0.0817**	0.0817	0.2573	0.0775	0.0559	0.0499	0.0669
4	**0.0208**	**0.0208**	**0.0208**	**0.0208**	0.0208	0.1600	0.0950	0.0457	0.0702	0.0259
5	**0.0453**	**0.0453**	**0.0453**	**0.0453**	0.0453	0.1021	0.1053	0.0687	0.0319	0.0198
6	**0.0317**	**0.0317**	**0.0317**	**0.0317**	0.0317	0.2866	0.0777	0.0629	0.0236	0.0149
7	**0.0429**	**0.0429**	**0.0429**	**0.0429**	0.0429	0.1696	0.0723	0.0915	0.0162	0.0260
8	**0.0250**	**0.0250**	**0.0250**	**0.0250**	0.0250	0.1136	0.0890	0.1821	0.0298	0.0303
9	**0.0210**	**0.0210**	**0.0210**	**0.0210**	0.0210	0.0892	0.0755	0.0965	0.0488	0.0278
10	**0.0169**	**0.0169**	**0.0169**	**0.0169**	0.0169	0.0750	0.0450	0.1142	0.0367	0.0255
11	**0.0157**	**0.0157**	**0.0157**	**0.0157**	0.0157	0.0557	0.0355	0.0939	0.1345	0.0271
12	**0.0130**	**0.0130**	**0.0130**	**0.0130**	0.0130	0.0936	0.0305	0.0628	0.0773	0.0237
13	**0.0090**	**0.0090**	**0.0090**	**0.0090**	0.0090	0.0295	0.0238	0.0522	0.0351	0.2742
14	**0.0069**	**0.0069**	**0.0069**	**0.0069**	0.0069	0.0247	0.0168	0.0374	0.0243	0.0595
15	**0**	**0.0060**	**0.0060**	**0.0060**	0.0060	0.0180	0.0188	0.0295	0.0231	0.0403
16	**0**	**0.0069**	**0.0069**	**0.0069**	0.0069	0.0138	0.0139	0.0291	0.0156	0.0224
17	**0**	**0.0088**	**0.0088**	**0.0088**	0.0088	0.0100	0.0185	0.0245	0.0124	0.0126
18	**0**	**0.0067**	**0.0067**	**0.0067**	0.0067	0.0089	0.0107	0.0170	0.0069	0.0098
19	**0**	**0.0067**	**0.0067**	**0.0067**	**0.0067**	**0.0089**	**0.0107**	**0.0170**	**0.0069**	**0.0098**
20	**0**	**0.0067**	**0.0067**	**0.0067**	**0.0067**	**0.0089**	**0.0107**	**0.0170**	**0.0069**	**0.0098**
21	**0**	**0.0067**	**0.0067**	**0.0067**	**0.0067**	**0.0089**	**0.0107**	**0.0170**	**0.0069**	**0.0098**
22	**0**	**0.0067**	**0.0067**	**0.0067**	**0.0067**	**0.0089**	**0.0107**	**0.0170**	**0.0069**	**0.0098**
Sum of AE homologue values = PEC_{stp}= 9.8µg/l										

Bold values are conservatively extrapolated. Values in normal text are based on the 90th percentile of the measured effluent concentrations.

4.1.2.4. Determination of local concentrations in the terrestrial environment

4.1.2.4.1 Determination of local concentrations in sewage sludge

In this assessment, $Clocal_{sludge}$ has been determined from monitoring data for total AE, as homologue-specific concentrations using the method of Dunphy et al (2001) are not available for AE in sewage sludge. However, Matthijs et al (2004) have carried out a monitoring exercise in which sludges from 17 sewage treatment works, located in Germany, Italy, The Netherlands, Spain, and the UK, have been collected and their AE contents measured. Thirteen activated sludge plants, three UK trickling filter plants, and one UK combined facility were covered in the monitoring study, serving populations ranging from 9,300 to 1,500,000, and with the percentage of industrial input ranging from 0 to 50%. Anaerobic digester sludge was collected from both digester inlet and outlet for 6 treatment plants, and digester outlet sludges only were collected from a further 8 treatment facilities. In addition, lagoon sludge was collected from a further three UK treatment facilities. As only digester sludge is now recommended for addition to agricultural soil, only the anaerobic digester results will be considered further.

The sludges described in Matthijs et al (2004) have been analysed by a new HPLC fluorescence detection method, which was ring tested in several laboratories. The method is stated to be able to detect C12 to C18 alkyl chainlengths with ethoxylate chains ranging from EO4 to EO20 in sewage sludges. The detection limit is given as 20-30mg/kg of total AEs in dry weight sludge. The method is said to over-estimate the concentration of AE present, due to the non-specific nature of the detection, but to be suitable for estimating AE concentrations in sludges, and thus the AE concentrations which are applied to soil.

The results from the six anaerobic digesters with inlet and outlet data both available indicated that a mean AE removal of 83% (range 61% to 92%) could be calculated, when inlet and outlet data were obtained on the same day. The mean AE concentration in the 14 digester outlet sludges was 168 mg/kg dry weight, with a range from less than 22 to 468 mg/kg dry weight. Based on this information, it has been decided to consider 468mg/kg dry weight of AE C12-18, EO4-20 as a worst case for the sum of the concentration of these AE homologues in sludges applied to soil.

It is possible to estimate the additional AE concentration in sludge which is due to AE homologues with hydrocarbon chainlengths of 8 and 9, 10, and 11, and EO chainlengths of 0 to 3 and 21-22, which are within the AE category considered in this HERA assessment, by using results from the EUSES calculations for AE homologues to extend the AE matrix. The procedure is somewhat different from that used for extension of the $Clocal_{dissolved}$ matrix, in that the AE adsorbed to sludge will be proportional to the initial tonnage of each homologue released into the environment. Thus the initial tonnage AE matrix (AISE/HERA 2003) has been used in the extension procedure.

Concentrations of AE homologues in undigested sludge were taken from the same EUSES calculations carried out to estimate a background concentration for the aquatic risk assessment. These sludge concentrations were then divided by the tonnage of the appropriate AE homologue, to obtain sludge concentrations per tonne of the AE

homologue for the representative AE homologues. These tonnage normalised sludge concentrations are given in bold text in Table 4.21. Normal text entries in Table 4.21 are interpolations between table entry values, or represent an extrapolation from the EO18 entries for EO19-22.

Table 4.21.EUSES estimates of AE concentrations in sewage sludge, in mg/kg sewage sludge, per tonne of AE released to sewer

C/ EO	9	10	11	12	13	14	15	16	18
0		**0.0143**	**0.0264**	**0.0443**	**0.0653**	**0.0845**	**0.0995**	**0.1095**	**0.1254**
1		0.0160	0.0295	0.0475	0.0680	0.0861	0.1009	0.1107	0.1267
2		0.0175	0.0325	0.0510	0.0710	0.0877	0.1023	0.1119	0.1280
3		**0.0189**	0.0355	0.0540	0.0740	0.0893	0.1037	0.1131	0.1293
4		0.0210	0.0390	0.0570	0.0770	0.0909	0.1051	0.1143	0.1306
5		0.0230	0.0415	0.0600	0.0800	0.0925	0.1065	0.1155	0.1319
6		**0.0246**	0.0440	0.0630	0.0830	0.0941	0.1079	0.1167	0.1332
7		0.0270	**0.0470**	**0.0659**	**0.0853**	0.0957	0.1093	0.1179	0.1345
8		0.0295	0.0500	0.0690	0.0875	0.0973	0.1107	0.1191	0.1358
9		**0.0315**	0.0525	0.0720	0.0895	**0.0989**	**0.1121**	0.1203	0.1371
10		0.0340	0.0550	0.0750	0.0915	0.1005	0.1132	0.1215	0.1384
11		0.0370	0.0575	0.0780	0.0935	0.1021	0.1143	0.1227	0.1397
12		0.0400	0.0600	0.0810	0.0955	0.1040	**0.1154**	**0.1234**	**0.1406**
13		0.0425	0.0625	0.0835	0.0975	0.1058	0.1165	0.1246	0.1416
14		**0.0452**	0.0650	**0.0856**	**0.0999**	0.1076	0.1177	0.1259	0.1427
15		0.0490	0.0680	0.0880	0.1015	0.1094	0.1188	0.1271	0.1437
16		0.0520	0.0720	0.0905	0.1032	0.1112	0.1200	0.1284	0.1448
17		0.0550	0.0750	0.0925	0.1047	0.1130	0.1214	0.1297	0.1458
18		**0.0574**	**0.0782**	**0.0946**	**0.1063**	**0.1146**	**0.1227**	0.1310	**0.1469**
19		0.0574	0.0782	0.0946	0.1063	0.1146	0.1227	0.1310	0.1469
20		0.0574	0.0782	0.0946	0.1063	0.1146	0.1227	0.1310	0.1469
21		0.0574	0.0782	0.0946	0.1063	0.1146	0.1227	0.1310	0.1469
22		0.0574	0.0782	0.0946	0.1063	0.1146	0.1227	0.1310	0.1469

Bold text entries obtained from EUSES calculations for the specified AE homologue. Normal text entries are interpolations from the calculated data, with extrapolations used to generate data for EO 19-22.

The AE homologue tonnage normalised sludge concentration matrix in Table 4.21 was then multiplied by the AE homologue tonnage matrix (AISE/HERA2003) to give sludge concentrations which would be expected if EUSES were used to calculate sludge concentrations for all the AE homologues. These sludge concentrations estimated using EUSES are shown in Table 4.22. In this table, as the C8 tonnage is very small, it has been combined with the C9 tonnage for this assessment. A conservative estimate of the C8 and C9 homologue concentrations has been estimated from the C10 data, by assuming similar sorption to C10 and a tonnage ratio of

C10/(C8+C9) of 1.9. This ratio, calculated from the information used to construct Table 3.7, assumes that the C8 tonnage is close to 1% of the overall AE tonnage.

Table 4.22 AE homologue concentrations in sewage sludge in mg/kg, as estimated with EUSES

C/ EO	C8 + C9*	10	11	12	13	14	15	16	18
0	8	**15**	**19**	**212**	**225**	**193**	**171**	**46**	**54**
1	6	12	14	154	159	133	118	31	37
2	10	19	24	253	254	208	182	49	57
3	13	**24**	30	305	301	241	210	56	66
4	15	28	35	342	332	260	226	60	71
5	17	33	40	380	365	280	243	64	76
6	17	**33**	40	378	359	270	233	61	72
7	20	38	**45**	**418**	**390**	290	249	66	77
8	22	41	47	426	389	287	246	64	76
9	22	**42**	477	433	387	284	242	63	75
10	23	45	486	438	385	280	237	62	73
11	22	43	448	402	347	251	212	55	65
12	20	39	39	348	295	213	**178**	**46**	**55**
13	18	34	34	301	253	182	151	39	46
14	16	**30**	44	**250**	**210**	150	123	32	38
15	13	25	23	196	163	117	95	25	29
16	8	16	15	124	102	73	59	15	18
17	9	17	16	127	104	74	60	16	18
18	5	**9**	**8**	**65**	**53**	**38**	**30**	8	**9**
19	5	9	8	65	53	38	30	8	9
20	5	9	8	65	53	38	30	8	9
21	5	9	8	65	53	38	30	8	9
22	5	9	8	65	53	38	30	8	9

* C9 concentrations have been estimated by dividing the C10 concentrations by 1.9, as a conservative estimate of the initial tonnage ratio. As C8 concentrations are very small, they have been combined with the C9 tonnages in this assessment.

The concentrations of the AE homologues calculated by EUSES are too high, both because degradation in the anaerobic digester may reduce the concentrations by up to an order of magnitude, but also due to other inherently conservative assumptions in the EUSES program. Therefore the experimental data of Matthijs et al (2004) has been used to scale the sludge concentrations. The ratio of the sum of the EUSES based sludge concentrations due to the AE homologues C12-18, EO4-20 to the total concentration due to C9-18, EO 0 to 22 has been calculated from the information calculated by EUSES given in Table 4.22, and found to be 0.70. Since 70% of the worst case measured concentration in anaerobically digested sludge is 468 mg/kg,

from the measured data of Matthijs et al (2004), the concentration of the C8-18, EO0-22 homologues should be 667 mg/kg. Thus

$$\mathbf{C_{local\text{-}sludge} = 667\ mg/kg\ dry\ weight}$$

There are many conservative factors in the $C_{local\text{-}sludge}$ calculation described above. The AE sludge homologue matrix has been extended in a conservative manner, with respect to both the hydrocarbon chain and ethylene oxide chainlengths. In addition, the monitoring data of Matthijs et al (2004) has been analysed in a conservative manner which includes as AE any other substance which gave an HPLC signal in the same region of the HPLC spectrum. By comparison, the CAS study of Wind et al (2006), which used the homologue-specific AE analytical methodology of Dunphy et al (2001) and which was able to subtract any signals in the AE region due to non-AE material by using a control CAS unit without added AE, found an AE sludge concentration of 33 mg/kg, for the AE homologues from C12 -18 and EO 0 to 18. Even after scale-up to account for the lower hydrocarbon chain and higher ethylene oxide chain material not covered in the analytical method, this value is less than 10% of the conservative value of 667 mg/kg dry weight sludge which is used in this HERA report. Thus the $\mathbf{C_{local\text{-}sludge}}$ calculation, based on the highest sludge concentration observed in the monitoring exercise, may be overly conservative.

4.1.2.4.2 Determination of PEC_{soil}

The TGD methodology has been followed in determining the $C_{Local\text{-}soil}$ that results from a single annual application of 5000 kg/ha dry weight sludge to agricultural soil. The initial soil concentration, using a plowing depth of 0.2m and $C_{local\text{-}sludge}$ of 667 mg/kg is

$$\mathbf{C_{soil\text{-}initial} = 0.98\ mg/kg\ wet\ weight\ soil.}$$

The TGD then refines this initial C_{soil} by considering biodegradation of AE within soil for a 30 day period, and by adding background AE which may have been deposited by wet and dry deposition from local, regional, and continental sources (TGD 2003, equation 55, page 81). For this assessment, it is assumed that the background concentration of AE is very low, due to the low volatility of most AE homologues. Thus the background concentration has been neglected in this AE risk assessment. A half life in soil of 5 days has been used, as in the EUSES calculations for the representative AE homologues. Supporting evidence showing a half-life of less than 5 days for a radio-labelled dodecyl AE in two soils with and without crops is given in Knaebel and Vestal (1992). The resulting PEClocal $_{soil}$ value is:

$$\mathbf{PEC_{soil\ local\ (30\ days)} = 0.24 mg/kg\ wet\ weight}$$

The environmental risk assessment uses this PEC in agricultural soil averaged over the first 30 days after sludge spreading to calculate the local PEC/PNEC ratio for soil organisms.

It is possible to use the AE homologue distribution in sewage sludge, as estimated in Table 4.22 using results based on EUSES calculations, to estimate the concentrations of the individual AE homologues in soil, averaged over the first 30 days after sludge

deposition. The EUSES calculations, although based on appropriate initial tonnage and Koc information, give values which are too high by at least a factor of three, even if the neglect of removal in the anaerobic digester in the EUSES model is taken into account. Conservative estimations in EUSES may not affect all AE homologues equally, and there is thus no guarantee that bias will not occur in the calculated AE homologue distribution. Also, conservative procedures have been used, both in the method of analysis used to obtain the sludge monitoring data for the C12-18 EO4-20 homologues, and in extrapolating this data to C8-11, EO0-3, and EO21-22. Thus the estimated AE homologue concentrations in soil, averaged over the first 30 days

Table 4.23 $PEClocal_{soil}$ averaged over 30 days, in µg/kg wet weight, distributed according to EUSES-based estimations given in Table 4.22.

C/ EO	C8 + C9	10	11	12	13	14	15	16	18
0	0.08	0.16	0.19	2.16	2.30	1.97	1.74	0.47	0.55
1	0.06	0.12	0.15	1.57	1.62	1.36	1.20	0.32	0.38
2	0.10	0.20	0.25	2.58	2.59	2.12	1.86	0.50	0.59
3	0.13	0.24	0.31	3.11	3.07	2.46	2.15	0.57	0.67
4	0.15	0.29	0.36	3.49	3.39	2.65	2.31	0.61	0.72
5	0.18	0.33	0.41	3.88	3.73	2.86	2.48	0.65	0.77
6	0.18	0.34	0.41	3.86	3.66	2.75	2.37	0.63	0.74
7	0.21	0.39	0.46	4.26	3.98	2.96	2.54	0.67	0.79
8	0.22	0.42	0.48	4.35	3.97	2.93	2.51	0.66	0.77
9	0.23	0.43	4.87	4.41	3.95	2.90	2.47	0.65	0.76
10	0.24	0.45	4.96	4.47	3.93	2.86	2.42	0.63	0.75
11	0.23	0.44	4.57	4.10	3.54	2.56	2.16	0.56	0.66
12	0.21	0.39	0.40	3.55	3.01	2.18	1.82	0.47	0.56
13	0.18	0.35	0.35	3.07	2.58	1.86	1.54	0.40	0.47
14	0.16	0.30	0.45	2.55	2.14	1.53	1.26	0.33	0.38
15	0.13	0.25	0.23	2.00	1.66	1.19	0.97	0.25	0.30
16	0.09	0.16	0.15	1.27	1.04	0.74	0.60	0.16	0.18
17	0.09	0.17	0.16	1.30	1.06	0.76	0.61	0.16	0.19
18	0.05	0.09	0.08	0.66	0.54	0.38	0.31	0.08	0.09
19	0.05	0.09	0.08	0.66	0.54	0.38	0.31	0.08	0.09
20	0.05	0.09	0.08	0.66	0.54	0.38	0.31	0.08	0.09
21	0.05	0.09	0.08	0.66	0.54	0.38	0.31	0.08	0.09
22	0.05	0.09	0.08	0.66	0.54	0.38	0.31	0.08	0.09
Sum of AE homologues = $PEC_{soil\ local\ (30\ days)}$ = 0.24mg/kg wet weight									

following sludge deposition and shown in Table 4.23, should be used as a guide only. However, Table 4.23 can provide a first step in informing decision – making strategy, for example, on the possible need for further testing or environmental monitoring.

4.1.2.5 Summary of PEC Values

A summary of the PEC values determined for the different environmental compartments is given below. In addition, table numbers are given for the appropriate AE homologue distributions. This information in these tables is used in the AE risk assessment in Section 4.3.

Environmental Compartment	**PEC value for sum of AE homologues**	***Table for AE homologue values***
Surface water	$PEClocal_{dissolved}$ = 1.01µg/l	*Table 4.18*
Sediment	$PEClocal_{sediment}$ = 1.01 mg/kg wet weight	*Table 4.19*
Sewage treatment plant	PEC_{stp} = 9.8µg/l	*Table 4.20*
Soil	$PEC_{soil\ local\ (30\ days)}$ = *0.24mg/kg wet weight*	*Table 4.23*

4.2. Effects assessment

4.2.1. Aquatic effects

The HERA aquatic effects assessment is based on the results of chronic single species and probabilistic QSARS which have been derived from chronic test data obtained with AE mixtures of known homologue distribution. This enables an effects matrix to be produced which contains the appropriate eco-toxicity entry for each AE homologue. This matrix is then used, together with the matrix containing the PEC values for each AE homologue, in order to carry out the risk assessment process. Using this homologue-based approach overcomes difficulties caused by the changes in the AE homologue distribution caused by the unequal action of degradative and sorptive environmental and treatment processes on the various AE homologues. This results in different homologue distributions between the original AE products and environmental AE resulting from their use.

The aquatic effects assessment is based on chronic AE effects data which has been determined for linear alcohol ethoxylates. However, acute effects data is available for branched AE which establishes that they are not more toxic than the linear AEs with the same number of carbon atoms in the hydrocarbon chain. This data is also presented to establish that the use of linear AE data only in the chronic risk assessment should be an appropriate or conservative approximation for the essentially linear and branched alcohol ethoxylates also covered in this HERA assessment.

The aquatic effects assessment first presents the available acute and chronic test data, and then describes the QSARs based on the chronic data which have been used in the HERA assessment.

4.2.1.1. Acute ecotoxicity information for alcohol ethoxylates

Acute aquatic effects data is presented in this HERA report only to establish that the essentially linear and branched AE used in household detergents are not more toxic than the linear AE which have the same uses. The toxicity mechanism for AE is accepted to be non-polar narcosis (Boeije et al 2006), in which the AE homologues

with longer hydrocarbon chains and higher logKow are more efficient at penetration of the cell membrane, and thus more toxic. However, the AE homologues must be sufficiently soluble in water to allow a toxic concentration to reach the target organism. For the long chain alcohols, generally the least soluble and the most toxic of the AE homologues, the long chain alcohol SIAR (SIAR 2006) shows that the toxicity is restricted by solubility considerations at hydrocarbon chainlengths of 15 and above. Although the addition of ethylene oxide groups makes the other AE homologues more soluble, solubility considerations may reduce the toxicity of several higher hydrocarbon chainlength and lower EO chainlength AE. The available acute toxicity data follows this generally accepted pattern, and also indicates that the linear, essentially linear and branches AE are of similar toxicity.

The Danish EPA review of alcohol ethoxylates (Danish EPA 2001) has reviewed the laboratory test data available in 2001 for algae, invertebrates, and fish. In the review, a distinction is made between linear AE and materials containing some branching, such as the essentially linear AE and those AE with a somewhat higher degree of branching which may be used in some detergent products covered by this HERA assessment. The Danish EPA review concludes that algae are somewhat more sensitive to AE than invertebrates or fish. In addition, the review identifies the long chain alcohols as the most toxic of the AE homologues, and states that products with a narrow range of EO distribution are likely to contain less long chain alcohol, and thus be less toxic.

4.2.1.1.1. Acute toxicity to algae

The Danish EPA (2001) found that the acute toxicity of linear and several branched AE to algae was in the same general range, with EC50 values from 0.05 to 50 mg/l. The review recognized that the reason for the differences in test results may be due to different test conditions and different test species as well as the differences in chemical structure.

The review concludes that, for the linear AE, the toxicity increases with increasing hydrocarbon chain length (comparison of C_{13} EO7-8 and C_{15} EO7-8, Table 4.24) and decreasing EO chain length (comparison of C_{12-14} with 4 to13 EO, Table 4.24). The review also concludes that the toxicity of AE to algae tends to decrease with increasing degree of branching[10], although the data used to reach this conclusion is from specially synthesized AE which have been shown to have a significantly higher toxicity than the AE made from a technical alcohol which are used commercially (Kaluza and Taeger, 1996).

The Danish EPA table containing the data on which these conclusions have been based is given below for linear (and essentially linear) AE. Some chronic data, i. e.

[10] The degree of branching is defined, in Kaluza and Taeger (1996), as 100 minus (% linearity), where the % linearity of the hydrocarbon chain is defined as 100 times the number of carbon atoms in the longest linear chain up to the first site of branching, divided by the total number of carbon atoms in that chain.

algal NOEC values, are also given in the table. The data are given a Klimisch[11] score of 4, as being from a secondary reference, unless the papers have been evaluated as part of this HERA assessment. The Danish EPA table containing data for branched compounds is not appropriate for products used in HERA applications. However, literature data for branched AE may be found in table 4.26.

Table 4.24
Effects of linear AE to algae. (Danish EPA 2001), (Klimisch score 4, unless otherwise stated)

Species	**AE**	**EC50 (mg/l)**	**Test Duration**	**Reference**[†]
Selenastrum capricornutum	C_{12-14} EO4	2-4	48 h	Yamane *et al.* 1984
Selenastrum capricornutum	C_{12-14} EO9	4-8	48 h	Yamane *et al.* 1984
Selenastrum capricornutum	C_{12-14} EO13	10	48 h	Yamane *et al.* 1984
Nitzschia fonticula	C_{12-14} EO9	5-10	48 h	Yamane *et al.* 1984
Microcystis aeruginosa	C_{12-14} EO9	10-50	72 h	Yamane *et al.* 1984
Scenedesmus subspicatus	C_{12-14} EO7	0.5 (EC_b50)	72 h	Kaluza and Taeger 1996 (Klimisch score 2)
Selenastrum capricornutum	C_{12-15} EO7	0.85 (95% confidence interval 0.84-0.85) NOEC:0.50	72 h	Madsen *et al.* 1996b
Scenedesmus subspicatus	*C_{13} EO7-8	0.5 (EC_b50)	72 h	Kaluza and Taeger 1996 (Klimisch score 2)
Scenedesmus subspicatus	C_{13-15} EO7-8	0.5 (EC_b50)	72 h	Kaluza and Taeger 1996 (Klimisch score 2)

[11] Klimisch Scores are 1 = valid without restriction; 2 = valid with restriction; 3 = not reliable; 4 = not assignable. Further information and guidance on the selection of data for HERA reports is given in Appendix C of the HERA Methodology Document (HERA 2005).

Species	AE	EC50 (mg/l)	Test Duration	Reference[†]
Scenedesmus subspicatus	C_{14-15} EO6	0.09	96 h	Lewis and Hamm 1986
Microcystis aeruginosa	C_{14-15} EO6	0.60	96 h	Lewis and Hamm 1986
Navicula pelliculosa	C_{14-15} EO6	0.28	96 h	Lewis and Hamm 1986
Scenedesmus subspicatus	*C_{15} EO7-8	0.05 (EC_b50)	72 h	Kaluza and Taeger 1996 (Klimisch score 2)
Selenastrum capricornutum	**C_{12-15} EO9	0.7	96 h	Dorn *et al.* 1993 (Klimisch score 2)

[†] Further information concerning the references may be found in Danish EPA (2001). reference information

* especially synthezised linear lab sample

** described by Dorn et al. as an essentially linear AE

The Danish EPA (2001) data can be supplemented by other publications and unpublished company data which also establish that the toxicity of linear and essentially linear AE to algae is very similar, especially if the variability between different test conditions is taken into consideration. The toxicity of branched AE to algae is lower. These data are given in tables 4.25 and 4.26, below.

Table 4.25. Acute and chronic algal ecotoxicity data for linear alcohol ethoxylates

AE C Chain description	AE EO chain description	Test Method	EC_b50 and/or EC_r50 mg/l	NOEC mg/l	Reference (Klimisch score)
C8	EO4 mean	72 hour Growth rate for the marine alga *Sleletonema costatum*	EC_r50 = 43.72	Not Given	Cognis/ Henkel 1998c (2)
C10	EO8 Specific homologue	*Scenedesmus subspicatus* growth. 72 hr 92/69/EEC	EC_b50 = 14 EC_r50 = 45	10	Cognis/ Henkel 1997f (2)

AE C Chain description	AE EO chain description	Test Method	EC_b50 and/or EC_r50 mg/l	NOEC mg/l	Reference (Klimisch score)
C11	EO5 mean	*Selenastrum capricornutum* growth after 96 hours (biomass)	EC_b50 = 2.9 and 3.5 (two distinct tests)	2.1 and 1.2 (two distinct tests)	Dorn et al 1988 (2)
C12	EO2 Specific homologue	*Scenedesmus subspicatus* growth. 72 hr 92/69/EEC	EC_b50 = 0.18 EC_r50 = 0.43	0.09	Cognis/ Henkel 1997c (2)
C12	EO4 Specific homologue	*Scenedesmus subspicatus* growth. 72 hr 92/69/EEC	EC_b50 = 0.92 EC_r50 = 1.6	0.26	Cognis/ Henkel 1997b (2)
C12	EO 4 mean	*Scenedesmus subspicatus* growth 92/69/EEC	EC_b50 = 1.4 EC_r50 = 1.8	0.4	Sasol 1997a (2)
C12	EO8 Specific homologue	*Scenedesmus subspicatus* growth. 72 hr 92/69/EEC	EC_b50 = 2.2 EC_r50 = 15	<0.15	Cognis/ Henkel 1997 a (2)
C12, 14	EO 2 mean	*Scenedesmus subspicatus* growth. 72 hr 92/69/EEC	EC_b50 = 0.13 EC_r50 = 0.57	0.035	Cognis/ Henkel (1996a) (2)
C12, 14	EO 2 mean	*Scenedesmus subspicatus* growth	$^{P}EC_b50$ 0.1937 $^{P}EC_r50$ <0.250 $^{A}EC_b50$ 0.134	0.05(P) 0.0365 (A)	Sasol 1993d (2)
C12, 14	EO3 mean	*Scenedesmus subspicatus* growth. 72 hr 92/69/EEC	EC_b50 = 0.46 EC_r50 = 1.2	0.088	Cognis/ Henkel 1998d (2)
C12, 14	EO3 mean	*Scenedesmus subspicatus* growth 92/69/EEC	EC_b50 = 0.3 EC_r50 = 0.5	<0.1	Sasol 1997b (2)

AE C Chain description	AE EO chain description	Test Method	EC_b50 and/or EC_r50 mg/l	NOEC mg/l	Reference (Klimisch score)
C12, 14	EO4 mean	*Scenedesmus subspicatus* growth, 72 hr EU 92/69/EWG	EC_b50 = 0.53 EC_r50 = 0.87	0.22	Cognis/ Henkel 1998e (2)
C12, 14	EO4 mean	*Scenedesmus subspicatus* growth	$^{P}EC_b50$ 0.39 $^{P}EC_r50$ 0.70 $^{A}EC_b50$ 0.35 $^{A}EC_r50$ 0.62	0.1(P) 0.09 (A)	Sasol 1993c (2)
C12, 14	EO6 mean	*Scenedesmus subspicatus* growth, 72 hr 92/69/EEC	EC_b50 = 0.7 EC_r50 = 1.3	0.19	Cognis/ Henkel (1998a) (2)
C12, 14	EO6 mean	*Scenedesmus subspicatus* growth 92/69/EEC	EC_b50 0.92 EC_r50 1.5	0.4	Sasol 1997c (2)
C12, 14	EO6 mean (Different product blend from above)	*Selenastrum capricornutum* Growth OECD 201	EC_r50 3.8	EC10 = 1.5	Sasol 1994d (2)
C12, 14	EO7 mean	*Scenedesmus subspicatus* growth, 72 hr 92/69/EEC	EC_{50} = 3.1 mg/L EC_b50 1.7 EC_r50 3.1	0.85	Cognis/ Henkel (1998b) (2)
C12, 14	EO7 mean	*Scenedesmus subspicatus* growth	$^{P}EC_b50$ 0.89 $^{P}EC_r50$ 1.23 $^{A}EC_b50$ 0.85 $^{A}EC_r50$ 1.18	0.36(P) 0.32 (A)	Sasol 1993b (2)
C12, 14	EO9 mean	*Scenedesmus subspicatus* growth. 72 hr	EC_b50 3.5 EC_r50 6.5	1.4	Cognis/ Henkel (1996b) (2)

AE C Chain description	AE EO chain description	Test Method	EC_b50 and/or EC_r50 mg/l	NOEC mg/l	Reference (Klimisch score)
C12, 14	EO9 mean	*Scenedesmus subspicatus* growth	EC_b50 1.2 EC_r50 2.2	0.4	Sasol 1995a (2)
C12, 14	EO15 mean	*Scenedesmus subspicatus* growth, 72 hr EU 92/69/EWG	EC_b50 = 2.6 EC_r50 = 6.5	0.97	Cognis/ Henkel (1998f) (2)
C16	EO2 Specific homologue	*Scenedesmus subspicatus* growth. 72 hr 92/69/EEC	EC_b50 >100 EC_r50 >100	100	Cognis/ Henkel 1997 d (2)
C16	EO8 Specific homologue	*Scenedesmus subspicatus* growth. 72 hr 92/69/EEC	EC_b50 0.35 EC_r50 0.64	0.25	Cognis/ Henkel 1997 e (2)
C16, 18	11 EO mean	*Scenedesmus subspicatus* growth	EC_b50 0.85 EC_r50 0.37	0.1	Sasol 1993e (2)
C16, 18	25 EO mean	*Scenedesmus subspicatus* growth	EC_b50 >990 EC_r50 >990	>990	Sasol 1994c (2)

[A]Based on active substance concentration. [P]Based on product concentration.

Table 4.26. Acute and chronic algal ecotoxicity data for essentially linear and branched alcohol ethoxylates used in household detergents

AE Hydrocarbon Chain description	AE EO chain description	Test Method	EC50 mg/l	NOEC mg/l	Reference (Reliability)
C9-11 essentially linear	Alcohol (EO = 0)	50% reduction in *Selenastrum capricornutum* relative growth rate between days 2 and 4	2.7		Shell Research Ltd 1982c (2)

AE Hydrocarbon Chain description	**AE EO chain description**	**Test Method**	**EC50 mg/l**	**NOEC mg/l**	**Reference (Reliability)**
C9-11 essentially linear	2.5 EO mean	50% reduction in *Selenastrum capricornutum* relative growth rate at day 4	1.4		Shell Research Ltd 1984 (2)
C9-11 essentially linear	8 EO mean	50% reduction in *Selenastrum capricornutum* relative growth rate between days 2 and 4	47		Shell Research Ltd 1981a (2)
C12-15 essentially linear	3 EO mean	50% reduction in *Selenastrum capricornutum* growth rate between days 2 and 4	0.74		Shell Research Ltd 1982d (2)
C12-15 essentially linear	7 EO mean	50% reduction in *Selenastrum capricornutum* relative growth rate between days 2 and 4 EPA-60/9-78-018	EC_r50 = 2.9 mg/L	1.0	Shell Research Ltd 1981b (2)
C12-15 Essentially linear	9 EO mean	*Selenastrum capricornutum* 96 hour test, cell count	0.7 mg/ L	1	Dorn et al. 1993 (2)
C12-15 essentially linear	EO9 mean	Microtox test using *Photobacterium phosphoreum*	5 minute EC50	1.5	Dorn et al. 1993 (2)
C13 branched	EO3 mean	*Scenedesmus subspicatus* growth 92/69/EEC	EC_b50 2.3 EC_r50 2.5	1.7	Sasol 1994e (2)
C13 branched	EO4 mean	*Scenedesmus subspicatus*	EC_b50 2.8 EC_r50	1.2	Sasol 1995b (2)

AE Hydrocarbon Chain description	AE EO chain description	Test Method	EC50 mg/l	NOEC mg/l	Reference (Reliability)
		growth 92/69/EEC	4.3		
C13 branched	EO6 mean	*Scenedesmus subspicatus* growth 92/69/EEC	EC_b50 7.2 EC_r50 8.2	2.4	Sasol 1994a (2)
C13 branched	EO 7-8 mean	*Scenedesmus subspicatus* growth DIN 38412 part 9	EC_b50 5		Kaluza andTaeger 1996 (2)
C13 branched	EO9 mean	*Scenedesmus subspicatus* growth 92/69/EEC	EC_b50 11 EC_r50 17	6	Sasol 1994b (2)
C13-15 essentially linear	7 EO mean	Scenedesmus *subspicatus*, 72h, OECD 201	EC_b50 0.623 mg/l EC_r50 1.1	0.039	BASF 1991a (2)
C13C15 oxo	7-8 EO	*Scenedesmus subspicatus* growth DIN 38412 part 9	EC_b50 0.5		Kaluza andTaeger 1996 (2)
C13-15 essentially linear	11 EO mean	Scenedesmus *subspicatus* , 72h, OECD 201	EC_b50 = 1.27 ECr50 = 3.77 mg/l	0.078	BASF 1991b (2)
C14, C15 Essentially linear	7 EO mean	*Selenastrum capricornutum* growth rate reduction between days 2 and 4	ECr50 = 0.95 mg/l		Shell Research Ltd. 1982e (4)

AE Hydrocarbon Chain description	AE EO chain description	Test Method	EC50 mg/l	NOEC mg/l	Reference (Reliability)
C14, C15 essentially linear	EO8 mean	*Scenedesmus subspicatus* growth	EC_b50 0.63 EC_r50 1.31	0.1	Sasol 1993a (2)
C14, C15 essentially linear	EO8 mean	*Selenastrum capricornutum* Growth OECD 201	EC_b50 0.38 EC_r50 0.76	<0.1	Sasol 1999f (2)
C14, C15 Essentially linear	EO11 mean	*Selenastrum capricornutum* growth ratebetween days 2 and 4	EC_r50 4.2		Shell Research Ltd. 1981d (4)

In conclusion, the available acute data for algae show that the branched AE which may be used in household detergent products do not have a higher toxicity than that of the linear and essentially linear AE containing the same number of carbon atoms in their hydrophobic chains and similar numbers of EO groups. Thus it would be expected that use of eco-toxicity data derived from linear AE would be appropriate for both the essentially linear AE and for the branched AE, in the environmental risk assessment process. In addition, the comparison of the C8EO4 and the C10EO8 indicates that, as expected from theoretical considerations, C8EO4 should not be more toxic than C10EO8. Thus it would be appropriate to group the low tonnage C8 material with the higher tonnage C9 AE in the risk assessment procedure.

4.2.1.1.2. Acute toxicity to Invertebrates

The Danish EPA (2001) found that the acute toxicity of AE to invertebrates varies, with EC50 values from 0.1 mg/l to more than 100 mg/l for linear AE and from 0.5 mg/l to 50 mg/l for branched AE. The toxicity is species specific and may vary between 0.29 mg/l and 270 mg/l for the same linear AE (Lewis and Suprenant 1983, quoted in Danish EPA 2001). The most commonly used invertebrates for testing are *Daphnia magna* and *Daphnia pulex*, and they are also among the most sensitive invertebrates to AE. The Danish EPA (2001) found that some AE are very toxic to invertebrates, i.e., linear AE of C_{12-15} EO1-8 and branched AE with a low degree of branching, i.e. < 10-25%. They concluded that branching of the alkyl chain reduces the toxicity of AE to invertebrates, as also observed for algae (Danish EPA 2001). However, the data used to reach this conclusion is from specially synthesized AE which have been shown to have a significantly higher toxicity than the AE made from a technical alcohol which are used commercially (Kaluza and Taeger, 1996).

The Danish EPA table containing the data on which these conclusions have been based for linear AE is given in Table 4.27. The data are given a Klimisch score of 4,

as being from a secondary reference, unless the papers have been studied as part of this HERA assessment. Any AE which are not used in household detergent products have been removed from the Danish EPA (2001) tables. The Danish EPA table containing the data on which these conclusions have been based is given below for linear AE. The Danish EPA table containing data for branched compounds is not appropriate for products used in HERA applications. However, literature data for branched AE used in household detergents may be found in table 4.29.

Table 4.27 *Effects of linear AE to invertebrates.Table from Danish EPA 2001. (Klimisch score 4, unless otherwise stated)*

Species	**AE**	**EC/LC50 (mg/l)**	**Test Duration**	**Danish EPA Reference**[††]
Hyalella azteca	C_{9-11} EO6	14	10 d	[†]Dorn *et al*. 1997
Chironomus tentans	C_{9-11} EO6	5.7	10 d	[†]Dorn *et al*. 1997
Mysidopsis bahia	C_{10} EO4	5.6	48 h	Hall *et al*. 1989
Daphnia magna	C_{12-13} EO5 C_{12-13} EO4.5-6 C_{12-13} EO6.5	0.46 (0.39-0.56)* 0.59 (0.42-0.83)* 0.74 (0.63-0.86)*	48 h 48 h 48 h	[†]Wong *et al*. 1997** (2)
Daphnia magna	C_{12-14} EO7-8	0.5	48 h	Kaluza and Taeger 1996 (2)
Daphnia pulex	C_{12-15} EO7	0.76	48 h	Salanitro *et al*. 1988
Daphnia magna	C_{12-15} EO7	1.0-2.0	48 h	Madsen *et al*. 1996b
Daphnia magna	C_{12-15} EO9	1.3 (1.1-1.4)* NOEC: 1.0	48 h	[†]Dorn *et al*. 1993** (2); Kravetz *et al*. 1991
Daphnia magna	C_{13} EO7-8	0.5	48 h	Kaluza and Taeger 1996 (2)
Mysidopsis bahia	C_{13} EO10	2.2	48 h	Hall *et al*. 1989

Species	**AE**	**EC/LC50 (mg/l)**	**Test Duration**	**Danish EPA Reference**[††]
Daphnia magna	C_{13-15} EO7-8	0.5	48 h	Kaluza and Taeger 1996 (2)
Daphnia magna	C_{14} EO1 C_{14} EO2 C_{14} EO3 C_{14} EO4 C_{14} EO6 C_{14} EO9	0.83 1.53 0.73 1.76 4.17 10.07	48 h	Maki and Bishop 1979
Daphnia pulex	C_{14} EO1 C_{14} EO4	0.10 0.21	48 h	Maki and Bishop 1979
Daphnia magna	C_{14-15} EO7	0.29-0.4	48 h	Lewis and Perry 1981
Paratanytarus parthenogenica (midge)	C_{14-15} EO7	23	48 h	Lewis and Suprenant 1983
Gammarus sp. (amphipod)	C_{14-15} EO7	3.3	48 h	Lewis and Suprenant 1983
Asellus sp. (isopod)	C_{14-15} EO7	270	48 h	Lewis and Suprenant 1983
Dugesia sp. (flatworm)	C_{14-15} EO7	1.8	48 h	Lewis and Suprenant 1983
Dero sp. (oligochaete)	C_{14-15} EO7	1.7	48 h	Lewis and Suprenant 1983
Rhabditis sp. (nematode)	C_{14-15} EO7	16	48 h	Lewis and Suprenant 1983
Daphnia magna	C_{14-15} EO13	1.2 (0.65-1.9)*	48 h	[†]Wong *et al*. 1997 ** (2)
Daphnia magna	C_{15} EO7-8	0.5	48 h	Kaluza and Taeger 1996 ** (2)

Species	AE	EC/LC50 (mg/l)	Test Duration	Danish EPA Reference††
Daphnia	C_{16-18} EO2-4	20-100	-	Schöberl *et al*. 1988
Daphnia	C_{16-18} EO5-7	5-200	-	Schöberl *et al*. 1988
Daphnia	C_{16-18} EO10-14	40-60	-	Schöberl *et al*. 1988
Daphnia magna	C_{16-18} EO18	20	48 h	Talmage 1994
Daphnia magna	C_{16-18} EO30	18	48 h	Talmage 1994

* Parentheses indicate 95% confidence intervals. **Paper evaluated and given Klimisch score of 2 in this HERA assessment. †Note that these AE are essentially linear. †† Further information concerning these references may be found in Danish EPA (2001).

The Danish EPA (2001) data can be supplemented by company and literature data which also establishes that the toxicity of linear and essentially linear AE to invertebrates is very similar, especially if the variability between different test conditions is taken into consideration. The toxicity of the branched AE to invertebrates is not higher than that of the linear and essentially linear AE of similar hydrocarbon and EO chainlengths. These data are given in tables 4.28 and 4.29.

Table 4.28. Acute invertebrate ecotoxicity data for linear alcohol ethoxylates

AE C Chain description	AE EO chain description	Test Method	EC50 mg/l	Less than 10% effect, mg/l	Reference (Klimisch score)
C8	EO4 mean	OECD 202, part 1 (24 hr static test with *Daphnia magna)*	71	Not Given	Cognis/ Henkel D (4)
C8	EO4 mean	48 hr static test with *Acartia tonsa* (marine invertebrate)	17.2	Not Given	Cognis 2001 (2)
*C11	EO5 mean	48 hr static (with 24 hr renewal) test with *Daphnia magna*	4.1 and 3.6 (results of two tests)	Not Given	Dorn et al 1988 (2)
C12	4 EO mean	EG/92/69/EWG 48 hr test	EC50 = 0.91 mg/l (Range 0.7 – 1.2)	0.7	Sasol Germany GmbH 1997d (2)

AE C Chain description	AE EO chain description	Test Method	EC50 mg/l	Less than 10% effect, mg/l	Reference (Klimisch score)
C12, 14	EO 2 mean	48 hr. *Daphnia magna* immobilisation, static test EG 92/69/EWG	0.53	0.2	Sasol 1993f (2)
C12, 14	EO 2 mean	*Daphnia*	EC_{50} Range = 1 - 10 mg/L		Cognis R9801528 Unpublished report (4)
C12, 14	EO3 mean	48 hr. *Daphnia magna* immobilisation, static test EG 92/69/EWG	0.80 Range 0.6-1.0)	0.6	Sasol 1997f (2)
C12, 14	EO3 mean	*Daphnia*	EC_{50} = 1 - 10		Cognis/ Henkel A Unpublished report (4)
C12, 14	EO4 mean	48 hr. *Daphnia magna* immobilisation, static test EG 92/69/EWG	0.63 (95% confidence limits 0.53-0.75)	0.31	Sasol 1993g (2)
C12, 14	EO4 mean	*Daphnia*	EC_{50} = 1 - 10		Cognis / Henkel B Unpublished report (4)
C12, 14	EO6 mean	*Daphnia magna* Immobilisation, 48 hr static test, EU 92/69/EWG	EC_{50} = 1.2 mg/L;	EC_0 =0.22	Cognis/ Henkel 1999a (2)
C12, 14	EO6 mean	48 hr. *Daphnia magna* immobilisation, static test EG 92/69/EWG	1.4 (Range 1.2 – 1.7)	Less than lowest test concn. of 1.2	Sasol 1997e (2)
C12, 14	EO7 mean	*Daphnia magna* Immobilisation, 48 hr static test, OECD 202/1	EC_{50} = 1.4 mg/L;		Cognis/ Henkel 2000 (2)

AE C Chain description	AE EO chain description	Test Method	EC50 mg/l	Less than 10% effect, mg/l	Reference (Klimisch score)
C12, 14	EO7 mean	48 hr. *Daphnia magna* immobilisation, static test EG 92/69/EWG	1.2	1.0	Sasol 1993h (2)
C12, 14	EO9 mean	48 hr. *Daphnia magna* immobilisation, static test EG 92/69/EWG	1.9	1.4	Sasol 1995c (2)
C12-18	EO9 mean	48 hr static test with *Daphnia magna Straus* EU 92/69/EWG	2.7 mg/l	EC_0 = 0.92 mg/l	Cognis/ Henkel 1995b (2)
C16, 18	11 EO mean	48 hr. *Daphnia magna* immobilisation, static test EG 92/69/EWG	0.72	0.3	Sasol 1994f (2)
C16, 18	25 EO mean	48 hr. *Daphnia magna* immobilisation, static test EG 92/69/EWG	117 (95% confidence limits 85-161)	28	Sasol 1994g (2)

* not clear, if pure linear C-chain

Table 4.29 Acute invertebrate ecotoxicity data for essentially linear and branched alcohol ethoxylates used in detergent applications

AE C Chain description	AE EO chain description	Test Method	EC50 mg/l	Reference (Klimisch score)
C9-11 essentially linear	Alcohol (EO = 0)	48 hour static test with *Daphnia magna*	EC50 = 8.5 mg/l	Shell Research Ltd (1982c) (2)
C9-11 essentially linear	2.5 EO mean	48 hour static test with *Daphnia magna*	EC50 = 2.5 mg/l	Shell Research Ltd (1984d) (2)
C9-11 essentially linear	2.5 EO mean	96 hr semi-static test in synthetic sea water with *Crangon crangon*	LC50 = 9.9 mg/l	Shell Chemicals Ltd (1990a) (2)

AE C Chain description	AE EO chain description	Test Method	EC50 mg/l	Reference (Klimisch score)
C9-11 essentially linear	5 EO mean	48 hour static test with *Daphnia magna*	EC50 = 5.1 mg/l	Uniqema 1985d (4)
C9-11 essentially linear	6 EO mean	48 hr *Daphnia magna* static test based on standard ASTM and US-EPA methods	EC50 = 5.3 mg/l	Wong et al (1997) (2)
C9-11 essentially linear	2.5 EO mean	96 hr semi-static test in synthetic sea water with *Crangon crangon* (brown shrimp)	EC50 = 17 mg/l	Shell Chemicals Ltd (1990b) (2)
C9-11 essentially linear	8 EO mean	48 hr *Daphnia magna* static test based on standard ASTM and US-EPA methods	EC50 = 12 mg/l	Wong et al (1997) (2)
C9-11 essentially linear	8 EO mean	48 hour static test with *Daphnia magna*	EC50 = 9 mg/l	Shell Resarch Ltd (1991c) (1)
C9-11 essentially linear	8 EO mean	48 hour static test with *Daphnia magna*	EC50 = 0.7 mg/l	Shell Research Ltd (1981a) (2)
C9-11 essentially linear	8 EO mean	48 hour static test with *Daphnia magna*	EC50 = 13.4 mg/l	Uniqema 1985e (2)
C9-11 essentially linear	10 EO mean	48 hour static test with *Daphnia magna*	EC50 = 13.4 mg/l	Uniqema 1985f (2)
C12-13 essentially linear	4.5 EO mean	48 hr *Daphnia magna* static test based on standard ASTM and US-EPA methods	EC50 = 0.59 mg/L	Wong et al (1997) (2)
C12-13 essentially linear	5 EO mean	48 hr *Daphnia magna* static test based on standard ASTM and US-EPA methods	EC50 =0.46 mg/L	Wong et al (1997) (2)
C12-13 essentially linear	6.5 EO mean	48 hr *Daphnia magna* static test based on standard ASTM and US-EPA methods	EC50 =0.74 mg/L	Wong et al (1997) (2)

AE C Chain description	AE EO chain description	Test Method	EC50 mg/l	Reference (Klimisch score)
C12-13 essentially linear	7 EO mean	48 or 72 hour static test	EC50 = 1.9 mg/L;	Sasol 1993k (4)
C12-15 essentially linear	3 EO mean	48 hr static test with *Daphnia magna*	EC50 =0.14 mg/L	Shell Research Ltd 1982d (2)
C12-15 essentially linear	7 EO mean	OECD 202 48 hr static test with *Daphnia magna*	EC50 = 4.3 mg/L;	Sasol 1993 l (2)
C12-15 essentially linear	7 EO mean	48 hr static test with *Daphnia magna*	EC50 =0.4 mg/L	Shell Research Ltd 1981b (2)
C12-15 essentially linear	9 EO mean	48 hour static test with *Daphnia magna* EG 92/69 EWG	EC_{50} = 1.9 mg/L;	Sasol 1995m (2)
C12-15 essentially linear	9 EO mean	48 hr static test with daily solution replacement	EC50 =1.3 mg/L	Dorn et al (1993) (2)
C12-15 essentially linear	12 EO mean	48 hr *Daphnia magna* static test based on standard ASTM and US-EPA methods	EC50 =1.4 mg/L	Wong et al (1997) (2)
C13 branched	3 EO mean	OECD 202 – 48 hr test with *Daphnia magna*	EC50 = 1.5 mg/l	Sasol 1994j (2)
C13 branched	4 EO mean	OECD 202 – 48 hr test with *Daphnia magna*	EC50 = 1.2 mg/l	Sasol 1995e (2)
C13 branched	6 EO mean	OECD 202 – 48 hr test with *Daphnia magna*	EC50 = 2.5 mg/l	Sasol 1994k (2)
C13 branched	7-8 EO mean	EC/79/381/EEC 48 hour test with *Daphnia magna*	EC50 = 5 mg/l	Kaluza and Taeger (1996) (2)
C13 branched	9 EO mean	OECD 202 – 48 hr test with *Daphnia magna*	EC50 = 4.7 mg/l	Sasol 1994i (2)
C13-15 essentially linear	3 EO mean	OECD 202 – 48 hr static test with *Daphnia magna Straus*	EC50 = 0.406 mg/l	BASF 1990a (2)

AE C Chain description	AE EO chain description	Test Method	EC50 mg/l	Reference (Klimisch score)
C13-15 mixture of linear, essentially linear, and branched	3 EO mean	OECD 202 – 48 hr test with *Daphnia magna*	EC50 = 0.67 mg/l (nominal)	Sasol 2001c (2)
C13-15 mixture of linear, essentially linear, and branched	6 EO mean	OECD 202 – 48 hr test with *Daphnia magna*	EC50 = 1.32 mg/l (nominal)	Sasol 2001d (2)
C13C15 oxo	7-8 EO mean	EC/79/381/EEC 48 hour test with *Daphnia magna*	EC50 = 0.5 mg/l	Kaluza and Taeger (1996) (2)
C13-15 mixture of linear, essentially linear, and branched	9 EO mean	OECD 202 – 48 hr test with *Daphnia magna*	EC50 = 1.96 mg/l (nominal)	Sasol 2001e (2)
C13-15 essentially linear	7 EO mean	OECD 202 – 48 hr static test with *Daphnia magna Straus*	EC50 = 0.94 mg/l	BASF 1990b (2)
C13-15 essentially linear	11 EO mean	OECD 202 – 48 hr static test with *Daphnia magna Straus*	EC50 = 1.5 mg/l	BASF 1992 (2)
C14-15 essentially linear	7 EO mean	48 hr static test without renewal, using *Daphnia magna*	EC50 = 0.24 mg/l	Shell Research Ltd. 1982e (4)
C 14-15 mixture of linear and essentially linear	8 EO mean	OECD 202 – 48 hr test with *Daphnia magna*	EC50 = 1.06 mg/l	Sasol 1999e (2)
C14-15 essentially linear	11 EO mean	48 hour static test without renewal, using *Daphnia magna*	EC 50 = 1.1 mg/l	Shell Research Ltd. 1981d (4)

In conclusion, the toxicity of linear and essentially linear AE to invertebrates is similar, especially if the variability between different test conditions is taken into consideration. In addition, branched AE are not more toxic to invertebrates. Thus it would be expected that use of eco-toxicity data derived from linear AE would be appropriate for the essentially linear AE and branched AE, in the environmental risk assessment process. In addition, the acute data for linear C8 and essentially linear C9-

11 AE indicate the lower toxicity of the C8 AE. Thus it is conservative to include the low tonnage of C8 AE as part of the C9 tonnage in the risk assessment.

4.1.1.1.3. Acute toxicity to fish

The Danish EPA (2001) found that the acute toxicity of AE to fish varies, with LC50 values from 0.4 mg/l to more than 100 mg/l for linear AE and from 0.25 mg/l to 40 mg/l for branched AE. For linear AE the toxicity was found to decrease with increasing numbers of EO units. The Danish EPA found only a few data on the toxicity of branched AE to fish, and none of these are used in household cleaning applications. Data in table 4.30 is given the Klimisch score of 4, as it is from a secondary reference, unless the paper has been reviewed as part of the HERA process.

Table 4.30 Effects of linear AE to fish. Table from Danish EPA 2001. (Klimisch score 4, unless otherwise stated)

Species	AE	LC50 (mg/l)	Test Duration	Reference[†]
Bluegill sunfish (*Lepomis macrochirus)*	C_{10-12} EO6	6.4	96 h	Macek and Krzeminski 1975
Fathead minnow (*Pimephales promelas)*	C_{12-13} EO5 C_{12-13} EO4.5-6 C_{12-13} EO6.5	1.0 (0.84-1.3)[A] 0.96 (0.73-1.6)[A] 1.3 (0.72-2.7)[A]	96 h	Wong *et al*. 1997 (2)
Brown trout *(Salmo trutta)*	C_{12-14} EO8 C_{12-14} EO10-11	0.8 0.8	96 h	Reiff *et al.* 1979
Golden orfe *(Idus idus melanotus)*	C_{12-14} EO8 C_{12-14} EO10-11	1.8 4.1	96 h	Reiff *et al.* 1979
Harlequin fish *(Rasbora heteromorpha)*	C_{12-14} EO10-11	1.6-2.8	96 h	Reiff *et al.* 1979
Zebra fish *(Brachydanio rerio)*	C_{12-15} EO7	1.0-2.0	96 h	Madsen *et al.* 1996
Bluegill sunfish	C_{12-15} EO3	1.5	96 h	Macek and Krzeminski 1975
Fathead minnow	C_{12-15} EO7	0.48	96 h	Salanitro *et al.* 1988
Fathead minnow	C_{12-15} EO9	1.6 (1.3-1.8)[A] NOEC: 0.4	96 h	Dorn *et al.* 1993 (2); Kravetz *et al.*

Species	**AE**	**LC50 (mg/l)**	**Test Duration**	**Reference**[†]
				1991
Bluegill sunfish	C_{12-15} EO9	2.1	96 h	Macek and Krzeminski 1975
Atlantic salmon (*Salmo salar*)	C_{12} EO4 C_{12} EO23	1.5 25.0	96 h	Wildish 1972
Bluegill sunfish	C_{13} EO9	7.5	96 h	Macek and Krzeminski 1975
Rainbow trout *(Salmo gairdneri)*	C_{14-15} EO7	0.78	96 h	Turner *et al.* 1985
Rainbow trout	C_{14-15} EO11	1.08	96 h	Turner *et al.* 1985
Rainbow trout	C_{14-15} EO18	5.0-6.3	96 h	Talmage 1994
Bluegill sunfish	C_{14-15} EO7	0.66	96 h	Lewis and Perry 1981
Bluegill sunfish	C_{14-15} EO7	0.7-1.12	96 h	Lewis and Suprenant 1983
Fathead minnow	C_{14-15} EO7	0.63-1.65	96 h	Lewis and Suprenant 1983
Fathead minnow	C_{14-15} EO13	1.0 (0.62-1.9)[A]	96 h	Wong *et al*. 1997 (2)
Not indicated	C_{16-18} EO2-4	> 100	-	Schöberl *et al.* 1988
Not indicated	C_{16-18} EO5-7	3-30	-	Schöberl *et al.* 1988
Not indicated	C_{16-18} EO10-14	1.7-3	-	Schöberl *et al.* 1988
Brown trout	Tallow EO14	0.4	96 h	Reiff *et al.* 1979
Golden orfe	Tallow EO14	2.3	96 h	Reiff *et al.* 1979

Species	AE	LC50 (mg/l)	Test Duration	Reference[†]
Harlequin fish	Tallow EO14	0.7	96 h	Reiff *et al.* 1979

[A] Parentheses indicate 95% confidence intervals. [†]Further information concerning these references may be found in Danish EPA(2001)

The Danish EPA (2001) data can be supplemented by published and company data which also establish that the toxicity of linear and essentially linear AE to fish is very similar, especially if the variability between different test conditions is taken into consideration. The branched AE do not show a higher toxicity to fish. These data are given in tables 4.31 and 4.32.

Table 4.31 Acute Fish Ecotoxicity Data for Linear Alcohol Ethoxylates

AE C Chain description	AE EO chain description	Test Method	EC50 mg/l	Reference (Klimisch score)
C8 linear	**4 EO mean**	ISO 7346/2 (semi static, 96 hour test with Zebra fish)	LC50 = 38 mg/l	Cognis 2006 (4)
C11	**5 EO mean**	96 hr test with *Pimephales promelas*, following US EPA and APHA guidelines	LC50 = 1.6 and 2.0 mg/l (two distinct tests)	Dorn et al (1988) (2)
C12 linear	**4 EO mean**	96h, Cyprinus carpio, EG/ 92/69 part C1	LC50 = 1.2 mg/l	Sasol 1997k (2)
C12-14 linear	**2 EO mean**		LC_{50} = 1 - 10 mg/L;	Cognis R9801527 Unpublished report (4)
C12-14 linear	**2 EO mean**	96h, *Brachydanio rerio*, semi-static EG 92/69 C1	LC_{50} = 1.2 mg/L;	Sasol 1995k (2)
C12-14 linear	**3 EO mean**		LC_{50} = 1 - 10 mg/L	Cognis R9601616 Unpublished report (4)
C12-14 linear	**3 EO mean**	96h, Cyprinus carpio, EG/ 92/69 part C1	LC_{50} = 0.8 mg/L	Sasol 1997m. (2)
C12-14 linear	**4 EO mean**		LC_{50} = 1 - 10 mg/L;	Cognis / Henkel C Unpublished report (4)
C12-14 linear	**4 EO mean**	96h, *Brachydanio rerio*, semi-static EG 92/69 C1	LC_{50} = 1.3 mg/L;	Sasol 1996a (2)
C12-14 linear	**6 EO mean**	96h, *Brachydanio rerio*, semi-static, ISO 7346/II	LC_{50} = 2.0 mg/L;	Cognis/ Henkel (1993) (2)

AE C Chain description	AE EO chain description	Test Method	EC50 mg/l	Reference (Klimisch score)
C12-14 linear	**6 EO mean**	96h, Cyprinus carpio, 92/69/EEC part C1	LC_{50} = 1.2 mg/L;	Sasol 1997l (2)
C12-14 linear (different product from above)	**6 EO mean**	96 hr test with zebrafish. OECD 203	LC_{50} = 1.5 mg/L	Sasol 1994l (2)
C12-14 linear	**7 EO mean**	96h, *Brachydanio rerio*, semi-static EG 92/69 C1	LC_{50} = 2.6 mg/L;	Sasol 1995l (2)
C12-14 linear	**9 EO mean**	96h, Cyprinus carpio, 92/69/EEC part C1	LC_{50} = 3 mg/L;	Sasol 1997i. (2)
C12-14 linear	**12 EO mean**	Semi static (daily change of test medium) 96h, Brachydanio rerio, EU 92/69/EWG	LC50 = 6.4 mg/l	Cognis/ Henkel (1997g) (2)
C16-18 linear	**25 EO mean**	96h, Brachydanio rerio, OECD 203	LC50 = 2.9 mg/l	Sasol 1995j (2)

Table 4.32 Acute Fish Ecotoxicity Data for Essentially Linear and Branched Alcohol Ethoxylates

AE C Chain description	AE EO chain description	Test Method	EC50 mg/l	Reference (Klimisch score)
C9-11 Essentially linear	Alcohol (EO = 0)	96 hr static test with *Salmo gairdneri*	EC50 value between 6 and 10 mg/l	Shell Research Ltd 1979c (2)
C9-11 Essentially linear	2.5 EO	96 hr static test with *Salmo gairdneri*	EC50 = 5-7 mg/l	Shell Research Ltd 1979d (2)
C9-11 Highly linear	3 EO	96 hour aerated static test with *Salmo gairdneri*	LC_{50} = 4.2 mg/l	Uniqema 1989 (2)
C9-11 Highly linear	4.5 EO	96 hour aerated static test with *Salmo gairdneri*	LC_{50} = 7.5 mg/l	Uniqema 1990 (2)
C9-11 Highly linear	5 EO	96 hour semi-static test with *Salmo gairdneri*	LC_{50} = 11.5 mg/l	Uniqema 1985a (2)
C9-11 Essentially linear	6 EO mean	96 hr static renewal test based on standard ASTM and US-EPA methods	EC50 = 8.5 mg/l	Wong et al (1997) (2)

AE C Chain description	AE EO chain description	Test Method	EC50 mg/l	Reference (Klimisch score)
C9-11 Essentially linear	8 EO mean	96 hr static renewal test based on standard ASTM and US-EPA methods	EC50 = 11 mg/l	Wong et al (1997) (2)
C9-11 Highly linear	8 EO	96 hour semi-static test with *Salmo gairdneri*	LC_{50} = 23.7 mg/l	Uniqema 1985b (2)
C9-11 Essentially linear	8 EO	96 hr static test with daily renewal of test solutions, using *Salmo gairdneri*	EC50 = 12 mg/l	Shell Research (1981a) (2)
C9-11 Highly linear	10 EO	96 hour semi-static test with *Salmo gairdneri*	LC_{50} = 20.9 mg/l	Uniqema 1985c (2)
C12-13 essentially linear	2 EO mean	96 hour static test with *Salmo gairdneri*	LC50 = 1 - 2 mg/L	Shell Research Ltd 1978f (2)
C12-13 essentially linear	4.5 EO mean		LC50 = 0.96 mg/L	Wong et al (1997) (2)
C12-13 essentially linear	5 EO mean		LC50 = 1.0 mg/L	Wong et al (1997) (2)
C12-13 essentially linear	6.5 EO mean		LC50 = 1.3 mg/L	Wong et al (1997) (2)
C12-13 essentially linear	7 EO mean		LC50 = 2,5 mg/L;	Sasol 93/537.A1 Unpublished report (4)
C12-15 essentially linear	3 EO mean	96 hour static test with *Salmo gairdneri*	LC50 = 1.3 - 1.7 mg/L	Shell Research Ltd 1978e (2)
C12-15 essentially linear	3 EO mean	96 hour static test with *Salmo gairdneri*	LC50 = 1.0 mg/L	Shell Research Ltd 1978g (2)
C12-15 essentially linear	7 EO mean	96 hour static test with daily renewal. *Salmo gairdneri*	LC50 = 1.1 mg/L	Shell Research Ltd 1981b (2)

AE C Chain description	AE EO chain description	Test Method	EC50 mg/l	Reference (Klimisch score)
C12-15 essentially linear	9 EO mean		LC_{50} = 2.8 mg/L;	Sasol 92/881 A2 Unpublished report (4)
C12-15 essentially linear	9 EO mean		LC50 = 1.6 mg/L	Dorn, et al (1993) (2)
C12-15 essentially linear	12 EO mean		LC50 = 1.4 mg/L	Wong et al (1997) (2)
C13 branched	3 EO mean	96h, Brachydanio rerio, OECD 203	LC50 = 3.0 mg/l	Sasol 1995g (2)
C13 branched	4 EO mean	96h, Brachydanio rerio, OECD 203	LC50 = 3.5 mg/l	Sasol 1997j (2)
C13 branched	6 EO mean	96h, Brachydanio rerio, OECD 203	LC50 = 5.8 mg/l	Sasol 1995h (2)
C13 branched	9 EO mean	96h, Brachydanio rerio, OECD 203	LC50 = 12 mg/l	Sasol 1995 (2)
C13-15 essentially linear	3 EO mean	Static test, 96h, Brachydanio rerio, OECD 203	LC50 = >2.2 <4.6 mg/l	BASF 1993a (2)
C13-15 essentially linear	7 EO mean	Static test, 96h, Brachydanio rerio, OECD 203	LC50 = 1.2 mg/l)	BASF 1993b (2)
C13-15 essentially linear	11 EO mean	Static test, 96h, Brachydanio rerio, OECD 203	LC50 = 2.2 mg/l	BASF 1993c (2)
C14-15 essentially linear	8 EO mean	96h, Brachydanio rerio, OECD 203	LC50 = 1.2 mg/L;	Sasol 1999f (2)
C14-15 linear and essentially linear	8 EO mean	96h, Brachydanio rerio, OECD 203	LC50 = 0.65mg/l	Sasol 1993j. (2)
C14-15 linear and essentially linear	8 EO mean	96h, Brachydanio rerio, OECD 203	LC50 = 0.72mg/l	Sasol 1993i. (2)
C14-15 essentially linear	11 EO mean	96 hr static test with daily renewal, with *Salmo gairdneri*	LC50 = 0.9 mg/l	Shell Research Ltd. 1981d (4)

In conclusion, the acute toxicity of linear and essentially linear AE to fish is similar, especially if the variability between different test conditions is taken into account. In addition, branched AE are shown not to be more toxic to fish. Thus fish data also indicate that use of eco-toxicity data derived from linear alcohols would be suitable for the branched alcohols, in the environmental risk assessment process. In addition, the acute data for linear C8 and essentially linear C9-11 AE indicate the lower toxicity of the C8 AE.

The acute fish, invertebrate, and algal acute eco-toxicity test results all confirm that the branched and essentially linear AE are not more toxic than linear AE of the same hydrocarbon chainlength and EO number. In addition, the acute data reflect the expected decreasing toxicity with increasing EO chain length and the increase in toxicity with increasing hydrocarbon chain length, so long as the AE remains soluble in water. These conclusions support the use of the QSARs based on chronic eco-toxicity tests to include all the linear, essentially linear, and branched AE, as discussed in the next section.

4.2.1.2. Chronic QSARs for alcohol ethoxylates and their use in deriving $PNEC_{aquatic}$

This section describes the development of the AE chronic QSARs for algae, daphnia, and fish, and their use in the development of a probabilistic QSAR which includes the available chronic data for other species. The QSAR developments are based on non-specific membrane disruption being the mode of action for all AE toxicity, which means that toxicity is related to the hydrophobicity and thus the Kow of the specific AE homologue (Boeije et al 2006). The chronic daphnia QSAR and the probabilistic QSAR are both used in the AE risk assessment.

4.2.1.2.1. Chronic eco-toxicity data for alcohol ethoxylates

Much of the acute data given in sections 4.1.1.1 refers to tests carried out using specific commercial AE products, each of which contains a different mixture of AE homologues. Chronic data for specific AE products, and for some specific AE homologues, is also available. In several cases, advances in analytical methodology have enabled good determinations to be obtained for both the hydrocarbon chainlength distribution and the EO chainlength distribution of the AE product mixtures which were used in these the chronic tests. Belanger et al (2006) have summarized the chronic information which has been obtained for these AE mixtures whose homologue distribution has been determined, and these data are reproduced in Table 4.33. This chronic data has been used to generate chronic QSARs for algae, daphnia, and fish. In some cases the original data has been used to calculate EC10 values, rather than the NOEC values reported in the original publications.

Table 4.33 includes fifty-nine studies of 17 different aquatic species which have been evaluated for chronic toxicity responses to alcohol ethoxylates. These studies have been summarized in aggregate by Belanger et al. (2006) and Belanger and Dorn (2004). Many of these studies also appeared as components of the review for the Dutch Risk Assessment of Surfactants, summarized in van de Plaasche et al. (1999). From the time of the van de Plaasche review to the present numerous additional studies have been added. Studies have previously been carefully reviewed and discussed in other regulatory contexts including reviews with Environment Canada as part of the Domestic Substance List categorization process (EC and HC 2005) and with the EC National Guidelines and Standards Office using criteria set forth under the protocol for developing Canadian water quality guidelines (CCME 1999). The assessment of the Klimisch[12] scores incorporated into Table 4.33 extends that of other related efforts specifically for HERA (Belanger 2006a).

Of the 59 studies that were reviewed, 37 were rated as Klimisch Score 1 and the remaining 22 as Klimisch score 2. Importantly, all studies used to develop structure

[12] Klimisch Scores are 1 = valid without restriction; 2 = valid with restriction; 3 = not reliable; 4 = not assignable. Further information and guidance on the selection of data for HERA reports is given in Appendix C of the HERA Methodology Document (HERA 2005).

activity relationships for algae (*Desmodesmus [=Scenedesmus] subspicatus*) and invertebrates (*Daphnia magna*) were 1 rated. The studies used to develop the fish SAR (Based on fathead minnow, *Pimephales promelas*) all received 2 ratings. Importantly for the fish SAR, however, is the fact that all the studies used in the SAR were performed in the same laboratory with full analytical support and verification and subsequently published in primary, peer-reviewed literature. Development of all three single species SARs were based on access to the full raw biological and analytical data (Boejie et al. 2006; Wind and Belanger 2006). Because many of the single species were often developed in the context of experimental stream mesocosm studies on the same alcohol ethoxylates, all the relevant stream mesocosm studies were also cited and reviewed as they contained relevant single species data (Belanger et al. 2000; Boeije et al. 2006). All mesocosm studies were rated as Klimisch Score 2 because there is no standard guideline for these studies. However, as pointed out by the conductors of these studies, they used extremely similar physical designs and analytical exposure verification played key roles in each (Belanger 2006a).

The strength of this database is exceptionally high. Of 59 studies reported here, 49 have been through the peer review process for publication and 37 were fully supported by a QA organization. A total of 54 of 59 studies were directly reflective of a present day guideline method or an equivalent. In addition, 58 of the 59 studies measured exposure concentrations during the conduct of the test and the single study that did not was the third representative of a taxon that was evaluated two additional times under full GLP (Belanger 2006a).

Key studies and taxa such as the rainbow trout, a mollusc (clam) and a series of studies on rotifers were amongst the most sensitive taxa, although there are relatively small differences in sensitivity between all species. This is thought to be a consequence of the mode of action of alcohol ethoxylates, i.e. general disruption of membrane function as a result of partitioning of AE into the lipid bi-layer. The most sensitive species in the data set were also amongst the most documented and controlled studies in addition to those used to develop structure-activity relationships (Belanger 2006a). The development of the chronic QSARs is discussed in the next sections.

Table 4.33 Chronic toxicity of alcohol ethoxylate (AE) to aquatic species [1]

Species name	Common name	Taxonomic Group for QSAR	Life stage	Compound	Most Sensitive Endpoint	Effect Statistic	Effect Conc (mg/L)	Citation[2] (Klimisch Score)[3]
Chlorella vulgaris	Green algae	Algae	Vegetative	C12-15EO3	Growth rate	EC10	2.179	Turner 1988 * (1)
Lemna minor	Duckweed	Algae	2-frond stage	C14-15EO7	Frond count	EC10	0.101	Bishop et al. 1980 * (1); Bishop et al. 1981 (1)
Microcystis aeruginosa	Blue-green algae	Algae	Vegetative	C14-15EO7	Cell density	EC10	0.154	Maziarz 1983a * (1); Lewis and Hamm 1986 (1)
Navicula pelliculosa	Diatom	Algae	Vegetative	C14-15EO7	Cell density	EC10	0.140	Maziarz 1983b * (1); Lewis and Hamm 1986 (1)
Scenedesmus subspicatus	Green algae	Algae	Vegetative	C10EO8	Growth rate	EC10	8.087	Rieche 1997 * (1)
Scenedesmus subspicatus	Green algae	Algae	Vegetative	C12E2	Growth rate	EC10	0.030	Kirch 1997a * (1)
Scenedesmus subspicatus	Green algae	Algae	Vegetative	C12E4	Growth rate	EC10	0.453	Werner 1997a * (1)
Scenedesmus subspicatus	Green algae	Algae	Vegetative	C12E8	Growth rate	EC10	0.325	Werner A. 1997b * (1)
Scenedesmus subspicatus	Green algae	Algae	Vegetative	C16E2	Growth rate	EC10	0.042	Geisel 1998 * (1)
Scenedesmus subspicatus	Green algae	Algae	Vegetative	C16E8	Growth rate	EC10	0.096	Kirch 1997b * (1)
Scenedesmus subspicatus	Green algae	Algae	Vegetative	C12-13E3	Growth rate	EC10	0.998	Neven 1993a * (1)
Scenedesmus subspicatus	Green algae	Algae	Vegetative	C16-18E7	Growth rate	EC10	5.831	Neven 1993b * (1)
Scendesmus subspicatus	Green algae	Algae	Vegetative	C12-13E3	Growth rate	EC10	0.204	Hantsveit and Oldersma 1993 * (1)
Scenedesmus	Green algae	Algae	Vegetative	C12-14E7	Growth rate	EC10	0.137	Neven 1993c * (1)

Species name	Common name	Taxonomic Group for QSAR	Life stage	Compound	Most Sensitive Endpoint	Effect Statistic	Effect Conc (mg/L)	Citation[2] (Klimisch Score)[3]
subspicatus								
Selenastrum capricornutum	Green algae	Algae	Vegetative	C8-10E5	Growth rate	EC10	9.791	Neven B. 1993d * (1)
Selenastrum capricornutum	Green algae	Algae	Vegetative	C14-15EO7	Cell density	EC10	0.092	Maziarz 1983c * (1); Lewis and Hamm 1986 (1)
Selenastrum capricornutum	Green algae	Algae	Vegetative	C12-14E9	Cell density	EC10	0.151	Yamane et al. 1984 (2)
Brachionus calyciflorus	Rotifer	Invertebrate	Neonate	C10EO6	Population size	EC10	2.015	Versteeg et al. 1997 (1); Morrall et al. 1999 (1)
Brachionus calyciflorus	Rotifer	Invertebrate	Neonate	C12EO6	Population size	EC10	0.562	Versteeg et al. 1997 (1); Morrall et al. 1999 (1)
Brachionus calyciflorus	Rotifer	Invertebrate	Neonate	C14EO4	Population size	EC10	0.207	Versteeg et al. 1997 (1); Morrall et al. 1999 (1)
Brachionus calyciflorus	Rotifer	Invertebrate	Neonate	C14EO6	Population size	EC10	0.112	Versteeg et al. 1997 (1); Morrall et al. 1999 (1)
Brachionus calyciflorus	Rotifer	Invertebrate	Neonate	C14EO8	Population size	EC10	0.169	Versteeg et al. 1997 (1); Morrall et al. 1999 (1)
Brachionus calyciflorus	Rotifer	Invertebrate	Neonate	C12E3	Population size	EC10	0.733	Versteeg and Shorter 2000 * (1)
Ceriodaphnia dubia	Water flea	Invertebrate	Neonate	C14-15EO7	Reproduction	EC10	0.328	Taylor 1984a, b * (1)
Ceriodaphnia dubia	Water flea	Invertebrate	Neonate	C14-15EO7	Reproduction	EC10	0.127	Taylor 1984a, b * (1)
Ceriodaphnia dubia	Water flea	Invertebrate	Neonate	C14-15EO7	Reproduction	EC10	0.464	Lewis 1989 * (1); Masters et al. 1991 (1)
Ceriodaphnia dubia	Water flea	Invertebrate	Neonate	C14-15EO7	Reproduction	EC10	0.236	Lewis 1989 * (2); Masters et al. 1991 (2)
Chironomus tentans	Insect	Invertebrate	Larvae	C9-11EO6	Survival	EC10	3.635	Dorn et al. 1997a (2)
Corbicula fluminea	Bivalve	Invertebrate	Juvenile	C12-15EO6	Length gain	EC10	0.062	Belanger et al. 1998 * (2); Belanger et al. 2000 (2)
Daphnia magna	Water flea	Invertebrate	Neonate	C14-15EO7	Reproduction	EC10	0.140	Mank and Krueger 1998a* (1); Morrall et al. 2003 (1)
Daphnia magna	Water flea	Invertebrate	Neonate	C13-15EO5	Reproduction	EC10	0.082	Mank et al. 1999 * (1); Morrall et al. 2003 (1)

Species name	Common name	Taxonomic Group for QSAR	Life stage	Compound	Most Sensitive Endpoint	Effect Statistic	Effect Conc (mg/L)	Citation[2] (Klimisch Score)[3]
Daphnia magna	Water flea	Invertebrate	Neonate	C12-15EO6	Reproduction	EC10	0.368	Mank and Krueger 1998b* (1); Morrall et al. 2003 (1)
Daphnia magna	Water flea	Invertebrate	Neonate	C12-13EO6.5	Reproduction	EC10	0.803	Gillespie et al. 1999 (1)
Daphnia magna	Water flea	Invertebrate	Neonate	C14-15EO7	Reproduction	NOEC	0.790	Gillespie et al. 1999 (1)
Daphnia magna	Water flea	Invertebrate	Neonate	C9-11EO6	Reproduction	EC10	2.579	Gillespie et al. 1999 (1)
Daphnia magna	Water flea	Invertebrate	Neonate	C12-15E9	Reproduction	EC10	0.167	Kroese 1994 * (1)
Daphnia magna	Water flea	Invertebrate	Neonate	C12-13EO6.5	Reproduction	EC10	0.355	Maki 1977 * (1); 1979 (1)
Daphnia magna	Water flea	Invertebrate	Neonate	C14-15EO7	Survival	EC10	0.255	Maki 1977 * (1); 1979 (1)
Elimia	Gastropod	Invertebrate	Junvenile	C12-15EO6	Weight gain	NOEC	0.259	Belanger et al. 1998 * (2); Belanger et al. 2000 (2)
Hyallela azteca	Amphipod	Invertebrate	Larval	C9-11EO6	Survival	EC10	3.882	Dorn et al. 1997b (2)
Dugesia gonocephala	Flatworm	Invertebrate	Immature	C14E10	Survival	EC10	0.840	Patzner and Adams 1979 (2)
Lepomis macrochirus	Bluegill	Fish	Juvenile	C9-11EO6	Survival	EC10	8.983	Dorn et al. 1997b (2)
Lepomis macrochirus	Bluegill	Fish	Juvenile	C12-13EO6.5	Reproduction	NOEC	0.880	Harrelson et al. 1997 (2)
Lepomis macrochirus	Bluegill	Fish	Juvenile	C14-15EO7	Survival	NOEC	0.160	Kline et al. 1996 (2)
Oncorhynchus mykiss	Rainbow trout	Fish	Egg - alevin	C12-15EO9	Dry weight	EC10	0.079	Wong et al. 2004 (1)
Pimephales promelas	Fathead minnow	Fish	Egg – juvenile	C9-11EO6	Survival	NOEC	4.350	Harrelson et al. 1997 (2)
Pimephales promelas	Fathead minnow	Fish	Egg – juvenile	C9-11EO6	Survival	NOEC	1.000	Harrelson et al. 1997 (2)
Pimephales promelas	Fathead minnow	Fish	Egg – juvenile	C9-11EO6	Reproduction	NOEC	0.730	Harrelson et al. 1997 (2)

Species name	Common name	Taxonomic Group for QSAR	Life stage	Compound	Most Sensitive Endpoint	Effect Statistic	Effect Conc (mg/L)	Citation[2] (Klimisch Score)[3]
Pimephales promelas	Fathead minnow	Fish	Egg – juvenile	C9-11EO6	Length	NOEC	1.010	Lizotte et al. 1999 (2)
Pimephales promelas	Fathead minnow	Fish	Egg – juvenile	C12-13EO6.5	Survival	EC10	0.213	Holman 1975 * (1); Maki 1979 (1)
Pimephales promelas	Fathead minnow	Fish	Egg – juvenile	C14-15EO7	Survival	EC10	0.121	Holman 1975 * (1); Maki 1979 (1)
Pimephales promelas	Fathead minnow	Fish	[4]Egg - juvenile	C12-13EO6.5	Survival	EC10	1.748	Lizotte et al. 1999 (2)
Pimephales promelas	Fathead minnow	Fish	Larva - juvenile	C12-13EO6.5	Survival	NOEC	0.880	Dorn, P.B et al. 1997b (2)
Pimephales promelas	Fathead minnow	Fish	Larva - juvenile	C12-13EO6.5	Survival	NOEC	0.880	Dorn, P.B et al. 1997b (2)
Pimephales promelas	Fathead minnow	Fish	[4]Egg - juvenile	C14-15EO7	Survival	EC10	1.441	Lizotte et al. 1999 (2)
Pimephales promelas	Fathead minnow	Fish	Larva - juvenile	C14-15EO7	Survival	NOEC	0.160	Kline et al. 1996 (2); Dorn et al. 1996 (2)
Pimephales promelas	Fathead minnow	Fish	Larval	C14-15EO7	Survival	NOEC	0.160	Kline et al. 1996 (2); Dorn et al. 1996 (2)
Pimephales promelas	Fathead minnow	Fish	Adult	C14-15EO7	Survival	NOEC	0.160	Kline et al. 1996; (2) Dorn et al. 1996 (2)
Pimephales promelas	Fathead minnow	Fish	Larval	C14-15EO7	Survival	NOEC	0.280	Kline et al. 1996 (2); Dorn et al. 1996 (2)

[1] Table taken from Belanger et al 2006, with additional Klimisch scores provided by Belanger (2006a) Klimisch Scores are 1 = valid without restriction; 2 = valid with restriction; 3 = not reliable; 4 = not assignable. Further information and guidance on the selection of data for HERA reports is given in Appendix C of the HERA Methodology Document (HERA 2005). Further information concerning the references is available in Belanger et al (2006).

[2] Unpublished internal industry reports are indicated with an asterisk.

[3] Klimisch evaluation scores are given in parentheses beside each reference. [4] Belanger (2006a, additional note).

4.2.1.2.2. Development of the chronic daphnia QSAR

The *Daphnia magna* QSAR developed by Boeije et al (2006) adds the individual toxicities of the homologues of an AE mixture, weighted by the fraction of that homologue present in the mixture, to determine the mixture toxicity. This is called the Toxic Units approach, and is represented by the following equation:

$$TOX_{mixture} = 1/\Sigma_i TU_i = 1/\Sigma_i (f_i / TOX_i)$$

In the equation above, the toxicity is defined as the reciprocal of the toxicity statistic which has been determined, such as the NOEC or the EC50 value, while TU_i is the fraction of the i^{th} AE homologue present in the mixture, and TOX_i is the reciprocal of the appropriate toxicity measure (e.g. NOEC or EC_{20} or EC_{50}) for that AE homologue. Experimental acute daphnia toxicities for several single AE homologues determined in Boeije et al (2006) show that this additivity method is more accurate than the alternative method involving a single averaged AE structure, as the additivity method gives appropriate weighting to the more toxic AE homologues present in the mixture.

The chronic *Daphnia magna* QSAR was developed from seven data sets measuring the most sensitive endpoint, survival, for six distinct AE homologue mixtures. For each of these AE homologue mixtures, the homologue distribution was known. For the C14-15 EO7 mixture, the geometric mean of the two reported results was used. The *Daphnia magna* data used are identified by grey flashes in the Citation column of Table 4.33. Good representation is available for the hydrocarbon range from C9 to C15, with good coverage of the ethylene oxide range.

The QSAR development method used the method of minimizing the sum of squared errors (SSE) to determine the best value of the individual AE homologue toxicities to give the six experimentally-based mixture toxicities. The QSAR calculates the EC20 in micromoles per litre for each AE homologue, and the initial data were converted into the EC_{20} format for this purpose. The EC_{20} was used, rather than the NOEC or the EC_{10}, as the QSAR developed with EC_{20} was statistically more robust, making this QSAR more reliable. Conversion from mg/l to µmol/L for the experimentally-based mixture measurements was carried out using the experimentally determined homologue distributions of the AE mixtures.

The available number of experimental data permitted the chronic *Daphnia magna* QSAR to have only one parameter, logKow, in addition to the intercept. The logKow data were determined using the method of Leo and Hansch (1979) as applied to surfactants by Roberts (1991), and by Roberts and Marshall (1995). The logKow values calculated for each AE homologue have then been used for each specific AE homologue, during the process of QSAR development in which the slope and intercept of the QSAR were determined by the SSE minimization procedure

The resultant QSAR,

$$EC_{20} = 10^{-0.532*logKow + 2.975} \qquad (\mu mol/L) \quad R^2 = 96.6 \qquad (4.7)$$

has then been used to generate an EC_{20} value in µmol/L for each AE homologue. It is also possible to generate the AE homologue EC_{20} matrix with units of mg/L, by using

the molecular weight of each AE homologue in the conversion. This has been done for each AE homologue EC_{20} concentration determined using equation 4.7, to generate the EC_{20} matrix for AE homologues given in Table 4.34.

The chronic *Daphnia magna* EC_{20} values calculated by the Boeije et al (2006) QSAR for the long chain alcohols can be compared with measured or calculated NOEC values given in the long chain alcohol SIAR (SIAR 2006). Considering the differences in endpoint (EC_{20} versus NOEC), the different QSARs used in the two approaches, possible impurities in the compounds, and the normal variability in the reproduction of experimental results, the agreement is quite acceptable. A summary of the information available in the long chain alcohol SIAR is given in Table 4.35.

Table 4.34 Chronic *Daphnia magna* EC_{20} values, in mg/l, calculated using the CSARA AE Workbook (ERASM 2005b) according to the method described in Boeije et al (2006)

C/ EO	C9	C10	C11	C12	C13	C14	C15	C16	C18
0	1.71E+00	9.71E-01	5.45E-01	3.04E-01	1.69E-01	9.33E-02	5.13E-02	2.81E-02	8.35E-03
1	2.13E+00	1.18E+00	6.52E-01	3.58E-01	1.96E-01	1.07E-01	5.83E-02	3.16E-02	9.24E-03
2	2.97E+00	1.63E+00	8.87E-01	4.83E-01	2.62E-01	1.42E-01	7.65E-02	4.12E-02	1.19E-02
3	4.00E+00	2.17E+00	1.17E+00	6.33E-01	3.41E-01	1.83E-01	9.85E-02	5.28E-02	1.51E-02
4	5.24E+00	2.82E+00	1.52E+00	8.15E-01	4.37E-01	2.34E-01	1.25E-01	6.67E-02	1.90E-02
5	6.74E+00	3.61E+00	1.93E+00	1.03E+00	5.51E-01	2.94E-01	1.57E-01	8.34E-02	2.35E-02
6	8.53E+00	4.56E+00	2.43E+00	1.29E+00	6.88E-01	3.66E-01	1.94E-01	1.03E-01	2.90E-02
7	1.07E+01	5.69E+00	3.02E+00	1.61E+00	8.52E-01	4.52E-01	2.39E-01	1.27E-01	3.55E-02
8	1.33E+01	7.03E+00	3.73E+00	1.98E+00	1.05E+00	5.54E-01	2.93E-01	1.55E-01	4.32E-02
9	1.63E+01	8.64E+00	4.57E+00	2.42E+00	1.28E+00	6.75E-01	3.56E-01	1.88E-01	5.22E-02
10	1.99E+01	1.05E+01	5.57E+00	2.94E+00	1.55E+00	8.17E-01	4.31E-01	2.27E-01	6.30E-02
11	2.42E+01	1.28E+01	6.74E+00	3.55E+00	1.87E+00	9.86E-01	5.19E-01	2.73E-01	7.56E-02
12	2.93E+01	1.54E+01	8.13E+00	4.28E+00	2.25E+00	1.18E+00	6.23E-01	3.27E-01	9.04E-02
13	3.53E+01	1.86E+01	9.77E+00	5.14E+00	2.70E+00	1.42E+00	7.45E-01	3.91E-01	1.08E-01
14	4.24E+01	2.23E+01	1.17E+01	6.14E+00	3.23E+00	1.69E+00	8.89E-01	4.66E-01	1.28E-01
15	5.06E+01	2.66E+01	1.40E+01	7.33E+00	3.84E+00	2.02E+00	1.06E+00	5.54E-01	1.52E-01
16	6.04E+01	3.17E+01	1.66E+01	8.71E+00	4.57E+00	2.39E+00	1.25E+00	6.57E-01	1.80E-01
17	7.18E+01	3.76E+01	1.97E+01	1.03E+01	5.41E+00	2.83E+00	1.48E+00	7.77E-01	2.13E-01
18	8.51E+01	4.46E+01	2.34E+01	1.22E+01	6.40E+00	3.35E+00	1.75E+00	9.17E-01	2.51E-01
19	1.01E+02	5.27E+01	2.76E+01	1.44E+01	7.56E+00	3.95E+00	2.07E+00	1.08E+00	2.95E-01
20	1.19E+02	6.23E+01	3.26E+01	1.70E+01	8.90E+00	4.65E+00	2.43E+00	1.27E+00	3.47E-01

The development of the *Daphnia magna* QSAR treats each AE homologue as fully soluble in water at the EC_{20} concentration. However, work by Schäfers et al (2006 in prep.) in the context of the SIAR on aliphatic alcohols shows that the C15 alcohol is not fully soluble at a concentration similar to that predicted for the EC_{20} for *Daphnia magna*, as predicted in table 4.34. Thus it is probable that the C15, C16, and C18 alcohols, and perhaps some of the C16 and C18 AE homologues with a low number of EO groups, will not be fully soluble at the EC_{20} concentrations predicted in Table 4.34. Using the data in Table 4.34, which assumes that all the AE homologues are soluble in water at the EC_{20}, is conservative, as some of the most toxic long chain, low EO AE may not be available to the organisms because of insolubility. However, this conservative methodology has been used in this HERA report. In addition, all the AE homologues are assumed to be soluble for the algal, fish, mesocosm, and probabilistic QSARs discussed below. Thus all the QSARs used in this report are conservative in this respect.

Further information on the development of the chronic *Daphnia magna* QSAR is available in Boeije et al (2006), and in the CSARA AE Workbook Documentation (ERASM 2005a). The QSAR is implemented in the CSARA AE Workbook (ERASM 2005b) which enables its easy use for AE risk assessment. The chronic *Daphnia magna* QSAR has also been used, transcribed to an EC_{10} and mg/l format, as part of the invertebrate section of the development of probabilistic QSARs described in section 4.2.1.2.6.

Table 4.35 Chronic Daphnia NOEC (Reproduction) values for several long chain alcohols, from the long chain alcohol SIAR (SIAR 2006)

C#	8	10*	11	12*	13	14*	15*†
NOEC, mg/l	1	0.11 - 0.35	0.44 - 0.17	0.014 – 0.16	0.006 – 0.046	0.0016 – 0.0098	0.008 – 0.056
Method	Meas.	Meas.	QSAR	Meas.	QSAR	Meas.	Meas.

* First number in range refers to averaged concentration over the duration of the experiment. Second number refers to the initial concentration. † Data excluded from Alcohol QSAR formation as not all material was soluble.

4.2.1.2.3. Development of the chronic algal QSAR

Unlike the other chronic QSARs discussed in the HERA AE environmental assessment, the algal QSAR developed by Wind and Belanger (2006) is based on data from pure AE homologues rather than from commercial mixtures. The six AE homologues ranged from 10 to 16 carbons in the hydrocarbon chain and from 2 to 8 ethylene oxide groups. The specific AE homologues and the EC20 and NOEC values determined for *Scenedesmus subspicatus* for both growth rate and biomass are shown in Table 4.36, where the data are expressed in millimoles per liter. The lower than

Table 4.36 Chronic 72-h EC_{20} endpoints (in mM) for various pure AE homologues from Wind and Belanger (2006)

AE homologue	72-h population growth rate			72-h yield or area under the curve		
	E_rC_{20}	95% Conf Limits	NOEC	E_bC_{20}	95% Conf Limits	NOEC
$C_{10}EO_8$	0.0282	0.0233-0.0341	0.0196	0.00902	0.00569-0.01412	0.0196
$C_{12}EO_2$	0.00518	0.00255-0.01051	0.000321	0.000201	0.000088-0.000471	0.000279
$C_{12}EO_4$	0.00318	0.00282-0.00356	0.000718	0.00174	0.00141-0.00218	0.000718
$C_{12}EO_8$	0.00493	0.00405-0.00600	0.000279	0.00121	0.00084-0.00173	0.000321
$C_{16}EO_2$	0.00133	0.000667-0.00276	0.000303	0.000273	0.00091-0.00636	0.000421
$C_{16}EO_8$	0.000370	0.000185-0.000741	0.000421	0.000253	0.000135-0.000438	0.000303

expected toxicity of the $C_{16}EO_2$ homologue is postulated to be due to possible sorption onto the algae, but the $C_{16}EO_2$ data has been kept in the dataset and used in the QSAR development. It is likely that it would not be possible to carry out experimental work with AE homologues with lower water solubility than that of $C_{16}EO_2$, however.

In contrast to the nominal concentrations used for most previous algal work, analytical confirmation of the algal concentrations has been carried out in the Wind and Belanger (2006) study. The more robust QSAR, the E_bC_{20}, is

$$\text{Log}(72\text{-h } E_bC_{20} \text{ in mM}) = -0.378*\log K_{ow} - 4.072 \qquad R^2 = 0.72 \qquad (4.8)$$

This QSAR is used for algae as part of the development of the chronic probabilistic QSAR described in section 4.2.1.2.6.

This algal work, carried out to the highest standard using specific AE homologues and analytical confirmation of the exposure concentrations, has resulted in a chronic algal QSAR with a slope (about -0.4) intermediate in value between the slope for the *Daphnia* QSAR (about -0.5 – see section 4.2.1.2.2) and the slope for the fish QSAR (about -0.3 – see section 4.2.1.2.4 below). In addition, the intercepts of the three QSARs are also in a similar range, from about -3 to about -4. Thus Wind and Belanger (2006) conclude that no one trophic group is uniquely sensitive or insensitive to AEs, based upon the chronic data. This result updates previously

obtained results, based on lower quality data, which indicated that algae were generally less sensitive than fish or invertebrates.

4.2.1.2.4. Development of the chronic fish QSAR

High quality data sets obtained by Lizotte et al. (1999) consisting of early life stage study results for fathead minnow (*Pimephales promelas*) exposed to three AE surfactant mixtures (C9-11EO6, C12-13 EO6.5, and C14-15EO7) have been used by Boeije et al (2006) to develop a limited chronic fish QSAR. Due to the limited number of data points, Boeije et al (2006) consider the QSAR to be of low reliability. The QSAR, given in

$$EC_{20} = 10^{-0.307*\log Kow + 2.08} \qquad (\mu mol/l) \qquad R^2 = 99.6 \qquad (4.9)$$

µmol/l units in equation 4.9, has been used, transcribed to an EC_{10} and mg/l format, as part of the fish section of the development of probabilistic QSARs described in section 4.2.1.2.6.

4.2.1.2.5. Development of the mesocosm QSAR

Boeije et al (2006) have developed a NOEC-based QSAR from the results of five high quality studies carried out using AE mixtures in experimental stream ecosystems, with three different experimental stream facilities. The mole-based QSAR is

$$NOEC = 10^{-0.740*\log kow + 3.22} \qquad (\mu mol/L) \qquad R^2=95.1 \qquad (4.10)$$

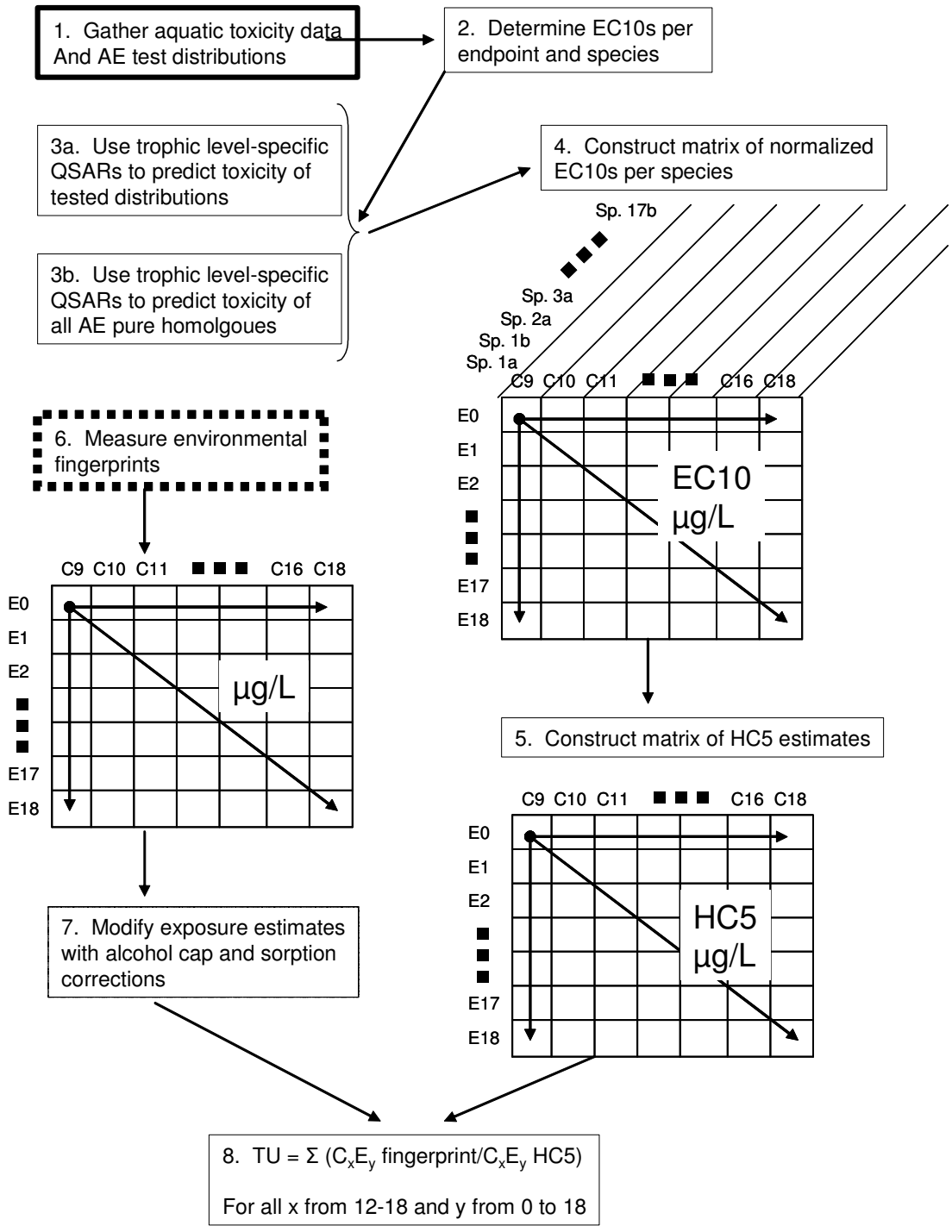
The goodness of fit of the mesocosm QSAR is not as good as that of the chronic *Daphnia magna* QSAR. In addition, it is not possible to develop an EC10 or an EC20 from mesocosm studies, as the conclusions are based on a combination of statistical findings, expert judgements, and the particular conditions of the test (e.g. species composition) (Boeije et al 2006). Thus, although the test results complement the results of the single species tests and give similar predictions of AE toxicity, they are used only as supporting information in this HERA assessment. However, as discussed in section 4.2.1.2.8, the chronic probabilistic QSAR and the mesocosm QSAR give similar results when similar distributions of AE homologues are considered. In a comparison of NOEC data from stream mesocosm studies of commercial C14-15EO7, C12-15EO9, C12-15EO6, C12-13EO6.5, and C9-11EO6 AE, Wong et al. (2004) showed that there is a general increase in surfactant toxicity with increased carbon chain length. Depending on the commercial mixture, species and endpoint reported, the studies show NOEC values ranging from 80 to >5000 ug/l, apart from one study (C12-15EO6) where there are two endpoints with more toxic endpoints (13 ug/l) than would have been predicted from SAR.

4.2.1.2.6. Development of the probabilistic chronic QSAR

Belanger et al (2006) have used the chronic data for the 17 species given in Table 4.33 to develop a probabilistic chronic QSAR for AE. As with the chronic *Daphnia*, algal, and fish QSARs, the logKow parameter has been used to estimate the hydrophobicity, and thus the toxicity, of both the AE product mixtures used in the tests and of the AE mixtures found in the environment.

The process used in the development of the probabilistic chronic QSAR for the AE homologues is shown schematically in Figure 4.3 (Belanger et al 2006). The first step in the process was the identification of the data in Table 4.33. Knowledge of the distribution of AE homologues used in each test was necessary for inclusion in the table. The second step involved the determination of an EC_{10} value for each endpoint and species, from the initial test data.

Figure 4.3 Information flow for the determination of toxic unit based assessment of alcohol ethoxylates (Belanger et al 2006). Bold boxes indicate the initial stages for fate or effects. Solid boxes are effects oriented portions of the assessment and dotted line boxes are environmental exposure oriented portions of the assessment.



In the third step, called the normalization step, the EC_{10} value obtained for the AE homologue distribution present in each eco-toxicity test is used, together with the appropriate chronic QSAR described in section 4.2.1.2.2 for *Daphnia magna*, 4.2.1.2.3 for *Scenedesmus subspicatus,* or 4.2.1.2.4 for *Pimephales promelas* to generate a table of EC_{10} values for each AE homologue which is specific to the species used in each original eco-toxicity test. This is carried out in the following manner:

1. The chronic QSAR most similar to the test species is chosen. This is carried out on a trophic level basis, with, for example, the *Scenedesmus subspicatus* based QSAR being used to predict the homologue-specific toxicity for all algae, and the *Daphnia magna* results calculated from equation 4.7 and given for each AE homologue in Table 4.34 being assumed to apply to all invertebrates. Table 4.33 shows which QSAR has been used for each test species. Note that these trophic level QSARs have been designed to predict EC_{20} values.
2. Using the appropriate trophic level QSAR and the AE homologue distribution which was used in the original eco-toxicity test, an EC_{20} value for the test can be calculated, using the toxic units approach. In the toxic units approach, toxic units, which result when the fraction of each AE homologue present in the distribution is multiplied by the toxicity of that AE homologue, are added together to determine the toxicity of the mixture as a whole. The resulting EC_{20} value is called ${}^{\text{Predicted}}EC_{20}$(test). In effect, this is the result predicted if the test had been carried out using the original AE homologue distribution, but with the species used to develop the chronic QSAR (e.g. *Daphnia magna* if the original test species were an invertebrate.) This is step 3a in Figure 4.3.
3. Next, the EC_{20} values for all the AE homologues in the appropriate QSAR are needed. These can be calculated, for *Daphnia magna*, *Scenedesmus subspicatus*, or *Pimephales promelas* by inserting the logKow values for each AE homologue into equations 4.7, 4.8, or 4.9 respectively. For *Daphnia magna*, these results are tabulated in mg/l units in Table 4.34. These results are called ${}^{\text{Predicted}}EC_{20}$(all). This is step 3b in figure 4.3.
4. The next step calculates, for the test species, the matrix of the toxicities of all the AE homologues which corresponds to the matrix calculated for *Daphnia magna*, *Scenedesmus subspicatus*, or *Pimephales promelas* above. This step uses the assumption (van de Plassche et al 1999, Belanger et al 2006) that, when the test results from two distributions are compared, the ratio of the predicted EC_{20} values and of the predicted EC_{10} values should be similar. This can be written as:

   $$ {}^{\text{Normalised}}EC_{10}(\text{all}) = {}^{\text{Reported}}EC_{10}(\text{test}) * {}^{\text{Predicted}}EC_{20}(\text{all})/{}^{\text{Predicted}}EC_{20}(\text{test}) \quad (4.11) $$

   In practical terms, the normalized matrix of all the EC_{10} values for all the AE homologues for the chosen test species is obtained by multiplying each element of the original chronic EC_{20}eco-toxicity matrix (e. g. *Daphnia magna*) by a number which is the ratio of the two numbers ${}^{\text{Reported}}EC_{10}$(test)/ ${}^{\text{Predicted}}EC_{20}$(test).
5. Next, the procedure in steps 2 to 4 above is repeated for each test result given in Table 4.33. If more than one test result is available for a given species, either because several tests have been carried out with the same AE homologue distribution or because different AE homologue distributions have

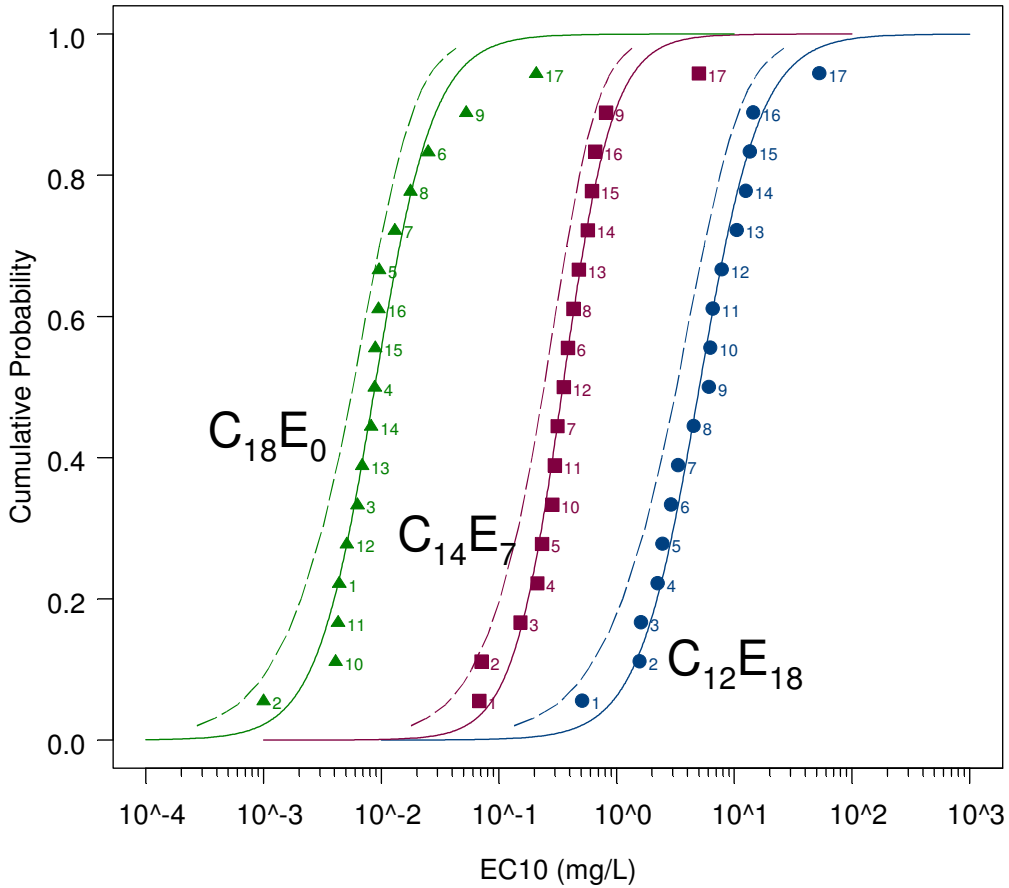
been used in different tests, then the geometric mean of the resulting EC_{10} values for each AE homologue is calculated, and this homologue specific geometric mean is used as the entry for each AE homologue in the EC_{10} matrix for that species. This process is shown schematically in box 4 of figure 4.3., with the result being an EC_{10} matrix covering each AE homologue for each of the 17 species shown in Table 4.33.

Following the generation of the homologue-specific EC_{10} matrix for each test species, the next step is to use probabilistic methods to generate a species-sensitivity distribution for each AE homologue. This is indicated in box 5 of figure 4.3. Belanger et al (2006) calculated the hazardous concentration predicted to be protective of 95% of species, the HC_5, using the methods of Aldenberg and Jaworska (2000) and Van Vlaardingen et al (2003). Examples of the cumulative probability vs EC_{10} values determined for three AE homologues are shown in Figure 4.4. The HC_5 values, in mg/l, which have been calculated from these cumulative distribution plots for each AE homologue are given in Table 4.37, which has been obtained from the CSARA AE Workbook (ERASM 2005b)

As indicated in box 8 of Figure 4.3, the HC_5 values obtained for each AE homologue can be combined with the predicted environmental concentration of each homologue resulting from use in household detergent and cleaning products to calculate the toxic unit value for each AE homologue. The sum of these toxic units is then used in the AE risk assessment.

4.2.1.2.7. Extension of the chronic Daphnia and probabilistic QSARs to C8, and EO21 and 22

The chronic *Daphnia* QSAR and the chronic probabilistic QSAR cover the hydrocarbon chain range from C8 to C18 and the EO range from 0 to 20. The HERA AE range includes a small amount of C8 material and a small amount of EO21 and 22 material in addition. The QSAR ranges have been extended in a conservative manner by assuming that the C8 toxicity is the same as the C9 toxicity for all C8 homologues which are present in the C8 EO range. Similarly, the toxicity of the EO20 homologues has been assumed to apply to EO 21 and 22. In all cases, the added AE homologues are less hydrophobic, and thus would be expected to be less toxic, than the AE homologues whose toxicity value has been used instead. The acute toxicity data in Tables 4.24 to 4.32 confirm this toxicity trend.



1. *Oncorhynchus mykiss*
2. *Corbicula fluminea*
3. *Lemna minor*
4. *Navicula pelliculosa*
5. *Microcystis aeruginosa*
6. *Pimephales promelas*
7. *Selenastrum capricornutum*
8. *Scenedesmus subspicatus*
9. *Lepomis macrochirus*
10. *Brachionus calyciflorus*
11. *Elimia*
12. *Daphnia magna*
13. *Ceriodaphnia dubia*
14. *Dugesia gonocephala*
15. *Chironomus tentans*
16. *Hyallela azteca*
17. *Chlorella vulgaris*

Figure 4.4. Comparison of species sensitivity distributions (SSD) for several alcohol ethoxylates (Belanger et al 2006). The relative spread of ethoxymer-specific SSDs and order of normalized chronic EC10 values for represented species are indicated

Table 4.37. HC_5 values, in mg/l, calculated for AE homologues (Belanger et al 2006)

	C#								
EO#	C9	C10	C11	C12	C13	C14	C15	C16	C18
0	1.24E-01	8.81E-02	6.07E-02	4.05E-02	2.60E-02	1.61E-02	9.63E-03	5.57E-03	1.71E-03
1	1.57E-01	1.09E-01	7.36E-02	4.82E-02	3.05E-02	1.86E-02	1.10E-02	6.28E-03	1.90E-03
2	2.10E-01	1.44E-01	9.67E-02	6.30E-02	3.97E-02	2.42E-02	1.43E-02	8.13E-03	2.44E-03
3	2.71E-01	1.85E-01	1.23E-01	8.01E-02	5.05E-02	3.07E-02	1.81E-02	1.03E-02	3.10E-03
4	3.39E-01	2.31E-01	1.54E-01	9.98E-02	6.29E-02	3.84E-02	2.26E-02	1.29E-02	3.89E-03
5	4.17E-01	2.83E-01	1.88E-01	1.22E-01	7.73E-02	4.72E-02	2.79E-02	1.60E-02	4.82E-03
6	5.06E-01	3.42E-01	2.28E-01	1.48E-01	9.37E-02	5.75E-02	3.41E-02	1.96E-02	5.93E-03
7	6.05E-01	4.09E-01	2.72E-01	1.77E-01	1.13E-01	6.93E-02	4.12E-02	2.37E-02	7.23E-03
8	7.17E-01	4.85E-01	3.23E-01	2.11E-01	1.34E-01	8.28E-02	4.95E-02	2.86E-02	8.76E-03
9	8.42E-01	5.69E-01	3.80E-01	2.48E-01	1.58E-01	9.83E-02	5.90E-02	3.42E-02	1.06E-02
10	9.83E-01	6.65E-01	4.43E-01	2.91E-01	1.86E-01	1.16E-01	6.98E-02	4.07E-02	1.27E-02
11	1.14E+00	7.71E-01	5.15E-01	3.38E-01	2.17E-01	1.36E-01	8.23E-02	4.82E-02	1.51E-02
12	1.32E+00	8.90E-01	5.95E-01	3.92E-01	2.53E-01	1.58E-01	9.65E-02	5.68E-02	1.79E-02
13	1.51E+00	1.02E+00	6.85E-01	4.52E-01	2.92E-01	1.84E-01	1.13E-01	6.66E-02	2.12E-02
14	1.73E+00	1.17E+00	7.85E-01	5.19E-01	3.36E-01	2.13E-01	1.31E-01	7.78E-02	2.50E-02
15	1.97E+00	1.34E+00	8.96E-01	5.93E-01	3.86E-01	2.45E-01	1.51E-01	9.06E-02	2.94E-02
16	2.24E+00	1.52E+00	1.02E+00	6.77E-01	4.41E-01	2.82E-01	1.75E-01	1.05E-01	3.44E-02
17	2.54E+00	1.72E+00	1.16E+00	7.69E-01	5.03E-01	3.22E-01	2.01E-01	1.21E-01	4.02E-02
18	2.87E+00	1.95E+00	1.31E+00	8.72E-01	5.72E-01	3.68E-01	2.30E-01	1.40E-01	4.69E-02
19	3.23E+00	2.19E+00	1.48E+00	9.87E-01	6.49E-01	4.18E-01	2.63E-01	1.61E-01	5.44E-02
20	3.63E+00	2.47E+00	1.67E+00	1.11E+00	7.33E-01	4.75E-01	3.00E-01	1.84E-01	6.30E-02

4.2.1.2.8. PNEC determination for aquatic species

Two alternative PNEC determinations are given in this section. The first is based on the use of the chronic *Daphnia magna* QSAR, while the second is based on the chronic probabilistic QSAR. In both cases, the QSARs have been extended to cover both C8 and EO21-22, as explained in section 4.2.1.2.7.

The chronic *Daphnia magna* QSAR developed by Boeije et al (2006) can be used, with an appropriate application factor, to determine the PNEC for each AE homologue. Of the three trophic level QSARs discussed here, the chronic *Daphnia magna* QSAR is the most robust. The algal QSAR work by Wind and Belanger (2006) has shown that AE toxicity to algae, daphnia, and fish are of similar magnitude, while the chronic AE toxicity data given in Table 4.33 indicates that *Daphnia magna* are among the most sensitive invertebrates.

According to the EU TGD (2003), an application factor of 10 is appropriate for the lowest of three chronic test values, in order to generate the PNEC. The EU TGD would accept application of a factor of 10 to a measured NOEC or EC_{10} value. Belanger et al. (2006) found EC_{10} to EC_{20}-ratios for all chronic endpoints were similar, with the EC_{20}-values being more robust. As a comparison with the results of the chronic probabilistic QSAR show that an application factor of 10 is conservative, this application factor has been used to calculate the PNEC for each AE homologue from the statistically more robust EC_{20}-values. The results are given in **Table 4.38**. Further justification of this application factor is given below, following the discussion of the chronic probabilistic PNEC.

Table 4.38 PNEC values in mg/l for AE homologues determined using the chronic *Daphnia magna* QSAR and an application factor of 10

C / EO	C8 +C9	C10	C11	C12	C13	C14	C15	C16	C18
0	1.71E-01	9.71E-02	5.45E-02	3.04E-02	1.69E-02	9.33E-03	5.13E-03	2.81E-03	8.35E-04
1	2.13E-01	1.18E-01	6.52E-02	3.58E-02	1.96E-02	1.07E-02	5.83E-03	3.16E-03	9.24E-04
2	2.97E-01	1.63E-01	8.87E-02	4.83E-02	2.62E-02	1.42E-02	7.65E-03	4.12E-03	1.19E-03
3	4.00E-01	2.17E-01	1.17E-01	6.33E-02	3.41E-02	1.83E-02	9.85E-03	5.28E-03	1.51E-03
4	5.24E-01	2.82E-01	1.52E-01	8.15E-02	4.37E-02	2.34E-02	1.25E-02	6.67E-03	1.90E-03
5	6.74E-01	3.61E-01	1.93E-01	1.03E-01	5.51E-02	2.94E-02	1.57E-02	8.34E-03	2.35E-03
6	8.53E-01	4.56E-01	2.43E-01	1.29E-01	6.88E-02	3.66E-02	1.94E-02	1.03E-02	2.90E-03
7	1.07E+00	5.69E-01	3.02E-01	1.61E-01	8.52E-02	4.52E-02	2.39E-02	1.27E-02	3.55E-03
8	1.33E+00	7.03E-01	3.73E-01	1.98E-01	1.05E-01	5.54E-02	2.93E-02	1.55E-02	4.32E-03
9	1.63E+00	8.64E-01	4.57E-01	2.42E-01	1.28E-01	6.75E-02	3.56E-02	1.88E-02	5.22E-03
10	1.99E+00	1.05E+00	5.57E-01	2.94E-01	1.55E-01	8.17E-02	4.31E-02	2.27E-02	6.30E-03
11	2.42E+00	1.28E+00	6.74E-01	3.55E-01	1.87E-01	9.86E-02	5.19E-02	2.73E-02	7.56E-03
12	2.93E+00	1.54E+00	8.13E-01	4.28E-01	2.25E-01	1.18E-01	6.23E-02	3.27E-02	9.04E-03
13	3.53E+00	1.86E+00	9.77E-01	5.14E-01	2.70E-01	1.42E-01	7.45E-02	3.91E-02	1.08E-02
14	4.24E+00	2.23E+00	1.17E+00	6.14E-01	3.23E-01	1.69E-01	8.89E-02	4.66E-02	1.28E-02
15	5.06E+00	2.66E+00	1.40E+00	7.33E-01	3.84E-01	2.02E-01	1.06E-01	5.54E-02	1.52E-02
16	6.04E+00	3.17E+00	1.66E+00	8.71E-01	4.57E-01	2.39E-01	1.25E-01	6.57E-02	1.80E-02
17	7.18E+00	3.76E+00	1.97E+00	1.03E+00	5.41E-01	2.83E-01	1.48E-01	7.77E-02	2.13E-02
18	8.51E+00	4.46E+00	2.34E+00	1.22E+00	6.40E-01	3.35E-01	1.75E-01	9.17E-02	2.51E-02
19	1.01E+01	5.27E+00	2.76E+00	1.44E+00	7.56E-01	3.95E-01	2.07E-01	1.08E-01	2.95E-02
20	1.19E+01	6.23E+00	3.26E+00	1.70E+00	8.90E-01	4.65E-01	2.43E-01	1.27E-01	3.47E-02
21	1.19E+01	6.23E+00	3.26E+00	1.70E+00	8.90E-01	4.65E-01	2.43E-01	1.27E-01	3.47E-02
22	1.19E+01	6.23E+00	3.26E+00	1.70E+00	8.90E-01	4.65E-01	2.43E-01	1.27E-01	3.47E-02

The TGD (2003) discusses factors which should be considered in determining the magnitude of the application factor which should be applied to a PNEC determined using statistical extrapolation techniques. These are given in Table 4.39, along with an evaluation of the alcohol ethoxylate data and methods used by Belanger et al (2006) to develop the chronic probabilistic QSAR (Belanger 2006b). The chronic probabilistic QSAR developed by Belanger et al (2006) contains chronic data from 17 species, compared with the minimum of 10 species and the recommended number of at least 15 species specified in the EU TGD (2003), following guidance developed at the Expert Consultation Workshop on Statistical Extrapolation Techniques for Environmental Effects Assessments, held in London on 17-18 January 2001. The data used in the QSAR development, shown in Table 4.33, are true chronic data, and in addition have been obtained for a known AE homologue distribution. The eight different taxonomic groups specified in the EU TGD (2003) are represented in the Belanger et al (2006) chronic

probabilistic QSAR, and differences in the life forms, feeding strategies and trophic levels of the organisms are also represented. Exceptional taxonomic diversity is present, along with measurable redundancy using the most commonly employed test species (i.e., the algae *Pseudokirchneriella* = *Selenastrum capricornutum* and *Desmodesmus* = *Scenedesmus subcapitata*; the daphnid *Daphnia magna* and the fish fathead minnow *Pimephales promelas*). Information indicating a similar toxicity mechanism has been provided, as AE toxicity is generally accepted to proceed via non-specific membrane disruption for all species. This theory is supported by the general similarity of the chronic deterministic QSARS derived from the data for algae, *Daphnia magna*, and fish.

Table 4.39 Justification of an AF of 1 for the AE (alcohol ethoxylate) data set (Belanger 2006b)

TGD (2003) Criterion	Standard for Acceptance	Adherence to Criterion for Alcohol Ethoxylates
Overall quality of the database	All data are generated from true chronic studies	All studies included have Klimisch scores of 1 or 2 • Of 59 studies reported here, 49 have been through the peer review process for publication • 37 were fully supported by a QA organization. • 54 of 59 studies were directly reflective of a present day guideline method or an equivalent. • 58 of 59 studies confirmed exposure concentrations during the conduct of the test and the single study that did not was the third representative of a taxon that was evaluated two additional times under full GLP.
	Chronic studies cover sensitive life stages	• The three most sensitive taxa contain representatives of fish, invertebrates, and plants • The most sensitive fish (rainbow trout) employed testing of the most sensitive life stages (egg, embryo, and juvenile) • The most sensitive invertebrate (the clam, *Corbicula*) was exposed over a long (56-day) duration to the juvenile stage, generally considered as equi-sensitive to late pediveliger stages (Belanger et al. 1986, 1990) • The most sensitive plant was *Lemna minor*, an aquatic macrophytes that grows vegetatively and the test begins with plants containing few fronds (early growth)
	Endpoints used as input	All endpoints are either NOECs or EC_{10}s for the most sensitive biological effect observed in each study
Taxonomic Groups Considered	Fish (at least two species)	Three species are considered, rainbow trout (a salmonid, 1 study), fathead minnow (14 studies including two complete life cycle studies) and bluegill (3 studies)

	Crustaceans (at least one species)	Studies on *Daphnia magna* (9 studies) and *Ceriodaphnia dubia* (4 studies) are included
	Insect (at least one species)	A study employing the midge, *Chironomus tentans*, is included
	Additional invertebrates (at least one additional phylum not represented by Insects or Crustceans)	Molluscs: two species, a bivalve (*Corbicula fluminea*) and a gastropod (*Elimia livescens*) are included Rotifers: A series of studies on *Brachionus calyciflorus* is presented Planarians: A study on *Dugesia gonocephala* is included
	Algae (number unspecified)	Studies on green algae (3 different species) diatoms (1 species), and blue-green algae (1 species) are present
	Higher plants (number unspecified)	A study of the aquatic macrophyte *Lemna minor* is present
Minimal Sample Size	10 NOECs, preferably more than 15 for different species	The AE database contains 17 species from 59 chronic studies
Treatment of Multiple Data for Each Species	Pre-Selected for Environmental Parameters Relevant to Europe	15 of 59 studies were actually conducted in Europe. The majority of studies were conducted in standard dilution waters and all were conducted with water qualities representative of some part of Europe (including soft water environments as low as 11 mg/L and near neutral pH)
	Use of Data on Most Sensitive Endpoints	Each study was evaluated and the most sensitive response from that study extracted for use. Studies were summarized as a single value per AE homologue by the normalization process described in Belanger et al. (2006) and by trophic level QSARs as developed in Boeije et al. (2006) and Wind and Belanger (2006). Multiple studies per taxon were compiled as the geometric mean of the most sensitive study-specific endpoints.
	Effect of Water Quality or Study Designs on Aggregating	Studies were evaluated by temperature, pH, hardness, alkalinity, conductivity, duration and exposure design. There were no patterns of general water quality parameters influencing test outcomes. When data developed on AE mixtures was normalized to a

	Data	set homologue for the purposes of comparing inter-study variability by taxon where >5 studies were available, invertebrates were least variable (coefficients of variation of 49-87), followed by fish and (CV of 89), followed by algae (111). Given the range of study types, lack of water quality and study design influences, and breadth of data it was considered reasonable to express these as a geometric mean per taxon.
Fit to A Distribution	Use of Log-Normal Underlying Distribution	Confirmed
	Anderson Darling Goodness of Fit	Range of the A-D Statistic for 171 homologue distributions (C_9EO_0 to $C_{18}EO_{18}$) is 0.510-0.715 such that normality is accepted at $\alpha = 0.05$
	Kolmogorov-Smirnov Goodness of Fit	Range of the K-S Statistic for 171 homologue distributions (C_9EO_0 to $C_{18}EO_{18}$) is 0.769-0.832 such that normality is accepted at $\alpha = 0.05$
	Cramer von Mises Goodness of Fit	Range of the CvM Statistic for 171 homologue distributions (C_9EO_0 to $C_{18}EO_{18}$) 0.068-0.112 such that normality is accepted at $\alpha = 0.05$
	Conclusion	A log-normal distribution is appropriate as described in Aldenberg et al. 2002. The data lack any evidence of bimodality.
Estimated Parameter	$SSD_{0.05}$ with 50% confidence interval should be derived	The $SSD_{0.05}$ and its accompanying lower and upper 50% confidence intervals (CI) are presented in Tables 4.37 and 4.40. The data shows that the CI has very strong stability across the entire range of homologues. Importantly, the CIs are widest at the least hydrophobic chain lengths which contribute least to the risk characterization. On average, the CI band is -31% (lower 50% CI) to +19% (upper 50% CI) relative to the $SSD_{0.05}$ for the span of AE homologues between $C_{12}EO_0$ to $C_{18}EO_{18}$. The narrowness of the CI band is due to the breadth and number of taxa in the SSD and the consistent adherence to underlying assumptions of the fitted distribution.
NOEC values below the $SSD_{0.05}$	Discuss values that fall below the $SSD_{0.05}$	Sensitivity of the overall SSD is influenced greatest by the least sensitive taxon which is consistently the green algae *Chlorella vulgaris*. By including this test

		result, the overall variance term of the SSD increases resulting in a corresponding reduction in the prediction (generally 20-25% for all homologues). Therefore, by including this taxon, predictions for sensitive taxa actually lie above the $SSD_{0.05}$.
Distribution of trophic levels within the SSD	Discuss trophic level influences	Trophic levels are randomly dispersed along the SSD and no one trophic level is uniquely sensitive. Belanger et al. (2006) presents statistical analyses to evaluate the trophic level dispersion of the data and showed no one group was more or less sensitive. Further support for this can be seen in the trophic-level specific SARs where similar slopes across taxa are observed
Knowledge of the Mode of Action	Discuss	The mode of ecotoxic action for surfactants is generally accepted to be non-specific, with exposure resulting in disruption of biological membrane integrity (Roberts, 1991; Roberts and Marshall, 1995). Roberts and Marshall (1995) state that the assumption of additivity (concentration addition model) for non-ionic surfactants, specifically alcohol ethoxylates, is valid. Escher *et al.* (2002a, 2002b) and Dyer *et al.* (2000) demonstrated that baseline toxicants and related alcohol-based surfactants also follow a concentration addition model.

Confidence intervals (or CIs) for the HC_5 values are given in Table 4.40 (Belanger 2006b), and demonstrate very strong stability across the entire range of homologues. The span of the 50 percentile confidence interval is consistent across the span of homologues and is relatively narrow compared to other published SSDs (Posthuma et al. 2002; Versteeg et al. 1999). Importantly, the confidence intervals are widest at the least hydrophobic chain lengths which contribute least to the risk characterization. On average, the CI band is -31% (lower 50% CI) to +19% (upper 50% CI) relative to the $SSD_{0.05}$ for the span of AE homologues between $C_{12}EO_0$ to $C_{18}EO_{18}$. The narrowness of the CI band is due to the breadth and number of taxa in the SSD and the consistent adherence to underlying assumptions of the fitted distribution. In addition, the treatment of multiple test data from a single species and the use of the Aldenberg and Jaworska (2000) method for HC_5 determination also follow the EU TGD (2003) recommendations. As Table 4.39 shows, the matrix of chronic HC_5 values determined using statistical extrapolation techniques by Belanger et al (2006) and given in Table 4.37 satisfies all of the TGD criteria. Thus confidence in the data is so high that no additional safety factor is required, and an application factor of 1 is justified when. determining PNEC values from these AE homologue specific HC_5 values.

Table 4.40 Predicted 50% lower confidence limit for the $SSD_{0.05}$ (mg/L) by homologue for alcohol ethoxylates (Belanger 2006b)

	C9	C10	C11	C12	C13	C14	C15	C16	C18
E0	0.081	0.059	0.042	0.028	0.018	0.011	0.007	0.004	0.001
E1	0.103	0.073	0.050	0.034	0.021	0.013	0.008	0.004	0.001
E2	0.136	0.096	0.066	0.044	0.028	0.017	0.010	0.006	0.002
E3	0.174	0.122	0.084	0.055	0.035	0.022	0.013	0.007	0.002
E4	0.217	0.152	0.104	0.069	0.044	0.027	0.016	0.009	0.003
E5	0.266	0.186	0.127	0.084	0.054	0.033	0.020	0.011	0.003
E6	0.320	0.223	0.153	0.102	0.065	0.040	0.024	0.014	0.004
E7	0.381	0.266	0.182	0.121	0.078	0.049	0.029	0.017	0.005
E8	0.448	0.313	0.214	0.143	0.093	0.058	0.035	0.020	0.006
E9	0.524	0.366	0.251	0.168	0.110	0.069	0.042	0.024	0.007
E10	0.607	0.424	0.292	0.196	0.128	0.081	0.049	0.029	0.009
E11	0.700	0.490	0.337	0.227	0.149	0.095	0.058	0.034	0.010
E12	0.802	0.562	0.388	0.262	0.173	0.110	0.068	0.040	0.012
E13	0.916	0.642	0.443	0.301	0.199	0.128	0.079	0.047	0.015
E14	1.040	0.730	0.505	0.344	0.228	0.147	0.092	0.055	0.017
E15	1.178	0.827	0.574	0.391	0.261	0.169	0.106	0.064	0.021
E16	1.329	0.934	0.649	0.444	0.297	0.194	0.122	0.074	0.024
E17	1.495	1.052	0.732	0.502	0.337	0.221	0.140	0.085	0.028
E18	1.678	1.182	0.824	0.566	0.382	0.251	0.160	0.098	0.033
E19	1.877	1.324	0.924	0.637	0.431	0.285	0.182	0.113	0.038
E 20	2.096	1.480	1.035	0.714	0.485	0.322	0.207	0.129	0.044

The PNEC values determined by applying an application factor of 1 are given for each AE homologue in **Table 4.41**. As the extensions to C8 and EO20 and 21 have been made in a conservative manner, i.e. assuming that these homologues, which would be expected to be less toxic, have the same toxicity as the C9 and EO20 homologues, their inclusion should not result in a higher application factor.

Table 4.41 PNEC values, in mg/l, calculated for AE homologues (Belanger et al 2006), using the application factor 1.

C / EO	C8 and C9	C10	C11	C12	C13	C14	C15	C16	C18
0	1.24E-01	8.81E-02	6.07E-02	4.05E-02	2.60E-02	1.61E-02	9.63E-03	5.57E-03	1.71E-03
1	1.57E-01	1.09E-01	7.36E-02	4.82E-02	3.05E-02	1.86E-02	1.10E-02	6.28E-03	1.90E-03
2	2.10E-01	1.44E-01	9.67E-02	6.30E-02	3.97E-02	2.42E-02	1.43E-02	8.13E-03	2.44E-03
3	2.71E-01	1.85E-01	1.23E-01	8.01E-02	5.05E-02	3.07E-02	1.81E-02	1.03E-02	3.10E-03
4	3.39E-01	2.31E-01	1.54E-01	9.98E-02	6.29E-02	3.84E-02	2.26E-02	1.29E-02	3.89E-03
5	4.17E-01	2.83E-01	1.88E-01	1.22E-01	7.73E-02	4.72E-02	2.79E-02	1.60E-02	4.82E-03
6	5.06E-01	3.42E-01	2.28E-01	1.48E-01	9.37E-02	5.75E-02	3.41E-02	1.96E-02	5.93E-03
7	6.05E-01	4.09E-01	2.72E-01	1.77E-01	1.13E-01	6.93E-02	4.12E-02	2.37E-02	7.23E-03
8	7.17E-01	4.85E-01	3.23E-01	2.11E-01	1.34E-01	8.28E-02	4.95E-02	2.86E-02	8.76E-03
9	8.42E-01	5.69E-01	3.80E-01	2.48E-01	1.58E-01	9.83E-02	5.90E-02	3.42E-02	1.06E-02
10	9.83E-01	6.65E-01	4.43E-01	2.91E-01	1.86E-01	1.16E-01	6.98E-02	4.07E-02	1.27E-02
11	1.14E+00	7.71E-01	5.15E-01	3.38E-01	2.17E-01	1.36E-01	8.23E-02	4.82E-02	1.51E-02
12	1.32E+00	8.90E-01	5.95E-01	3.92E-01	2.53E-01	1.58E-01	9.65E-02	5.68E-02	1.79E-02
13	1.51E+00	1.02E+00	6.85E-01	4.52E-01	2.92E-01	1.84E-01	1.13E-01	6.66E-02	2.12E-02
14	1.73E+00	1.17E+00	7.85E-01	5.19E-01	3.36E-01	2.13E-01	1.31E-01	7.78E-02	2.50E-02
15	1.97E+00	1.34E+00	8.96E-01	5.93E-01	3.86E-01	2.45E-01	1.51E-01	9.06E-02	2.94E-02
16	2.24E+00	1.52E+00	1.02E+00	6.77E-01	4.41E-01	2.82E-01	1.75E-01	1.05E-01	3.44E-02
17	2.54E+00	1.72E+00	1.16E+00	7.69E-01	5.03E-01	3.22E-01	2.01E-01	1.21E-01	4.02E-02
18	2.87E+00	1.95E+00	1.31E+00	8.72E-01	5.72E-01	3.68E-01	2.30E-01	1.40E-01	4.69E-02
19	3.23E+00	2.19E+00	1.48E+00	9.87E-01	6.49E-01	4.18E-01	2.63E-01	1.61E-01	5.44E-02
20	3.63E+00	2.47E+00	1.67E+00	1.11E+00	7.33E-01	4.75E-01	3.00E-01	1.84E-01	6.30E-02
21	3.63E+00	2.47E+00	1.67E+00	1.11E+00	7.33E-01	4.75E-01	3.00E-01	1.84E-01	6.30E-02
22	3.63E+00	2.47E+00	1.67E+00	1.11E+00	7.33E-01	4.75E-01	3.00E-01	1.84E-01	6.30E-02

In this HERA report, PNEC values calculated using both the chronic *Daphnia magna* QSAR with an application factor of 10 and the chronic probabilistic QSAR with an application factor of 1 will be used in the risk assessment. This is done because, in effect, both QSARs support each other. The existence of the probabilistic QSAR, which generally gives lower AE homologue toxicity predictions than the chronic *Daphnia magna* QSAR, supports the use of the application factor of 10 for the chronic *Daphnia magna* QSAR, rather than raise the possibility of a higher application factor as the *Daphnia magna* QSAR calculates an EC_{20} rather than an EC_{10} value. In addition, the existence of the chronic *Daphnia magna* QSAR supports the probabilistic QSAR, in that, by predicting somewhat greater toxicity, its use avoids the need to determine whether the application factor for the

probabilistic QSAR should be 1 or a slightly higher number. The use of both QSARs preserves the strengths of both, including the use of some sediment dwelling species in the probabilistic QSAR, and is in accord with the HERA principle of investigating the uncertainties involved in the risk assessment process (HERA 2005).
The mesocosm QSAR developed by Boeije et al (2006) and discussed in section 4.2.1.2.5 is also available as supporting evidence for the PNEC values based on either the chronic *Daphnia magna* QSAR or the chronic probabilistic QSAR. The AE mixtures used to develop the mesocosm QSAR reflect the AE mixtures used commercially, especially in the US, and the test studies focus on AE mixtures with a moderate level of ethoxylation (Belanger 2006b). However, the chronic probabilistic QSAR is derived from tests with an even broader distribution. Belanger (2006b) shows that it is possible to use the normalization process described in Belanger et al. (2006) to calculate predicted EC_{10} values for the algae, invertebrates, and fish present in each mesocosm study, and to use the mesocosm QSAR (Boeije et al 2006) to calculate the predicted mesocosm NOEC value for each mesocosm study. The results are shown in Table 4.42, where the measured mesocosm NOEC values and the predicted 5 percentile value ($SSD_{0.05}$) for the experimental distributions are also shown. It can be seen that the predicted mesocosm to chronic probabilistic QSAR ratio ranges from just more than 3 to about 0.8 for the five AE mixtures, while the measured mesocosm to chronic probabilistic QSAR ratio ranges from less than 3 to about 0.2 for the same AE mixtures. The average mesocosm:$SSD_{0.05}$ ratios for predicted and measured mesocosm results are 1.7 and 1.3, respectively. Thus, when the same AE distributions are considered, the results of the mesocosm and chronic probabilistic QSAR methods are very similar, especially when the constraints of the different methods are taken into account. On average, the chronic probabilistic QSAR

Table 4.42. Predictions of chronic toxicity based on the structure-activity relationships with subsequent species sensitivity distribution and mesocosm information (Belanger 2006b).

	Predicted values				$SSD_{0.05}$	Measured	Predicted	Measured
AE	Algae (EC_{10} mg/L)	Inverts (EC_{10} mg/L)	Fish (EC_{10} mg/L)	Mesocosm (NOEC mg/L)	prediction (mg/L)	Mesocosm (NOEC mg/L)	Mesocosm to SSD ratio	Mesocosm to SSD ratio
91-6	1.22	3.27	3.30	0.80	0.258	0.730	3.10	2.83
23-6.5	0.511	0.917	1.672	0.13	0.103	0.280	1.26	2.72
45-7	0.240	0.302	0.925	0.027	0.042	0.080	0.64	1.90
25-9	0.419	0.606	1.501	0.062	0.078	0.070	0.79	0.90
25-6	0.335	0.482	1.204	0.049	0.062	0.013	0.79	0.21

method is somewhat more conservative than the mesocosm-based predictions. Given the breadth of AE tested in the single species chronic toxicity context, these may be considered to be preferred especially at the ends of the hydrophobicity distributions. In conclusion, as SSD and mesocosm studies are consistent within the range that the work overlaps, and the greater breadth of information developed for the Species Sensitivity Distribution analysis supports the use of the SSD approach (i.e. the chronic probabilistic QSAR) to derive PNECs for the environment (Belanger 2006b).

4.2.2. Alcohol ethoxylate toxicity to sediment organisms

The EU TGD (2003) allows the equilibrium partitioning method to be used to predict the sediment PNEC from the aquatic PNEC, if no sediment data are available. The chronic data given in Table 4.33 and used to develop the probabilistic QSAR for aquatic species discussed in section 4.2.1.2.6. does contain aquatic test data for species which live in sediment, such as *Chironomus tentans* and *Corbicula fluminea,* and others which live near or in the top sediment surface such as *Hyallela azteca, Dugesia gonocephala*, *Chlorella vulgaris* and *Navicula pelliculosa.* Thus use of the equilibrium partitioning method to predict the PNEC for sediment dwelling organisms is appropriate.

Comparison of the chronic *Daphnia magna* data with that for the sediment dwelling organisms given in Table 4.33 shows that *Daphnia magna* is of similar sensitivity to the sediment dwelling organisms. As the deterministic chronic *Daphnia magna* QSAR is quite robust, it has been decided that the aquatic PNEC derived from

Table 4.43 Sediment PNEC values, in mg/kg, determined by the equilibrium partitioning method, based on the Deterministic method using the chronic *Daphnia magna* QSAR

C/ EO	C 8 and C9	C10	C11	C12	C13	C14	C15	C16	C18
0	2.01E+00	2.30E+00	2.67E+00	3.10E+00	3.60E+00	4.16E+00	4.80E+00	5.51E+00	7.21E+00
1	2.76E+00	3.11E+00	3.55E+00	4.06E+00	4.65E+00	5.32E+00	6.07E+00	6.91E+00	8.90E+00
2	4.27E+00	4.76E+00	5.37E+00	6.09E+00	6.91E+00	7.84E+00	8.88E+00	1.00E+01	1.28E+01
3	6.36E+00	7.05E+00	7.90E+00	8.90E+00	1.00E+01	1.13E+01	1.27E+01	1.43E+01	1.81E+01
4	9.23E+00	1.02E+01	1.14E+01	1.28E+01	1.43E+01	1.61E+01	1.80E+01	2.02E+01	2.53E+01
5	1.32E+01	1.45E+01	1.61E+01	1.80E+01	2.01E+01	2.25E+01	2.52E+01	2.81E+01	3.49E+01
6	1.85E+01	2.04E+01	2.26E+01	2.51E+01	2.80E+01	3.12E+01	3.48E+01	3.87E+01	4.80E+01
7	2.57E+01	2.83E+01	3.13E+01	3.47E+01	3.86E+01	4.29E+01	4.77E+01	5.30E+01	6.54E+01
8	3.55E+01	3.89E+01	4.30E+01	4.76E+01	5.28E+01	5.86E+01	6.50E+01	7.22E+01	8.86E+01
9	4.85E+01	5.31E+01	5.86E+01	6.48E+01	7.18E+01	7.96E+01	8.81E+01	9.76E+01	1.20E+02
10	6.59E+01	7.21E+01	7.95E+01	8.78E+01	9.71E+01	1.07E+02	1.19E+02	1.31E+02	1.61E+02
11	8.90E+01	9.74E+01	1.07E+02	1.18E+02	1.31E+02	1.44E+02	1.60E+02	1.76E+02	2.15E+02
12	1.20E+02	1.31E+02	1.44E+02	1.59E+02	1.75E+02	1.93E+02	2.13E+02	2.35E+02	2.86E+02
13	1.60E+02	1.75E+02	1.93E+02	2.12E+02	2.34E+02	2.58E+02	2.84E+02	3.13E+02	3.80E+02
14	2.14E+02	2.34E+02	2.57E+02	2.83E+02	3.12E+02	3.43E+02	3.78E+02	4.16E+02	5.04E+02
15	2.85E+02	3.11E+02	3.42E+02	3.76E+02	4.13E+02	4.55E+02	5.01E+02	5.51E+02	6.66E+02
16	3.77E+02	4.13E+02	4.53E+02	4.98E+02	5.47E+02	6.02E+02	6.62E+02	7.28E+02	8.79E+02
17	4.99E+02	5.46E+02	5.99E+02	6.58E+02	7.23E+02	7.94E+02	8.73E+02	9.59E+02	1.16E+03
18	6.59E+02	7.21E+02	7.90E+02	8.68E+02	9.53E+02	1.05E+03	1.15E+03	1.26E+03	1.52E+03
19	8.68E+02	9.50E+02	1.04E+03	1.14E+03	1.25E+03	1.38E+03	1.51E+03	1.66E+03	1.99E+03
20	1.14E+03	1.25E+03	1.37E+03	1.50E+03	1.65E+03	1.80E+03	1.98E+03	2.17E+03	2.61E+03
21	1.14E+03	1.25E+03	1.37E+03	1.50E+03	1.65E+03	1.80E+03	1.98E+03	2.17E+03	2.61E+03
22	1.14E+03	1.25E+03	1.37E+03	1.50E+03	1.65E+03	1.80E+03	1.98E+03	2.17E+03	2.61E+03

both the chronic *Daphnia magna* QSAR and the chronic probabilistic QSAR should be used with the equilibrium partitioning method to determine the PNEC sediment for all the AE homologues.

In order to account for uptake *via* sediment ingestion, the EU TGD (2003) also specifies that an additional application factor of 10 should be applied to the PNEC/PNEC ratio for

any chemicals with a logKow value greater than 5. This has been incorporated in the CSARA AE Workbook (Erasm, 2005c) for those AE homologues with logKow values greater than 5.

The TGD (2003, equation 70) calculates the PNEC sediment using the equilibrium partitioning method according to

$$PNEC_{sed} = (K_{susp\text{-}water} / Rho_{susp}) * PNEC\ water * 1000 \qquad \text{(TGD 70)}$$

Here Rho_{susp} is the density of wet suspended matter, and $K_{susp\text{-}water}$ is derived from TGD equations 23 and 24 as described in section 4.1.2.1.9 in the PEC sediment section of this HERA environmental assessment. The calculation of PNEC sediment

Table 4.44 Sediment PNEC values, in mg/kg, determined by the equilibrium partitioning method, based on the chronic probabilistic QSAR

C/ EO	C8 and C9	C10	C11	C12	C13	C14	C15	C16	C18
0	1.46E+00	2.09E+00	2.97E+00	4.12E+00	5.54E+00	7.19E+00	9.00E+00	1.09E+01	1.48E+01
1	2.04E+00	2.87E+00	4.01E+00	5.47E+00	7.24E+00	9.26E+00	1.14E+01	1.37E+01	1.83E+01
2	3.02E+00	4.22E+00	5.85E+00	7.95E+00	1.05E+01	1.34E+01	1.65E+01	1.98E+01	2.62E+01
3	4.30E+00	6.00E+00	8.30E+00	1.13E+01	1.49E+01	1.90E+01	2.34E+01	2.80E+01	3.71E+01
4	5.98E+00	8.33E+00	1.15E+01	1.56E+01	2.06E+01	2.64E+01	3.26E+01	3.91E+01	5.18E+01
5	8.16E+00	1.14E+01	1.57E+01	2.13E+01	2.82E+01	3.62E+01	4.48E+01	5.38E+01	7.16E+01
6	1.10E+01	1.53E+01	2.12E+01	2.88E+01	3.81E+01	4.90E+01	6.10E+01	7.34E+01	9.80E+01
7	1.46E+01	2.03E+01	2.82E+01	3.84E+01	5.10E+01	6.58E+01	8.22E+01	9.93E+01	1.33E+02
8	1.92E+01	2.68E+01	3.72E+01	5.07E+01	6.77E+01	8.77E+01	1.10E+02	1.33E+02	1.80E+02
9	2.50E+01	3.50E+01	4.87E+01	6.66E+01	8.91E+01	1.16E+02	1.46E+02	1.78E+02	2.42E+02
10	3.25E+01	4.55E+01	6.33E+01	8.69E+01	1.17E+02	1.52E+02	1.93E+02	2.36E+02	3.23E+02
11	4.19E+01	5.88E+01	8.19E+01	1.13E+02	1.52E+02	1.99E+02	2.53E+02	3.11E+02	4.29E+02
12	5.37E+01	7.55E+01	1.05E+02	1.45E+02	1.96E+02	2.59E+02	3.30E+02	4.08E+02	5.68E+02
13	6.86E+01	9.66E+01	1.35E+02	1.87E+02	2.53E+02	3.35E+02	4.30E+02	5.33E+02	7.49E+02
14	8.73E+01	1.23E+02	1.73E+02	2.39E+02	3.25E+02	4.31E+02	5.56E+02	6.94E+02	9.84E+02
15	1.11E+02	1.56E+02	2.19E+02	3.04E+02	4.15E+02	5.54E+02	7.17E+02	9.01E+02	1.29E+03
16	1.40E+02	1.98E+02	2.78E+02	3.87E+02	5.29E+02	7.08E+02	9.22E+02	1.16E+03	1.68E+03
17	1.76E+02	2.50E+02	3.52E+02	4.90E+02	6.72E+02	9.03E+02	1.18E+03	1.50E+03	2.19E+03
18	2.22E+02	3.15E+02	4.43E+02	6.19E+02	8.51E+02	1.15E+03	1.51E+03	1.92E+03	2.84E+03
19	2.78E+02	3.95E+02	5.58E+02	7.80E+02	1.08E+03	1.46E+03	1.92E+03	2.46E+03	3.67E+03
20	3.48E+02	4.95E+02	7.00E+02	9.80E+02	1.36E+03	1.84E+03	2.44E+03	3.15E+03	4.74E+03
21	3.48E+02	4.95E+02	7.00E+02	9.80E+02	1.36E+03	1.84E+03	2.44E+03	3.15E+03	4.74E+03
22	3.48E+02	4.95E+02	7.00E+02	9.80E+02	1.36E+03	1.84E+03	2.44E+03	3.15E+03	4.74E+03

has been implemented in the CSARA AE Workbook[13] (ERASM 2005c), with the input parameter PNECwater being taken either from the values determined for each AE homologue using the chronic Daphnia QSAR, given in Table 4.38, or from the probabilistic QSAR determined from the EC_{10} values of the species-sensitivity distributions for each AE homologue, given in Table 4.41. The PNEC sediment values, in mg/kg, obtained using each method are given in Tables 4.43 and 4.44. The results from the CSARA AE Workbook (ERASM 2005c) have been extended to C8 and EO21, 22 as described in section 4.2.1.2.7.

As explained in section 4.2.1.2.8., both of these PNEC tables will be used in the HERA AE sediment risk assessment.

4.2.3 Alcohol ethoxylate toxicity to sewage treatment plant organisms

Available data for AE toxicity to sewage treatment plant organisms indicates that this toxicity is relatively low. The available data for several commercial mixtures is given in Table 4.45. Appropriate bacterial toxicity data is available for some long chain alcohols, the EO=0 AE homologues, and this data is also shown in the table. As it can be assumed that the alcohols will be the most toxic AE homologues for each hydrocarbon chainlength, the alcohol data indicates that the AE homologues in the C8 to C11 hydrocarbon chainlength region will also have low toxicity. Information in the long chain alcohol SIAR (2006) and in Schäfers et al (2006 in prep.) indicates that lack of solubility influences the chronic toxicity results for *Daphnia magna* at hydrocarbon chainlengths of 15 and above, and thus it is possible that lack of solubility can account for the lower toxicity seen for the longer chain alcohols in Table 4.45.

The highest toxicity seen in Table 4.45 is the EC_{50} value of 140 mg/l seen in the activated sludge respiration test for a commercial C12EO4 mixture. An application factor of 100 should be applied to this test result, according to the TGD (EU 2003). Thus, if it is assumed that all AE homologues are as toxic as this "highest toxicity" AE mixture, the $PNEC_{micro\text{-}organisms}$ for AE will be 1.4mg/l. Although this is a conservative assumption,

$$\mathbf{PNEC_{micro\text{-}organisms} = 1.4\ mg/l}$$

will be used as the toxicity for the sum of all AE homologues in this HERA report.

[13] The CSARA AE Workbook (ERASM 2005b,c) is an EXCEL workbook which has been developed by the CSARA taskforce, sponsored by ERASM. It contains the basic data and calculation methods available to calculate the results of several ecotoxicity and sorption QSARs for each AE homologue from C9 -18 and EO 0 to 20. If environmental concentrations can be provided, the Workbook can enable the full risk assessment process to be carried out, with several choices of method available to the user. This enables more efficient use of QSARS including those developed by CSARA, in the AE risk assessment process.

Table 4.45 AE Toxicity to STP Organisms

AE Description	Test	Inoculum	EC50	Reference (Klimisch[14] score)
C12 EO 4 mean	Inhibition of sludge respiration (EC Guideline 88/302/EC)	Activated sludge at 1527 mg/l	140 mg/l	Sasol 1997h (2)
C12-14 EO3 mean	Inhibition of sludge respiration (EC Guideline 88/302/EC)	Activated sludge at 1425 mg/l	1000mg/l EC10= 2mg/l	Sasol 1997g (2)
C12-14 EO6 mean	Bacteria Toxicity test German Standard DIN 38412, part 8	Pseudomonas Putida	No inhibition of growth up to 10 g/l	Sasol 1996b (2)
C12-14 EO9 mean	Bacteria Toxicity test German Standard DIN 38412, part 8	Pseudomonas Putida	No inhibition of growth up to 10 g/l	Sasol 1995d (2)
C16-18 EO 25 mean	Bacteria Toxicity test German Standard DIN 38412, part 8	Pseudomonas Putida	No inhibition of growth up to 10 g/l	Sasol 1994h (2)
C8 alcohol	24-hr activated sludge	Activated sludge	200 mg/l	Tang et al (1990) (2)

[14]Klimisch Scores are 1 = valid without restriction; 2 = valid with restriction; 3 = not reliable; 4 = not assignable. Further information and guidance on the selection of data for HERA reports is given in Appendix C of the HERA Methodology Document (HERA 2005).

Table 4.45 continued

AE Description	Test	Inoculum	EC50	Reference (Klimisch score)
C10 alcohol	30 min. EC_0	Pseudomonas Putida	EC_0 = 10000mg/l	SIAR(2006)* (2)
C12 alcohol	30 min. EC_0	Pseudomonas Putida	EC_0 = 10000mg/l	SIAR(2006)** (1)
C12 -14 alcohols	30 min. EC_0	Pseudomonas Putida	EC_0 = 10000mg/l	SIAR(2006)* (4)
C14 alcohol	30 min. EC50	Pseudomonas Putida	>10000mg/l	Cognis/Henkel (1995a) (2)
C16-18 alcohol and C18 unsaturated	30 min. EC10	Pseudomonas Putida	EC_{10}>10000mg/l	SIAR(2006)* (2)
C18 alcohol	30 min. EC_0	Pseudomonas Putida	EC_0 = 10000mg/l	SIAR(2006)** (1)

*Klimisch scores are taken from the IUCLID for the data given in the SIAR, or **from the robust summary for the data given in the SIAR

4.2.4. AE toxicity to terrestrial species

Although some acute and chronic test results are available for commercial AE mixtures and for some pure AE homologues, this information does not cover the full AE homologue hydrocarbon chainlength or ethylene oxide range. In addition, the precise AE homologue distribution is not available for many of the commercial AE mixtures for which terrestrial toxicity test data is available. For these reasons the equilibrium partitioning method as described in the TGD (2003) has been used to determine $PNEC_{soil}$ values for the AE homologues. In addition, the available acute and chronic test data is compared with the equilibrium partitioning results, and is seen to provide support for the $PNEC_{soil}$ values as determined by the equilibrium partitioning method. These topics are described below.

4.2.4.1. $PNEC_{soil}$ calculated using equilibrium partitioning

For the terrestrial compartment, the TGD (2003) allows the equilibrium partitioning method, based on $PNEC_{water}$, to be used to calculate $PNEC_{soil}$, as a screening method to identify substances which require further testing. The method uses TGD equation 72,

$$PNEC_{soil} = (K_{soil\text{-}water} / RHO_{soil}) * PNEC_{water} * 1000 \qquad \text{(TGD 72)}$$

where RHO_{soil} is the bulk density of wet soil, with a value of 1700 kg/m^3, and $K_{soil\text{-}water}$ is determined by TGD equation 24, which becomes:

$$K_{soil\text{-}water} = Fwater_{soil} + Fsolid_{soil} * (Kp_{soil} / 1000) * RHO\ solid \qquad \text{(TGD 24)}$$

if there is no substance present in the air fraction of the soil, as is the case for all AE homologues. Here the fraction of water in the soil is 0.2, the fraction of solid in the soil is 0.6, the density of the solid phase present in soil is 2500 kg/m^3, and Kp_{soil} is related to the Koc of each AE homologue by:

$$Kp_{soil} = Foc_{soil} * Koc \qquad \text{(TGD 23)}$$

Table 4.46 $PNEC_{soil}$ values, in mg/kg soil, calculated for each AE homologue using the equilibrium partition method, based on the probabilistic chronic $PNEC_{water}$ with an application factor 1

C/ EO	8	9	10	11	12	13	14	15	16	18
0	0.54	1.12	1.65	2.38	3.33	4.49	5.83	7.32	8.88	12.01
1	0.76	1.57	2.28	3.22	4.42	5.86	7.50	9.31	11.16	14.87
2	1.13	2.34	3.35	4.71	6.43	8.51	10.88	13.49	16.10	21.28
3	1.62	3.36	4.80	6.68	9.11	12.06	15.38	19.03	22.73	30.13
4	2.25	4.68	6.67	9.31	12.65	16.73	21.43	26.47	31.72	42.13
5	3.08	6.41	9.10	12.66	17.23	22.91	29.36	36.42	43.83	58.17
6	4.16	8.67	12.25	17.11	23.29	30.94	39.85	49.60	59.83	79.75
7	5.53	11.54	16.32	22.74	31.04	41.58	53.51	66.77	80.62	108.35
8	7.30	15.23	21.56	30.09	41.23	54.94	71.25	89.39	108.41	146.28
9	9.54	19.91	28.17	39.44	54.00	72.18	94.25	118.73	144.45	197.23
10	12.40	25.89	36.68	51.23	70.60	94.69	123.93	156.51	191.55	263.32
11	16.00	33.44	47.38	66.36	91.36	123.09	161.90	205.63	252.77	348.86
12	20.63	43.13	60.93	85.42	118.07	159.91	209.59	268.67	331.92	460.82
13	26.28	54.96	77.79	109.57	151.69	205.65	271.97	350.56	433.67	608.15
14	33.52	70.14	99.42	139.90	194.08	263.68	350.82	452.85	564.49	799.12
15	42.51	88.98	126.86	177.93	247.09	337.53	449.64	581.65	732.50	1047.18
16	53.83	112.70	160.32	225.69	314.32	429.70	576.70	751.14	945.95	1365.31
17	67.99	142.37	202.13	285.98	397.83	546.12	733.75	961.34	1214.68	1777.87
18	85.56	179.21	255.33	359.86	502.66	692.01	934.42	1225.77	1566.05	2311.25
19	107.25	224.70	319.50	453.00	633.97	874.89	1182.68	1561.84	2006.79	2987.25
20	134.26	281.34	401.50	569.56	794.45	1101.06	1497.55	1985.19	2555.61	3854.91
21	149.56	313.45	447.35	634.63	885.24	1226.89	1668.71	2212.08	2847.69	4295.50
22	166.61	349.23	498.45	707.15	986.40	1367.11	1859.43	2464.90	3173.17	4786.45

where Foc_{soil}, the fraction of organic carbon in soil, has the TGD default value of 0.02 and the Koc values for each AE homologue are those determined by Van Compernolle et al (2006) and given in Table 4.2. By substituting the given values into TGD equations 23, 24, and 72, and using the $PNEC_{water}$ values determined by the probabilistic method with an application factor of 1 as given in Table 4.41, the $PNEC_{soil}$ values for each AE homologue can be calculated. These values, in mg/kg soil, are given in Table 4.46. Note that the TGD requires the PEC/PNEC ratio to be multiplied by an additional factor of 10 for all substances with a logKow value of 5 or greater. This correction will be applied to the soil PNEC values for the appropriate AE homologues in the risk assessment section.

The equilibrium partitioning calculation for $PNEC_{soil}$ has also been carried out using the $PNEC_{water}$ values determined by using the deterministic *Daphnia magna* QSAR with an application factor of 10. The results of this calculation are shown in Table 4.47 for each AE homologue.

Although the $PNEC_{soil}$ calculation using the equilibrium partitioning method is designed as a screening level tool, it gives values which are generally supported by

Table 4.47. $PNEC_{soil}$ values, in mg/kg soil, calculated for each AE homologue using the equilibrium partition method, based on the deterministic chronic *Daphnia magna* $PNEC_{water}$ values with an application factor 10

C/ EO	8	9	10	11	12	13	14	15	16	18
0	0.75	1.54	1.82	2.14	2.50	2.91	3.38	3.90	4.48	5.87
1	1.03	2.14	2.47	2.85	3.29	3.77	4.32	4.93	5.62	7.24
2	1.60	3.31	3.79	4.32	4.93	5.61	6.37	7.22	8.16	10.39
3	2.39	4.96	5.62	6.37	7.20	8.14	9.19	10.36	11.65	14.70
4	3.48	7.24	8.15	9.18	10.33	11.61	13.05	14.64	16.41	20.54
5	4.98	10.36	11.61	13.01	14.59	16.34	18.29	20.45	22.84	28.42
6	7.02	14.62	16.32	18.23	20.36	22.74	25.37	28.28	31.50	39.01
7	9.77	20.38	22.69	25.27	28.15	31.35	34.89	38.80	43.13	53.18
8	13.49	28.15	31.26	34.74	38.62	42.90	47.65	52.88	58.67	72.08
9	18.48	38.58	42.76	47.43	52.61	58.34	64.67	71.66	79.36	97.21
10	25.14	52.52	58.12	64.36	71.27	78.91	87.33	96.62	106.85	130.52
11	34.02	71.10	78.57	86.87	96.07	106.21	117.40	129.71	143.26	174.58
12	45.81	95.78	105.72	116.75	128.94	142.38	157.18	173.47	191.38	232.72
13	61.43	128.49	141.67	156.27	172.40	190.16	209.71	231.20	254.81	309.28
14	82.07	171.72	189.15	208.45	229.73	253.16	278.91	307.21	338.29	409.89
15	109.29	228.74	251.75	277.19	305.21	336.04	369.91	407.10	447.92	541.90
16	145.10	303.78	334.09	367.56	404.40	444.89	489.35	538.15	591.68	714.81
17	192.15	402.37	442.22	486.17	534.51	587.61	645.88	709.79	779.89	940.98
18	253.83	531.68	583.96	641.59	704.92	774.44	850.69	934.29	1025.93	1236.40
19	334.60	700.99	769.50	844.93	927.78	1018.66	1118.30	1227.50	1347.15	1621.77
20	440.19	922.38	1012.01	1110.60	1218.82	1337.49	1467.53	1609.98	1765.99	2123.89
21	490.34	1027.65	1127.59	1237.49	1358.11	1490.34	1635.25	1793.99	1967.83	2366.63
22	546.22	1144.94	1256.39	1378.89	1513.31	1660.67	1822.14	1999.03	2192.75	2637.13

the available acute and chronic terrestrial data. These data are discussed in the following sections.

4.2.4.2. Acute soil toxicity data for AE commercial products and homologues

Acute soil toxicity measurements for both plants and soil invertebrates are available from standard tests carried out on commercial AE mixtures, which contain differing distributions of AE homologues. The available data is shown in Table 4.48. Many of the tests have been carried out at concentrations of either 1000 or 100 mg/kg, with no intermediate concentrations being tested. The available earthworm tests either indicate that no mortality was observed at 1000 mg/kg soil, or that the LC_{50} value was greater than 1000 mg/kg. Most of the available plant tests also indicate no effect at the highest concentration tested, which was either 100 or 1000 mg/kg soil. However, one of the two entries for C13EO3 reports an effect on cress growth at 100 mg/kg soil, and thus reports

an LC_0 value as the next lower concentration tested, 10 mg/kg soil. A measured EC50 value of 300 mg/kg soil is also reported in Table 4.48 for a commercial C18 EO10 mixture.

Table 4.48 Acute Terrestrial Ecotox Data for AE mixtures

AE Mixture	Species Tested	Ecotoxological Endpoint	Value, mg/kg	Data Source or Owner	Klimisch score
Soil Invertebrate data					
C16-18 /18(1:2) EO10	*Eisenia foetida*	LC50 (14 day)	>1000 (Highest tested)	Cognis/Henkel 1990b	2
C16-18 /EO11	*Eisenia foetida*	LC0 (14 day)	>1000 (Highest tested)	Sasol 1994t	1
C13 (branched) EO3	*Eisenia foetida foetida*	(14 day) 88/302/EWG No mortality observed	>1000 (Highest concentration tested)	Sasol 1994u	1
C13 (branched) EO6	*Eisenia foetida foetida*	(14 day) 88/302/EWG No mortality observed	>1000 (Highest concentration tested)	Sasol 1994 v	1
C13 (branched) EO9	*Eisenia foetida foetida*	(14 day) 88/302/EWG No mortality observed	>1000 (Highest concentration tested)	Sasol 1995n	1
Terrestrial plant data					
C16-18 /18(1:2) EO10	*Avena sativa*	NOEC (21 day) EEC Higher plants test	1000 (Highest tested)	Cognis/Henkel (1990a)	2
C16-18 /18(1:2) EO10	*Lycopersicum esculentum*	NOEC (21 day) EEC Higher plants test	1000 (Highest tested)	Cognis/Henkel (1990a)	2
C16-18 /18(1:2) EO10	*Raphanus sativus* (radish)	NOEC (21 day early seedling growth)	300	Cognis/Henkel (1990a)	2

C16-18 EO11	*Triticum aestivum,*	No negative effect (17 days) OECD 208	100 (Highest tested)	Sasol 1994m	1
C16-18 EO11	*Brassica alba*	No negative effect (17 days) OECD 208	100 (Highest tested)	Sasol 1994m	1
C16-18 EO11	*Lepidium sativum*	No negative effect (17 days) OECD 208	100 (Highest tested)	Sasol 1994m	1
C12-14 EO2	*Triticum aestivum,*	No negative effect (19 days) OECD 208	100 (Highest tested)	Sasol 1994n	1
C12-14 EO2	*Brassica alba*	No negative effect (19 days) OECD 208	100 (Highest tested)	Sasol 1994n	1
C12-14 EO2	*Lepidium sativum*	No negative effect (19 days) OECD 208	100 (Highest tested)	Sasol 1994n	1
C12-14 EO4	*Triticum aestivum,*	No negative effect (19 days) OECD 208	100 (Highest tested)	Sasol 1994o	1
C12-14 EO4	*Brassica alba*	No negative effect (19 days) OECD 208	100 (Highest tested)	Sasol 1994o	1
C12-14 EO4	*Lepidium sativum*	No negative effect (19 days) OECD 208	100 (Highest tested)	Sasol 1994o	1

C12-14 EO7	*Triticum aestivum*	No negative effect (19 days) OECD 208	100 (Highest tested)	Sasol 1994p	1
C12-14 EO7	*Brassica alba*	No negative effect (19 days) OECD 208	100 (Highest tested)	Sasol 1994p	1
C12-14 EO7	*Lepidium sativum*	No negative effect (19 days) OECD 208	100 (Highest tested)	Sasol 1994p	1
C13 (branched) EO3	*Triticum aestivum,*	No negative effect (17 day test) OECD 208	100 (highest tested)	Sasol 1994q	1
C13 (branched) EO3	*Brassica alba*	No negative effect (17 day test) OECD 208	100 (highest tested)	Sasol 1994q	1
C13 (branched) EO3	*Lepidium sativum*	No negative effect (17 day test) OECD 208	10	Sasol 1994q	1
C13 (branched) EO6	*Triticum aestivum,*	No negative effect (17 day test) OECD 208	100 (highest tested)	Sasol 1994r	1
C13 (branched) EO6	*Brassica alba*	No negative effect (17 day test) OECD 208	100 (highest tested)	Sasol 1994r	1
C13 (branched) EO6	*Lepidium sativum*	No negative effect (17 day test) OECD 208	100 (highest tested)	Sasol 1994r	1

C13 (branched) EO9	*Triticum aestivum,*	No negative effect (17 day test) OECD 208	100 (highest tested)	Sasol 1994s	1
C13 (branched) EO9	*Brassica alba*	No negative effect (17 day test) OECD 208	100 (highest tested)	Sasol 1994s	1
C13 (branched) EO9	*Lepidium sativum*	No negative effect (17 day test) OECD 208	100 (highest tested)	Sasol 1994s	1

The highest toxicity seen in the acute data for commercial AE mixtures is that reported for *Lepidium sativum* (cress) exposed to C13EO3, in one of the two sets of reported plant toxicity data for this species and AE mixture. Examination of the test report shows that the effect at 100 mg/kg soil was a reduction of less than 50% in plant growth. Note that the concentration of C13EO3 used in the acute soil test considerably exceeds the C13EO3 concentration for these EO homologues expected in the environment, which is approximately 0.03 mg/kg, as shown in Table 4.23. As the TGD applies an application factor of 1000 to acute soil toxicity EC_{50} values, it would be possible to use 100 mg/kg as a conservative approximation of the EC_{50} value in this test, and then to apply the application factor of 1000 as required in the TGD (2003). This would give a PNEC for the C13EO3 mixture of 0.1 mg/kg soil. Comparison shows that the equilibrium partitioning based PNEC values derived from chronic aquatic tests for C13 AE homologues are higher by factors of 30 or more than those derived from acute data with an assessment factor of 1000. This indicates significantly reduced toxicity compared to the toxicity predicted from the acute data. However, this is consistent with the use of more conservative application factors for data from acute tests, and the fact that higher PNEC values are often derived from chronic tests. As supporting information, Ahlers et al (2006) have noted that a narcotic mode of action, as is seen for AE toxicity, is associated with a low acute to chronic toxicity ratio.

In addition to the acute soil toxicity data for commercial mixtures, a series of acute soil toxicity studies has been carried out for soil invertebrates with C8EO4, C12EO4, C12EO10, and for C10 and C14 alcohols (Shell Research Ltd. 2004a). Except for C12EO10, which was a commercial product, these were all single homologues. The tests were carried out for nematodes (*C. elegans*), springtails ((*F. candida*), earthworms (*E. fetida*), and Ostracods (*Heterocypris incongruens*).

The earthworm acute test was an adaptation of OECD Guideline 207 which used juvenile worms instead of mature ones, 100 rather than 500g of treated soil, and a reduced exposure period of one week. The worms, which were not fed during the test, were counted at the beginning and end of the test to determine survival. The springtail acute test follows that given in Lokke and van Gestel (1998). The test used 10 springtails (10-12

days old) per treatment jar, and food (dried yeast) was added to the 30 g of treated soil at the beginning of the 7 day test. Survival was the test endpoint. The nematode tests were based on methods developed by Donkin and Dusenbery (1993), adapted to use only 1 g treated soil per test vessel. Ten adult nematodes were placed in the soil for 72 hours, and then all nematodes present were recovered. The test endpoint was the number of nematodes recovered. The ostracod test used a commercial test kit, Ostracodtoxkit F. Each test well contained 10 newly hatched ostracods, an algal suspension for food, and treated soil. After 6 days incubation (25C in the dark) the ostracods were removed from the soil for growth and survival determination. The test results are given in Table 4.49.

Table 4.49. Acute test results for single AE homologues, and for one commercial AE mixture tested simultaneously.

AE	EC_{50} values, in mg/kg dry weight soil, for the soil invertebrates listed below. 95% confidence limits are given in parentheses. All tests are given a Klimisch[15] score of 2.			
	C. elegans	*F. candida*	*E. foetida*	*H. incongruens* (mortality)
C8EO4	440 (350-470)	700 (500-1300)	440 (350-580)	450 (350-630)
C10 alcohol	98 (89-100)	320 (240-440)	170 (100-300)	150 (130-170)
C12EO4	460 (380-550)	600 (480-760)	850 (640-1000)	270 (210-340)
C12EO10 (commercial mixture)	360 (270-550)	>1000	500 (380-680)	430 (350-550)
C14 alcohol	>1000	530 (420-660)	>1000	>1000

The highest toxicity single homologue result of 98mg/kg in Table 4.49 is comparable to the result obtained for technical mixtures as shown in Table 4.48. As the TGD applies an application factor of 1000 to acute soil toxicity EC_{50} values, this would also give a PNEC value of 0.1 mg/kg soil. Comparison shows that the equilibrium partitioning based PNEC values derived from chronic aquatic tests for C10 alcohol are about 15 times higher than those derived from acute data with an assessment factor of 1000. This again indicates significantly reduced toxicity compared to the toxicity predicted from the acute data. In addition, this is consistent with the use of more conservative application factors for data from acute tests, and the fact that higher PNEC values are often derived from chronic tests. Also, a low acute to chronic toxicity ratio has been associated with a narcotic mode of action (Ahlers et al, 2006), which applies to AE toxicity.

[15] Klimisch Scores are 1 = valid without restriction; 2 = valid with restriction; 3 = not reliable; 4 = not assignable. Further information and guidance on the selection of data for HERA reports is given in Appendix C of the HERA Methodology Document (HERA 2005).

This comparison should be considered together with the chronic data presented in section 4.2.4.3 below to determine the appropriateness of the application factor selected, and the support the data in Table 4.49 gives for the $PNEC_{soil}$ values determined by equilibrium partitioning.

4.2.4.3. Chronic soil toxicity data for AE homologues.

A range of chronic soil tests has been carried out for two pure AE homologues, C12EO4 and C16EO4 (Shell Research Ltd. 2004b). The two AE homologues chosen for the chronic tests span the hydrocarbon chainlength range of high tonnage AE, and have an EO chainlength value at which the toxicity is expected to be in the high range for each hydrocarbon chainlength.

The chronic tests, to assess the survival and reproduction of earthworms (*Eisenia fetida fetida)*, springtails (*Folsomia candida*), and nematodes (*Caenorhabditas elegans*), were carried out using a natural sandy loam soil, with an organic carbon content of 1.3%, which could be assumed to be free of pesticide (Shell Research Ltd. 2004a). Dimethoate was used as a reference compound.

The earthworm reproduction tests followed methods given in the draft OECD Guideline 222 (2002). The springtail reproduction tests followed methods described in ISO standard 11276 – Inhibition of reproduction of Collembola by soil pollutants (1999). The nematode tests were based on methods developed by Donkin and Dusenbery (1993).

The test results for C12EO4 and C16EO4 are given in Table 4.50. These chronic results are given a Klimisch score of 2. If an application factor of 10 were applied to the lowest chronic NOEC value to determine a PNEC, a PNEC of 22 mg/kg would result for C12EO4 and a PNEC of 46mg/kg would result for the C16EO4 AE homologue. Comparison of the acute test data with the equilibrium partition soil PNEC values shown in Tables 4.46 and 4.47 (dark gray background), shows very

Table 4.50 Chronic effects of two AE homologues on terrestrial organisms

Toxicity Measure	AE Homo-logue	Soil organism test results. All results are given a Klimisch score of 2.					
		E. foetida		*F. candida*		*C. elegans*	
		Adult mortality, mg/kg	Number of juveniles, mg/kg	Adult mortality, mg/kg	Number of juveniles, mg/kg	Adult mortality, mg/kg	Number of juveniles, mg/kg
LC/EC_{50}	C12EO4	>1000	270 (200-360)	>1000	>1000	220-460*	220-460*
NOEC		460	220	1000	1000	220	220
LOEC		1000	460	-	-	460	460
LC/EC_{50}	C16EO4	>1000	No estimate	>1000	>1000	No data	No data
NOEC		1000	460	1000	1000	No data	No data
LOEC		-	1000	-	-	No data	No data

* Range determined by visual inspection of data as LC/EC_{50} unable to be calculated

similar values. Thus the chronic soil toxicity results for C12EO4 and C16EO4 support the validity of the PNEC values determined by equilibrium partitioning.

Shell Research Ltd. (2004b) notes that the chronic toxicity of C12EO4 is similar to, or only slightly lower than, the acute toxicity for soil invertebrates. Certainly the differences between the acute EC_{50} and chronic NOEC values are less than a factor of 4 for this AE homologue, rather than a factor of 100 as would be predicted by the EU TGD (2003) default application factors. The acute soil toxicity EC_{50} values span an order of magnitude between the different test species and test chemicals, with nematodes generally being among the more sensitive species in acute tests, but with other species showing similar sensitivity. As the chronic and acute results are so similar, it would be appropriate to apply an application factor of 50 or less to the acute test EC_{50} data, rather than a factor of 1000 as specified in the TGD (EU 2003). If this is done, the PNEC values determined from the lowest EC_{50} data in Table 4.49 can be shown to conservatively support the PNEC determined by equilibrium partitioning as given in Tables 4.46 and 4.47, for those AE homologues (C10 and C14 alcohol, C12EO4 and C8 EO4) for which acute data is available.

In conclusion, the PNEC calculated by equilibrium partitioning for each AE homologue given in Tables 4.46 and 4.47 is supported by chronic data for two AE homologues. Information showing the relatively small difference between acute and chronic toxicity for soil organisms means that available single homologue acute data also supports the PNEC calculated by equilibrium partitioning from aquatic monitoring data. In addition, available data for AE mixtures is consistent with the equilibrium partitioning PNEC values.

4.2.5 Summary of PNEC Information Location

The location of the PNEC information derived in section 4.2 is indicated in the Summary Table below. This information will be used, together with the PEC information summarized in Section 4.1.2.5, in the AE risk assessment (Section 4.3).

PNEC	**Basis of PNEC Determination**	**Table or page**
Aquatic	Chronic Probabilistic QSAR	Table 4.41
	Chronic Daphnia QSAR	Table 4.38
Sediment	Equilibrium partitioning using Chronic Probabilistic QSAR	Table 4.44
	Equilibrium partitioning using Chronic Daphnia QSAR	Table 4.43
STP organisms	AE toxicity data – No homologue distribution. $PNEC_{micro\text{-}organisms}$ = **1.4 mg/l**	Section 4.2.3, page 108
Soil	Equilibrium partitioning using Chronic Probabilistic QSAR	Table 4.46
	Equilibrium partitioning using Chronic Daphnia QSAR	Table 4.47

4.3 Risk assessment

The AE risk assessments are carried out, as specified in the EU TGD 2003, by dividing the Predicted Environmental Concentration (PEC) by the Predicted No Effect Concentration (PNEC). For the aquatic, sediment, and terrestrial environments, this is carried out for each AE homologue, and the resulting sum of the toxic units (or individual homologue PEC/PNEC values), gives one Risk Quotient, (or overall PEC/PNEC value) for the overall environmental AE distribution.

4.3.1. Risk assessment for aquatic species

The risk assessment for aquatic species dwelling in surface waters is based on the $PEClocal_{dissolved}$ values given for each AE homologue in Table 4.17, section 4.1.2.1.8, and the PNEC values determined for each AE homologue using both the chronic *Daphnia magna* QSAR with an application factor of 10 given in Table 4.38 and the chronic probabilistic QSAR with an application factor of 1 given in Table 4.41, as described in section 4.2.1.2.8. For the *Daphnia magna* QSAR, the application factor of 10 is used as three chronic QSARs are available for AE homologues. The *Daphnia magna* QSAR has

Table 4.51 PEC/PNEC values for AE homologues determined using the chronic *Daphnia magna* QSAR with an application factor of 10

C/ EO	C8* and C9	C10	C11	C12	C13	C14	C15	C16	C18
0	1.56E-04	2.76E-04	4.87E-04	8.65E-04	2.03E-03	1.29E-03	2.96E-03	5.00E-03	2.44E-03
1	2.91E-05	5.25E-05	9.43E-05	1.70E-04	1.03E-03	5.03E-04	6.61E-04	1.97E-04	7.72E-04
2	1.57E-05	2.88E-05	5.23E-05	9.53E-05	5.87E-04	1.01E-04	1.26E-03	3.78E-04	7.71E-04
3	2.35E-05	4.33E-05	8.01E-05	1.47E-04	8.35E-04	4.44E-04	5.54E-04	8.27E-04	2.50E-03
4	4.65E-06	8.63E-06	1.61E-05	2.96E-05	4.04E-04	4.28E-04	3.60E-04	9.04E-04	6.99E-04
5	7.75E-06	1.45E-05	2.70E-05	5.01E-05	2.05E-04	3.74E-04	4.24E-04	3.24E-04	4.33E-04
6	4.80E-06	8.98E-06	1.63E-05	3.00E-05	4.82E-04	2.33E-04	3.08E-04	1.93E-04	2.49E-04
7	5.82E-06	1.09E-05	2.03E-05	3.64E-05	2.29E-04	1.75E-04	3.58E-04	1.08E-04	3.19E-04
8	2.79E-06	5.26E-06	9.79E-06	1.75E-05	1.25E-04	1.72E-04	5.77E-04	1.56E-04	3.10E-04
9	1.91E-06	3.60E-06	6.71E-06	1.19E-05	8.01E-05	1.17E-04	2.46E-04	2.01E-04	2.17E-04
10	1.26E-06	2.39E-06	4.46E-06	7.87E-06	5.53E-05	5.57E-05	2.38E-04	1.20E-04	1.47E-04
11	9.16E-07	1.74E-06	3.25E-06	5.70E-06	3.37E-05	3.60E-05	1.62E-04	3.61E-04	1.23E-04
12	6.56E-07	1.25E-06	2.33E-06	4.04E-06	4.70E-05	2.53E-05	8.92E-05	1.68E-04	7.98E-05
13	3.77E-07	7.17E-07	1.34E-06	2.30E-06	1.23E-05	1.64E-05	6.00E-05	6.23E-05	7.64E-04
14	2.45E-07	4.59E-07	8.74E-07	1.47E-06	8.48E-06	9.83E-06	3.56E-05	3.41E-05	1.29E-04
15	1.75E-07	3.29E-07	6.28E-07	1.06E-06	5.26E-06	8.81E-06	2.27E-05	2.68E-05	6.77E-05
16	1.72E-07	3.23E-07	6.15E-07	1.04E-06	3.39E-06	5.57E-06	1.83E-05	1.45E-05	2.86E-05
17	2.05E-07	3.91E-07	6.61E-07	1.11E-06	2.13E-06	6.24E-06	1.27E-05	9.73E-06	1.47E-05
18	1.30E-07	2.48E-07	4.19E-07	7.07E-07	1.60E-06	3.17E-06	7.60E-06	4.71E-06	8.29E-06
19	1.10E-07	2.09E-07	3.54E-07	5.98E-07	1.36E-06	2.69E-06	6.44E-06	4.00E-06	7.04E-06
20	9.28E-08	1.77E-07	3.00E-07	5.07E-07	1.15E-06	2.28E-06	5.47E-06	3.40E-06	5.99E-06
21	9.28E-08	1.77E-07	3.00E-07	5.07E-07	1.15E-06	2.28E-06	5.47E-06	3.40E-06	5.99E-06
22	9.28E-08	1.77E-07	3.00E-07	5.07E-07	1.15E-06	2.28E-06	5.47E-06	3.40E-06	5.99E-06
Sum of PEC/PNEC values for AE homologues = 0.041									

*C8 values are for EO =0 to EO=14 only, as higher C8 EO homologues are not released to the environment by products which come within the scope of HERA

been chosen as it is the most robust of these three chronic QSARs, and has the most sensitive endpoint over much of the range of AE homologues. Further information is given in section 4.2.1.2.2. The chronic probabilistic QSAR gives the 95th percentile of a species sensitive distribution curve containing data from 17 species, described in section 4.2.1.2.6. Justification for the application factor of 1 is given in section 4.2.1.2.8. The resulting PEC/PNEC values for each AE homologue are shown in Table 4.51 when the chronic *Daphnia magna* QSAR is used to determine the PNEC for each AE homologue, and in Table **4.52** when the chronic probabilistic QSAR is used to determine the PNEC.

When the chronic *Daphnia magna* QSAR is used to determine the PNEC, the sum of the toxic units, equivalent to the PEC/PNEC ratio for the distribution of AE homologues found in the environment, is 0.041. When the chronic probabilistic QSAR is used to determine the PNEC, the PEC/PNEC ratio for the distribution of AE homologues found in the environment is 0.024.

Table 4.52 PEC/PNEC values for AE homologues determined using the probabilistic chronic QSAR

C/ EO	C8* and C9	C10	C11	C12	C13	C14	C15	C16	C18
0	2.15E-04	3.04E-04	4.38E-04	6.51E-04	1.32E-03	7.44E-04	1.58E-03	2.52E-03	1.19E-03
1	3.94E-05	5.69E-05	8.35E-05	1.26E-04	6.63E-04	2.89E-04	3.50E-04	9.93E-05	3.77E-04
2	2.23E-05	3.25E-05	4.80E-05	7.30E-05	3.87E-04	5.91E-05	6.76E-04	1.92E-04	3.75E-04
3	3.47E-05	5.09E-05	7.63E-05	1.16E-04	5.64E-04	2.65E-04	3.01E-04	4.23E-04	1.22E-03
4	7.18E-06	1.06E-05	1.59E-05	2.42E-05	2.80E-04	2.61E-04	1.99E-04	4.67E-04	3.41E-04
5	1.25E-05	1.85E-05	2.77E-05	4.23E-05	1.46E-04	2.33E-04	2.38E-04	1.69E-04	2.12E-04
6	8.09E-06	1.20E-05	1.74E-05	2.62E-05	3.54E-04	1.49E-04	1.76E-04	1.02E-04	1.22E-04
7	1.03E-05	1.52E-05	2.25E-05	3.29E-05	1.73E-04	1.14E-04	2.08E-04	5.75E-05	1.57E-04
8	5.16E-06	7.64E-06	1.13E-05	1.64E-05	9.76E-05	1.15E-04	3.42E-04	8.44E-05	1.53E-04
9	3.69E-06	5.46E-06	8.08E-06	1.16E-05	6.46E-05	8.05E-05	1.49E-04	1.10E-04	1.07E-04
10	2.56E-06	3.79E-06	5.60E-06	7.96E-06	4.60E-05	3.93E-05	1.47E-04	6.70E-05	7.33E-05
11	1.95E-06	2.88E-06	4.25E-06	5.99E-06	2.90E-05	2.61E-05	1.02E-04	2.04E-04	6.14E-05
12	1.46E-06	2.16E-06	3.19E-06	4.41E-06	4.19E-05	1.89E-05	5.76E-05	9.70E-05	4.02E-05
13	8.81E-07	1.30E-06	1.92E-06	2.61E-06	1.14E-05	1.27E-05	3.97E-05	3.66E-05	3.88E-04
14	5.99E-07	8.73E-07	1.30E-06	1.74E-06	8.14E-06	7.82E-06	2.42E-05	2.04E-05	6.59E-05
15	4.51E-07	6.56E-07	9.77E-07	1.30E-06	5.24E-06	7.24E-06	1.58E-05	1.64E-05	3.50E-05
16	4.63E-07	6.73E-07	1.00E-06	1.33E-06	3.50E-06	4.73E-06	1.31E-05	9.08E-06	1.49E-05
17	5.80E-07	8.55E-07	1.13E-06	1.50E-06	2.29E-06	5.49E-06	9.39E-06	6.23E-06	7.75E-06
18	3.85E-07	5.67E-07	7.46E-07	9.90E-07	1.79E-06	2.89E-06	5.78E-06	3.09E-06	4.44E-06
19	3.42E-07	5.03E-07	6.61E-07	8.76E-07	1.58E-06	2.54E-06	5.06E-06	2.69E-06	3.82E-06
20	3.04E-07	4.47E-07	5.87E-07	7.76E-07	1.40E-06	2.24E-06	4.44E-06	2.35E-06	3.30E-06
21	3.04E-07	4.47E-07	5.87E-07	7.76E-07	1.40E-06	2.24E-06	4.44E-06	2.35E-06	3.30E-06
22	3.04E-07	4.47E-07	5.87E-07	7.76E-07	1.40E-06	2.24E-06	4.44E-06	2.35E-06	3.30E-06
Sum of PEC/PNEC values for AE homologues = 0.024									

*C8 values are for EO =0 to EO=14 only, as higher C8 EO homologues are not released to the environment by products which come within the scope of HERA

As the sum of the toxic units (or PEC/PNEC values) for AE homologues determined with both complementary PNEC prediction methods is much less than 1, the environmental concentration and distribution of AE homologues does not pose a risk to the aquatic environment.

Summary: RCR* values for aquatic species (surface waters)

QSAR used for PNEC	RCR*
Daphnia magna	0.041
Probabilistic	0.024

*The Risk Characterisation Ratio RCR is equal to the sum of the PEC/PNEC values for all the AE homologues

4.3.2 Risk assessment for sediment species

The risk assessment for sediment species is based on the $PEClocal_{sediment}$ values given for each AE homologue in Table 4.19, section 4.1.2.1.9, and the $PNEC_{sediment}$ values determined for each AE homologue with both the chronic *Daphnia magna* QSAR as given in **Table 4.38** and the chronic probabilistic QSAR as given in Table 4.41. The equilibrium partitioning method has been used to determine both the $PEClocal_{sediment}$ and the $PNEC_{sediment}$ values. The resulting PEC/PNEC ratios for each AE homologue are shown in

Table 4.53 Sediment PEC/PNEC values determined by the equilibrium partitioning method, based on the Deterministic method using the chronic *Daphnia magna* QSAR

C/ EO	C8* and C9	C10	C11	C12	C13	C14	C15	C16	C18
0	1.75E-04	2.75E-04	4.87E-04	8.58E-03	2.00E-02	1.25E-02	2.78E-02	4.40E-02	1.53E-02
1	4.07E-05	5.24E-05	9.38E-05	1.69E-03	1.01E-02	4.87E-03	6.17E-03	1.71E-03	4.64E-03
2	3.24E-05	2.88E-05	5.21E-05	9.46E-04	5.77E-03	9.72E-04	1.17E-02	3.24E-03	4.42E-03
3	2.67E-05	4.33E-05	7.97E-05	1.46E-03	8.18E-03	4.26E-03	5.08E-03	6.96E-03	1.37E-02
4	9.91E-06	8.61E-06	1.60E-05	2.93E-05	3.95E-03	4.09E-03	3.27E-03	7.48E-03	3.64E-03
5	1.09E-05	1.44E-05	2.68E-05	4.95E-05	2.00E-03	3.55E-03	3.81E-03	2.63E-03	2.14E-03
6	8.64E-06	8.94E-06	1.62E-05	2.96E-05	4.69E-03	2.20E-03	2.74E-03	1.53E-03	1.16E-03
7	7.62E-06	1.09E-05	2.01E-05	3.57E-05	2.22E-03	1.64E-03	3.14E-03	8.35E-04	1.40E-03
8	4.02E-06	5.25E-06	9.71E-06	1.72E-05	1.21E-03	1.61E-03	5.00E-03	1.18E-03	1.28E-03
9	2.72E-06	3.58E-06	6.65E-06	1.17E-05	7.71E-05	1.08E-03	2.10E-03	1.48E-03	8.40E-04
10	1.85E-06	2.37E-06	4.42E-06	7.71E-06	5.30E-05	5.10E-04	2.00E-03	8.59E-04	5.33E-04
11	1.33E-06	1.72E-06	3.22E-06	5.57E-06	3.21E-05	3.27E-04	1.33E-03	2.50E-03	4.14E-04
12	8.94E-07	1.24E-06	2.30E-06	3.94E-06	4.46E-05	2.27E-04	7.21E-04	1.12E-03	2.50E-04
13	5.31E-07	7.13E-07	1.33E-06	2.23E-06	1.16E-05	1.46E-04	4.75E-04	4.01E-04	2.22E-03
14	3.54E-07	4.57E-07	8.60E-07	1.42E-06	7.95E-06	8.62E-05	2.75E-04	2.11E-04	3.46E-04
15	1.75E-07	3.28E-07	6.17E-07	1.02E-06	4.90E-06	7.62E-06	1.71E-04	1.59E-04	1.68E-04
16	1.71E-07	3.20E-07	6.05E-07	9.98E-07	3.13E-06	4.74E-06	1.34E-04	8.23E-05	6.54E-05
17	2.04E-07	3.88E-07	6.48E-07	1.07E-06	1.95E-06	5.23E-06	9.04E-05	5.25E-05	3.08E-05
18	1.29E-07	2.44E-07	4.09E-07	6.73E-07	1.45E-06	2.61E-06	5.23E-05	2.41E-05	1.60E-05
19	1.09E-07	2.06E-07	3.45E-07	5.68E-07	1.22E-06	2.17E-06	4.28E-05	1.94E-05	1.24E-05
20	9.20E-08	1.75E-07	2.92E-07	4.78E-07	1.02E-06	1.80E-06	3.50E-06	1.56E-05	9.66E-06
21	1.02E-07	1.95E-07	3.24E-07	5.29E-07	1.12E-06	1.96E-06	3.75E-06	1.64E-05	9.83E-06
22	1.14E-07	2.16E-07	3.60E-07	5.85E-07	1.23E-06	2.13E-06	4.00E-06	1.71E-05	9.98E-06
Sum of PEC/PNEC values for AE homologues = 0.316									

*C8 values are for EO =0 to EO=14 only, as higher C8 EO homologues are not released to the environment by products which come within the scope of HERA

Table 4.53 when the chronic *Daphnia magna* QSAR is used to determine the sediment PNEC for each AE homologue, and in Table 4.54 when the chronic probabilistic QSAR is used to determine the sediment PNEC. The additional factor of 10 is included for each AE homologue with a log Kow value greater than 5, as required in the TGD (2003) to account for ingestion of sorbed material.

Table 4.54 Sediment PEC/PNEC values determined by the equilibrium partitioning method, based on the chronic probabilistic QSAR

C/ EO	C8* and C9	C10	C11	C12	C13	C14	C15	C16	C18
0	2.41E-04	3.03E-04	4.38E-04	6.46E-03	1.30E-02	7.22E-03	1.48E-02	2.22E-02	7.46E-03
1	5.51E-05	5.68E-05	8.31E-05	1.25E-03	6.52E-03	2.80E-03	3.27E-03	8.63E-04	2.26E-03
2	4.58E-05	3.25E-05	4.78E-05	7.24E-04	3.80E-03	5.69E-04	6.26E-03	1.64E-03	2.16E-03
3	3.94E-05	5.08E-05	7.59E-05	1.15E-03	5.53E-03	2.54E-03	2.77E-03	3.56E-03	6.66E-03
4	1.53E-05	1.05E-05	1.58E-05	2.39E-05	2.74E-03	2.49E-03	1.81E-03	3.86E-03	1.78E-03
5	1.77E-05	1.84E-05	2.76E-05	4.18E-05	1.43E-03	2.21E-03	2.14E-03	1.37E-03	1.04E-03
6	1.46E-05	1.19E-05	1.73E-05	2.58E-05	3.44E-03	1.40E-03	1.56E-03	8.10E-04	5.68E-04
7	1.35E-05	1.51E-05	2.24E-05	3.23E-05	1.68E-03	1.07E-03	1.83E-03	4.46E-04	6.89E-04
8	7.44E-06	7.61E-06	1.12E-05	1.61E-05	9.43E-04	1.07E-03	2.96E-03	6.39E-04	6.32E-04
9	5.26E-06	5.42E-06	8.01E-06	1.14E-05	6.22E-05	7.44E-04	1.27E-03	8.13E-04	4.16E-04
10	3.74E-06	3.76E-06	5.54E-06	7.79E-06	4.41E-05	3.60E-04	1.24E-03	4.79E-04	2.65E-04
11	2.83E-06	2.86E-06	4.21E-06	5.85E-06	2.77E-05	2.37E-04	8.41E-04	1.41E-03	2.07E-04
12	1.99E-06	2.15E-06	3.15E-06	4.30E-06	3.98E-05	1.70E-04	4.66E-04	6.48E-04	1.26E-04
13	1.24E-06	1.29E-06	1.89E-06	2.54E-06	1.08E-05	1.13E-04	3.14E-04	2.36E-04	1.13E-03
14	8.68E-07	8.69E-07	1.28E-06	1.69E-06	7.63E-06	6.86E-05	1.87E-04	1.26E-04	1.77E-04
15	4.49E-07	6.52E-07	9.62E-07	1.26E-06	4.88E-06	6.26E-06	1.19E-04	9.71E-05	8.70E-05
16	4.61E-07	6.67E-07	9.85E-07	1.28E-06	3.24E-06	4.03E-06	9.60E-05	5.14E-05	3.42E-05
17	5.78E-07	8.48E-07	1.10E-06	1.43E-06	2.10E-06	4.60E-06	6.68E-05	3.36E-05	1.63E-05
18	3.83E-07	5.59E-07	7.28E-07	9.44E-07	1.62E-06	2.38E-06	3.98E-05	1.58E-05	8.56E-06
19	3.40E-07	4.96E-07	6.44E-07	8.31E-07	1.42E-06	2.05E-06	3.36E-05	1.30E-05	6.74E-06
20	3.01E-07	4.40E-07	5.70E-07	7.31E-07	1.24E-06	1.76E-06	2.84E-06	1.08E-05	5.32E-06
21	3.36E-07	4.91E-07	6.33E-07	8.10E-07	1.36E-06	1.92E-06	3.04E-06	1.13E-05	5.41E-06
22	3.73E-07	5.45E-07	7.03E-07	8.96E-07	1.50E-06	2.08E-06	3.25E-06	1.18E-05	5.49E-06
Sum of PEC/PNEC values for AE homologues = 0.181									

*C8 values are for EO =0 to EO=14 only, as higher C8 EO homologues are not released to the environment by products which come within the scope of HERA

When the chronic *Daphnia magna* QSAR is used to determine the PNEC for sediment dwelling organisms, the sum of the toxic units, equivalent to the PEC/PNEC ratio for the distribution of AE homologues found in the sediment environment, is 0.316. When the chronic probabilistic QSAR is used to determine the PNEC for sediment dwelling organisms, the PEC/PNEC ratio for the distribution of AE homologues found in the environment is 0.181.

As the sum of the toxic units (or PEC/PNEC values) determined with both complementary PNEC prediction methods are less than 1, the environmental concentration and distribution of AE homologues does not pose a risk to the sediment compartment of the environment.

Summary: RCR* values for sediment species

QSAR used for PNEC	RCR*
Daphnia magna	0.316
Probabilistic	0.181

*The Risk Characterisation Ratio RCR is equal to the sum of the PEC/PNEC values for all the AE homologues

4.3.3 Risk assessment for STP micro-organisms

The risk assessment for sewage treatment plant organisms has not been carried out for each AE homologue, as the low effluent concentrations and low toxicity both indicate that the risk of AE to sewage treatment plant micro-organisms is low. Instead, the overall $PEC_{micro\text{-}organisms}$ developed in section 4.1.2.3 has been compared with the most toxic $PNEC_{micro\text{-}organisms}$ value found in section 4.2.3 in order to carry out the risk assessment. Thus

$$\mathbf{PEC_{micro\text{-}organisms} / PNEC_{micro\text{-}organisms} = 9.8\mu g/l / 1.4mg/l = 0.007}$$

indicating no risk to sewage treatment plant micro-organisms due to AE concentrations found in sewage treatment facilities. As the conservative approximation for $PNEC_{micro\text{-}organisms}$ used here results in a low value for the PEC/PNEC ratio, further refinement of $PNEC_{micro\text{-}organisms}$ on a homologue-specific basis has not been felt to be appropriate.

4.3.4 Risk assessment for terrestrial species

The risk assessment for terrestrial species uses the PEC information based on monitored sludge data and an AE distribution calculated in accordance with the TGD and given in Table 4.22 to determine the PEC_{soil} values for each AE homologue given in Table 4.23. The soil PNEC has been determined by equilibrium partitioning from the aquatic PNEC, where two different methods, a deterministic QSAR based on chronic *Daphnia magna* data with an application factor of 10 (see Table 4.47) and a probabilistic QSAR based on chronic data from 17 species and an application factor of 1 (see Table 4.46) have both been presented. The additional factor of 10 is included for each AE homologue with a log Kow value greater than 5, as required in the TGD (2003) to account for ingestion of sorbed material. The resulting PEC/PNEC values for each AE homologue are given in Table 4.55 and Table 4.56.

The sum of the toxic units (i.e. PEC/PNEC values) in Table 4.55, in which the chronic *Daphnia magna* QSAR forms the basis of the PNEC calculation, is 0.103. Thus the sum of these PEC PNEC values, also called the risk characterization ratio or RCR, is less than 1.

The sum of the toxic units in Table 4.56, in which the chronic *probabilistic* QSAR forms the basis of the PNEC calculation, is 0.068. Thus this RCR is also less than 1.

Table 4.55 PEC/PNEC values determined for the terrestrial environment, with PNEC values based on the Deterministic method using the chronic *Daphnia magna* QSAR

C / EO	8	9	10	11	12	13	14	15	16	18
0	1.1E-04	5.2E-05	8.8E-05	8.9E-05	8.6E-03	7.9E-03	5.8E-03	4.5E-03	1.0E-03	9.4E-04
1	5.8E-05	2.8E-05	4.9E-05	5.3E-05	4.8E-03	4.3E-03	3.2E-03	2.4E-03	5.7E-04	5.3E-04
2	6.3E-05	3.0E-05	5.3E-05	5.8E-05	5.2E-03	4.6E-03	3.3E-03	2.6E-03	6.1E-04	5.7E-04
3	5.4E-05	2.6E-05	4.3E-05	4.9E-05	4.3E-03	3.8E-03	2.7E-03	2.1E-03	4.9E-04	4.6E-04
4	4.3E-05	2.1E-05	3.6E-05	3.9E-05	3.4E-04	2.9E-03	2.0E-03	1.6E-03	3.7E-04	3.5E-04
5	3.6E-05	1.7E-05	2.8E-05	3.2E-05	2.7E-04	2.3E-03	1.6E-03	1.2E-03	2.8E-04	2.7E-04
6	2.6E-05	1.2E-05	2.1E-05	2.2E-05	1.9E-04	1.6E-03	1.1E-03	8.4E-04	2.0E-04	1.9E-04
7	2.1E-05	1.0E-05	1.7E-05	1.8E-05	1.5E-04	1.3E-03	8.5E-04	6.6E-04	1.6E-04	1.5E-04
8	1.6E-05	7.8E-06	1.3E-05	1.4E-05	1.1E-04	9.2E-04	6.1E-04	4.7E-04	1.1E-04	1.1E-04
9	1.2E-05	6.0E-06	1.0E-05	1.0E-04	8.4E-05	6.8E-05	4.5E-04	3.4E-04	8.2E-05	7.8E-05
10	9.6E-06	4.6E-06	7.8E-06	7.7E-05	6.3E-05	5.0E-05	3.3E-04	2.5E-04	5.9E-05	5.7E-05
11	6.8E-06	3.2E-06	5.6E-06	5.3E-05	4.3E-05	3.3E-05	2.2E-04	1.7E-04	3.9E-05	3.8E-05
12	4.6E-06	2.2E-06	3.7E-06	3.4E-06	2.8E-05	2.1E-05	1.4E-04	1.0E-04	2.5E-05	2.4E-05
13	2.9E-06	1.4E-06	2.5E-06	2.2E-06	1.8E-05	1.4E-05	8.9E-05	6.7E-05	1.6E-05	1.5E-05
14	1.9E-06	9.3E-07	1.6E-06	2.2E-06	1.1E-05	8.4E-06	5.5E-05	4.1E-05	9.8E-06	9.3E-06
15	1.2E-06	5.7E-07	9.9E-07	8.3E-07	6.5E-06	4.9E-06	3.2E-06	2.4E-05	5.6E-06	5.5E-06
16	6.2E-07	3.0E-07	4.8E-07	4.1E-07	3.1E-06	2.3E-06	1.5E-06	1.1E-05	2.7E-06	2.5E-06
17	4.7E-07	2.2E-07	3.8E-07	3.3E-07	2.4E-06	1.8E-06	1.2E-06	8.6E-06	2.1E-06	2.0E-06
18	2.0E-07	9.4E-08	1.5E-07	1.2E-07	9.4E-07	7.0E-07	4.5E-07	3.3E-06	7.8E-07	7.3E-07
19	1.5E-07	7.1E-08	1.2E-07	9.5E-08	7.1E-07	5.3E-07	3.4E-07	2.5E-06	5.9E-07	5.6E-07
20	1.1E-07	5.4E-08	8.9E-08	7.2E-08	5.4E-07	4.0E-07	2.6E-07	1.9E-07	4.5E-07	4.2E-07
21	1.0E-07	4.9E-08	8.0E-08	6.5E-08	4.9E-07	3.6E-07	2.3E-07	1.7E-07	4.1E-07	3.8E-07
22	9.2E-08	4.4E-08	7.2E-08	5.8E-08	4.4E-07	3.3E-07	2.1E-07	1.6E-07	3.7E-07	3.4E-07
Sum of PEC/PNEC values for AE homologues = 0.103										

As the sum of the PEC/PNEC values for the AE homologues is less that 1 for both methods using the equilibrium partitioning method to determine the soil PNEC from PNEC aquatic, the TGD recommends that further testing using soil organisms is not indicated. This recommendation is strengthened by the support which available chronic test data for two AE homologues give to the soil PNEC values determined from the probabilistic aquatic data, and by the conservative nature of the PEC determination. Thus the environmental concentration and distribution of AE homologues does not pose a risk to the soil compartment of the environment.

Table 4.56 PEC/PNEC values determined for the terrestrial environment, with PNEC values based on the Probabilistic method with an application factor of 1

C / EO	8	9	10	11	12	13	14	15	16	18
0	1.5E-04	7.2E-05	9.7E-05	8.0E-05	6.5E-03	5.1E-03	3.4E-03	2.4E-03	5.3E-04	4.6E-04
1	7.9E-05	3.8E-05	5.3E-05	4.7E-05	3.6E-03	2.8E-03	1.8E-03	1.3E-03	2.9E-04	2.6E-04
2	8.9E-05	4.3E-05	6.0E-05	5.3E-05	4.0E-03	3.0E-03	1.9E-03	1.4E-03	3.1E-04	2.8E-04
3	8.0E-05	3.9E-05	5.0E-05	4.6E-05	3.4E-03	2.5E-03	1.6E-03	1.1E-03	2.5E-04	2.2E-04
4	6.7E-05	3.2E-05	4.3E-05	3.9E-05	2.8E-04	2.0E-03	1.2E-03	8.7E-04	1.9E-04	1.7E-04
5	5.8E-05	2.8E-05	3.6E-05	3.2E-05	2.3E-04	1.6E-03	9.7E-04	6.8E-04	1.5E-04	1.3E-04
6	4.3E-05	2.1E-05	2.8E-05	2.4E-05	1.7E-04	1.2E-03	6.9E-04	4.8E-04	1.1E-04	9.3E-05
7	3.8E-05	1.8E-05	2.4E-05	2.0E-05	1.4E-04	9.6E-04	5.5E-04	3.8E-04	8.3E-05	7.3E-05
8	3.0E-05	1.4E-05	1.9E-05	1.6E-05	1.1E-04	7.2E-04	4.1E-04	2.8E-04	6.1E-05	5.3E-05
9	2.4E-05	1.2E-05	1.5E-05	1.2E-04	8.2E-05	5.5E-05	3.1E-04	2.1E-04	4.5E-05	3.9E-05
10	1.9E-05	9.3E-06	1.2E-05	9.7E-05	6.3E-05	4.2E-05	2.3E-04	1.5E-04	3.3E-05	2.8E-05
11	1.4E-05	6.9E-06	9.3E-06	6.9E-05	4.5E-05	2.9E-05	1.6E-04	1.1E-04	2.2E-05	1.9E-05
12	1.0E-05	4.9E-06	6.4E-06	4.7E-06	3.0E-05	1.9E-05	1.0E-04	6.8E-05	1.4E-05	1.2E-05
13	6.8E-06	3.3E-06	4.5E-06	3.2E-06	2.0E-05	1.3E-05	6.8E-05	4.4E-05	9.2E-06	7.7E-06
14	4.8E-06	2.3E-06	3.0E-06	3.2E-06	1.3E-05	8.1E-06	4.4E-05	2.8E-05	5.8E-06	4.8E-06
15	3.1E-06	1.5E-06	2.0E-06	1.3E-06	8.1E-06	4.9E-06	2.6E-06	1.7E-05	3.4E-06	2.9E-06
16	1.7E-06	8.0E-07	1.0E-06	6.6E-07	4.0E-06	2.4E-06	1.3E-06	8.0E-06	1.7E-06	1.3E-06
17	1.3E-06	6.3E-07	8.4E-07	5.6E-07	3.3E-06	1.9E-06	1.0E-06	6.3E-06	1.3E-06	1.1E-06
18	5.8E-07	2.8E-07	3.5E-07	2.2E-07	1.3E-06	7.8E-07	4.1E-07	2.5E-06	5.1E-07	3.9E-07
19	4.7E-07	2.2E-07	2.8E-07	1.8E-07	1.0E-06	6.2E-07	3.2E-07	2.0E-06	4.0E-07	3.0E-07
20	3.7E-07	1.8E-07	2.2E-07	1.4E-07	8.3E-07	4.9E-07	2.5E-07	1.6E-07	3.1E-07	2.3E-07
21	3.3E-07	1.6E-07	2.0E-07	1.3E-07	7.5E-07	4.4E-07	2.3E-07	1.4E-07	2.8E-07	2.1E-07
22	3.0E-07	1.4E-07	1.8E-07	1.1E-07	6.7E-07	3.9E-07	2.0E-07	1.3E-07	2.5E-07	1.9E-07
Sum of PEC/PNEC values for AE homologues = 0.068										

Summary: RCR values for terrestrial species

QSAR used for PNEC	RCR*
Daphnia magna	0.103
Probabilistic	0.068

*The Risk Characterisation Ratio RCR is equal to the sum of the PEC/PNEC values for all the AE homologues.

4.3.5. Risk assessment summary

The information presented in the environmental section of this report shows that the AE homologues from C8 to C18 and from EO=0 to 22 which are used in household detergent products do not constitute a risk to the aquatic environment, to sediment, to soil, or to the sewage treatment plant. In addition, no risk to the atmosphere is expected, due to the low volatility of the AE homologues. The risk characterization ratio RCR, equal to the sum of the PEC/PNEC values for all the AE homologues used in household detergents, is given for each environmental compartment in the overall summary table below.

Overall Summary: RCR* values for total AE in the environment

Environmental Compartment	Method used for PNEC Determination	RCR *	Section and Table reference
Surface water	Chronic *Daphnia magna* QSAR with AF = 10**	0.041	Section 4.3.1 Table 4.51
	Chronic probabilistic QSAR with AF = 1***	0.024	Section 4.3.1 Table 4.52
Sediment	Chronic *Daphnia magna* QSAR with AF = 10**	0.316	Section 4.3.2 Table 4.53
	Chronic probabilistic QSAR with AF = 1***	0.181	Section 4.3.2 Table 4.54
Sewage treatment plant	Most conservative data applied to all AE	0.007	Section 4.33
Soil	Chronic *Daphnia magna* QSAR with AF = 10**	0.103	Section 4.3.4 Table 4.55
	Chronic probabilistic QSAR with AF = 1***	0.068	Section 4.3.4 Table 4.56

*The Risk Characterisation Ratio RCR is equal to the sum of the PEC/PNEC values for all the AE homologues. **The application factor of 10 is used as three chronic QSARs are available for AE homologues. The *Daphnia magna* QSAR is the most robust of these QSARs, and has the most sensitive endpoint over much of the range of AE homologues. Further information is given in section 4.2.1.2.2. ***The probabilistic QSAR gives the 95th percentile of a species sensitive distribution curve containing data from 17 species, described in section 4.2.1.2.6. Justification for the application factor of 1 is given in section 4.2.1.2.8.

5. Human health assessment

5.1 Consumer exposure

5.1.1 Product types

In line with the objectives of the HERA initiative, this human health assessment will focus on the use of alcohol ethoxylates (AE) in household cleaning products. Table 5.1 lists household cleaning applications and typical finished product concentration ranges of all AEs used in household products.

Table 5.1: Household applications and finished product concentrations of the different alcohol ethoxylates (AE) (AISE, Unpublished data)

Product application	Range of AE level in finished product	Typical content of AE in finished product
Regular laundry detergents	0.0 - 15.0%	0.0 - 13.5%
Compact laundry detergents	0.0 - 24.0%	0.0 - 24.0%
Fabric conditioners	0.0 - 2.0%	0.0 - 1.5%
Laundry additives	0.0 - 11.5%	0.0 - 11.5%
Hand dishwashing detergent	0.0 - 6.0%	0.0 - 5.0%
Machine dishwashing detergent	0.0 - 14.5%	0.0 - 14.0%
Surface cleaners	0.0 - 22.0%	0.0 - 7.0%
Toilet cleaner	0.0 - 16%	0.0 - 16.0%

5.1.2 Consumer contact scenarios

For the use of alcohol ethoxylates the following consumer exposure scenarios were identified and assessed:

1. Direct skin contact with neat (*e.g.*, laundry pre-treatment) or diluted consumer product (*e.g.*, hand-washed laundry, hand dishwashing, surface cleaning).
2. Indirect skin contact via release from clothes fibres to skin.
3. Inhalation of detergent dust during washing process or aerosols generated by spray cleaners.
4. Oral ingestion of residues deposited on dishes.
5. Accidental or intentional overexposure.

5.1.3 Consumer exposure estimates

There is a consolidated overview concerning habits and practices of use of detergents and surface cleaners in Western Europe which is tabulated and issued by the European Soap and Detergent Industry Association, AISE (AISE, 2002). This table reflects consumers' use of detergents in g/task, 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 laundry), additional H&P information for a targeted exposure assessment is directly provided by the member companies of AISE. The calculations of the estimated consumer exposures are based on the highest relevant concentrations that consumers can be exposed to.

5.1.3.1 Direct skin contact from hand-washing laundry

Hand-washing laundry has been identified as a common consumer habit. In this task, the AE containing laundry solution comes in direct contact with the skin of hands and forearms. A hand washing task is expected to take 10 minutes (AISE, 2002). The dermal systemic exposure (Exp_{sys}) to AE can be estimated according to the following algorithm from the HERA guidance document:

$$\mathbf{Exp_{sys} = F_1 \times C \times Kp \times t \times S_{der} \times n / BW} \quad \mathbf{(1)}$$

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

F_1	percentage weight fraction of substance in product	***24%*** (Table 1; compact laundry, gel; worst case)
C	product concentration	***10 mg/cm^3*** (AISE, 2002)
Kp	dermal penetration coefficient	***9.2 x 10^{-6} cm/h*** (Drotman, 1980; see section 5.2.10.3 for the derivation of K_p)
t	duration of exposure or contact	***10 min*** (AISE, 2002)
S_{der}	surface area of exposed skin	***1,980 cm^2*** (TGD, 2003)
n	product use frequency (tasks per day)	***1.4*** (AISE, 2002)
BW	body weight	***60 kg*** (TGD, 2003)

$\mathbf{Exp_{sys}}$ = [0.24 x (10 mg/cm^3) x (0.0000092 cm/h) x (0.17 h) x 1.4 x (1980 cm²)] / 60 kg
= 0.17 µg/kg bw/day

5.1.3.2 Direct skin contact from pre-treatment of laundry

Consumers typically spot-treat stains on the laundry 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, at most the skin surface of both hands is exposed and the time taken for the task is typically less than 10 minutes. Algorithm (1) is used to calculate the systemic exposure resulting from the pre-treatment of laundry. The following assumptions are considered to represent a conservative reflection of this scenario:

F_1	percentage weight fraction of substance in product	***24%*** (Table 1; compact laundry, gel; worst case)
C	product concentration	***1,000 mg/cm^3*** (AISE, 2002)
Kp	dermal penetration coefficient	***9.2 x 10^{-6} cm/h*** (Drotman, 1980; see section 5.2.10.3)
t	duration of exposure or contact	***10 min*** (AISE, 2002)
S_{der}	surface area of exposed skin	***840 cm^2*** (TGD, 2003)
n	product use frequency (tasks per day)	***1*** (AISE, 2002)
BW	body weight	***60 kg*** (TGD, 2003)

$\mathbf{Exp_{sys}}$ = [0.24 x (1000 mg/cm^3) x (0.0000092 cm/h) x (0.17 h) x (840 cm²)] / 60 kg
= 5.25 µg/kg bw/day

The above exposure estimate can be regarded to be very conservative. 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. The assumption that the consumer is exposed to the concentrated laundry product is therefore a worst case assumption. It should also be considered that only a fraction of the hands' skin will actually be exposed to the product. The assumption that both hands will be fully immersed in the product is a likely overestimate of the true exposure.

5.1.3.3 Direct skin contact from hand dishwashing

To calculate the dermal systemic exposure from direct contact of the skin to dishwashing detergent algorithm (1) is adapted. The determination of alcohol ethoxylate exposure from hand dishwashing is conducted in a manner very similar to that of hand-washed laundry. The following assumptions were made to address a reasonable worst case scenario:

F_1	percentage weight fraction of substance in product	***6%*** (Table 1; liquid concentrate; worst case)
C	product concentration	***2 mg/cm^3*** (AISE, 2002)
Kp	dermal penetration coefficient	***9.2 x 10^{-6} cm/h*** (Drotman, 1980; see section 5.2.10.3)
t	duration of exposure or contact	***45 min*** (AISE, 2002)
S_{der}	surface area of exposed skin	***1,980 cm^2*** (TGD, 2003)
n	product use frequency (tasks per day)	***3*** (AISE, 2002)
BW	body weight	***60 kg*** (TGD, 2003)

$\mathbf{Exp_{sys}}$ = [0.06 x (2 mg/cm^3) x (0.0000092 cm/h) x (0.75 h) x (1980 cm²) x 3] / 60 kg
= 0.082 µg/kg bw/day

5.1.3.4 Direct skin contact from surface cleaners

During this task, the AE containing hard surface cleaning solution comes into direct contact with the skin of the hands. A surface cleaning task takes at maximum 20 minutes (AISE, 2002). Algorithm (1) is used to calculate the dermal systemic exposure to AEs via hard surface cleaner applications. This calculation is very conservative as the percentage of AE in the product is based on a concentrated formulation which is diluted to the same extent as a regular liquid. It was assumed that the concentrate is a liquid and all the assumptions where used from the AISE habits and practices table for liquids.

The terms are defined with following values for the calculation of a worst case exposure estimate:

F_1	percentage weight fraction of substance in product	***22%*** (Table 1, Liquid concentrate)
C	product concentration	***22 mg/cm^3*** (AISE, 2002)
Kp	dermal penetration coefficient	***9.2 x 10^{-6} cm/h*** (Drotman, 1980; see section 5.2.10.3)
t	duration of exposure or contact	***20 min*** (AISE, 2002)
S_{der}	surface area of exposed skin	***840 cm^2*** (TGD, 2003)
n	product use frequency (tasks per day)	***1*** (AISE, 2002)
BW	body weight	***60 kg*** (TGD, 2003)

$$\mathbf{Exp_{sys}} = [0.22 \times (22\ mg/cm^3) \times (0.0000092\ cm/h) \times (0.334\ h) \times 1 \times (840\ cm^2)] / 60\ kg$$
$$\mathbf{= 0.21\ \mu g/kg\ bw/day}$$

5.1.3.5 Indirect skin contact from wearing clothes

The consumer can also be exposed to detergent residues via the skin by wearing clothes that have been laundered. No data are available measuring the alcohol ethoxylate deposit on the fabric following a wash process. Typical alcohol ethoxylates used in laundry detergents are highly water soluble and taking into account their non-ionic structure, it is likely that alcohol ethoxylates are to a large extent removed with the wash water and not absorbed to the negatively polarised fabric fibres. In this exposure scenario, it is assumed that the concentration of substance available for deposition before spinning is decreased to less than 2.5% of the initial concentration in the wash-liquor (ZVEI and IKW, 1999). The indirect skin exposure resulting from alcohol ethoxylate residues in clothes can be estimated with algorithm (2) listed below. This algorithm has been slightly modified versus the algorithm for calculation of the dermal exposure to detergent residues in the fabric recommended in the HERA guidance document (AISE, 2002) to account for the absence of real alcohol ethoxylate deposition data.

$$\mathbf{Exp_{sys} = F_1 \times (M \times (F' / V) \times FD \times FL) \times S_{der} \times F_2 \times F_3 \times F_4 / BW} \qquad \mathbf{(2)}$$

The terms used in this algorithm are defined as follows:

F_1	percentage weight fraction of substance in product	***24%*** (Table 1, compact detergent, gel)
M	amount of undiluted product used	***200 g*** (compact detergent; AISE, 2002)
F'	Concentration of water soluble ingredient in wash liquor	***2.5%*** (ZVEI and IKW, 1999)
V	volume of wash liquor	***15 L*** (assumption)
FD	fabric density	***10 mg/cm^2*** (Procter and Gamble, 1996)
FL	percentage liquor after final spinning	***60%*** (Henkel, unpublished data)

S_{der}	surface area of exposed skin	***17,600 cm²*** (TDG, 2003)
F_2	percent weight fraction transferred to skin	***1%*** (Vermeire *et al*., 1993)
F_3	percent weight fraction remaining on skin	***100%*** (worst case)
F_4	percent weight fraction absorbed via skin	**2%** (Drotman, 1980)
BW	body weight	***60 kg*** (TGD, 2003)

$$\mathbf{Exp_{sys\ (indirect\ skin\ contact)}} = [0.24 \times [(200000\ mg) \times (0.025 / 15000000\ mg) \times (10\ mg/cm^2) \times 0.6] \times (17600\ cm^2) \times 0.01 \times 1 \times 0.02] / 60\ kg$$
$$= \mathbf{0.028\ \mu g/kg\ bw/day}$$

5.1.3.6 Inhalation of detergent dust during washing processes

Filling powder into the washing machine dispenser can result in some detergent dust being generated. Studies determined an average release of about 0.27 µg dust per cup of product (*i.e.*, laundry powder) used for machine laundering (van de Plassche *et al*., 1998). Alcohol ethoxylates are present in laundry powder detergents at a maximum level of 10.8%. Exposure to detergent dust particles containing AE can be calculated by algorithm (3) derived from the HERA guidance document. It should be pointed out that the task is of short duration (less than 1 minute) and the assumptions made in this scenario, *i.e*., that all dust particles are respirable and present in the breathing zone, are worst case and highly unrealistic. Moreover, washing powders are granulated to minimize the formation of respirable dust.

$$\mathbf{Exp_{sys\ (inhalation)} = F_1 \times n \times F_5 \times F_6 / BW} \qquad \mathbf{(4)}$$

The variables are explained below with the relevant values which represent worst case exposure for this task:

F_1	percentage weight fraction of substance in product	***10.8%*** (Table 1; Compact laundry powder; worst case)
n	product use frequency (tasks per day)	***2.6*** (AISE, 2002)
F_5	amount of inhalable dust per task	***0.27 µg*** (van de Plassche *et al*., 1998)
F_6	percentage (%) weight fraction absorbed or inhaled	***100%*** (worst case)
BW	body weight	***60 kg*** (TGD, 2003)

$$\mathbf{Exp_{sys\ (inhalation)}} = [0.108 \times 2.6 \times (0.27\ \mu g) \times 1] / 60\ kg$$
$$= \mathbf{0.0013\ \mu g\ /kg\ bw/\ day}$$

5.1.3.7 Inhalation of aerosols from cleaning sprays

Alcohol ethoxylates are also present in surface cleaning sprays. The HERA guidance document specifies the algorithm to be used for calculation of consumers' worst-case exposure to AE containing aerosols generated by the spray cleaner:

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

The terms used in this algorithm are defined as follows:

F_1	percentage weight fraction of substance in product	***7%*** (Table 1; Cleaning spray)
C`	product concentration in air:	***0.35 mg/m³*** * (Procter and Gamble, 1996)
Q_{inh}	ventilation rate	***0.8 m³/h*** (TGD, 2003)
t	duration of exposure	***10 min*** (AISE, 2002)
n	product use frequency (tasks per day)	***1*** (AISE, 2002)
F_7	weight fraction of respirable particles	***100%*** (worst case)
F_8	weight fraction absorbed or bioavailable	***75%*** (TGD, 2003)
BW	body weight	***60 kg*** (TGD, 2003)

** 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.*

$$\mathbf{Exp_{(inhalation)}} = [0.07 \times (0.35\ mg/m^3) \times (0.8\ m^3/h) \times (0.17\ h) \times 1 \times 1 \times 0.75] / 60\ kg$$
$$= \mathbf{0.042\ \mu g/kg\ bw/day}$$

5.1.3.8 Oral exposures to alcohol ethoxylates

Oral exposure to alcohol ethoxylates can originate from residues on eating utensils and dishes as well as from exposure to residues found in water and food.

The daily exposure to AE from eating with utensils and dishware that have been washed with AE-containing dishwashing liquids can be estimated according to the following algorithm from the HERA guidance document:

$$\mathbf{Exp_{sys} = [F_1 \times C` \times T_{a'} \times S_a / BW] \times A}$$

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	***14.5%*** (Table 1; machine dishwashing liquid, worst case)
C`	concentration of product in dish wash solution:	***1 mg/cm³*** (AISE, 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	***5,400 cm²*** (TGD, 2003)
BW	body weight	***60 kg*** (TGD, 2003)
A	oral absorption	***100%*** (worst case)

$Exp_{sys\ (oral\ dish\ deposition)}$ = [[0.145 x (1 mg/cm^3) x (5.5 x 10^{-5} ml/cm^2) x (5,400 cm^2)] / 60 kg]
= 0.72 µg/kg bw/day

The indirect oral exposure from drinking water will be added after completion of the environmental risk assessment. Considering the profile of environmental fate of AE, it is expected that indirect exposures via the environment, including drinking water, is insignificant.

5.1.3.9 Accidental or intentional overexposure

Accidental or intentional overexposure to alcohol ethoxylates may occur via swallowing of solid detergents or drinking of liquid washing solutions. Typically, one would estimate that no more than 5 g of powder detergent (equals a maximum of 1.2 g. of alcohol ethoxylate) or 20 ml of dishwashing liquid (equals a maximum of 1 g. of alcohol ethoxylate) would be swallowed. Studies of acute oral toxicity demonstrate that the toxic dose of alcohol ethoxylates is many times higher than this, even for a toddler (see 5.2.1.1.1 acute oral toxicity).

The German Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV, 1999) published a report on products involved in poisoning cases. No fatal case of poisoning with detergents is reported. Detergent products are not mentioned as dangerous products with a high incidence of poisoning.

Accidental contact with the eyes is possible by splashes of dilute washing solutions or to low amounts of the detergent powder from hands into the eyes. Also, spillage of undiluted detergents products may lead to inadvertent skin contact. Therefore, the skin and eye irritation potential has to be considered when assessing the risks of accidental exposures.

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

5.1.3.10 Total exposure

In the unlikely event of maximum worst case exposure from all sources, the total exposure to AEs from its use in cleaning products would be 6.48 µg/kg bw/day. The individual sources of exposures leading to the overall exposure are summarized in Table 5.2.

Table 5.2: Worst case exposure estimates for the different consumer contact scenarios

Task	Worst case exposure estimate (EXP_{sys}) [µg/kg bw/day]
Direct contact from hand washing laundry	0.17
Direct skin contact from pre-treatment of laundry	5.25
Direct skin contact from hand dishwashing	0.082
Direct skin contact from surface cleaners	0.21
Indirect skin contact from wearing laundered clothes	0.028
Inhalation of laundry powder dust	0.0013
Inhalation of aerosol particles	0.042
Oral exposure to alcohol ethoxylates	0.7
Total exposure	**6.48 µg/kg bw/day**

5.2 Hazard assessment

5.2.1 Summary of the available toxicological data

An extensive toxicology data base exists for the category of alcohol ethoxylates (AEs), covering studies dating back to the 1960s as well as very recent studies performed under good laboratory practice (GLP) and conforming to the relevant OECD guidelines. Several in-depth reviews have been published on AEs bringing together the state of the science and clearly outlining the hazards associated with these compounds (Little, 1977, 1981; Talmage, 1994; Shell Chemicals Ltd., 2002; Exxon, unpublished report; Shell Chemical Company, unpublished report). This assessment aims to incorporate the current knowledge and report on new studies and in detail where deemed necessary.

The key studies are available in form of robust study summaries and available upon request. A justification allowing to assess alcohol ethoxylates as a single group is presented in Annex II to this report.

5.2.1.1 Acute toxicity

Many high quality studies investigating the acute toxicity of AEs have shown that in terms of oral and dermal toxicity the use of these compounds are of low concern. They are of low oral, dermal and inhalational toxicity. From a structural activity point of view, the length of the alkyl chain did not exert any meaningful influence on acute toxicity. The degree of ethoxylation of the AE appear to be the only factor found to be of relevance in acute oral toxicity with the compounds with ethoxylate chains between 5 and 14 being more toxic by oral consumption than those with less than 4 or more than 21 ethoxy units.

5.2.1.1.1 Acute oral toxicity

The acute oral toxicity of alcohol ethoxylates (AEs) has been extensively evaluated in numerous studies with rats, but also in dogs and monkeys. The oral LD_{50} values for rats were found to range from 0.6 g/kg to more than 10 g/kg bodyweight. The variation in the reported results is a reflection of the degree of ethoxylation of the study compound and differences in the study design. AEs can be classed as slightly to moderately toxic based on the toxicity rating scale by Gosselin *et al*. (1976).

Several reviews are available on this subject (Little, 1977, 1981; Talmage, 1994; Shell Chemicals Ltd., 2002; Exxon). Talmage (1994) reported a higher toxicity of alcohol ethoxylates as the length of the ethoxylate chain increased but found a levelling off at around 12 ethoxylate units. A similar trend was found by Arthur D. Little (1981). Both reviews report that the length of the alkyl chain exerts only a negligible effect on toxicity. It was also found that in some studies data for males and females animals were evaluated separately. Females appear to be more sensitive to AEs than males.

The test materials were typically solutions containing 0.1 to 100% w/v active ingredient diluted in de-ionized water or corn oil which were administered at dose levels of 0.19 g/kg to 16 g/kg bodyweight through gavage. Several studies conformed to OECD guidelines and/or EC method B1 and a number of studies were in compliance with GLP regulation. However, most of the reported values are from non-guideline compliant and pre-GLP studies.

The following summarizes the ranges of outcome of some key guideline and GLP compliant acute oral toxicity studies which aim to demonstrate the impact of changing ethoxy chain length on acute oral toxicity. Where appropriate, comments on specific studies are given. The data reported here were chosen from the most reliable studies although the critical aspect of current testing standards was not always met (*e.g.*, 5 animals from each sex per dose group).

- $C_xAE_{1\text{-}3}$ – The acute oral LD_{50} values ranged from 4 to greater than 10 g/kg, for both sexes combined (Hüls AG, 1986a, 1986b; Imperial Chemicals Industries, Ltd., 1975; Lifestream Laboratories, 1968, Shell Research Ltd., 1978a, 1978b, 1979a, 1979b, 1980a; Uniqema Ltd., 1975). From all available studies, only one study was GLP-compliant, but not in full compliance with OECD guidelines (Shell Research Ltd., 1980a).
    - In this study, five dosage levels up to 10 g/kg of $C_{9\text{-}11}AE_{2.5}$ were tested with groups of eight (4 male and 4 female) fasted rats. The acute oral LD_{50} was calculated to be greater than 4 g/kg and less than 10 g/kg. Seven of the eight study animals died at the highest dosage level. No necropsies were conducted to determine the cause of death. Pilo-erection was observed as the overt clinical sign of poisoning at the two highest dose levels. All surviving animals gained weight over the course of the study.
- $C_xAE_{4\text{-}6}$ – A substantial number of studies were conducted on AE's calling into this class. The determined oral LD_{50} ranged from 1.2 to greater than 10 g/kg (BASF AG, 1979, 1983a, 1989a; Hüls AG, 1985, 1986c; Lifestream Laboratories, 1966a; Shell Development Company, 1981a; Shell International BV, 1996a; Shell Oil Company, 1990; Shell Research Ltd., 1969, 1971, 1975a, 1978c, 1980a, 1986, 1990a, 1990b; Shell Toxicology Laboratory, 1977).
    - Two studies determining the acute oral toxicity of $C_{12\text{-}13}AE_{6.5}$ were in compliance with GLP regulations, and resulted in an LD_{50} value of 2.1 g/kg for both sexes combined (Shell Oil Company, 1990) and 2.5 g/kg for males and 1.7 g/kg for females (Shell Research Ltd., 1986).
    - In another GLP study, following OECD guidelines, groups of five female rats received a dose of 1.3 or 1.6 g/kg $C_{7\text{-}9}AE_6$ and a group of five males and five females received 2 g/kg. There was one decedent in each of the

treated groups. All deaths occurred within 48 hours after dosing. The clinical observations indicated compound related toxicity in all treated groups. Prone posture, ataxia and changes of breathing (*e.g.*, hyperpnoea, tachypnoea, rales or gasping) were the principal signs of reaction to treatment. The signs generally appeared within 4 hours after dosing, but recovery was complete by day 3. No macroscopic changes were apparent in the majority of rats subject to necropsy on day 15. Those changes that were apparent (*e.g.*, pallor of the lungs in one rat dosed at 1.3 g/kg, red foci on the thymus in three females treated at 1.6 g/kg and pale foci on the spleen of one male dosed at 2 g/kg) showed no treatment-related trend. For both sexes combined, the LD_{50} was determined to be larger than 2 g/kg (Shell International BV., 1996).

- C_xAE_{7-9} – LD_{50} values ranged from 1.1 and 3.4 g/kg with most studies reporting values below 2 g/kg (BASF AG, 1978, 1983b, 1984a, 1989b; Imperial Chemical Industries PLC, 1978; Lifestream Laboratories, 1967; Shell Oil Company, 1979a, 1979b; Shell Research Ltd., 1970, 1972a, 1972b, 1975b, 1976a, 1976b, 1980a, 1984a, 1991a, 1992; Wil Research Laboratories Inc., 1993a).
    - In a GLP studies conforming to OECD method 401, an LD_{50} value of 1.1 g/kg was calculated for $C_{11}AE_9$ (Wil Research Laboratories Inc., 1993a). In this study groups of 5 male and 5 female fasted rats were tested at 3 different dosages up to 2 g/kg. All deaths occurred within two days of dosing. There were 4/10, 6/10 and 9/10 deaths in the 0.89, 1.5 and 2.0 g/kg dose groups, respectively. Females were more sensitive and in the low dose all mortalities were female rats. The majority of the rats (*i.e.*, 25 out of 30) were hypoactive following dosing, of these, 14 rats were also ataxic. Hypothermia, prostration and/or laboured respiration were noted for 12 out of 19 animals that died. Gastro-intestinal and lung abnormalities, foamy tracheal contents and kidney changes were observed at in rats that were found dead. There were no test-related gross findings in animals at necropsy.
    - In a GLP study with $C_{12-15}AE_7$, five rats of each sex were given doses up to 5 g/kg and the acute oral LD_{50} was determined to be 1.7 g/kg (Shell Research Ltd., 1984).
    - In another OECD method 401 compliant study, the acute oral LD_{50} of $C_{9-11}AE_8$ in fasted rats was found to be 1.2 g/kg (Shell Research Ltd., 1991a). Signs of toxicity were seen within 24 hours of administration with subsequent recovery being fairly rapid.
- C_xAE_{10-12} – LD_{50} values ranged from 0.6 to 2.5 g/kg (BASF AG, 1983c, 1989c; Hüls AG, 1987a; Imperial Chemical Industries PLC, 1984; Lifestream Laboratories, 1966b; Shell Oil Company, 1979c; Shell Research Ltd., 1975c, 1976c, 1984b, 1986, 1990c). Considering only GLP-compliant studies, the LD_{50} ranged from 0.72 g/kg to 1.8 g/kg.
    - Two GLP studies were performed with $C_{14-15}AE_{11}$; however, the LD_{50} values were different at 0.72 g/kg and 1.8 g/kg (Shell Research Ltd., 1984b; 1986). It should be noted that the test compound in Shell Research Ltd. (1984b) was administered as neat product whereas in the other study it was

given as 50% (m/v) solution in corn oil. Both LD_{50} values were calculated using probit analysis.

- In an OECD guideline and GLP-compliant study, dose levels of 1 and 2 g/kg of $C_{12\text{-}15}AE_{11}$ were tested in 5 male and 5 female fasted rats (Imperial Chemical Industries PLC, 1984). The acute oral LD_{50} was calculated to be larger than 2 g/kg for males, and between 1 and 2 g/kg for females. One male and four females died following exposure to a dose of 2 g/kg. None of the animals died after exposure to a dose of 1 g/kg. Decreased activity, dehydration, pilo-erection and urinary incontinence were observed at the highest dosage administered in male and female rats. No abnormalities were observed in any of the animals that were examined by necropsy at the conclusion of the study.
- Of all acute oral toxicity studies, the lowest LD_{50} values of 0.6 g/kg for males and 0.5 g/kg for females were determined for $C_{15\text{-}16}AE_{10}$ (Shell Research Ltd., 1976c). It should be noted that this study was not in conducted in compliance with OECD guidelines and GLP regulations. $C_{15\text{-}16}AE_{10}$ was administered to four rats of each sex as a 19% w/v solution in water. Diarrhea and lethargy were observed at the highest dose of 1.5 g/kg within 24 hours. These signs had subsided in surviving animals within 48 hours. On the 9th day, two females were underweight; these animals subsequently died on days 12 and 15. Necropsies at study termination were not conducted.

- C_xAE_{13+} - For this group of AEs, LD_{50} values ranged from 1.0 to greater than 10 g/kg (BASF AG, 1984b; Huntingdon Research Centre, 1978a; Imperial Chemical Industries PLC, 1986a; Hüls AG, 1986d, 1986e; Shell Oil Company, 1979d, 1979e).
  - In the only GLP study in this range, acute oral exposure to $C_{18}AE_{21}$ did not result in significant signs of acute toxicity. For this compound, the LD_{50} was larger than 2 g/kg (Imperial Chemical Industries PLC, 1986a).
  - The LD_{50} values for $C_{14\text{-}15}AE_{13}$ were 1.1 and 1.0 g/kg in two separate studies, not GLP-compliant, but following OECD guidelines (Shell Oil Company, 1979d, 1979e). In both studies, all animals (5 males and 5 females) died after exposure to the undiluted material. In-life observations included diarrhoea, dilated pupil, pilo-erection, polyuria, salivation, chromodacryorrhea, lacrimation, ptosis, epistaxis, bright yellow urine, activity decrease, lethargy and tremors. Clinical and necropsy findings included diarrhoea, polyuria, epistaxis, salivation, oral and nasal discharge, discoloration of the adrenal glands and mesenteric lymph nodes, discoloration of the stomach and intestinal contents, ulcerations on stomach, discoloration of the liver and spleen, pronounced serosal blood vessels, discoloration of the kidneys, gastrointestinal tract distended with gas, discoloration of abdominal fat, and variations thereof.
  - The compounds with more than 15 ethoxy units (*i.e.*, 15, 20, 21 and 30 ethoxy units) were less toxic with acute oral LD_{50} values exceeding 4 g/kg.

The acute oral toxicity of AEs to rats appears to be related to the degree of ethoxylation (Figure 5.2-1a). When plotting the determined acute oral LD_{50} values versus the ethoxylation degree, a parabolic curve is obtained with lower toxicity at the low and high end of the ethoxy units. Products containing more than 5 and less than 14 ethoxy units appear to be of higher acute oral toxicity. For example, LD_{50} values for $C_{9\text{-}11}$ alcohol ethoxylates with 2.5 ethoxy units ranged from 2.7 to 10 g/kg (Shell Research Ltd., 1979b, 1980) compared to 1.2 to 2.7 g/kg for $C_{9\text{-}11}$ alcohol ethoxylates with 8 ethoxy units (BASF AG, 1983b; Shell Research Ltd., 1976b, 1980a, 1991). The same trend was observed for $C_{12\text{-}14}$ and $C_{13\text{-}15}$ alcohol ethoxylate LD_{50} values; $C_{12\text{-}14}AE_3$ (LD_{50} 9.35 g/kg) was less acutely toxic than $C_{12\text{-}14}AE_{10}$ (LD_{50} 2.82 g/kg) (Hüls AG, 1986a, 1987), and $C_{13\text{-}15}AE_4$ (LD_{50} > 5g/kg) was less acutely toxic than $C_{13\text{-}15}AE_{11}$ (LD_{50} 2.45 g/kg) (BASF AG, 1979, 1983c). The contribution of chain length to oral toxicity was examined and no relationship was observed (Figure 5.2-1b).

Figure 5.2-1a **Figure 5.2-1b**

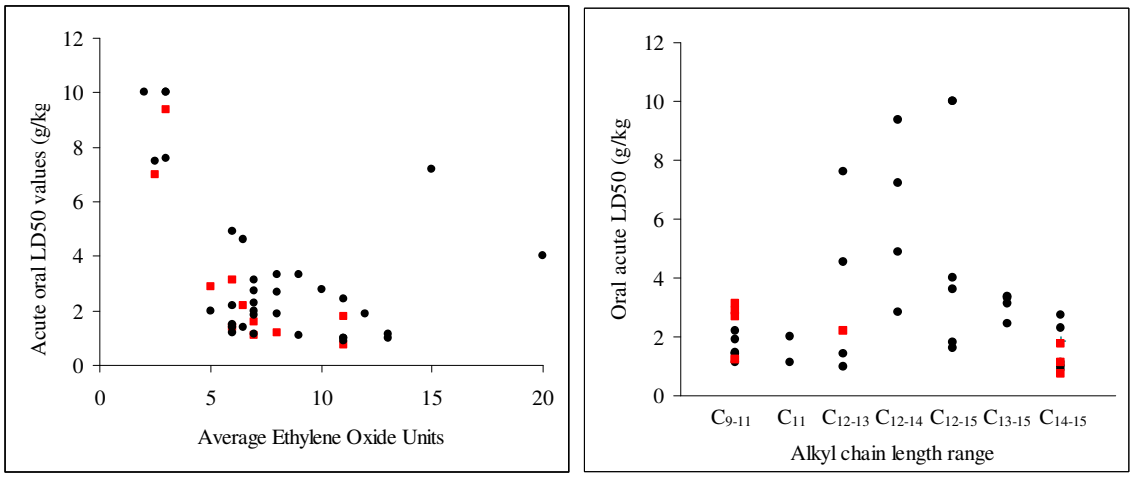


Figure 5.2-1: Acute Oral LD_{50} values in the rat versus ethoxylate chain length (a) and alkyl chain length (b)

■ – GLP study; ● – non-GLP study

In most of the studies, the test animals were observed after administration of the test compounds and signs of toxicity typically occurred within 2 hours post-exposure but had seized at study termination. Clinical effects were observed at the high end doses with diarrhoea being the most frequently reported effect of treatment after oral administration. Other clinical signs included pilo-erection, lethargy, ataxia, abnormal posture, difficult laboured breathing, salivation, lacrimation, bloody noses and in a few cases chromodacryorrhoea. Yellow urogenital staining was frequently observed in dead animals, but not in surviving animals. Necropsies revealed one or more of the following: congestion of the kidneys, adrenals, liver, lungs and gastro-intestinal tract, haemorrhage of the gastric mucosa, adhesions of the abdominal viscera, and reddish coloured urine in the bladder, discoloration of liver and heart and ulcerations of the stomach. The latter is a typical sign of a gastrointestinal irritant in rats (Shell Chemicals Ltd., 2002).

Most acute oral studies were conducted with rats, however, other animals have been tested and LD_{50} values were within the range reported above. Acute oral studies in Beagles at 1.65 g/kg of $C_{12\text{-}13}AE_{6.5}$ and monkeys at up to 6.7 g/kg of $C_{14\text{-}15}AE_7$ showed no effects other than emesis and diarrhoea (Benke *et al*., 1977). One of two monkeys administered 10 g/kg of $C_{14\text{-}15}AE_7$ died.

Conclusion

Alcohol ethoxylates have been shown to have a low to moderate order of acute oral toxicity in the rat with LD_{50} values ranging between 0.6 to more than 10 g/kg. The structure of the test compound influenced acute toxicity determined by the relative number of ethoxy units, whereas, carbon chain length was not correlated with the acute oral toxicity. The degree of ethoxylation of the AE appeared to be the only factor found to be of relevance in acute oral toxicity with the compounds with ethoxylate chains between 5 and 14 being more toxic by oral consumption than those with less than 4 or more than 21 ethoxy units. Clinical findings observed in the test animals after treatment were indicative of gastrointestinal irritation such as ulcerations of the stomach, pilo-erection, diarrhoea and lethargy and may be linked with administration of a bolus dose, in particular in cases where the test item was administered undiluted.

There is further an apparent sex difference for a group ethoxylates with LD50 values below 2,000 mg/kg, with females being more susceptible to the acute oral toxicity than males. It should be noted that there is unpublished information suggesting that this is not a sex specific phenomenon, but an effect related to body weight; lighter animals being more susceptible than heavier animals.

5.2.1.1.2 Acute inhalation toxicity

Few studies evaluated the inhalation toxicity of alcohol ethoxylates in rats. None of these studies followed the principles of OECD guideline nor were they GLP compliant.

In one study, five rats of each sex were exposed for 4 hours to two different concentrations of $C_{9\text{-}11}AE_5$ generated as a mist (Shell Research Ltd., 1980b). In the first exposure, the mass median diameter of the particles was 3.4 ± 2.0 µm and in the second exposure the median diameter was 3.0 ± 2.2 µm. The acute 4h-LC_{50} was determined to be greater than 0.22 mg/L. There were no mortalities or signs of toxicity observed during the study.

Talmage (1994) reported that alcohol ethoxylates were not acutely toxic to rats at concentrations less than or equal to their saturated vapour concentrations in air. Acute toxic thresholds were reached only when animals were exposed to the undiluted test chemical in the form of a respirable mist or aerosol. Under these conditions, 1- or 4-hour inhalation LC_{50} values ranged from 1.5 to 20.7 mg/L. Some studies reported no mortalities (1-hours LC_{50}-study) occurred at concentrations as high as 52 mg/L.

Talmage (1994) further reported about treatment-related effects observed in acutely exposed animals such as laboured breathing, inactivity and bloody nasal discharge. Gross necropsies revealed corneal opacities, congestion and mottling of the lungs, and in some cases, paleness or congestion of the liver, kidneys, and adrenals. Necropsy findings in surviving animals were typical of healthy animals. No details on methodology and time point of observations were provided.

Conclusion

Alcohol ethoxylates are considered to be of low acute inhalation toxicity to rats with LC_{50} values exceeding the saturated vapour concentration in air. Acute toxic thresholds were

reached only when animals were exposed to the undiluted test chemical in form of a respirable mist or aerosol.

5.2.1.1.3 Acute dermal toxicity

The acute dermal toxicity of alcohol ethoxylates has been extensively evaluated in numerous studies with rats and rabbits. Lethal doses in rats were in most cases above the maximum dose levels tested, *i.e.*, larger than 0.7 g/kg to 5 g/kg, with most values in the >2 to >5 g/kg range. In rabbits dermal LD_{50} values were determined to be in the range of 2 g/kg to 5.2 g/kg. On the basis of these results, alcohol ethoxylates can be considered to be slightly to practically non-toxic by the dermal route of application (Weiss, 1980). The test materials applied were typically solutions containing 19 to 100% w/v active ingredient, in the concentration range from 1.3 to 10.2 g/kg. Most of the reported values are from pre-GLP studies, however, several studies conform to OECD guidelines and some studies complied with GLP regulations. There was no apparent relationship between dermal toxicity and chemical structure with regard to alkyl chain length and degree of ethoxylation for AEs (Little, 1977, 1981; Talmage, 1994; Shell Chemicals Ltd., 2002; Exxon).

For the determination of the dermal LD_{50}, the trunk of test animals was clipped and wrapped in a semi-occlusive dressing. The test product, neat or in solution, was applied to the intact skin under the wrap with a syringe. After an exposure period of 24 hours, the skin was washed to remove any residual test material and the animals were observed for skin reactions and mortalities up to 14 days following treatment. The data reported below were identified to be of the highest standard available. These studies reported the methodologies and results in sufficient detail to allow a reasonable assessment of the potential dermal toxicity of the tested AEs in laboratory animals.

Findings and determined LD_{50} ranges are summarized in the following:

- $C_{7\text{-}9}AE_n$ –An LD_{50} value of greater than 2 g/kg has been determined, for both sexes combined (Shell International BV., 1995a) in a GLP study with $C_{7\text{-}9}AE_6$. No animal died and there were no treatment related clinical signs of toxicity.
- $C_{9\text{-}11}AE_n$ – Dermal LD_{50} values for AEs with an alkyl chain length of 9 – 11 carbon atoms were determined in three rat studies and ranged from greater than 2 to greater than 4 g/kg (Shell Research Ltd., 1976b, 1978c, 1979b). No signs of intoxication or mortalities were detected in these studies.
    - In one GLP compliant study in rabbits, undiluted $C_{9\text{-}11}AE_6$ applied to abraded exposure sites of 8 (4 per sex) rabbits' backs at a dose of 2 g/kg for 24 hours did not result in any mortalities or clinical signs of toxicity (Shell Development Company, 1981b). Necropsy of the rabbits at study termination did not reveal any consistent treatment related findings.
    - In another, non-guideline conform study, 5 rabbits out of 8 (4 per sex) died after a dermal exposure of 5 g/kg of undiluted $C_{9\text{-}11}AE_6$ and the acute dermal LD_{50} was determined to be less than 5 g/kg (Shell Oil Company, 1979f). In-life observations included little or no urine, little or no faeces, lethargy, diarrhoea, ataxia, muscle tremors and activity decrease.

- $C_{12-14}AE_n$ – An LD_{50} value of more than 2 g/kg was determined for $C_{12-14}AE_3$ and $C_{12-14}AE_6$ in two studies compliant with GLP and following OECD 402 guidelines (Hüls AG, 1997a, 1997b).
    - In both studies groups of ten rats (five males and five females) were given a single dermal application of respectively $C_{12-14}AE_3$ and $C_{12-14}AE_6$ at a dose level of 2 g/kg. No deaths or signs of toxicity were observed.
- $C_{12-15}AE_n$ – LD_{50} values in the rat ranged from greater than 0.9 g/kg to greater than 5 g/kg (Huntingdon Research Centre, 1978a, 1978b; Imperial Chemical Industries Ltd., 1975; Shell Research Ltd., 1972b, 1976a, 1978a, 1978b, 1984a).
    - In one GLP-compliant study, five rats of each sex were given doses up to 2 g/kg. The acute dermal LD_{50} of $C_{12-15}AE_7$ was determined to be greater than 2 g/kg (Shell Research Ltd., 1984a). The only signs of toxicity observed in both sexes were wet appearance of the fur and inflammation of the treated site.
    - $C_{13-15}AE_{11}$ was applied as a 40% suspension in corn oil and administered at a maximum dose of 2.3 mL/kg to twelve rats (6 male and 6 female). At the maximum dose of 0.92 g/kg bodyweight, all findings were normal (*i.e.,* no mortalities or signs of toxicity). The dermal LD_{50} was therefore greater than the maximum practical dose (Huntingdon Research Centre, 1978b). Observations of the application site showed slight oedema in 7 of the treated animals but this dermal reaction was ameliorated by day eight.
- $C_{15-16}AE_n$ - Only one study was conducted with $C_{15-16}AE_{10}$ (Shell Research Ltd., 1976c). The study was not performed according to GLP or OECD guidelines. The acute LD_{50} value was greater than 0.8 g/kg in rats. $C_{15-16}AE_{10}$ was administered to four rats of each sex as a 19% w/v solution in water as a single dose. There were no deaths or signs of toxicity observed at this low concentration.

As discussed before (Little, 1977, 1981; Talmage, 1994; Shell Chemicals Ltd., 2002; Exxon), the chemical structure did not appear to influence the acute dermal toxicity. For example, for AEs with 3 to 5 ethoxy units the LD_{50} values were greater than 2 g/kg and similar results are observed for highly ethoxylated compounds (E_{12} to E_{20}) with LD_{50} values exceeding 1 and 2 g/kg. However, in most studies the concentration range tested was too low to see any signs of systemic toxicity, and often the highest concentration tested reflected the compound solubility limit in water. No sex differences could be identified with regard to acute dermal toxicity. The test animals were observed up to 14 days after administration of the test compounds. Most reported observations included damaged skin at the site of application with varying degrees of dryness and sloughing (*e.g.*, erythema and oedema). In most cases these symptoms had disappeared by the end of the observation period. Other in-life observations included wet appearance of the fur, diarrhoea, little or no urine, difficult and laboured breathing, small and few or no faeces, activity decrease and in a few dying animals, pilo-erection, lethargy and abnormal gait. Necropsies revealed one or more of the following: haemorrhage of subcutaneous tissues and small to moderate hyperaemia of the small intestine.

Talmage (1994) thoroughly reviewed unpublished data from the Union Carbide Corporation (1981) that investigated the effects of large doses of AE applied dermally for 24 hours. The data showed that very high doses of AEs (exceeding 16 g/kg) could lead to severe skin irritation in rabbits, ataxia and lung lesions.

Conclusion

Alcohol ethoxylates were shown to have a low order of acute dermal toxicity in the rat and rabbit with LD_{50} values typically greater than the maximum applied dose, ranging from greater than 0.8 to greater than 5 g/kg in rats. LD50 values in rabbits were greater than 2 g/kg but less than 5 g/kg. There was no relationship between compound structure and dermal toxicity.

5.2.1.2 Corrosiveness/irritation

High quality studies investigating the skin and eye irritation potential of alcohol ethoxylates have shown that the use of these compounds in household cleaning products is of low concern. When tested undiluted AEs were found to be slightly too severely irritating to skin in rabbits and rats and mildly to severely irritating to the rabbit eye. However, if the skin or eye irritation potential was investigated at in-use concentrations, AEs were only mildly irritating to skin and eyes.

5.2.1.2.1 Skin irritation

The potential of alcohol ethoxylates to cause skin irritation has been evaluated in numerous studies with rabbits. Skin irritation studies were conducted with different concentrations ranging from 0.1% to 100%, exposure times ranging from 4 hours to up to 4 weeks and patch conditions such as open applications, semi-occlusive, and fully occlusive conditions. Most of the reported values are from pre-GLP studies. Several studies, however, were in compliance with OECD guidelines and GLP regulations.

- *Studies with 4 hour exposure*
  Several studies following current OECD 404 guidelines, and in most cases in compliance with GLP regulations, were conducted in rabbits with different alcohol ethoxylates (Shell International BV., 1995b, 1996b; Shell Oil Company, 1993; Shell Research Ltd., 1984a, 1984b, 1992, 1993a, 1993b, 1993c, 1993d, 1993e, 1993f, 1993g). In these studies, the test product was applied as a single dose under a semi-occlusive dressing for 4 hours to the shorn intact skin of three to six rabbits. Twenty four, 48 and 72 hours after removal of the patch, the skin reaction was evaluated for erythema (*i.e.*, reddening) and oedema (*i.e.*, swelling). Each of the two factors was scored on a basis of 0 (no change) to 4 (severe reaction) and these scores were combined to give a maximum value of 8. A primary irritation index 'PII' was calculated as the average of the scores assigned at three time periods (ECETOC, 1995). Products tested ranged between $C_{9\text{-}11}$ to $C_{23\text{-}25}$ with 2.5 to 20 ethoxylate units. The following summarises some key high quality studies sorted by increasing ethoxylation degree:

  A 4-hour semi-occluded topical application of undiluted $C_{12\text{-}15}AE_3$ to the clipped dorsal skin of three rabbits caused very slight erythema at all dermal test sites one hour of post-dosing and well-defined erythema at two of the dermal test sites within 24 hours after patch removal (Shell Research Ltd., 1993d). All erythema resolved within 14 days after dosing. Desquamation affected all dermal test sites on the eighth day of observation but this dermal change resolved before termination of the study. A primary skin irritation index (PII) of 1.3 was calculated on the basis of the data presented indicating that under the chosen testing conditions undiluted

$C_{12-15}AE_3$ should be considered as slightly irritating to rabbit skin. This GLP study followed EC guidelines and the principles of OECD guideline 404.

Semi-occluded topical application of undiluted $C_{9-11}AE_8$ to the clipped dorsal skin of three rabbits for 4 hours following OECD guideline 404 caused very slight erythema one hour after treatment at one of the three dermal test sites (Shell Research Ltd., 1993g). On the following day, 2 dermal test sites showed very slight or well-defined erythema. Resolution of the erythematous response was completed seven days after conclusion of treatment. No other dermal changes were observed. A PII of 0.6 was calculated on the basis of the data presented indicating that under the testing conditions undiluted $C_{9-11}AE_8$ was slightly irritating to rabbit skin. Dermal irritation reactions following semi-occluded topical applications of 10 and 25% m/v aqueous solutions of $C_{9-11}AE_8$ were limited to very slight erythema. This resolved within 72 hours after treatment. A PII of 0.2 was calculated for both dilutions, indicating minimal irritating potential of the diluted test substance.

In another GLP study, following OECD 404 guidelines, dermal exposure to the undiluted $C_{11}AE_9$ under occlusive dressings resulted in slight erythema and very slight oedema in all six rabbits tested (Shell Oil Company, 1993). Desquamation was noted on one site at 72 hours and persisted through day 5. Oedema had subsided by the 48-hour observation period and all signs indicative of dermal irritation were completely subsided at study termination on day 6. Under the testing conditions and based on a PII of 1.3, undiluted $C_{11}AE_9$ can be considered to be slightly irritating. Dermal exposure to 1, 10 and 25% w/v aqueous dilutions of the test material induced only very slight erythema on all animals. Oedema resolved by 72 hours, all other effects resolved by day 5. Based on a PII of 0.0 (1%), 0.1 (10%) and 0.7 (25%), diluted test material received a rating classification of minimally irritating.

A series of OECD 404 compliant studies, investigated the dermal irritation potential of a range of undiluted AEs with varying ethoxylation degree. Under the testing conditions (*i.e.*, 4hr exposure under fully occlusive conditions), the responses to the test materials ranged from slightly irritating (PII – 0.6 to 1.6; $C_{13}AE_{20}$ and $C_{12-14}AE_{15}$) through moderately irritating (PII – 4.1 to 5.6; $C_{12-14}AE_{10}$, $C_{13}AE_6$, and $C_{13}AE_{5-6.5}$) to extremely irritating (PII – 6.3 to 7.1; $C_{12-14}AE_6$, $C_{12-14}AE_3$, and $C_{13}AE_3$) (Hüls AG, 1986f, 1986g, 1986h, 1986i, 1986j, 1986k, 1987b, 1987c) to rabbit skin. Redness extended over the application region and was accompanied with dry skin in the application area when the substance was classed as a moderate to severe irritant. No signs of necrosis were observed. These data indicate the trend that with increasing ethoxylation degree, AEs become less irritating. The effects caused by the slightly irritating materials reversed six days after exposure. For those material that resulted in moderate to severe irritations signs of irritation such as fissures and scaly skin persisted until the end of the observation period of 14 days.

- *Studies with 24 hours exposure*
  Most studies conducted to investigate skin irritation of various dilutions of alcohol ethoxylates after 24 hour exposure are not compliant with GLP or OECD guidelines. In the only GLP study, $C_{9-11}AE_6$ was evaluated to be severely irritating to the rabbit skin, with a primary irritation score of 5.3 based on observations made at 24 and 72 hours (Shell Development Company, 1981c). Rabbits' backs were

shaved 24 hours prior to exposure. There were two intact and two abraded skin sites per animal. Each test site was treated with 0.5 mL of the undiluted test material after which they were occluded for 24 hours. Severe erythema and oedema were observed at 24 and 72 hours after treatment. At day 7, oedemas were reduced or absent, while erythema scores were both increased and decreased at some sites compared to the 72-hour observations.

The remaining 24-hour dermal exposure studies were more investigative in nature and examined the skin irritation effects of various undiluted AEs (Huntingdon Research Centre, 1977; Lifestream Laboratories, 1966a, 1966b, 1967; Shell Oil Company, 1979g, 1979h, 1979i, 1979j, 1979k; Shell Research Ltd., 1978a, 1978b, 1979a, 1979b). The examined undiluted alcohol ethoxylates covered the range of C_9 to C_{15} with 2 to 13 ethoxylate groups and were under the testing conditions determined to be mild to severely irritating. Primary irritation indexes were calculated based on 24- and 72-hour observations. For example, a single 24-hour application of undiluted $C_{13\text{-}15}AE_3$ to occluded rabbit skin induced well-defined erythema with or without very slight to slight oedema on the intact and abraded sites of all three tested animals after 24 hours. After 72 hours well-defined to moderate erythema with or without slight or moderate oedema was observed. Hyperkeratinization of the skin was seen in only one animal after 72 hours. The PII was estimated to be 3.9, indicating the test material was moderately irritating (Huntingdon Research Centre, 1977a). Dermal exposure to undiluted $C_{14\text{-}15}AE_7$ produced throughout the study slight to moderate erythema and moderate to severe oedema, resulting in a PII of 6.42 (Shell Oil Company, 1979g).

The skin irritation potential of AEs appears to be related to the degree of ethoxylation (Figure 5.2-2a). When plotting the determined primary irritation indexes versus the ethoxylation degree, a minor trend is obtained with lower irritation potential for the AEs with longer ethoxylation degree. This trend becomes more apparent when AEs with varying ethoxylation degree were investigated under exactly the same testing conditions. No trend in irritation potential related to the length of the alkyl chain of the test compounds was observed (Figure 5.2-2b).

Figure 5.2-2a

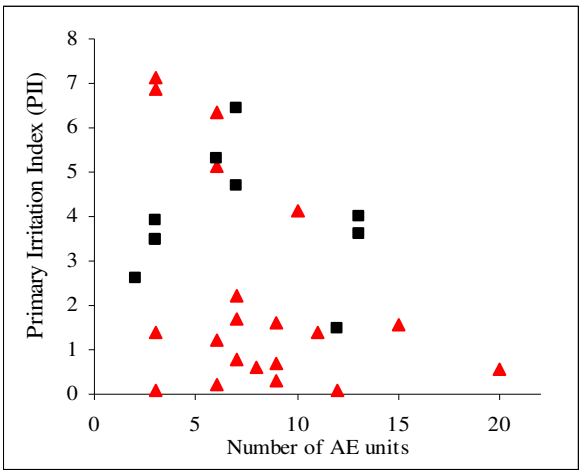


Figure 5.2-2b

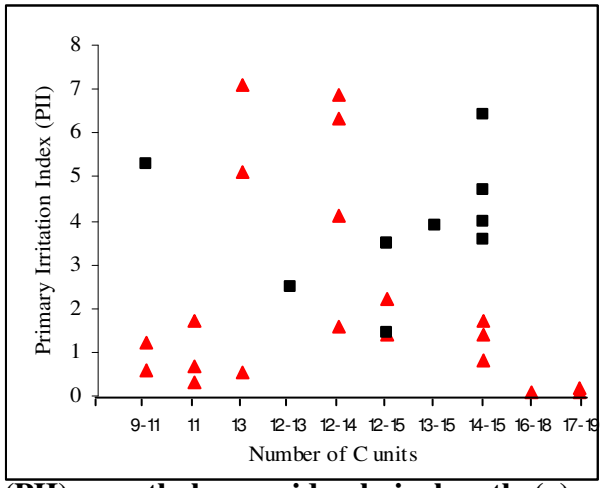


Figure 5.2-2: Primary irritation index (PII) vs. ethylene oxide chain length (a) and alkyl chain length (b).

▲ 4 hours exposure time (PII calculated based on 24, 48 and 72-hour observations)

■ 24 hours exposure time (PII calculated based on 24 and 72-hour observations)

- *Studies with exposure in 3 periods of 6 hours*

  Alcohol ethoxylates were repeatedly applied to the skin of rabbits in 3 periods of 6 hours in several non-guideline compliant studies (Shell Research Ltd., 1971, 1972a, 1972b, 1975b, 1975c, 1976a, 1976b, 1976c, 1978a, 1978c, 1978d). These studies provide useful information with regard to the cumulative skin irritation potential of alcohol ethoxylates. The materials tested cover the alkyl chain length range of C_9 to C_{16} with E_3 to E_{18} ethoxylate units. In general, shorn backs of 8 rabbits (4 per sex) were exposed to test material under occlusive dressing for 6 hours on three subsequent days. Under the testing conditions, undiluted alcohol ethoxylates were found to cause moderate to severe irritation (PII between 3.1 and 6.5). In the same studies, the authors also investigated aqueous solutions of the same materials, which produced lesser effects. In general, irritation was moderate at 10% w/v (PII 3.1-5.0), slight with 1% w/v (PII 0.6-1.5) and absent at 0.1% (PII 0.0).

- *Studies with repeated skin application over 12 days*

  Three female rats with shaved backs were treated with six applications of $C_{12-15}AE_3$ on alternate days over a twelve day period starting with the first application at day 1 (Huntingdon Research Centre, 1977b). The test material was applied undiluted or as a 0.5% active solution in distilled water and the treated sites were occluded for 24 hours. On days 2, 4, 6, 8, 10 and 12, the patches were removed and the treated area of each animal washed with 1% cetrimide solution. Local reactions resulting from the treatment were assessed each day. Repeated application of the undiluted product under fully occlusive conditions elicited distinct dermal irritation by day seven of the test. Similar levels of erythema and oedema were observed in one animal throughout the remainder of the study (days 7-12; erythema and oedema scores of 2) but in the other two animals the reactions increased, and moderate erythema and oedema had developed by day 10 (day 7; erythema and oedema scores of 2 and 3; days 10-12 erythema and oedema scores of 3). Cracking, scaling and scab formation was observed in all three animals throughout the latter half of the study. The rats

exposed to the diluted test material showed no response to treatment throughout the 12 days observation period.

In a further study with $C_{13-15}AE_3$, the same experimental procedure was used to test the neat material under occluded and non-occluded conditions (Huntingdon Research Centre, 1977a). In both cases, the repeated application over twelve days of the neat surfactant elicited moderate dermal irritation.

In a similar study, the irritant properties of $C_{13-15}AE_7$ was evaluated in three female rabbits that received 6 doses of 0.1 ml of either the neat or a 0.5% active solution under occlusive dressing (Huntingdon Research Centre, 1977c). Slight to distinct erythema and oedema accompanied by cracking and scaling developed in all animals by day seven and similar levels were seen until the end of the study in animals treated with the neat product. None of the animals in the 0.5% treatment group showed any signs of dermal irritation at the end of the study. The above studies following a 12-day repeated dose skin irritation test protocol were not in compliance with GLP regulations or OECD guidelines. However, the studies were assessed to be scientifically sound and well conducted.

- *Four and half (4½) weeks skin irritation*
  Several 4½ week skin irritation studies were conducted under non-occluded conditions in rabbits and guinea pigs with different AEs covering a range of ethoxy and carbon chain lengths (*i.e.*, $C_{12-15}AE_7$, $C_{14-15}AE_7$, $C_{9-11}AE_8$, $C_{14-15}AE_{11}$, $C_{14-15}AE_{18}$) (Shell Research Ltd., 1975b, 1975c, 1976a, 1976b, 1978d). In all studies, the test compounds were applied 5 times per week for a total of 23 applications to five guinea pigs and one rabbit of each sex. The amount of test material applied was 0.5 mL for guinea pigs and 1.0 mL for rabbits. Visual assessment of the skin reaction was made on a daily basis. In the series tested here, no relationship between compound structure and skin irritation could be established.

  $C_{12-15}AE_7$ was repeatedly applied at dilutions of 0.1%, 1% and 10% of neat product according to the protocol described before (Shell Research Ltd., 1976a). Moderate erythema with some fissuring and lifting of roughened skin was observed with the 10% dilution in guinea pigs and rabbits. After two weeks, the superficial skin layer of the guinea pig was almost completely lifted to expose the hardened underlying skin. Slight flaking of regenerated epidermis was observed in four male guinea pigs after four weeks (*i.e.*, mean erythema score of 2 in guinea pigs and rabbits). The 1% dilution caused mild erythema and roughing of the skin in the test animals and this response persisted for 4 weeks after the test period (*i.e.*, mean erythema score of 0.7 in guinea pigs and 1.8 in rabbits). After 4½ weeks the 0.1% dilution was assessed to be not irritating to rabbit and guinea pig skin.

  $C_{14-15}AE_7$ was applied neat and in dilutions of 0.1%, 1.0% and 10% to the back of rabbits and guinea pigs (Shell Research Ltd., 1975b). Under the testing conditions, the repeated application of neat product was severely irritating to the skin of rabbits and the test was terminated after only 5 applications of the test material. The 0.1% dilution was non-irritating whereas the 1% and 10% dilution caused mild to moderate irritations responses (*i.e.*, mean erythema score of 0.5 to 1.5).

The no observed effect concentration with $C_{9-11}AE_8$ was 1% aqueous dilution of the neat product (Shell Research Ltd., 1976b). The 10% and 100% treatment levels caused mild to severe reactions. Guinea pig skin exposed to the neat product after 2 weeks showed decreased sensitivity due to accommodation or hardening of the regenerated epithelium (*i.e.*, mean erythema score after 2 weeks of 1 and 2, respectively).

$C_{14-15}AE_{11}$ and $C_{14-15}AE_{18}$ were tested under the conditions described before neat and in dilutions of 10%, 1.0% and 0.1% (Shell Research Ltd., 1975c, 1978d). For both products the 0.1% dilutions were not irritating to skin, whereas, all other concentrations caused dilution dependant reactions from mild (*i.e.*, in the case of the 1.0% dilution) to severe irritation (*i.e.*, in the case of the 100% dilution).

Conclusion

Alcohol ethoxylates with varying carbon chain lengths and ethoxylation degree were found to be slightly to severely irritating to skin in rabbits and rats. The degree of irritation was dependant on the type of patches used (*i.e.*, open application versus full occlusions), the exposure time as well the concentration of test material. Generally, undiluted materials were moderate to severe skin irritants, whereas 1% aqueous solutions were mildly irritating and 0.1% to 0.5% aqueous solutions were usually not irritating. As can be expected, open applications led to a lower degree of irritation response and longer exposure times to higher degree of irritation. There is also a trend observable that the degree of ethoxylation impacts the skin irritation potential of AE's. Alcohol ethoxylates with lower ethoxylation degree (*i.e.*, 1-3 EO-units) appeared to be more irritating than AE's with more than 4 ethoxy units. In the acute 4 hour dermal irritation studies with neat materials, effects of AE's which were considered to be slightly irritating disappeared about six days after exposure. Signs of irritation for those materials considered to be moderately to severely irritating persisted until the end of the observation period of 14 days.

5.2.1.2.2 Eye irritation

The potential for AEs to produce eye irritation has been widely examined in rabbits. Most of the reported values are from pre-GLP studies, however, several studies conformed to OECD guidelines or EC method B5 and a number of studies were GLP compliant. Although most tests are standardized, there were subtle variations in grading or scoring procedure varying from laboratory to laboratory and from individual to individual. Moreover, in older studies the test substances were applied in volumes of 0.2 mL, in more recent studies a volume of only 0.1 mL was applied. This could explain some of the observed variation. Several reviews (Little, 1977, 1981; Talmage, 1994) found that all undiluted products were either moderate or severe irritants whereas diluted AEs in a concentration of 0.1% were generally not irritating.

The studies reported and examined in this summary demonstrated that under the testing conditions of the Draize eye irritation study and the low volume eye irritation test, undiluted alcohol ethoxylates produced varying degrees of eye irritation in rabbits. In most studies, a dose of 0.1 mL of the liquid test substance was administered into the conjunctival sac of one eye of each of three rabbits over 24 hours. The other eye remained untreated to serve as a control. Initial pain reactions to the product were recorded and the eyes were examined for ocular reaction up to 21 days after the instillation of the test

material using the Draize method (Draize *et al*., 1944). The Kay and Calandra rating was used to assign an eye irritation score 'EII' from 0 to 110 (Kay and Calandra, 1962). This method uses the criteria of incidence, extent and persistence of injury to the cornea, iris and conjunctivae to determine the irritation potential of a product.

Several AE surfactants (*i.e.,* $C_{12-15}AE_3$, $C_{14-15}AE_7$, $C_{7-9}AE_{12}$, $C_{12-14}AE_{15}$, $C_{14-15}AE_{18}$ and $C_{13}AE_{20)}$ were considered to be practically not to minimally irritating as they all produced an EII between 0.5 and 15 (Hüls, AG, 1986l, 1986m; Shell International BV, 1996c; Shell Oil Company, 1979l, 1979m; Shell Research Ltd., 1978a, 1978b, 1978d, 1979a 1992). A few exemplar studies are presented below to illustrate the range of effects observed.

- Undiluted $C_{12-13}AE_2$ which was tested according to the procedure outlined above was the only AE that was non-irritating to rabbit eyes (EII 0.0) in a pre-GLP study (Shell Research Ltd., 1979a). No initial pain response was observed immediately after instillation into the eyes. The only effect noted effect was a slight induction of redness (score of 0.5) 1-2 hours after instillation of the product, which resolved completely by day 1.
- Undiluted $C_{7-9}AE_{12}$ was tested in a GLP and OECD method 405-compliant study (Shell International BV, 1996c). Eye exposure to $C_{7-9}AE_{12}$ induced chemosis and redness of the conjunctival tissue in all rabbits during the first 24 hours. The highest score was a score 3 for chemosis in one rabbit. Corneal opacity was seen at 24 hours in one animal but this regressed rapidly as was the case with all other treatment related effects. By day 7 all reactions to treatment had resolved. Based on the overall mean 24, 48 and 72 hours scores an EII of 10.1 was determined, indicating $C_{7-9}AE_{12}$ was minimally irritating to rabbit eyes.
- Undiluted $C_{14-15}AE_7$ was tested following the Draize method, in two pre-GLP studies (Shell Oil Company, 1979l, 1979m). Corneal ulcerations were observed in respectively 5 and 3 animals. Based on 24, 48 and 72 hours scores, EIIs of 12.9 and 14.2 were established. Signs of irritation were still visible at the end of the observation period of 7 days.
- Undiluted $C_{13}AE_3$ was found to be mildly irritating to rabbit eyes in an OECD method 405 compliant study (Hüls, AG, 1986n). The Eye Irritation Index was calculated to be 21.6 indicating $C_{13}AE_3$ is mildly irritating to rabbit eye. However, there were still effects observed at the end of the observation period in cornea, iris and conjunctivae in one animal.

Most alcohol ethoxylates tested as the undiluted neat product were considered to be moderately to severely irritating with an eye irritation index ranging from > 25 to 50. AEs tested covered the range of C_9 to C_{19} with 2.5 to 15 ethoxy units (Hüls, AG, 1986o, 1986p, 1986q; 1987d, 1987e; Huntingdon Research Centre, 1977c, 1978b; Imperial Chemical Industries PLC, 1984; Lifestream Laboratories, 1966a, 1966b, 1967; Shell Development Company, 1981d; Shell International BV, 1995c; Shell Oil Company, 1979n, 1979o, 1979p; Shell Research Ltd., 1968, 1975c, 1976b, 1978c, 1979b, 1984a, 1984b). A few exemplar studies are presented below to illustrate the range of effects observed.

- In a GLP-compliant study, six rabbits were treated with undiluted $C_{9-11}AE_6$ (Shell Development Company, 1981d). All nine animals developed corneal opacities which were not completely cleared within 14 days after treatment. Consequently $C_{9-11}AE_6$ was assessed to be moderate to severely irritating to the rabbit eye with

an overall eye irritation index of 41.3 and a maximum irritation score of 53.5 on day 3.

- $C_{12-14}AE_3$, $C_{12-14}AE_6$, $C_{13}AE_{5-6.5}$, $C_{13}AE_6$ and $C_{12-14}AE_{10}$, tested in a series of OECD compliant studies were found to be moderately to severely irritating to rabbits eyes. They produced an eye irritation index ranging between 27.1 and 44.2 (Hüls, AG, 1986o, 1986p, 1986q, 1987d; 1987e). Effects were still seen for $C_{13}AE_6$ (cornea and conjunctivae in 1 animal), $C_{13}AE_{5-6.5}$ (cornea, iris and conjunctivae in 2 animals), and $C_{12-14}AE_{10}$ (cornea, iris and conjunctivae in all 3 animals) at the end of the observation period of 21 days.
- Following installation of undiluted $C_{12-15}AE_{11}$ into the rabbit's eyes, all of the animals showed signs of slight or moderate initial pain (Imperial Chemical Industries PLC, 1984). Within 24 hours, slight to moderate redness of the conjunctivae, slight to mild chemosis and slight to severe discharge were seen. In addition, slight iritis and corneal opacity were observed in one animal which was subsequently killed. Based on the observations and an EII score of 39, $C_{12-15}AE_{11}$ was considered to be moderately to severely irritating. The study was performed in compliance with GLP.
- $C_{7-9}AE_6$ was tested in a GLP compliant study for its eye irritation potential. Although the study was conducted in only one animal, it was considered to be compliant with OECD guidelines (Shell Int. BV., 1995c). The first response to the test material centred upon effects in the cornea. Initial extensive corneal damage was followed by regeneration of the corneal epithelium. On day 7, neo-vascularization of the cornea developed which became marked by day 11. As a consequence of this irreversible damage, the study was terminated and the test animal sacrificed.
- Undiluted $C_{14-15}AE_{11}$ was tested for eye irritation properties in a GLP-compliant study (Shell Research Ltd., 1984b). The installation of the test material into the conjunctival sac of one eye of each of six rabbits resulted in moderate initial pain. All rabbits showed iritis at one or more observation time which had cleared by 7 days post-dosing. However, at this time, all rabbits had vascularization of the cornea. As this is considered to be a permanent effect, the animals were killed. Based on the mean 24, 48 and 72 hours scores an EII of 35.2 has been calculated and $C_{14-15}AE_{11}$ was considered to be moderately to severely irritating.

The eye irritation data available do not allow establishing a clear relationship between chemical structure and eye irritation potency (Figure 5.2-3). Undiluted products were found to induce response from non-irritating to severely irritating but the products did not appear to fall into any particular irritation class based on ethoxy or carbon chain length.

Figure 5.2-3a

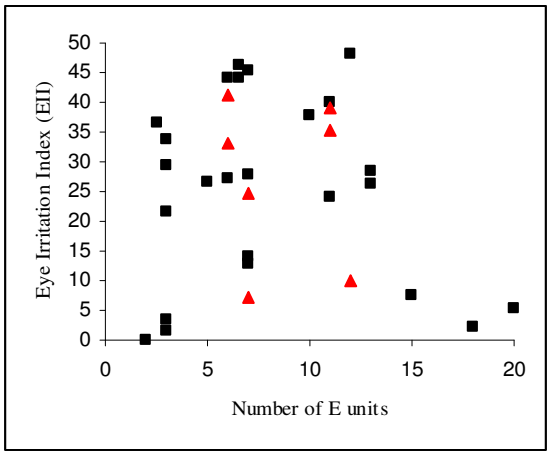


Figure 5.2-3b

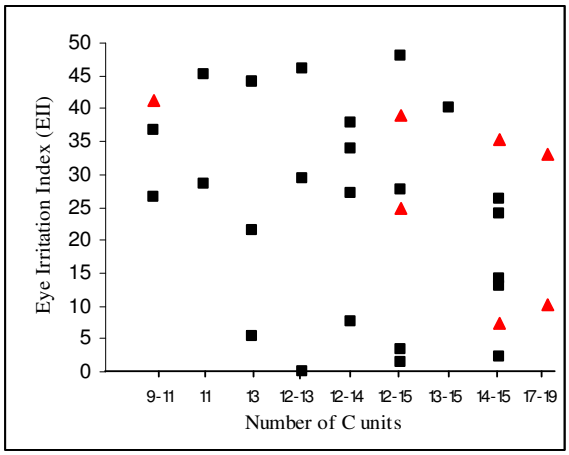


Figure 5.2-3: Primary irritation index (PII) vs. ethylene oxide chain length (a) and alkyl chain length (b).
▲ GLP compliant study
■ Non-GLP compliant study

Since many of the alcohol ethoxylates tested were moderately to severely irritating to the rabbit eye, additional tests were conducted to determine whether the irritation potential of these test materials could be reduced by rinsing the eyes after installation. Products tested covered the range of C_9 to C_{18} with 3 to 20 ethoxy groups (Henkel KGaA, 1983, 1986; Imperial Chemical Industries Ltd., 1975; Shell Development Company, 1981d; Shell Oil Company, 1979m, 1979n, 1979o). Rinsing the eye after application with tap water 30 seconds after exposure may reduce the severity of the eye effects. The following studies illustrate the effects of eye rinsing.

- In a GLP-compliant study, 0.1 mL of $C_{9\text{-}11}AE_6$ was placed into the right eyes of a total of nine rabbits. The eyes of rabbits were flushed with tap water 30 seconds after exposure. The eyes were examined and scored for irritation one hour and 1, 2, 3, 7, and 14 days after treatment. In the non-rinse group a maximum mean irritation score of 53.5 was observed at 3 days after exposure. In the rinse group, a maximum mean irritation score of 32 was seen at 3 days after exposure (Shell Development Company, 1981d).
- The effects of rinsing rabbits' eyes after AE exposure were investigated in a series of eye irritation studies with $C_{14\text{-}15}AE_7$, $C_{14\text{-}15}AE_{11}$, and $C_{14\text{-}15}AE_{13}$. A volume of 0.1 mL of each of the undiluted test materials was applied in the conjunctival sac of the right eye of nine animals. The treated eyes of three animals were each washed with 300 mL of tap water 30 seconds after treatment. The undiluted test material $C_{14\text{-}15}AE_7$ produced in unwashed eyes a maximum average irritation score of 18 at twenty-four hours after treatment. In the washed eyes a maximum average irritation score of 12 was observed at one hour after treatment. The respective score at twenty four hours was 7.3. Under the same conditions, $C_{14\text{-}15}AE_{11}$ produced a maximum score of 30.7 at 7 days after treatment in the unwashed eyes, while producing a maximum score of 32 at 7 days after treatment in the rinsed eyes. $C_{14\text{-}15}AE_{13}$ produced a maximum irritation score of 28.5 at 72 hours after treatment in the unwashed eyes and 31.3 at seven days after treatment for washed

eyes (Shell Development Company, 1981d; Shell Oil Company, 1979m, 1979n, 1979o).

The severity of irritation caused by eye exposure to AEs is concentration dependent. Talmage (1994) provided a comprehensive overview on the effect of dilution on the eye irritation potential of alcohol ethoxylates. Generally concentrations of 0.1% can be considered as virtually non-irritating, and concentrations of 1 to 10% ranged from slight to moderately irritating. A few exemplar studies are presented below to demonstrate the impact of concentration on the severity of effects.

- They eye irritation potential of Dobanol 23-6.5 (*i.e.*, $C_{12\text{-}13}AE_{6.5}$) has been investigated at concentrations of 0.1, 1, 10 and 100%. For the purpose of this investigation, 0.2 mL of the respective test solution was applied into the conjunctival sac of one eye of each rabbit. A total of two rabbits were used. The undiluted sample was severely irritating to rabbits' eyes causing conjunctivitis and corneal opacity in both animals 24 hours after application. In view of the severity of the effects the rabbits were killed before the end of the experiment. The 10% aqueous solutions were moderately irritating causing some redness and discharge, but both eyes were normal after 7 days. The 1 and 0.1% aqueous solutions of Dobanol 23-6.5 were non-irritant to the eyes of the rabbits under the test conditions (Shell Research Ltd., 1975a).
- They eye irritation potential of $C_{12\text{-}15}AE_7$, $C_{13\text{-}15}AE_{11}$, and $C_{13\text{-}15}AE_{20}$.were investigated undiluted and in a 0.5% aqueous solution. For this purpose, 0.1 mL of the test solutions was instilled into one eye of a rabbit. A total of 3 rabbits were used for this investigation. For undiluted $C_{12\text{-}15}AE_7$ a maximum irritation score of 36.3 was determined at day 2 and an eye irritation index (EII) of 27.8. The 0.5% solution resulted in a maximum irritation score of 0.7 at day 1 and an EII of 0.2. Exposure to undiluted $C_{13\text{-}15}AE_{11}$ resulted in a maximum irritation score of 53.7 was determined at day 7 and an eye irritation index (EII) of 40.1. Exposure to the 0.5% solution showed some minor signs of irritation at the 1 hour reading point. For undiluted $C_{13\text{-}15}AE_{20}$ a maximum irritation score of 33.3 was determined at day 3 and an eye irritation index (EII) of 29.6. The 0.5% solution resulted in a maximum irritation score of 2 at the 1-hr reading and an EII of 0.2 (Huntingdon Research Centre, 1977c, 1978a, 1978b).
- They eye irritation potential of Dobanol 23-6.5 (*i.e.*, $C_{12\text{-}13}AE_{6.5}$) has further been investigated at concentrations of 0.1, 5, 15, 30 and 45%. In this study, 0.1 mL of the test solutions was instilled into one eye of a rabbit. Two rabbits were used per dose group. Exposure to the 45% solution resulted in a maximum eye irritation score (MEI) of 71 on day 6 and an EII of 40. The 30% solution resulted in a MEI score of 59.7 on day 6 and an EII of 42.8. For the 15%, 5% and 0.1% solutions, the MEI scores were 62.7 (day 6), 28 (day 8), and 2.5 (1 hour) and the EII's were 35.3, 22.1, and 0 respectively (Shell Research Ltd., 1975b). The exact reason for this variation of response is unclear.

Conclusion

Alcohol ethoxylates range from mildly to severely irritating to rabbit eyes. In some of the tested AEs, the eyes of the treated animals recovered a few days after exposure. In others, exposure caused irreversible damages to the eyes. Rinsing the eyes directly after product

application with distilled water for 20 to 30 seconds reduced the severity of the effects such that these products produced only mildly irritating effects. The degree of irritation is concentration dependant as dilutions in water cause proportionally lower irritation. Generally, concentrations of 0.1% were non-irritating, and concentrations of 1 to 10% ranged from slight to moderately irritating. Also, the severe effect did not persist when the rabbit eye was flushed with water. No relationship could be established between the chemical structures of the tested AEs and their eye irritation responses.

5.2.1.3 Sensitization

The skin sensitization potential of the whole range of alcohol ethoxylates was evaluated in the guinea pig maximisation test according to the Magnusson Kligman protocol (Huntingdon Research Centre, 1977a, 1977b, 1977c, 1978a, 1978b; Imperial Chemical Industries PLC, 1986b; Shell International BV, 1996d, 1996e; Shell Research Ltd., 1975b, 1975c, 1976a, 1976b, 1976c, 1978a, 1978c, 1978d, 1979a, 1979b, 1981c, 1983a, 1983b, 1984a, 1984b, 1986, 1992) and in the non-adjuvant Buehler test protocol (Hüls, AG. 1997c, 1997d; Huntingdon Research Centre, 1975; Shell Development Company, 1978a, 1978b, 1982a; Shell Oil Company, 1979q, 1979r, 1979s, 1979t, 1979u, 1979v; Wil Research Laboratories Inc., 1993b) in guinea pigs.

In summary, out of the 25 studies conducted on different AEs (C_9 to C_{21} with 2 to 21 ethoxy units) according to the Magnusson-Kligman protocol, 22 studies revealed no evidence for skin sensitization, two studies concluded that there was 'essentially' no evidence for skin sensitization (*i.e.*, for $C_{12\text{-}15}AE_7$, $C_{14\text{-}15}AE_7$) and only one AEs (*i.e.*, $C_{7\text{-}9}AE_6$) was found to exert weak skin sensitization potential. In the latter three studies, there was no evidence that impurities might have contributed to the responses observed. Although preliminary dose-range finding studies were conducted to determine the appropriate dose levels for intradermal and topical induction and topical challenge, it is conceivable that these minor signs of erythema in a few animals at the 24 hours reading after the removal of the challenge patch may be signs of irritation and not of sensitization.

All 13 available Buehler studies covering the range of C_9 to C_{15} with 3 to 13 ethoxy units revealed no evidence for skin sensitization. The majority of the studies were not conducted in full compliance with or according to OECD guidelines, and GLP standards. Nevertheless, most studies appeared to be scientifically well conducted and the results can be included in the overall evaluation of the skin sensitization potential of AEs. The following paragraphs will report studies of highest quality and relevance.

The skin sensitization potential of $C_{12\text{-}15}AE_3$ was evaluated in the Magnusson-Kligman guinea pig maximization test in a good quality and OECD compliant study (Shell Research Ltd., 1983a). Prior to the main study, dose range finding studies were conducted to determine the concentration of test material to be used for intradermal induction, topical induction and topical challenge without causing untoward toxicity. In the main study, a test group of ten male and ten female guinea pigs, and a control group of five males and five females were used in the study. In the induction phase, the treatment group was intradermally injected 3 pairs of 0.1 mL volume (injection 1: Freund's complete adjuvant (FCA); injection 2: 0.05% test substance solved in corn oil; injection 3: 0.05% test substance in a 1:1 mixture FCA) on the shoulder region. A week later, a patch containing 50% 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. Two weeks after the induction phase, the flanks of the treated and the control animals were shaved and an occlusive 'challenge' patch containing 2% of the test substance (or corn oil 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 evaluated according to the Magnusson-Kligman grading scale. Under the test conditions, $C_{12-15}AE_3$ did not cause skin sensitization in guinea pigs.

$C_{18}AE_{21}$ was assessed in a GLP-compliant study using the maximization test of Magnusson and Kligman (Imperial Chemical Industries PLC., 1986b). The dose levels to be used for intradermal and topical induction in the main study were determined on the basis of a screening study. Thirty female guinea pigs (i.e., 20 test and 10 control animals) were used for the study. In the induction phase, animals received an intradermal injection of a 5% w/v emulsion of the test sample in de-ionized water or de-ionized water/FCA followed by topical application of a 30% w/v emulsion of the test sample in de-ionized water. In the challenge phase, an occlusive patch containing a 10% w/v emulsion of the test sample in de-ionized water was topically applied to the shaved flanks of the animals. Challenge sites were examined 48 and 72 hours from the start of the challenge application. $C_{18}E_{21}$ did not elicit any sensitization response in guinea pigs under the conditions of the test.

In a GLP/OECD compliant study, the skin sensitization potential of $C_{7-9}AE_6$ was evaluated according to the Magnusson-Kligman protocol. Based on the results of a preliminary screening study, the following dose levels were chosen: a. intradermal injection – 1% m/v in water for injection and/or adjuvant; topical induction – undiluted test article; challenge application – 60% m/v in distilled water. Twenty test and ten control animals were used in this study. Slight erythema was apparent in eight test animals out of 20 up to 24 or 48 hours and in one animal out of 20 at 24 and 48 hours after the challenge application (Shell International BV., 1996d). A concurrent study using identical conditions at the same testing facility, but a challenge concentration of 30% m/v showed a response rate of 20% (2 of 10 animals) in the control group, indicating that the challenge concentration may have caused irritation and therefore may have contributed to the observed response with $C_{7-9}AE_6$ (Shell International BV., 1996d). The investigators, however, concluded that the response in eight animals was considered to be positive evidence indicative of delayed contact hypersensitivity. From the study documentation, no information is available that would have indicated that test substance impurities may have triggered the response. The issue was not investigated further.

In two further good quality studies, the skin sensitization potential of alcohol ethoxylates $C_{12-15}AE_7$ and $C_{14-15}AE_7$ was evaluated according to the Magnuson Kligman protocol. The concentrations used for induction and challenge were based on the outcome of preliminary dose range finding studies. For the main study, twenty test and ten control animals were used in both investigations. In each of the studies one of the test animals died for treatment-unrelated reasons. Only two (for $C_{12-15}AE_7$) or one out (for $C_{14-15}AE_7$) of the 19 animals of the test groups showed slight erythema 24 hours after the challenge patch removal. No positive responses were recorded at the 48 hours reading (Shell Research Ltd., 1984a, 1992). This response was considered to be within the normal background incidence of spontaneous irritation (*i.e.*, up to 20%) that has been observed in the testing facility (Shell, 2005, personal communication).

The skin sensitization of $C_{9-11}AE_6$ was evaluated in a GLP-compliant study (Shell Development Company, 1982a) using the Buehler method. In this study, groups of 5 male and 5 female guinea pigs were treated with 0.5 mL of a 1% solution of $C_{9-11}AE_6$ in de-ionized water. The exposure sites were shaved and depilated prior to exposure. The test material was applied at the highest non-irritating concentration topically under occlusive bandages one day a week, six hours per day, for 3 consecutive weeks. After a two-week rest period following the last exposure, a challenge dose was given in the same manner as the induction dose on the original site and on a virgin site. Simultaneously a separate group was treated with 0.5 mL 1% w/v $C_{9-11}AE_6$ in de-ionized water to serve as an irritation control. The results did not reveal any potential of $C_{9-11}AE_6$ to cause skin sensitization under the conditions of the test.

In another GLP and OECD guideline compliant study, the skin sensitization potential of $C_{11}AE_9$ was evaluated in a modified Buehler study (Wil Research Laboratories, 1993b). Groups of 5 male and 5 female guinea pigs were treated with undiluted $C_{11}AE_9$. The exposure sites were shaved the day prior to exposure. The test materials were applied topically at the highest non-irritating concentration under occlusive patches one day a week, six hours per day, for 3 consecutive weeks. After a two-week rest period a challenge patch was applied in the same manner as the induction patch to previously unexposed areas of skin. One week after challenge, test group animals were re-challenged with a 25% concentration of $C_{11}AE_9$ in de-ionized water to confirm the initial challenge results. Based on these combined challenge and re-challenge data, it was concluded that $C_{11}AE_9$ was not a skin sensitizer in guinea pigs under the test conditions.

Further reviews on skin sensitization state that AEs should not be considered skin sensitizers (Little, 1977, 1981; Talmage, 1994; Shell Chemicals Ltd., 2002; Exxon; Shell Chemical Company). Only one review reported a very weak response for $C_{12-13}AE_3$ but this response could not be reproduced in another sample of the same product were the score was negative (Shell Research Ltd., 1983a).

Conclusion

Based on a weight of evidence approach and considering quality criteria in evaluating the studies, alcohol ethoxylates are not considered to be skin sensitizers. The overwhelming majority of available guinea pig studies in which AEs were tested for skin sensitization properties demonstrated the absence of skin sensitization potential with both the Magnusson and Kligman and Buehler protocol. Only one study following the Magnusson and Kligman protocol indicated a weak sensitization potential of selected AE. No follow-up work was conducted to further investigate the relevance of the observation. However, for structurally similar products the sensitization reaction was not seen and therefore it must be taken into consideration that the observed reactions may have been confounded with irritation reactions.

5.2.1.4 Repeated dose toxicity

5.2.1.4.1 Oral route

A series of five studies investigated the oral toxicity of alcohol ethoxylates with C_{13-15} and 3, 7, 11, or 20 ethoxy units (Huntingdon Research Centre, 1978a, 1978b; Imperial Chemical Industries Ltd., 1975; Imperial Chemical Industries PLC, 1978; Uniqema Ltd.,

1974). The test materials were administered by gavage in aqueous solutions once daily over a period of 14 days to 10 male and female rats at doses of 100, 250 or 500 mg/kg bw/d. At the end of the dosing period, half of the animals were sacrificed and examined. The remaining animals were observed for another 7 days before necropsies. The examination in the course and at termination of the study included clinical symptoms, haematology, biochemistry, urinalysis, histopathology, and microscopy. Except for some reversible mild gastric irritation which was observed in a few test animals and probably related to the irritant properties of the test materials and the invasive dosing procedure, no treatment-related effects were observed. Thus, under the study conditions the systemic NOAELs were greater than the respective applied dose levels ranging from 100 mg/kg bw/d to greater than 500 mg/kg bw/d indicating a low level of toxicity. This series of studies did not comply with OECD guidelines, or GLP regulations.

The repeated dose toxicity of 5 different AEs (*i.e.*, $C_{12\text{-}15}AE_3$, $C_{12\text{-}14}AE_7$, $C_{12\text{-}15}AE_7$, $C_{12\text{-}15}AE_{11}$, $C_{16\text{-}20}AE_{18}$) was evaluated on the basis of a repeated dose 21-day oral toxicity assay (Unilever, 1977a, 1977b, 1977c, 1977d, 1977e). All compounds were tested at dietary concentrations of 0%, 0.023%, 0.047%, 0.094%, 0.188%, 0.375%, 0.75%, 1.00%, and 1.50%. Three (3) Colworth Wistar-derived rats per sex per dose and 6 animals of each sex in the control group were used in these investigations. In all studies the growth of the experimental animals was retarded at the higher dosages of 0.75% to 1.5%. Changes in plasma protein concentration and organ weights (*i.e.*, heart, liver and spleen) were associated with this effect on growth. The liver appeared the major target organ for these compounds. The observed changes in the liver are indicative of an adaptive response rather than a true adverse effect. On the basis of observed increases in liver weight and hepatocytic hypertrophy, the lowest observed effect level (LOEL) in all these studies was established at the 0.75% dietary level. No treatment related effects were observed at the 0.375% dietary level leading to the establishment of the NOAEL at this exposure level, which is equivalent to a dose of about 433 mg/kg bw/d for females and 579 mg/kg bw/d for males. The studies were not conducted according to OECD guidelines nor were they GLP compliant. However, the methodology used was similar to the OECD method 407 with the exception that the exposure duration in the OECD protocol is at 28 days and that at least 5 animals per dose and sex are required.

Several 28-day studies conducted with rats are reviewed elsewhere (Exxon) Reported NOELs or NOAELs ranged from 100 mg/kg bw/d for $C_{12\text{-}14}AE_2$ through 300 mg/kg bw/d for $C_{11\text{-}13}AE_7$ to 588 mg/kg bw/d for $C_{12}AE_7$. At dose levels greater than 100 mg/kg bw/d, $C_{12\text{-}14}AE_2$ induced a treatment-related increase in haematocrit, mean cell volume, erythrocyte and leukocyte count. Biochemical investigations revealed compound related increases in GPT, glucose urea and creatinine values. At necropsy treatment-related mucosal lesions of the forestomach were observed. More study details were not available.

In a 90-day oral feeding study, $C_{10}AE_5$ was fed to rats at doses of 125, 250 or 500 mg/kg bw/d (Procter and Gamble Ltd., 1978). In this study, 20 female Sprague-Dawley rats were used per treatment group. The in-life observations included the physical condition, behaviour, body weight and food intake for each rat. At necropsy, major organs were taken for histological and clinical chemical examinations. No mortalities were recorded during the study. The treatment with $C_{10}AE_5$ was not associated with any gross or histological lesions in the tissue examined. Clinical examinations did not indicate treatment-related effects which were considered to be of biological significance. No statistically significant differences between control and treated animals were determined

for body weight gain, food consumption or feed efficiency. The only treatment-related effect was a slight increase in absolute liver weights as well as a trend toward a dose-dependant increase in the liver weight/body weight ratio, with a statistically significant increase in the high dose group. However, the histological evaluation did not reveal any indication of hepatotoxicity and therefore the increase in liver weights was not interpreted to be a toxicological effect. It can be considered to be an adaptive response as a result of extensive metabolism of the test compound by the liver. The investigators did not report a NOEL or NOAEL. On the basis of the results reported, a NOAEL can be established at 500 mg/kg bw/d under the assumption that the increased absolute and relative liver weights are of adaptive nature and not indicative of a toxic effect. Taking a more conservative approach, a NOEL can be established at 250 mg/kg bw/d. Although the study was pre-GLP and not in full compliance with OECD guidelines, the study provided sufficient information and was judged to be scientifically reliable.

$C_{12-15}AE_7$ and $C_{12-14}AE_7$ were tested in a 90-day dietary feeding study at dose levels of 0%, 0.0313%, 0.0625%, 0.125%, 0.25%, 0.5% and 1.0% active material in Colworth Wistar rats (Unilever, 1978a, 1978b). In both studies, the body weight gain was significantly compromised in male and female rats that were fed at doses above 0.25%. This observation was associated with marked decreases in food and water consumption of these animals. Significant increases in relative liver weights were recorded in male rats fed at the 0.5 and 1.0% dose level and in female rats fed diets containing 0.25, 0.5, and 1.0% of the surfactants. The histological examination of the liver at necropsy revealed hepatocytic enlargement, suggesting an increased liver metabolism on the basis of increased alkaline phosphatase activity at the higher dose levels. No effects have been observed on the organs of the reproductive system. The NOAELs were established on the basis of hepatic histology at the 0.125% level, corresponding to a daily intake of $C_{12-15}AE_7$ of 102 mg/kg bw/d and of $C_{12-14}AE_7$ of 110 mg/kg bw/d. Other changes in haematological, urinary and pathological parameters were not treatment related or occurred above the NOAEL. These studies were conducted pre-GLP and followed the principles of OECD guidelines.

In a further good quality 90-day oral feeding study, $C_{14-15}AE_7$ was fed to Wistar rats at dietary concentrations of 0, 300, 1,000, 3,000, and 10,000 ppm of active ingredient (Shell Research Ltd., 1982a). During the study, male and female rats (*i.e.*, 6 per dose group and 12 in the control group) were observed for general health and behaviour, body weight and food intake. At necropsy, major organs including those of the reproductive system were weighed and specified tissues were examined histologically. Terminal blood samples were taken for haematological and clinical chemical evaluations. All animals survived until their scheduled necropsy date. Significant treatment-related effects on body weight (*i.e.*, reduced mean body weights in males at 10,000 ppm and in females at 3,000 ppm), food intake (*i.e.*, reduced intake in both sexes at 10,000 ppm and at 3,000 ppm for females), organ weights (*i.e.*, increased relative liver weight in both sexes at 3,000 and 10,000ppm and in females also at 1,000 ppm; increased spleen weight in males at 10,000 ppm; clinical chemistry (*i.e.*, confined to 10,000 ppm dose groups; significantly higher urea, chloride and potassium levels in males; significantly higher urea, chloride and cholesterol levels in females) and haematology (*i.e.*, in both sexes at 10,000 ppm and in males also at 3,000 ppm increased total leukocytes and lymphocytes; females at 10,000 ppm showed depression in numbers of neutrophils, mean cell volume and mean cell haemoglobin) were identified in one or both sexes fed with dietary concentrations of 3,000 and 10,000 ppm. Histopathologically, there were no compound-related effects at any dose level. No effects

were observed on the organs of the reproductive system. Minor, but statistically significant changes in liver weight, kidney weights and plasma urea concentration were recorded in female rats in the 1,000 ppm group were not of toxicological significance. Taking a conservative approach, the NOEL for $C_{14-15}AE_7$ can be established at a dietary level 300 ppm (15 mg/kg/day). No adverse effects were reported at 1,000 ppm (equivalent to ca. 50 mg/kg/day).

In another 90-day study with $C_{14-15}AE_7$, the material was fed via the diet to three groups of young albino rats each consisting of 20 males and 20 females with control group consisting of an equal number of rats (Procter and Gamble Ltd., 1974a). The surfactant was incorporated in the diet at concentrations of 0.1%, 0.5% and 1%. During the in-life phase, standard haematological and biochemical parameters, and complete urinalyses were performed. At 28 days, five male and five female rats from each group were sacrificed and thirty tissues from each one of them were examined histological. At termination of the experiment, all remaining rats were autopsied. There were no treatment-related changes in body weight, food intake, and organ weights including those of the reproductive system, clinical chemistry and haematology at the 0.1%. 0.5% or 1.0% dietary intake level. The individual mean exposure for the high level males was 700 mg/kg bw/d of $C_{14-15}AE_7$. The corresponding individual mean exposure for the high level females was 785 mg/kg bw/d. As there were no treatment-related findings, the NOEL was established at the highest exposure level. This study followed the principles of OECD methodology, but was not compliant with GLP regulations.

$C_{16-18}AE_{10}$ was tested for systemic toxicity in a 90-day study at repeated doses by oral gavage of 0, 20, 100, and 500 mg/kg bw/d in an application volume of 10 mL/kg bw (Cognis Germany GmbH, 1983). Ten male and female rats were used for each dose. Five 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. The highest dosage resulted in delayed growth of the male animals and caused damage to forestomach and kidneys in both male and female rats. No effects were observed on the organs of the reproductive system. Inflammatory changes in the forestomach, seen in the animals in the middle dosage range (*i.e.*, 100 mg/kg/day) were less obvious and were reversible. These effects were most likely due to the gavage administration of an irritant concentration of the test substance as similar observations were not made in the dietary studies. According to these findings, the limit of systemic compatibility for $C_{16-18}AE_{10}$ was 100 mg/kg bw/d for male rats. On the basis of the observations made in this study, a NOAEL of 100 mg/kg bw/d can be established. The study followed the OECD guideline method 408. GLP compliance was not indicated in the study report.

The toxicity of oral application of 20, 100 and 500 mg/kg bw/d of $C_{16-18}AE_{10}$ was evaluated in a 90-day oral feeding study (Cognis Germany GmbH, 1981a). The following enzyme and clinical parameters were monitored: kidney levels of ammonia, creatinine and liver levels of several enzymes. No significant dose-response relationships were established with any of the measured factors and fed levels of $C_{16-18}AE_{10}$. All results were within the normal range for the measured parameters in the tested rat strain. Since no detailed information on the design and outcome of this investigation was available, a NOEL or NOAEL could not be established.

The subchronic toxicity of $C_{9-11}AE_6$ was evaluated in a 90-day feeding study at dietary concentrations of 0, 125, 250, 500, 1,000 and 3,000 ppm (Shell Research Ltd., 1973). The

general health and behaviour of the rats were observed daily and body weight and food intake recorded weekly. After 13 weeks, the animals were autopsied and a wide range of tissues weighed and taken for histological examination. Terminal blood samples were taken for haematological and clinical chemical determinations. The results showed that oral exposure to up to 3,000 ppm C_9AE_{11} in the diets to rats produced no significant signs of toxicity. A NOEL or NOAEL was not established by the investigators, but based on the information presented the NOAEL could be established at the 3,000 ppm dose level. This value translates into an exposure of approximately 150 mg/kg bw/d. This study was conducted pre-GLP but the procedures followed the principles of OECD protocol. With 12 animals per sex and dose group, the number of animals used per treatment group was even higher than the recommended 10 per sex per dose.

$C_{9\text{-}11}AE_8$ was tested for systemic toxicity in a 90-day feeding study at dietary concentrations of 0.04, 0.2 and 1.0% (Procter and Gamble Ltd., 1976). Groups of 20 male and 20 female Charles River rats were used for each dose. The criteria evaluated for compound effects were clinical signs, mortality rates, opthalmoscopic findings, organ weight data, and gross and microscopic pathology. Organs of the reproductive system were included in the investigation. No effects attributable to the administration of the test compound were noted in these examinations. In addition, no compound-related gross or histomorphologic tissue alterations were noted in any of the treated animals. When compared to the control group, lower body weight gain and decreased food consumption were noted in the high dose males and females and in the middle dose females from week 1 through the end of the study. Further statistical analyses revealed a significant decrease in the mean body weight gain noted in the high dose females and the decreases in mean food consumption noted in the high dose males and females. The differences noted in the mid dose females were not statistically significant. The investigators considered these observations to be the result of poor palatability of the test substance. However, since there was no further information to verify this information, it is suggested to take a conservative approach and to establish the NOEL at 0.2% dose level. This reflects a daily intake of about 80 mg/kg bw/d. This study was not indicated to be GLP or OECD compliant but should be regarded as suitable as the study followed the principles and procedures of the OECD guidelines.

Talmage (1994) cites a number of subchronic 90-day toxicity studies with alcohol ethoxylates. Oral exposures were up to 500 mg/kg bw/d and produced no significant adverse effects.

No unusual findings of systemic toxicity were noted in a two year chronic feeding study in rats fed $C_{12\text{-}13}AE_{6.5}$ or $C_{14\text{-}15}AE_7$ in the diet at levels of 0%, 0.1%, 0.5% and 1% (*i.e.*, equals about 500 mg/kg bw/d) (Exxon; Talmage, 1994). Reduced food consumption at the higher dose levels (*i.e.*, 0.5 and 1% for females and 1% for males) resulted in a lower body weight gain compared to the control group. After 104 weeks, elevated organ to body weight ratios were observed for females fed with the 0.5 and 1% dose (*i.e.*, liver, kidney and brain), females fed with the 1% dose (*i.e.*, heart), and males fed at the 1% dose level (*i.e.*, liver). In male rats, dose related focal myocarditis was the only pathology observed. Although this is a common spontaneous type of lesion in aging rats, incidences were higher in the treated group than in the control group. No tumours or other treatment-related lesions were observed. On the basis of the observed relative organ weight increase at the 0.5% dietary intake level, the LOAEL, the NOAEL can be established at the 0.1% level. This reflects a daily intake of about 50 mg/kg bw/d.

In a second chronic oral feeding study, Charles River rats were fed with $C_{14-15}AE_7$ containing diet at dose levels of 0, 0.1, 0.5 and 1% (Little, 1981; Talmage, 1994). Dose related body weight depression in females in the upper two treatment levels and in males at the 1% dose level was probably due to poor palatability of the diet. At termination, elevated organ-to-body weight ratios were noted for the liver, kidney, heart and thyroid/parathyroid glands at the highest exposure level. The only significant histopathological finding prevalent in all dose groups was a dose related increase in incidence of focal myocarditis at 12 months but not at study termination at 2 years. No other treatment-related histopathology and no increase in tumour incidence were reported. On the basis of these observations the NOAEL was set at the 0.5% level. This converts to a daily exposure of about 190 mg/kg bw/d for female rats and 162 mg/kg bw/d for male rats.

5.2.1.4.2 Inhalation

Long-term inhalation studies on alcohol ethoxylates were not available.

5.2.1.4.3 Dermal route

A 90-day dermal toxicity study was conducted with a 2.5% aqueous solution of $C_{14-15}AE_7$ in rabbits (Procter and Gamble Ltd., 1974b). The animals (*i.e.*, 3 animals of each sex per treatment) received a total of 65 exposures during a 13-week treatment period. The test solution was applied 5 days a week for 6 hours at dosage levels of 2 mL/kg bw/d. Three animals of the treatment group died in the course of the study. The cause of death in all 3 animals was a result of an infectious disease (also observed in the control group) in combination with the stress produced by the treatment regimens. In the surviving animals moderate localised test compound induced dermal irritation, indicated amongst other signs by erythema and oedema, was noted in all surviving animals of both test groups during each week of treatment. A NOAEL was not established as it was not possible to differentiate between treatment effects and other factors such as disease of the animals in this study, especially as only few animals remained at the end of the study.

Dermal treatment of 10 rats per sex per group for 90-days with 1%, 10% and 25% $C_{9-11}AE_6$ did not result in any significant compound related effects (Gingell and Lu, 1991). In-life observations included clinical observations for e.g., skin irritation, body weights, urine and blood collection and analysis. At necropsy organs and tissues collected were preserved in buffered formalin and histopathologically examined. Scores for signs of irritation at the application site throughout the study were zero but at 10% and 25% dry and flaky skin was noted. Relative kidney weights were increased in both sexes at the 25% treatment level, but no histological lesions could be determined. As a result of the observation of the increases in relative kidney weight, the NOAEL was established at the 10% level. This exposure level reflects a dose of about 80 mg/kg bw/d. This study followed the principles of the OECD procedure 411 and was GLP compliant.

No treatment-related lesions were observed when $C_{12-13}AE_{6.5}$ was applied to the backs of ICR Swiss mice three times a week at dilutions of 0, 0.2, 1.0 or 5.0% for 18 month (Talmage, 1994). The 5% level is approximately equivalent to 270 mg/kg bw/d assuming that the mouse weight averages over the study was 75 g. No more detailed study information was available.

5.2.1.4.4 Other routes of exposure

Arthur D. Little (1977) and Talmage (1944) cited a study from Berberian *et al.* (1965a, 1965b), in which repeated applications of $C_{12}AE_9$ to the vaginal mucosa of dogs was evaluated. In one group, 5 mL of a 15% aqueous solution of $C_{12}AE_9$ was introduced in to the vagina once daily, five days a week for two consecutive weeks. No irritation was observed. Similarly, another group was treated with 10 mL of a 15% $C_{12}AE_9$ aerosol cream formulation three times weekly for six months (79 vaginal exposures/dogs). No changes were observed in test animals when compared to controls treated with the aerosol formulation without $C_{12}AE_9$.

The following table 5.2.1 summarizes all available repeated dose toxicity studies with alcohol ethoxylates.

Table 5.2-1: Summary table of the repeated dose toxicity studies with AEs

Animal	Route	Duration	Test Material	Estimated NOAEL/NOEL	Dosage level	Reference
			Subacute			
Alderley Park rat N=30	Oral gavage	14 days	$C_{13\text{-}15}AE_3$	> 500 mg/kg bw/d	0.5 mL/kg/day	Imperial Chemical Industries Ltd. (1975)
Alderley Park rat N=30	Oral gavage	14 days	$C_{13\text{-}15}AE_7$ or $C_{13\text{-}15}AE_{7.5}$	> 500 mg/kg bw/d	0.5 and 0.2 mL/kg	Imperial Chemical Industries PLC (1978); Uniqema Ltd. (1974)
Alderley Park rat N=30	Oral gavage	14 days	$C_{14\text{-}15}AE_7$	> 250 mg/kg bw/d	0.25 mL/kg	Imperial Chemical Industries PLC (1978)
CFY rat N=30	Oral gavage	14 days	$C_{13\text{-}15}AE_{11}$	> 100 mg/kg bw/d	0.1 g/kg/day	Huntingdon Research Centre (1978b)
CFY rat N=30	Oral gavage	14 days	$C_{13\text{-}15}AE_{20}$	> 100 mg/kg bw/d	0.1 g/kg/day	Huntingdon Research Centre (1978a)
Colworth-Wistar rat N=60	Oral feeding	21 days	$C_{12\text{-}15}AE_3$	471 mg/kg bw/d	0, 0.023, 0.047, 0.094, 0.188, **0.375**, 0.75, 1.00, 1.50%	Unilever (1977a)
Colworth-Wistar rat N=60	Oral feeding	21 days	$C_{12\text{-}14}AE_7$	459 mg/kg bw/d	0, 0.023, 0.047, 0.094, 0.188, **0.375**, 0.75, 1.00, 1.50%	Unilever (1977c)
Colworth-Wistar rat N=60	Oral feeding	21 days	$C_{12\text{-}15}AE_7$	502 mg/kg bw/d	0, 0.023, 0.047, 0.094, 0.188, **0.375**, 0.75, 1.00, 1.50%	Unilever (1977d)

Animal	Route	Duration	Test Material	Estimated NOAEL/NOEL	Dosage level	Reference
Colworth-Wistar rat N=60	Oral feeding	21 days	$C_{12-15}E_{11}$	519 mg/kg bw/d	0, 0.023, 0.047, 0.094, 0.188, 0.375, 0.75, 1.00, 1.50%	Unilever (1977b)
Colworth-Wistar rat N=60	Oral feeding	21 days	$C_{16-20}AE_{18}$	433 mg/kg bw/d	0, 0.023, 0.047, 0.094, 0.188, **0.375**, 0.75, 1.00, 1.50%	Unilever (1977e)
			Subchronic			
Cox rats N=80	Oral feeding	90 days	$C_{10}AE_5$	250 mg/kg bw/d	0, 125,250, 500 mg/kg/day	Procter and Gamble Ltd. (1978)
Colworth Wistar rats N=160	Oral feeding	90 days	$C_{12-14}E_7$	110 mg/kg bw/d	0, 0.03, 0.063, **0.125**, 0.25, 0.5, 1.0%	Unilever (1978a)
Colworth Wistar rats N=160	Oral feeding	90 days	$C_{12-15}E_7$	102 mg/kg bw/d	0, 0.03, 0.063, **0.125**, 0.25, 0.5, 1.0%	Unilever (1978b)
Wistar rats N=144	Oral feeding	90 days	$C_{14-15}E_7$	50 mg/kg bw/d	0, 300, **1,000,** 3,000, 10,000 ppm	Shell Research Ltd. (1982a)
Sprague-Dawley rats N=160	Oral feeding	90 days	$C_{14-15}E_7$	700 mg/kg bw/d	0., 0.1, 0.5, **1.0%**	Procter and Gamble Ltd. (1974a)
CFE rats N=181	Oral feeding	90 days	$C_{9-11}AE_6$	150 mg/kg bw/d	0, 125, 250, 500, 1,000, **3,000** ppm	Shell Research Ltd. (1973)
Wistar rats N=80	Oral gavage	90 days	$C_{16-18}AE_{10}$	100 mg/kg bw/d	0, 20, 100, 500 mg/kg/day	Cognis Germany GmbH, (1983)
Rats N=80	Oral feeding	90-days	$C_{16-18}AE_{10}$	> 500 mg/kg bw/d	20, 100 and 500 mg/kg/day	Cognis Germany GmbH (1981)
Charles River rats N=160	Oral feeding	90 days	$C_{9-11}AE_8$	400 mg/kg bw/d	0, 0.04, 0.2, 1.0%	Procter and Gamble Ltd. (1976)
Fisher rats N=80	Dermal application	90 days	$C_{9-11}AE_6$	80 mg/kg bw/d	0, 1, **10** and 25%	Gingell and Lu (1991)
			Long term studies (chronic)			
Sprague-Drawley rats N=	Oral feeding	104 weeks	$C_{12-13}AE_{6.5}$ or $C_{14-15}AE_7$	50 mg/kg bw/d	0, **0.1**, 0.5, 1%	Exxon; Talmage (1994)
Charles River rats N=520	Oral feeding	1-2 year	$C_{14-15}E_7$	160 mg/kg bw/d	0, 0.1, **0.5**, 1%	Little (1981); Talmage (1994)

Conclusion

The subacute, subchronic and chronic oral and dermal toxicity studies on alcohol ethoxylates provide a coherent picture of their systemic toxicity profile. In two chronic long-term toxicity studies which also investigated the carcinogenic potential of AEs, no adverse effects were observed up to a dose level of 50 mg/kg/day. In several dermal and oral subchronic studies over 90 days the range of NOELs/NOAELs was 50 to 700 mg/kg/day.

In a series of 14-days repeated dose toxicity studies, and one 90-day study the test compounds were administered by oral gavage and local treatment related effects were observed in the forestomach of the test animals. These effects were generally 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 was considered to be a response secondary to the irritant properties of the study compound 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. In all cases, the NOAEL for these studies was established at the highest dose (*i.e.*, greater than 100 mg/kg bw/d).

In a series of 21-day oral feeding studies, various alcohol ethoxylates were examined for repeated dose toxicity. The established NOAELs ranged from 433 to 519 mg/kg bw/d. The organ mostly affected in these studies was the liver, indicated by increased liver weight and hepatic hypertrophy at higher doses. This series of oral feeding studies can serve to evaluate the impact of changing ethoxylation degree on the systemic toxicity of AEs. Since for all studies the NOAELs were at the same exposure level, an impact of the ethoxylation degree on toxicity could not be determined in these feeding studies with AEs.

Most of the 90-day oral feeding studies were in many respects similar to OECD test method 407. Two studies, one dermal and one oral repeated dose studies were conducted in compliance with GLP regulations. In the oral GLP-compliant study with $C_{14\text{-}15}AE_7$, the NOEL was established at the 50 mg/kg bw/d exposure level. However, the same product was tested in a non-GLP 90-day oral feeding study and the NOAEL was determined to be at the highest exposure level of 700 mg/kg bw/d. $C_{14\text{-}15}AE_7$ was also examined in two 2-year feeding studies. Dose related body weight depressions in females in the upper two treatment levels were seen. At termination, elevated organ-to-body weight ratios were noted in the liver, kidney and heart. No effects have been observed on the organs of the reproductive system. Moreover, no treatment-related histopathology and no increase in tumour incidence were reported. It was concluded that the NOAEL should be established at the 0.5% level which converts to a dose of about 190 mg/kg bw/d for female rats. In the other long term study dose related body-weight depression were observed in females in the upper two treatment levels (*i.e.*, 100 and 250 mg/kg bw/d).

Based on these findings, the NOAEL was established at the 50 mg/kg/d exposure level. In a 2-year feeding study with $C_{12\text{-}14}AE_{6.5}$ the NOAEL was established to be 50 mg/kg bw/d. At the higher dose levels (*i.e.*, 250 and 500 mg/kg bw/d) reduced food consumption and body weight gain was observed. At study termination, elevated organ-to-body weight ratios were noted for the liver, kidney and brain in females at the 250 and 500 mg/kg bw/d dose levels. These differences were not accompanied by histological changes in the organs

examined. This study was not indicated to be GLP or OECD compliant but should be regarded as suitable as the study was conducted following the principles and procedures of the OECD guideline.

A number of different alcohol ethoxylates with different structural characteristics were evaluated (*e.g.*, carbon chains raging in length from C_9 to C_{14-16} and ethoxy unit length from 3 to 20). Despite differences in protocols and study periods the overall toxicological response was qualitatively and quantitatively similar and a contribution of structural characteristics to toxicity could not be established. No clear trends in the toxicity after repeated exposure with structural components of the test material could therefore be determined.

When given by gavage the most prominent finding was local irritation in the gastro-intestinal tract. In repeated dose feeding studies the liver was the most prominent target organ. AEs induced increased relative liver weights and in some cases liver hypertrophy. This effect could however be related to an induction of liver metabolism and would normally considered an adaptive rather than an adverse effect. The NOAEL in the chronic toxicity studies is based on reduced body weight gain and increased relative organ weights only. The NOAEL of 50 mg/kg bw/d that is taken forward to the risk characterisation is based on the lowest NOAEL in a chronic oral feeding study in rats which was equal to the lowest NOAELs in subchronic feeding studies in rats.

5.2.1.5 Genetic toxicity

5.2.1.5.1 *In vitro*

Bacterial tests

More than thirteen reliable and well documented and GLP compliant studies covering the whole spectrum of alcohol ethoxylates were conducted to assess their potential to induce reverse mutations in the presence and absence of a metabolic activation system in the so-called Ames test (Ames *et al*., 1975) (Cognis Germany GmbH, 1994; Henkel KgaA, 1988a, 1988b, 1994a, 1994b, 1997a; Hüls AG, 1994a, 1994b, 1997e, 1997f; Shell Development Company, 1981e; Shell International BV, 1996f; Shell Research Ltd., 1982b, 1991b). The range of evaluated surfactants spanned $C_{7-9}AE_2$ to $C_{22}AE_{10}$ and for ethoxy units a similarly broad range was evaluated $C_{12-14}AE_3$ to $C_{16-18}AE_{20}$. A few typical studies will be presented here to illustrate the methodology used to determine the *in vitro* mutagenicity in bacterial systems and the results obtained. Most studies evaluated the mutagenic potential of AEs in at least five of the *Salmonella typhimurium* strains TA98, TA100, TA102, TA104, TA1535, TA1537, and TA1538 and in either *Escherichia coli* strain WP2 or WP2uvrA.

In recent GLP compliant studies, the mutagenic activity of $C_{14-15}AE_7$ was investigated in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 and *Escherichia coli* WP_2 uvrA pKM101 (Shell Research Ltd., 1982b, 1991b). The dose levels covered the range from 1 to 5000 µg/plate. The tests were conducted in triplicate both with and without the addition of a metabolizing system (*i.e.*, Aroclor 1254 induced rat liver S9 mix). All 5 bacterial strains exhibited mutagenic response to the positive control substance. For the solvent controls the mean numbers of spontaneous revertants were in the 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 given test conditions $C_{14-15}AE_7$ is not a bacterial mutagen.

Two more recent studies also evaluated *S. typhimurium* TA102 and TA104 with the procedure described above testing $C_{7-9}AE_6$ and $C_{18}AE_{20}$ (Henkel, KGaA, 1994a; Shell International BV, 1996f). The control substances confirmed the activity and sensitivity of the test system and it was found that these AEs were not mutagenic in any of the tested strains.

Several reviews further confirmed the absence of mutagenic activity in bacterial systems (Little, 1977, 1981; Talmage, 1994; Shell Chemicals Ltd., 2002; Exxon; Shell Chemical Company).

From the presented information it can be concluded that alcohol ethoxylates do not induce reverse gene mutations in *Salmonella typhimurium* and *Escherichia coli* test systems.

Non-bacterial tests

The mutagenic potential of $C_{14-15}AE_7$ was further evaluated in a good quality *Saccharomyces cerevisiae* gene conversion assay (Shell Research Ltd., 1982b). The assay was carried out with and without metabolic activation. It was concluded that the addition of $C_{14-15}AE_7$ to liquid suspension of *Saccharomyces cerevisiae* did not induced mitotic gene conversion in yeast. The same results were obtained in a non-GLP study conducted with $C_{12-15}AE_3$ (Shell Research Ltd., 1980c).

$C_{12-14}AE_{21}$ was evaluated in the *in vitro* mammalian cytogenetic test with Chinese Hamster V79 cells for its potential to induce chromosomal aberrations (Henkel, KGaA, 1997b). There was no indication of an increase in the frequency of polyploid metaphases after the treatment with the test substance compared to the negative controls. In conclusion, the test substance did not induce chromosomal aberrations in the V79 Chinese hamster cell line under the tested conditions. This study was conducted according to GLP and OECD guideline 473.

In another GLP study $C_{14}AE_{12}$ was tested in the absence and in the presence of metabolic activation by a liver microsome fraction (*i.e.*, S-9) for its ability to induce chromosome aberrations in the Chinese Hamster ovary (CHO) cells cultured *in vitro* (Shell Development Company, 1982b*)*. Medium controls, solvent controls and positive controls were run concurrently with the test chemical. The test chemical did not demonstrate an effect on cytogenetic parameters under the conditions tested, although the highest concentration resulted in cytotoxic effects.

Two *in vitro* mouse lymphoma tests were reported investigating the mutagenic potential of AEs. Dose levels of 40 µg/mL and 30 µL/mL were tested, respectively (Raymond *et al.*, 1987; Myhr and Caspary, 1991). In both tests, it was shown that AEs are not mutagenic. In the absence of more details on the protocol and also the results, the reliability of the studies could not be evaluated.

Talmage (1994) reported an assay testing the ability of $C_{14-15}AE_7$ to induce chromosome aberrations in rat liver cells. In slide cultures exposed to culture medium containing C_{14-}

$_{15}AE_7$ at concentrations of 10, 15, 20, and 25 µL/mL the frequency of chromatic and chromosome aberrations did not differ significantly from that of control cultures.

Further Talmage (1994) cited a series of other short-term *in vitro* assays with mammalian cells that have been conducted to assess the genotoxicity of AEs ($C_{14-15}AE_7$, $C_{12-15}AE_3$, $C_{12-14}AE_3$ and $C_{12-14}AE_9$), including rat liver cells and human leucocytes. In none of these assays, alcohol ethoxylate surfactants induced genetic damage.

5.2.1.5.2 *In vivo*

The potential of $C_{13-15}AE_7$ to induce chromosome damage in Chinese hamster bone marrow cells after an acute oral dose was evaluated in an *in vivo* cytogenetics assay (Unilever, 1978c). In this study, $C_{13-15}AE_7$ was administered as a 20% aqueous solution at doses of 3.4 g/kg and 1.7 g/kg active ingredient. In both cases, cyclophosphamide was used as the positive control and saline as the negative control. There were eight male and eight female animals at each dose level which were killed 24 hours after compound administration. Chromosome preparations were prepared from the bone marrow and ten slides from each animal were scored for metaphase aberrations. No evidence was found that $C_{13-15}AE_7$ damages bone marrow chromosomes under the conditions of the experiments. This study was not indicated to be GLP or OECD compliant but should be regarded as suitable as the study was conducted following the principles and procedures of the OECD guideline.

In a similar experiment, $C_{12-14}AE_7$ was administered as a 10% aqueous solution to male and female Chinese hamsters by oral intubation at two dose levels, 1.25 g/kg and 2.5 g/kg active ingredient (Unilever, 1979). Chromosome preparations were made from the bone marrow 24h after administration. Metaphase divisions were scored for aberrations and there was no indication that the tested product damaged chromosomes under the given test conditions. Although the study was pre-GLP and not in full compliance with current OECD guidelines, the study provided sufficient information and was judged to be scientifically reliable.

$C_{14-15}AE_7$ was also administered orally to 5 male and female Tunstall Wistar rats at doses of 250, 500 and 1,000 mg/kg in a GLP compliant study. Bone marrow smears were prepared 24 hours after and were processed for chromosome analysis. The test material did not show any potential for clastogenicity under the given test conditions (Shell Research, 1982b).

Two *in vivo* studies testing for chromosome damage with $C_{12-15}AE_3$ and $C_{12-14}AE_9$ in a mouse micronucleus test with CD-1 mice bone marrow cells were reviewed by Talmage (1994). No chromosome abnormalities were observed in these studies at an intraperitoneal dose of up to 100 and 50 mg/kg, respectively. No more detailed study information was available.

Conclusion

In all available *in vitro* and *in vivo* genotoxicity assays, there was no indication of genetic toxicity of broad range of structurally different alcohol ethoxylates. Most of the studies were performed in accordance with GLP and following OECD guideline methodologies. The remaining *in vitro* and *in vivo* studies were well documented and conducted. The

structure of alcohol ethoxylates are not of concern for potential genotoxicity. Based on the presented data, it is therefore concluded that there is no evidence that AEs are either mutagenic or genotoxic.

5.2.1.6 Carcinogenicity

The carcinogenic potential of $C_{14-15}AE_7$ in rats has been evaluated in a one- to two-year oral feeding study (Procter and Gamble Ltd., 1979). $C_{14-15}AE_7$ was administered at dietary levels of 0, 0.1, 0.5 and 1% to four groups of Charles River rats (*i.e.*, 65 of each sex) for a period of one or two years. Fifteen males and females from the control and the 0.5% dose group, 15 males and 14 females from the 0.1% dose group, and 14 males and 15 females from the 1% dose group were sacrificed after an interim of 1 year exposure. The remaining animals were treated for the full 2-year period. Administration of $C_{14-15}AE_7$ for a period of 1 or 2 years did not produce any compound related changes in general behaviour and appearance. The survival rate of the test animals was comparable if not better than the controls. Body weights of females fed with 0.5% $C_{14-15}AE_7$ and males and females fed with 1% $C_{14-15}AE_7$ had significantly lower weight gains than the control. At necropsy, no compound related effects were observed in organ to body weight determinations. In conclusion, there was no evidence to indicate that treatment related changes of a carcinogenic nature were produced in rats by repeated ingestion of 0.1, 0.5 and 1% $C_{14-15}AE_7$. Although the study was pre-GLP and not in full compliance with OECD guidelines, the study provided sufficient information and was judged to be scientifically reliable.

In another chronic feeding study reported in Shell Chemicals Ltd., (2002), Sprague-Dawley rats (*i.e.*, 100 of each sex) were fed with $C_{14-15}AE_7$ at 0.1, 0.5 and 1% in the diet for two years. No further testing details were reported. A treatment-related body weight depression was observed in females at the two highest treatment levels and in males at the 1% dose level, probably due to the poor palatability of the diet. There was no evidence for any carcinogenic activity of $C_{14-15}AE_7$. The study was not in compliance with GLP regulations or current guidelines, but well conducted and the data are considered to be scientifically supportable.

No carcinogenic effects were observed in a two-year study in which 100 Sprague-Dawley rats were fed with $C_{12-13}AE_{6.5}$ containing diet at doses up to 1% (*i.e.*, 500 mg/kg bw/d) (Exxon; Talmage, 1994). Reduced food consumption was noted at the higher dose levels (*i.e.*, 0.5 and 1% for females and 1% for males), resulting in a lower body weight gain compared to the control group. No treatment-related histopathology was found and no increase in tumour incidence was observed. Thus, on the basis of this study, $C_{12-13}AE_{6.5}$ is not considered to be carcinogenic. No more detailed study information was provided.

No treatment-related lesions were observed when $C_{12-13}AE_{6.5}$ was applied to the backs of ICR Swiss mice three times a week at 0, 0.2, 1.0 or 5.0% for 18 month (Shell Chemicals Ltd., 2002; Talmage, 1994). No more detailed study information was provided.

Conclusion

The available oral and dermal long term toxicity/carcinogenicity studies, even if not performed according to the accepted guidelines for carcinogenicity bioassays, appear to be scientifically well conducted and documented. It should be noted that some more sensitive

physiological endpoints of these same studies were discussed in the section on repeated dose toxicity (*i.e.*, 5.2.1.4). On the basis of the information presented it can be concluded that alcohol ethoxylates are not carcinogenic. This assessment is further supported by the absence of any mutagenic or genotoxic activity of this compound class as elaborated in the section on genotoxicity (*i.e.*, 5.2.1.5).

5.2.1.7 Reproductive toxicity

In a two-generation study conducted in Charles River CD rats, the reproductive toxicity and developmental effects of $C_{14\text{-}15}AE_7$ were evaluated at dietary levels of 0.05%, 0.1% and 0.5% (*i.e.*, about 25, 50 and 250 mg/kg bw/d) (Procter and Gamble Ltd., 1977). The control group and the six treated groups comprised of 25 male and 25 female animals. Three of the groups received the compound continuously during the study. In the other three groups the females received the compound only during the 6th through the 15th day of gestation and the males were untreated. Detailed physical examinations, body weight, food consumption and mortalities were recorded weekly. Specific observations for the reproduction phase of this study included observations for fertility, litter size, numbers of male and female pups, viability of the newborn, survival of pups to weaning and growth of the pups. No treatment-related changes in behaviour or appearance were observed in the parental rats or pups throughout the study. Female rats from the 0.5% continuous treatment group gained slightly less body weight compared to control females. No other consistent differences in body weight were observed. Food consumption was similar for control and treated rats. No compound related differences were seen between control and treated rats with respect to fertility, gestation or viability indices. The average 21-day body weights for pups at the 0.5% continuous treatment group were significantly lower as compared to the average pup body weights in the controls. No other compound-related changes in body weight were observed. None of the deaths of parental rats during the study was considered to be compound-related. Examination of organ weight values revealed that compound-related effects were limited to increased group mean relative liver weights of male and female F_1 from the 0.5% continuous feeding group at the 91-day sacrifice, and increases in group mean relative liver weights of males from the 0.5% continuous feeding group of the F_2 generation at the 60-day and caesarean section sacrifices. No compound-related histopathological lesions were observed in any of the tissues examined from rats for the F_0 and F_1 generations. In conclusion, the compound did not show any potential for reproductive toxicity at the tested dose levels. The NOAEL for reproductive effects was greater than the highest tested dose of 0.5%, which equals an exposure level of 250 mg/kg bw/d. Although the study was pre-GLP and not in full compliance with current OECD guidelines, the study provided sufficient information and was assessed to be scientifically reliable.

The reproductive toxicity and developmental effects of $C_{12}AE_6$ was evaluated in a feeding study using a similar experimental design as described above (Little, 1977; Shell Chemicals Ltd., 2002; Talmage, 1994). Rats were exposed in a two-generation study to the compound at dose levels of 25, 50 or 250 mg/kg bw/d. No treatment related effects in the parents or pups on general behaviour, appearance or survival were observed. Fertility of treated groups was comparable with the controls. The only observation was related to a reduced weight gain of parental rats and pups relative to the control at the highest dose level (*i.e.*, 250 mg/kg bw/d). The NOAEL for reproduction was therefore set at the highest dose level which was 0.5% dietary level greater than 250 mg/kg bw/d. More detailed study information was not available.

In another GLP-compliant two-generation reproduction study, groups of 30 weanling Fisher 344 rats of each sex were dermally exposed to 1 mL/kg bw $C_{9-11}AE_6$ at concentrations of 0, 1, 10 or 25% w/v three times a week except during the mating periods (Shell Development Company, 1985). This treatment equals exposure levels of about 0, 10, 100 or 250 mg/kg bw/d. No mortalities were observed in the F_0 generation, and the five deaths in the F_1 adult males and females in the control and treatment groups were not considered to be compound-related. In the highest dose group, body weights of both males and females in both treated generations were sporadically decreased compared to controls. There was no effect on maternal body weight during gestational and lactational periods in both generations. At necropsy organ weight differences in liver, lung, kidney and heart were observed in the F_1 generation. However no pathological findings were associated with these affected organs. There were no compound-related effects on mating and fertility indices and mean gestational length in both generations. No effects on testicular weights, sperm counts and LDH-X activities in F_0 and F_1 male adults were observed. Macroscopic and microscopic examination of the reproductive organs did not reveal significant differences in the treated groups compared to the controls. Based on these observations the NOAEL for reproductive and developmental toxicity can be established at 250 mg/kg bw/d, the highest dermal tested dose.

Further evidence for the lack of reproductive toxicity of alcohol ethoxylates has been provided by a range of subchronic oral feeding studies which investigated also any potential effects on the organs of the reproductive system (see Chapter 5.2.1.4.1). None of these studies revealed any adverse effects of exposure to AEs on the reproductive system.

Conclusion

There was only limited information on reproductive toxicity available. Two oral and one dermal study of AEs were identified. The oral studies were not performed in accordance with GLP or OECD protocol. However, the studies were judged to be of good quality and reliable. The presented information indicates that the investigated AEs did not cause reproductive toxicity when applied orally or dermally and the NOAEL for reproductive toxicity is greater than 250 mg/kg bw/d for selected AEs. The NOAEL for systemic toxicity was established to be 50 mg/kg bw/d. This conclusion is supported by the fact that none of the AEs investigated in seven subchronic toxicity studies caused any adverse effects on the reproductive system.

5.2.1.8 Developmental toxicity/teratogenicity

In a two-generation developmental and teratogenicity study groups of 25 Charles River CD rats of both sexes were fed $C_{14-15}AE_7$ in the diet at dosage levels of 0.05, 0.1 and 0.5% (*i.e.*, about 25, 50 and 250 mg/kg bw/d) (Procter and Gamble Ltd., 1977). The control group and the six treated groups comprised of 25 male and 25 female. Three of the groups received the test compound continuously during the study. In the other 3 groups the females received the compound only during the 6^{th} through the 15^{th} day of gestation and the males were untreated. No compound related differences were seen between control and treated rats with respect to fertility, gestation or viability indices and the NOAEL for reproduction was assessed to be greater than 0.5% which equals the dose of about 250 mg/kg bw/d. On the 13th day of the gestation period a representative number of female rats from each treatment group of the FC generation (*i.e.*, pups from the 3rd mating of the

F0 and F1 parental generation) were sacrificed. Laparotomies were performed and the uterus was examined for uterine abnormalities, normal implantation and resorption sites. Remaining females were sacrificed on the 21st day of gestation. Corpora lutea of pregnancy were counted and the presence and distribution of live and dead foetuses were recorded. The foetuses were removed and examined for external anomalies, sexed and weighed. Various maternal and foetal parameters showed occasional values that were significantly different from the corresponding controls. However these were not considered related to the material tested as none occurred at the high feeding level and no dose response for these parameters was apparent. With respect to body weight gains, parental female rats and pups of the high dose group did not gain as much body weight as the control rats. Examination of organ weight values reveal compound related effects were limited to increased group mean liver weighs of male and female P1 generation from the 0.5% continuous feeding group at the 91 day sacrifice and increase in group mean relative liver weights of males of the 0.5% continuous feeding group of the P2 generation at the 60 day section sacrifices. Hence the NOAEL for maternal and developmental toxicity was established at the 50 mg/kg bw/d dose level. Although the study was pre-GLP and not in full compliance with current OECD guidelines, the study provided sufficient information and was judged to be scientifically reliable.

The same protocol as above was used to evaluate the developmental toxicity of $C_{12}AE_6$ in a 2-generation study (Talmage, 1994). Groups of 25 Charles River rats of both sexes were fed $C_{12}AE_6$ in the diet at dosage levels of 0.05, 0.1 and 0.5% which equals an exposure of about 25, 50 and 250 mg/kg bw/d. General behaviour, appearance and survival were not affected by treatment. At the 0.5% dose level, adults and pups gained less weight than the control rats. In the 0.5% dose group, there was a statistical increase in embryo lethality and soft tissue anomalies and at the 0.1% there was a statistical decrease in mean foetal liver weight. Neither of these effects was considered to be treatment-related by the authors as they showed no dose response characteristics. Although not specifically reported, it appeared that NOAEL for maternal toxicity was 50 mg/kg bw/d. The NOAEL for developmental and teratogenic toxicity was set at the 0.1% dose level, equalling an exposure of about 50 mg/kg bw/d. No more detailed study information was provided.

In a study with $C_{12}AE_6$, twenty-five female rabbits were orally administered doses of 0, 50, 100 or 200 mg/kg bw/d from day 2 to day 16 of gestation (NOT GIVEN)(Little, 1977; Shell Chemicals Ltd., 2002; Talmage, 1994). Caesareans were performed on the 28th day of pregnancy. A definite increase in maternal toxicity, evidenced by ataxia and a slight decrease in body weight was observed at 100 and 200 mg/kg bw/d. No effects were observed for parameters such as corpora lutea, implantations, number of live foetuses and spontaneous abortions. Nine control rabbits and 31 treated rabbits died during the study. Surviving rabbits at the 200 mg/kg bw/d dose level generally showed slight losses of body weight. In seven treated and two control rabbits early deliveries were recorded. The NOAEL for this study based on the maternal toxicity was therefore assumed to be greater than 50 mg/kg bw/d level. No more detailed study information was available.

In another GLP-compliant 2-generation reproduction study, groups of thirty weanling rats of each sex were treated dermally with 1 mL/kg bw of $C_{9\text{-}11}AE_6$ at concentration of 0, 1, 10 or 25% w/v (0, 10, 100 or 250 mg/kg bw/d) three times a week except during the mating periods (Shell Development Company, 1985). The complete study protocol has been described under 5.2.1.7. No compound related effects on litter size, number of live pubs and sex ratio of pups in the F_1 and F_2 generations were observed. Low incidences of

foetal malformations were observed, but these were not dose related and considered to be of spontaneous nature. At necropsy, no effects were observed in the F_1 pups. In the F_2 pups, a significantly higher carcass weight in the females of the 250 mg/kg bw/d dose group was noted and some minor organ weight differences. Due to the lack of a dose-response and no associated morphological findings, these effects were considered to be of no toxicological significance. It was concluded that dermal application of $C_{9\text{-}11}AE_6$ to rats did not induce any adverse effects on the growth and development of the offspring during two generations. The NOAEL of $C_{9\text{-}11}AE_6$ with respect to developmental and teratogenic toxicity can be assumed to be higher than the highest dose level dermally applied in this study (*i.e.*, 250 mg/kg bw/d).

Talmage (1994) cited two unpublished studies were the test compound was dermally applied to rats and rabbits during gestation. Studies were not outlined in great detail but the NOAEL for developmental and teratogenicity of $C_{12}AE_4$ was greater than 240 mg/kg bw/d for rats and 310 mg/kg bw/d for rabbits.

Conclusion

The available oral and dermal developmental and teratogenicity studies appeared to be scientifically well conducted and documented in an acceptable and thorough manner. In the studies where the test compounds was administered orally, a NOAEL greater than 50 mg/kg bw/d can be estimated for developmental toxicity. At higher exposure levels a reduced pup body weight was observed in the second generation. When applied dermally, no adverse effects on the growth and development of the offspring was observed during two generations. Following dermal exposure, the NOAEL can be assumed to be higher than the highest tested dose of 250 mg/kg bw/d.

The following table 5.2.-2 summarizes the design and results of available reproductive and developmental toxicity studies.

Table 5.2-2: Summary of reproductive and developmental toxicity studies

Study type	Species	AE	Endpoint	Exposure	Result	References
Two generation dietary feeding	Charles River Rat	$C_{14\text{-}15}AE_7$	Reproductive and developmental toxicity	0, 25, 50, 250 mg/kg bw/d	$NOAEL_{repro}$ = 250 mg/kg bw/d NOEL F_1 = 50 mg/kg bw/d	Procter and Gamble Ltd. (1977)
Two generation dietary feeding	Charles River Rat	$C_{12}AE_6$	Reproductive and developmental toxicity	0, 25, 50, 250 mg/kg bw/d	$NOAEL_{repro}$ = 250 mg/kg bw/d NOAEL F_1 = 50 mg/kg bw/d	Talmage, (1994)
Two generation dietary feeding	rabbit	$C_{12}AE_6$	Developmental toxicity	0, 50, 100, 200 mg/kg bw/d	NOAEL > 50 mg/kg bw/d	Talmage, (1994)

Study type	Species	AE	Endpoint	Exposure	Result	References
Two generation dermal study	Fisher Rat	$C_{9\text{-}11}AE_6$	Reproductive and developmental toxicity	0, 10, 100, 250 mg/kg bw/d	$NOAEL_{repro}$ = 250 mg/kg bw/d NOAEL F1 = 250 mg/kg bw/d	Shell Development Company (1985)

5.2.1.9 Toxicokinetics

Oral and dermal absorption, distribution, metabolism and excretion (ADME) of three ^{14}C-labelled alcohol ethoxylates (*i.e.*, $C_{12}AE_3$, $C_{12}AE_6$ and $C_{12}AE_{10}$) were determined in female Colworth Wistar rats (Unilever, 1978d). The tracer was located in the alkyl chain. A specification of the location of the tracer within the chain was not provided. For the ADME studies, the test solution was administered through oral intubation, intraperitoneal injection or subcutaneous injection. Following administration, the rats were placed in a metabolism chamber for 4 days and the faeces, urine and air were monitored for ^{14}C activity. At the end of the period, the study was terminated and various tissues and organs were removed and analysed for radioactivity. In summary, ^{14}C was excreted by the rats mainly in the urine after oral or parental administration of the compound. The relative proportions of compounds found in the urine, faeces, air and carcass did not differ with the route of application and the recoveries were close to 100% for all routes. Small proportions were recovered as $^{14}CO_2$ and in the faeces (see table 5.2-3). These proportions increased with longer ethoxylate length. The results suggest an almost complete absorption from the alimentary tract. There were indications that some of the longer ethoxylate chain compounds may be excreted via the bile or excreted into the intestine by other routes. Each detergent gave rise to two distinct polar metabolites in the urine and no parent compound. It was hypothesized that the alcohol chain was oxidized and the ethoxylate residue remained intact.

Table 5.2-3: Recoveries (%) of ^{14}C from rats administered $^{14}C_{12}AE_X$ alcohol ethoxylates through oral intubation, intraperitoneal injection or subcutaneous injection (Unilever, 1978d) four days after administration

Ethoxylate length	Urine (%)	Faeces (%)	Expired Air (%)	Carcass (%)	Total Recovery (%)
Oral					
E3	78.3	6.9	6.5	2.5	94.3 ± 9.2
E6	76.3	11.8	8.1	1.8	98.2 ± 1.5
E10	49.8	17.4	12.4	4.5	84.2 ± 6.8
Intraperitoneal					
E3	84.5	2.1	6.7	1.8	95.3 ± 5.8
E6	85.1	9.1	4.1	0.8	99.4 ±4.4
E10	61.5	18.2	14.2	3.2	97.1 ±3.3
Subcutaneous					
E3	87.5	4.4	4.3	3.7	99.8 ± 4.6
E6	83.5	10.2	4.6	2.9	101.2 ±3.3
E10	61.2	19.9	11.7	4.9	97.7 ± 2.2

In the same study, the skin penetration of a range of alcohol ethoxylates which were applied in 1% solutions was evaluated after a series of wash and rinse procedures (study results for $C_{12}AE_3$ presented in table 5.2-4). Considerable proportions of the administered dose penetrated the skin. Shorter chained ethoxylated were absorbed more readily than longer ones. The penetration of $C_{12}AE_3$ and $C_{12}AE_6$ were 4-5 µg/cm^2 rat skin after a single 5 minute wash with 1% (w/v) AE solutions. Only 0.85 µg $C_{12}AE_{10}$/cm^2 penetrated from a similar test solution applied for 5 minutes. Penetration of all three compounds was proportional to the test concentration in the test solution and the penetration rate was increased by longer durations of contact and multiple applications (*e.g.*, highest penetration rate of 8.4 µg/cm^2 was observed after a 20 minute contact to $C_{12}AE_3$).

Table 5.2-4: Dermal flux of ^{14}C from rats washed with test solutions of $^{14}C_{12}AE_3$ (Unilever, 1978d)

Amount applied (µg/10 cm^2)	Duration of contact (min)	Dermal flux (µg/cm^2)
340 (0.25%)	5	0.7
900 (0.6%)	5	1.9
1,800 (1.2%)	5	4.4
3,000 (2%)	1	3.1
3,000 (2%)	5	4.3
3,000 (2%)	10	5.9
3,000 (2%)	20	8.4
750	5	5.9
2,463	5	16.5
750 x 4	5 x 4	10.4

In another study, the elimination and resorption of ^{14}C labelled $C_{14-18}AE_{10}$ was monitored over 72 hours after a single oral gavage application at doses of 20, 40, 100, 200, 500 and 1,000 mg/kg bw to Wistar rats (Cognis Germany GmbH, 1981b). From the 40, 200 and 1,000 mg/kg bw dose group (*i.e.,* four male rats) one animal was placed in a closed metabolism cage to monitor exhaled $^{14}CO_2$ whereas the other rats were kept in a non-closed system. Urine and faeces were monitored daily over 4 days and gastrointestinal tract, liver, oesophagus, kidney and blood were monitored for ^{14}C activity. Most of the administered compound was resorbed in the intestine (*i.e.*, about 80-90%) of that approx. 30% was excreted via the gall and 2% was excreted as $^{14}CO_2$ in air. Within 72 hours about 98-99% of the compound was rapidly eliminated with 90% being excreted within the first 24 hours. The test compound was excreted in the urine and in the faeces (*i.e.*, about 40-50%) to equal amounts. Very low levels of residual radioactivity (*i.e.*, about 1%) were noted in the liver and to an even lower extent in the kidney. No dose-dependant differences in elimination were observed. The test substance was excreted rapidly even at quite high doses. The highest dose did not cause any symptoms of toxicity within the test rats.

^{14}C-labelled $C_{12-15}AE_6$ and $C_{12-15}AE_7$ were applied orally and dermally to rats to evaluate the intake (absorption) and excretion in rats. The label was either in the hydroxyl-bearing carbon or the α carbon of the alkyl group. The orally dosed material was absorbed quickly and extensively (>75% of the dose). The percutaneous doses were absorbed slowly and incompletely (about 50% in 72 hours). In most of the experiments about half of the ^{14}C that was absorbed by either route was excreted promptly in the urine; smaller amounts appeared in the faeces and CO2. Much of the ^{14}C in the faeces probably resulted from

biliary excretion. The greatest amount of radioactivity was found in faeces, urine and expired air, whereas very little radioactivity remained in tissues (Drotman, 1980).

Biotransformation
The major degradation pathway of AEs appears to be the degradation of the ether linkage and oxidation of the alkyl chain to form lower molecular weight polyethylene glycol-like materials and ultimately carbon dioxide and water. Studies with radio-labelled compounds showed that both the alkyl and the ethoxy groups are sites of attack. AE surfactants labelled either with ^{14}C in the α-carbon of the alkyl group or the hydroxyl-bearing position of the ethoxylate moiety showed that distribution and excretion of ethoxylate groups of varying length was similar but the metabolism of their alkyl chains was a function of chain length. Metabolism of the alkyl chain seemed to change as the alkyl chain length increased with longer alkyl chains giving rise to a higher percentage of $^{14}CO_2$ into expired air, and a lower percentage in urine. Distribution of label following dermal application followed a similar but slower pattern. Studies with radio-labelled compounds showed that both the alkyl chain and the ethoxylate groups are sites of attack (Drotman, 1980; Talmage, 1994) Drotman investigated also the absorption, distribution, metabolism and excretion of ^{14}C-labelled $C_{12\text{-}15}AE_6$ and $C_{12\text{-}15}AE_7$ following oral and dermal exposure in humans. The findings are presented in section 5.2.1.10.3.

Conclusion

In rats, alcohol ethoxylates are readily absorbed in the gastrointestinal tract (*i.e.*, oral absorption has been estimated to be >75%) and rapidly excreted via the urine and faeces after oral application. The alkyl chain length appears to have an impact on the metabolism. AEs with longer alkyl chains are excreted at a higher proportion into expired air and less in urine. Also, ethoxy chain length impacts the proportions excreted via the urine, the faeces and the expired air with more being excreted via the faeces and expired in the air with longer ethoxy chain length.

The same trends were observed when AEs were administered dermally, with the only difference being that adsorption was slower and less of the total administered compound was absorbed.

5.2.1.10 Experience with human exposure

5.2.1.10.1 Skin irritation

Basketter et al. assembled the results of six AEs (*i.e.*, $C_{11}AE_3$, $C_{11}AE_7$, $C_{12\text{-}15}AE_5$, $C_{12\text{-}15}AE_5$, $C_{16\text{-}18}AE_5$, $C_{16\text{-}18}AE_{14}$) which were tested undiluted in a standard human 4 hour patch test. The protocol is described elsewhere (Basketter et al., 1997), but in brief the patch test procedure involved the application of 0.2ml on a Hill Top Chamber containing a Webril pad, moistened for solid test materials, to the skin of the upper outer arm of 30 human volunteers for up to 4h. The treatment sites were assessed for the presence of irritation using a 4 point scale, ranging from no reaction to strongly positive reaction (strong often spreading erythema with oedema) at 24, 48 and 72 hours. The interpretation of the results in terms of EU classification was done by statistical comparison of the data with a concurrent positive control (*i.e.*, 20% sodium dodecyl sulphate). The results, which were presented in form of the EU classifications for skin irritation (R38) were compared with existing EU classifications of the same AEs, which were predominantly based on

animal studies. The information presented clearly demonstrated that when tested in the human 4 hour patch test none of the alcohol ethoxylates would warrant a classification for skin irritation (Basketter et al., 2004).

In a 24 hour patch test several alcohol ethoxylates (*i.e.*, $C_{12-14}AE_3$, $C_{14-15}AE_4$, $C_{16-18}AE_5$, $C_{15}AE_7$, $C_{16-18}AE_8$, $C_{16-18}AE_{14}$ were evaluated under COLIPA and GLP testing guidelines (Henkel, KGaA, 1997c). The surfactants were applied in aqueous solutions at test concentrations of 0.1% to the backs of 50 male and female volunteers for 24 hours under fully occlusive condition. After removal of the plasters, the skin was monitored for 6, 24, 48 and 72h and examined for erythema, oedema and scaling. The surfactants tested caused slight but quickly reversible redness in some individual test panellists. No scaling or oedema was observed in any of the test subjects.

In a similar GLP-compliant study according to COLIPA testing guidelines, the skin irritation potential of neat and 20% diluted $C_{16-18}AE_{12}$ and $C_{16-18}AE_{20}$ was tested over a 24 hour application on 20 volunteers (Henkel, KGaA, 2000). Skin reactions were evaluated 6, 24, 48 and 72 hours after removal of the patch for erythema, oedema and scaling. The test substance $C_{16-18}AE_{20}$ was very slightly irritating. Two out of the 20 persons tested with $C_{16-18}AE_{20}$ developed very mild erythema which had cleared by the end of the 72 hours observation period. $C_{16-18}AE_{12}$ applied neat did not cause any skin reaction, however 1 person developed slight erythema resulting from application of the 20% diluted surfactant. The erythema cleared very quickly in all cases.

Talmage (1994) reported several studies evaluating skin irritation properties of AEs in humans. Using the Draize patch test, AE surfactants at dilutions of ≤ 60% produced no to slight skin irritation in human subjects. Arthur D. Little (1977) cites a study in which ten human volunteers were exposed for 4 hours a day on 3 alternate days to undiluted or a 25% aqueous solution of $C_{14-15}AE_7$ under an occlusive patch. Only slight to negligible skin irritation was noted. In another study, slight skin irritation was observed in 8 subjects exposed for 24 hours to an occluded patch containing a 10% aqueous solution of $C_{12-13}AE_{6.5}$.

5.2.1.10.2 Allergic contact sensitization

Alcohol ethoxylates $C_{12-15}AE_7$ and $C_{12-15}AE_9$ were evaluated in a Human Repeated Insult Patch Test (HRIPT) to determine their cumulative skin irritation and skin sensitizing properties (Shell Chemical Company, 1969). Each test material was evaluated as an aqueous solution at various concentrations of 5% w/v, 10% w/v up to 25% w/v. A patch with 0.03 mL of the test material was allowed to contact the skin for 24 hours after which time it was removed and the skin site graded for irritation. The site was then left for 24 hours after which the second patch was placed on the same site for 24 hours. This was repeated nine times followed by a 2 week rest period. After the resting period, a final 24h challenge patch was applied to an alternative site to determine if a sensitizing reaction to the test material occurred. During the induction phase, the patches containing the highest concentration of the test materials (*i.e.*, 25%) caused very slight primary skin irritation. Six out of 108 subjects reacted to $C_{12-15}AE_7$ with slight erythema and 14/108 experienced dryness and itching. Similar observations were recorded for $C_{12-15}AE_9$ with 15/108 subjects exhibiting mild erythema and one test subject was recorded with well defined erythema and 26/108 persons displayed dryness and itching. At lower concentrations (*i.e.*, 5%) fewer skin reactions were noted 1/108 for $C_{12-15}AE_7$ and 5/108 for $C_{12-15}AE_9$ resulted

in very slight erythema. The evaluation of the skin sites after challenge revealed no evidence of skin sensitization for neither of the materials. It was therefore concluded that under the conditions of the test, the test materials did not possess skin sensitizing properties.

$C_{12-13}AE_{6.5}$ and $C_{12-15}AE_{12}$ were also evaluated in the HRIPT at aqueous dilutions of 5% and 15% w/v (Shell Chemical Company, 1969). Nine patches containing 0.03 mL of test material were placed on each of twelve subjects (*i.e.*, per test material) over the test period of 18 days. Following a resting period of 2 weeks, a 24-hour challenge patch was applied to each of the test panellists to detect any skin sensitization potential of the test materials. At the highest concentration tested, 1/108 and 12/108 subjects developed very mild erythema and dryness and itching, following exposure to $C_{12-15}AE_{6.5}$ and similar incidences of reactions were noted with the more dilute material. None of the patients applied with the $C_{12-15}AE_{12}$ solutions developed skin reactions. There was no evidence that any of the test materials possessed any skin sensitizing properties. The above experiments tested a series of compounds with the same C_{12-15} carbon chain length and varying ethoxy groups from 6.5 to 12. These compounds caused only mild skin reactions even at the highest dilutions tested. The fact that those test panellists exposed to $C_{12-15}AE_{12}$ developed no or a lower cumulative irritation response compared to those exposed to $C_{12-13}AE_{6.5}$ indicates a lower irritation potential of AEs with a higher ethoxylation degree.

Another Human Repeated Insult Patch Test was conducted with $C_{12-13}AE_{6.5}$ and $C_{12-15}AE_9$ (Shell Chemical Company, 1967). Test materials were evaluated at a concentration of 1% w/v aqueous solutions. In the induction phase, nine patches containing the test substances were placed on each of twelve subjects. After a resting period of 2 weeks, the test panellists were challenged with a 24h patch. During the induction phase, very slight primary skin irritating properties were observed for $C_{12-13}AE_{6.5}$ with one subject reacting at four time points with very slight erythema. There was no evidence that under the conditions of the test the product possessed any skin sensitizing properties as a result of the challenge patch. $C_{12-15}AE_9$ did not possess any significant primary skin irritating properties. There was also no evidence that the test material possessed any skin sensitizing properties. These results are in line with what was reported for the higher dilutions of the same products tested.

Talmage (1994) reports several other dermal sensitizations studies with AEs different than those discussed before. In general, it was concluded in this review that AE surfactants produced were not skin sensitizers. It was reported that $C_{12}AE_9$ tested at 10, 15 and 20% in an aerosol cream was well tolerated in an HRIPT at all concentrations and none of the observed reactions were indicative of a skin sensitization reaction. Volunteers wore patches containing 2.5% aqueous solutions of $C_{14-15}AE_7$ (144 subjects) and $C_{12-13}AE_{6.5}$ (176 subjects) for up to three weeks and were then subjected to a challenge test 17 days later. Skin hyper-reactivity occurred only to one subject exposed to $C_{12-13}AE_{6.5}$. However, subsequent home usage tests with formulations containing these surfactants indicated not significant skin irritation. In another HRIPT test, 0.1 mL of the 25% solution containing $C_{12}AE_{23}$ was placed on the backs of 168 males and female panellists. No irritation or sensitization was reported. Similar results (*i.e.*, no reaction) with this material were obtained in an HRIPT examining 3% and 5% aqueous solutions on 103 and 150 volunteers, respectively. In similar experiments $C_{18}AE_2$ (60%), $C_{18}AE_{10}$ (60%), $C_{18}AE_{20}$ (60%) were tested on 200 subjects and under the conditions of the studies these products were neither primary irritants nor sensitizers.

5.2.1.10.3 Toxicokinetics

The absorption, distribution and excretion of ^{14}C labelled $C_{12}AE_6$ and $C_{13}AE_6$ (labelled in the carbon chain or ethoxy chain), was examined in human male volunteers (Drotman, 1980). Six adult human males (*i.e.*, 60-90 kg) per treatment group were given a capsule containing 50 mg of the radio-labelled surfactant. Blood samples were taken at 0.5, 1, 1.5, 2, 4, 6, 8, 12, 24, 48 and 144 hours after dosing. Urine and faeces were collected at 0-6 h, 6-24 hours and thereafter during each 24 hour period up to 144 hours. Expired CO_2 was collected continuously over the first 6 hours and after that for 15 minutes at 8, 12, 24, 30, 48 and 72 hours. Most of the radioactivity (*i.e.*, about 83-89%) for both compounds was recovered after 144 hours in the urine, faeces and air. Amounts in the blood were very low and never exceeded 1%. In general, metabolism of these compounds in humans closely patterns the disposition in rats, namely that most of the radioactivity 75% was excreted via the urine within the first 24 hours whereas faecal and air elimination were lower: 5% and 4%, respectively. As seen in the rats $C_{13}AE_6$ and $C_{12}AE_6$ distribution and excretion of the ethoxylate groups of different AEs was similar, but the metabolism of their alkyl chains was function of chain length, with the longer chained compounds giving rise to more CO_2 and less urinary elimination products.

The same authors investigated the dermal penetration of $C_{12}AE_6$ in human male volunteers. One hundred mg ^{14}C-labelled $C_{12}AE_6$ dissolved in 1 mL ethanol was applied dermally over a 90 cm^2 area to the outer part of the forearm of 2 male subjects. The treated skin was protected by a non-occlusive metal shield for 8 hours. After 144 hours the application site was repeatedly washed to remove any remaining product and the blood, urine, faeces and expired air were monitored as described for the oral application. Most of the activity applied to the skin was removed by cleaning the application site with alcohol soaked gauze (*i.e.*, 73.9% in subject 1; 87.5% in subject 2) and less than 2% (*i.e.*, 1.82% in subject 1; 1.03% in subject 2) was detected in the urine. No radioactivity was found in the faeces or in form of CO_2. Radioactivity in the blood was barely detectable. In subject 2, it was equivalent to 0.14 µg/g at 8 hours, 0.02 µg/g at 12 hours and 0.01 µg/g at 24 hours indicating that the vast majority of dermally absorbed AE was absorbed within the first 24 hours. The total recovery of radioactivity was 82.4% for subject 1 and 94.7% for subject 2. Urinary excretion was the primary route of elimination following dermal absorption. In summary, although there are some limitations in the reporting of the study details and also with regard to the small number of test panellists, the study clearly demonstrated that, $C_{12}AE_6$ was absorbed only poorly through human skin and clearly less readily than rat skin. This study was therefore considered to represent more reliably the systemic availability of AEs in humans following dermal exposures to AE containing cleaning products. Thus, the dermal penetration coefficient used in section 5.1 to calculate systemic availability of AEs under in-use conditions was derived from this study (Drotman, 1980).

Derivation of Dermal penetration coefficient (K_p)
The dermal penetration rate for alcohol ethoxylates was calculated on the basis of a dermal penetration study with ^{14}C-labeled $C_{12}AE_6$ in two human volunteers (Drotman, 1980) calculated according to the following algorithm: Kp = dermal flux / exposure time x concentration of test solution; Kp = 0.022 mg/cm^2 / (24 hours x 100 mg/cm^3) = 0.0000092 cm/h. This penetration rate is derived from measured data and assumes –conservatively- 2% absorption within the first 24 hours following dermal application. In the study, however, the maximum systemically available $C_{12}AE_6$ after 144 hours exposure was

determined to be 1.82%. It should be noted that the study was performed only on few test subjects and that reporting was limited. However, the study clearly demonstrated that AEs penetrate poorly through human skin and clearly less readily than through rat skin. The human study was therefore judged to represent more reliably the systemic availability of AEs in humans following dermal exposures to AE containing cleaning products. It should also be noted that rat studies (Unilever, 1978d) have shown that short chain AEs (C_8-C_{14} and E_3-E_7) penetrate the skin more readily than longer chained AEs (i.e., >C_{14}, >E_7). Thus, calculating dermal exposures to the whole range of AEs on the basis of a dermal penetration rate derived from a short chain AE such as $C_{12}AE_6$ can be considered as a conservative scenario.

Conclusion

Human data on a range of alcohol ethoxylates with simple patch tests showed that the tested surfactants were only mildly irritating to human skin even when tested neat. Skin reactions were restricted to very slight erythema which cleared quickly. In some cases the response was not dose-related which indicated that other factors than the test compound might have caused the slight skin reaction. Human HRIPT data clearly indicate that the AEs do not produce skin sensitization reactions. This supports the observations seen in the guinea pig assays.

Following oral exposure, the metabolism and excretion of radio-labelled alcohol ethoxylates resembled the disposition in rats. Seventy five percent of the radioactivity was excreted in the urine within the first 24 hours whereas excretion via faeces and respired air was 5% and 4% respectively. The dermally applied product was less readily available compared to ingested AEs. After dermal absorption, the highest proportion of compound was also excreted via the urinary route.

5.2.2 Identification of critical endpoints

5.2.2.1 Overview on hazard identification

Alcohol ethoxylates are of low to moderate acute oral toxicity with LD_{50} values ranging from 0.6 to 10 g/kg body weight. The median LD_{50} value was 2 g/kg suggesting that most AEs are of low toxicity. The number of ethoxy groups appeared to impact the acute oral toxicity which was highest for compounds with ethoxy unit length between 5 and 15. A similar trend was not found for compounds with varying alkyl chain lengths.

Acute dermal toxicity was very low with values typically greater than 2 g/kg. The low acute dermal toxicity is consistent with the low oral toxicity even though dermal absorption in animals is high compared to humans. In most studies the concentrations tested were too low to cause any mortality of the study animals. Given the absence of toxicity under the testing conditions, a relationship between compound structure and toxicity could not be determined.

In rats, AEs can be considered to be of low acute inhalation toxicity with LD_{50} values exceeding the saturated concentration in air. Acute toxic thresholds were reached only when animals were exposed to the AE in form of a respirable mist or aerosol. Consumers could inhale AE containing detergents while filling powder into the washing machine dispenser resulting in the generation of detergent dust. Under normal and foreseeable use

conditions this is, however, exposures are very low (see chapter 5.1.3.9). Hence, no human health issues are to be expected.

Alcohol ethoxylates range from mildly to severely irritating to rabbit eyes. Rinsing the eyes directly after exposure with water for 20 to 30 seconds greatly reduced the severity of the effects such that these products produced only mildly irritating effects. The degree of irritation is concentration dependant as dilutions in water cause proportionally lower irritation. Generally, concentration of 0.1% were non-irritating, and concentrations of 1 to 10% ranged from slight to moderately irritating. No relationship could be established between the chemical structures of the tested AEs and their eye irritation responses.

Alcohol ethoxylates with varying alkyl chain lengths and ethoxylation degree were found to be slightly to severely irritating to skin in rabbits and rats. The degree of irritation was dependant on the type of patches used (open application versus full occlusion), the exposure time as well as the concentration of the test material. In humans, AEs are less irritating to skin than in animals. Neat applications of a range AEs in a 4h human patch test did not warrant these chemicals to be classified as skin irritants under EU legislation, while the same AEs would have been classified for skin irritation on the basis of animal data.

Alcohol ethoxylates should not be considered as skin sensitizers. A substantial amount of skin sensitization studies in guinea pigs following either the Magnusson Kligman maximization or the Buehler testing protocol are available to evaluate the skin sensitization potential of AEs. Although a mild skin sensitization reaction was observed in a study following the Magnusson Kligman protocol, the weight of evidence clearly supports the assessment that AEs should not be considered as skin sensitizers. This is further supported by clinical and market data that demonstrate the absence skin sensitization responses to AEs when tested under the conditions of the HRIPT or when used in AE containing consumer products.

A wide variety of AEs were evaluated in scientifically well-designed oral and dermal repeated dose toxicity studies. This included subacute (*i.e.*, exposure up to 28 days), subchronic (*i.e.*, exposure up to 91 days) and chronic toxicity studies (*i.e.*, exposure up to 2 years). The outcome of these studies provided a comprehensive toxicity profile of AE's. In five subacute 21-day oral gavage studies with rats, no systemic toxicity was observed at the highest tested concentrations of 100 mg/kg bw/d up to 500 mg/kg bw/d. In another series of five 21-day oral feeding studies, NOAELs were established between 433 and 579 mg/kg bw/d. Adverse effects observed at the LOAEL were that of hepatocytic hypertrophy.

A total of 8 subchronic oral, 3 subchronic dermal and 3 chronic oral studies with AEs tested at different dilutions in aqueous media or admixed in the diet did not reveal adverse systemic effects below 100 mg/kg bw/d. However, a few studies revealed lower NOAELs. The lowest reported NOAEL for systemic toxicity was determined in a 90-day oral feeding study with Wistar rats. In this study rats fed with $C_{14\text{-}15}AE_7$ showed minor dose-related, but significant changes in liver weight, kidney weights and plasma urea concentration in female rats dosed at 50 mg/kg bw/d. These changes were, however, not accompanied by histopathological changes. Taking a conservative approach, the NOEL for this study was established to be 50 mg/kg bw/d. However, the same test compound (*i.e.*, $C_{14\text{-}15}AE_7$) was evaluated in a 90-day and a 2-year oral feeding studies which revealed

NOAEL of 700 mg/kg bw/d and 190 mg/kg bw/day respectively. In the 90-day study, there were no treatment-related findings leading to the establishment of the NOAEL at the highest dose level. In the 2-year oral feeding study, effects observed at the LOAEL were related to significantly elevated organ-to-body weight ratios for liver, kidney and heart. A similar test compound (*i.e.*, $C_{12\text{-}13}AE_{6.5}$) was further evaluated in a 2-year feeding study revealing a NOAEL of 50 mg/kg bw/d. Also in this study, the effects observed at the LOAEL were related to significantly elevated organ-to-body weight ratios for liver, kidney and heart.

Dermal treatment of rats with a daily dose of 8, 80, 200 mg/kg bw/d of AE resulted in an increase in relative kidney weights at the 200 mg/kg bw/d level. No histological lesions were observed at this level. However, taking a conservative approach, the NOAEL was established to be 80 mg/kg bw/d.

AEs are not considered to be mutagenic, genotoxic or carcinogenic. They do not possess structural elements that are of concerns for genotoxicity or carcinogenicity. Although a number of studies addressing endpoints of mutagenicity and genotoxicity were not performed according to current guidelines, the study protocols were scientifically sound and well conducted. They provided a coherent picture with many representative AEs being tested. In all available *in vitro* and *in vivo* genotoxicity assays, there was no indication of genetic toxicity of a broad range of structurally different AEs. Long term carcinogenicity studies did not indicate the potential of alcohol ethoxylates to induce tumours.

Several AEs have been evaluated for reproductive and developmental/teratogenicity. A NOAEL of 50 mg/kg bw/d was established for developmental toxicity on the basis of an oral two-generation toxicity assay. At the next highest tested dose (*i.e.*, 250 mg/kg bw/d), a slight but statistically significant decrease of the pup weight of the F_1 generation was observed. This effect is, however, not considered to be relevant because maternal toxicity was seen at this exposure level. Based on the available information from two 2-generation studies, there was no evidence that exposure to AEs caused reproductive toxicity.

5.2.2.2 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 must be considered for the human health risk assessment. Substantial amounts of data are available addressing skin irritation and skin sensitization potential of AEs solutions and AEs containing consumer product formulations. While AEs penetrate rat skin fairly well, dermal penetration studies in humans have shown that AEs have only limited potential to penetrate the skin to become systemically available. The majority of the studies involving repeated exposures used the oral route of exposure. Although fewer dermal studies are available, the profile of the systemic toxicity after oral and dermal administration is similar justifying the use of oral repeated dose toxicity studies in experimental animals to assess potential human exposure via the dermal route.

5.2.2.3 Adverse effects related to accidental exposure

The acute oral and dermal toxicity of neat alcohol ethoxylates is considered to be moderate to low. AE can be present in detergent formulations at a maximum of 24%. 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 1% 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 components in the formulation might contribute to these effects. Therefore, in case of accidental eye contact, immediate rinsing with plenty of water is recommended. In animal experiments, this immediate action has been shown to minimize irritation effects.

5.2.3 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 alcohol ethoxylates.

A substantial number of AEs of different structures with regard to the length of the alkyl chain and the degree of ethoxylation were evaluated in oral and dermal repeated dose toxicity studies. The NOAEL of AEs for systemic toxicity was established to be 50 mg/kg bw/d on the basis of a scientifically sound and well conducted 2-year oral feeding study in rats with $C_{12\text{-}13}AE_{6.5}$. Effects observed at the LOAEL were related to significantly elevated organ-to-body weight ratios for liver, kidney and heart. No adverse histopathological changes were observed at the LOAEL. Therefore, the established NOAEL ensures an appropriate and high level of protection which was based on a scientifically sound and well conducted 2-year rat study. Moreover, this NOAEL is consistent with the outcome of the majority of existing chronic and subchronic studies determined for further AEs most commonly used in consumer products. Only one 90-day study revealed some minor effects at a dose level of 50 mg/kg bw/d. The study investigators did not consider these effects to be of toxicological significance which suggest also for this study a NOAEL of 50 mg/kg bw/d.

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 NOAEL of 50 mg/kg bw/day as a basis to calculate the Margin of Exposure.

5.3 Risk assessment

5.3.1. Margin of exposure calculation

The margin of exposure (MOE) is the ratio of the No Observed Adverse Effect Level (NOAEL) or an appropriate substitute (*e.g.* NOEL) to the estimated or actual level of human exposure to a substance. The NOAEL of alcohol ethoxylates for systemic toxicity was established to be 50 mg/kg bw/d on the basis of a scientifically sound and well conducted 2-year oral feeding study in rats with $C_{12\text{-}13}AE_{6.5}$. Although no individual toxicokinetic data are available indicating the systemic availability of AEs in rats following oral exposure, several investigators considered AEs to be readily absorbed from the gastro-intestinal tract with oral absorption rates to be >75%. Thus, taking a conservative approach by considering a bioavailability of alcohol ethoxylates of 75% following gastrointestinal absorption, a systemic NOAEL of 37.5 mg/kg bw/d was used to calculate the MOE values for the different exposure scenarios.

5.3.1.1 Exposure scenario: direct skin contact from hand-washed laundry

For calculation of the MOE, the systemic NOAEL of 37.5 mg/kg bw/day was divided by the daily systemic dose of 0.17 µg/kg bw/day which was estimated for the dermal exposure to AE from hand-washed laundry.

$$\mathbf{MOE_{direct\ skin\ hand\text{-}washed\ laundry}} = 37{,}500/0.17\ [\mu g/kg\ bw/day] = \mathbf{220{,}600}$$

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

The MOE was calculated by dividing the systemic NOAEL of 37.5 mg/kg bw/day by the estimated exposure from pre-treatment of clothes of 5.25 µg/kg bw/day.

$$\mathbf{MOE_{direct\ skin\ pre\text{-}treatment}} = 37{,}500/5.25\ [\mu g/kg\ bw/day] = 7{,}\mathbf{100}$$

5.3.1.3 Exposure scenario: direct skin contact from hand dishwashing

The MOE was calculated by dividing the systemic NOAEL of 37.5 mg/kg bw/day by the estimated exposure from hand dishwashing of 0.082 µg/kg bw/day.

$$\mathbf{MOE_{direct\ skin\ \ hand\ dishwashing}} = 37{,}500/0.082\ [\mu g/kg\ bw/day] = \mathbf{457{,}300}$$

5.3.1.4 Exposure scenario: direct skin contact from hard surface cleaning

Based on the calculations presented in chapter 5.1.3.6, the systemic dose from skin contact during hard surface cleaning was estimated to be 0.21 µg/kg bw/day. The MOE was calculated by dividing the systemic NOAEL of 37.5 mg/kg bw/day by the estimated exposure from hard surface cleaning of 0.21 µg/kg bw/day.

$$\mathbf{MOE_{direct\ skin\ hard\ surface\ cleaning}} = 37{,}500/0.21\ [\mu g/kg\ bw/day] = \mathbf{178{,}600}$$

5.3.1.5 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 0.028 µg/kg bw/day. The MOE was calculated by dividing the systemic NOAEL of 37.5 mg/kg bw/day by the estimated exposure from hard surface cleaning of 0.028 µg/kg bw/day.

$$\mathbf{MOE_{indirect\ skin\ exposure\ clothes}} = 37{,}500/0.028\ [\mu g/kg\ bw/day] = \mathbf{1{,}339{,}300}$$

5.3.1.6 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 0.0013 µg/kg bw/day. The MOE that could be calculated from this low exposure is much greater than 1,000,000. The described exposure does not significantly add to the overall AE exposure and will therefore not be considered in the risk assessment.

5.3.1.7. Exposure scenario: inhalation of aerosols from cleaning sprays

For calculation of the MOE, the systemic NOEL of 37.5 mg/kg bw/day was divided by the daily systemic dose of 0.042 µg/kg which was estimated for the inhalation of AE-containing aerosols in spray cleaning applications. This exposure results in a very large MOE (>> 100,000) and does not significantly add to the overall exposure. It will therefore not be considered in the risk assessment

5.3.1.8 Exposure scenario: oral route from residues left on dinnerware

The MOE was calculated by dividing the systemic NOAEL of 37.5 mg/kg bw/day by the estimated oral exposure from AE residues left on eating utensils and dinnerware of 0.72 µg/kg bw/day.

MOE $_{\text{oral route}}$ = 37,500/0.72 [µg/kg bw/day] = **52,100**

5.3.1.9 Exposure scenario: oral route from accidental ingestion and eye contact

Accidental ingestion of a few milligrams of AE 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 AE in particular. This view is supported not only by available toxicological information from animal studies, but also by the fact that national poison control centres 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 AE as a major surfactant block at a concentration between 15-24% are expected to cause mild to moderate irritation which is fully reversible shortly after the accidental exposure. This assessment is supported by poison control centre data demonstrating that accidental eye contact with AE 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 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 AE containing solutions under usage conditions (*e.g.*, in hand-washed laundry or hand dishwashing) are not expected to cause more than a very mild irritation.

5.3.1.10 Total Consumer Exposure

In a worst case scenario, the consumer exposure from direct and indirect skin contact of neat or diluted AE containing products, inhalation of AE containing aerosols from spray cleaner applications and from the oral route via AES residues on eating utensils and dinnerware, results in an estimated systemic AE exposure of 6.48 µg/kg bw/day. The MOE can be calculated by dividing the systemic NOAEL of 37,500 µg/kg bw/day by the total exposure:

MOE $_{\text{total}}$ = 37,500/6.48 [µg/kg bw/day] = **5,800**

5.3.2 Risk characterisation

5.3.2.1 Systemic toxicity

Consumers are exposed to alcohol ethoxylates through their use in household laundry and cleaning products. All potential exposure scenarios were identified, quantified and assessed by comparing the estimated systemic exposure values with the systemic NOAEL determined in a chronic oral feeding study. The MOE for the systemic dose resulting from the total consumer exposure is 5,800. This MOE calculation reflects the aggregate of all possible exposure scenarios using worst case assumptions, an exposure situation which is very unlikely to occur. The lowest MOE for an individual exposure scenario was with 7,100 the direct skin contact with neat product during the pre-treatment of laundry. As elaborated already in Chapter 5.1.3.3 'Direct skin contact from pre-treatment of laundry', this exposure estimate must be regarded as overly conservative. This is because consumers typically pre-wet the laundry before applying the detergent for pre-treatment or conduct the pre-treatment under running tap water. Both practices lead to significant dilutions which were not accounted for in the exposure calculation. This exposure estimate is an important driver of the total MOE.

Taking into account the conservatism in the exposure calculations and the assigned systemic NOAEL for the group of alcohol ethoxylates, the determined MOE is certainly large enough to account for the inherent uncertainty of the hazard data and inter species and intra species extrapolations which are conventionally accounted for by a factor of 100.

The available toxicological information indicates that alcohol ethoxylates are not mutagenic, genotoxic or carcinogenic, nor was there any evidence for reproductive toxicity, developmental or teratogenic effects in animals at doses that were not maternally toxic. The systemic effects observed in subchronic and chronic oral feeding studies at dose levels above the NOAEL included elevated organ-to-body weight ratios for liver, kidney and heart; these changes in organ weight could not be associated with any microscopic changes.

Some concerns were raised due to the presence of traces of 1,4-dioxane in some AEs. 1,4-dioxane is a chemical classified as possibly carcinogenic (2B) by IARC (IARC, 1999); in the EU 1,4-dioxane has classified as a carcinogen of Category 3. 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.

AEs may contain residual levels of ethylene oxide (EO) with Talmage (1994) quoting a maximum level of 10 ppm. EO has been classified as by IARC as a carcinogen of Category 1 (IARC, 1994); in the EU has been classified EO as a carcinogen Category 2 (R45) and mutagen of Category 2 (R46).

Exposure to low levels of EO can result from the use of products containing AE, but formation of EO also occurs in humans as an intermediate metabolite of ethylene. Ethylene is a natural body constituent formed from lipid peroxidation, breakdown of certain amino acids or intestinal bacteria (Filser *et al.*, 1994) For an assessment of the

potential risk of low levels of EO in household cleaning products two approaches are being considered here. Both are based on the calculated exposure from the worst-case scenario considered in this risk assessment [direct skin contact during laundry pre-treatment, presented in section 5.1.3.3]:

1. The systemic exposure calculated from direct skin contact, assuming a skin penetration coefficient 10 times higher than that of AE, is 5.25 x 10^{-4} µg/kg/day. The amount of EO is formed endogenously is equivalent to approximately 0.2 µg/kg/day, based on biochemical and kinetic data (Filser *et al*., 1994). The additional body burden of EO due to the use of household cleaning agents is negligible in comparison to the unavoidable internal/physiological body burden of EO.
2. Alternatively, the concentration of EO in air associated with a cancer risk of 1 in 1,000,000 was estimated to be 37 ug/m^3 (Kirman *et al*. 2004). The calculated 'virtual safe dose' (VSD) based on this airborne level of EO is 12 µg/kg/day, assuming a 100% uptake from the air, a respiratory volume of 20 m^3/day and a body weight of 60 kg. A comparison of the VSD and the EO exposure estimated for the scenario of direct skin contact during laundry pre-treatment (see above) indicates that the use of AEs in household cleaning products containing residual levels of 10 ppm EO is at least 4 orders of magnitude below the acceptable level of adverse health risks.

A large proportion of the total systemic AE exposure results from percutaneous absorption of AE in applications involving skin contact. The percutaneous penetration coefficient used in the exposure calculations was determined on the basis of a percutaneous absorption study with ^{14}C-labeled $C_{12}AE_6$ in two human volunteers. Although there are some limitations in the reporting of the study details and also with regard to the small number of test panellists, the study clearly demonstrated that, $C_{12}AE_6$ was absorbed only poorly through human skin and clearly less readily than rat skin. This study was therefore considered to represent more reliably the systemic availability of AEs in humans following dermal exposures to AE containing cleaning products. Generally, rat studies (Unilever, 1978d) have shown that the short chain AEs (C_8-C_{14} and E_3-E_7) penetrate the skin more readily than the longer chained AEs (*i.e.,* >C_{14}, >E_7). Thus, calculating dermal exposures of the whole range of AEs on the basis of a dermal penetration rate derived from a short chain AE such as $C_{12}AE_6$ can be considered as a worst case estimate.

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

5.3.2.2 Local toxicity

Alcohol ethoxylates are not skin sensitizers and their irritation potential is concentration dependent. Under normal use conditions with direct skin contact (*e.g*., hand laundering; hand dishwashing) the consumer is exposed to diluted detergent solutions containing 0.0 – 0.1% AEs. At these concentration levels, alcohol ethoxylates are virtually non-irritating to skin and eyes. This has been demonstrated in clinical as well as in animal studies.

Short-term contact with neat or concentrated detergent formulations (*e.g*., pre-treatment of clothes) may result in minor signs of localised, superficial irritation of the skin of the hands (*e.g.,* palms and fingers). The initial high AE concentration is usually diluted out

rapidly in the course of the pre-treatment task thereby preventing the skin irritation. Potential irritation of the respiratory tract is not a concern given the very low levels of airborne AE generated as a consequence of cleaning spray aerosols or laundry powder detergent.

AEs are present in laundry and cleaning products at concentrations between 0 – 24%. While animal data suggest that without rinsing exposure to such concentrations could cause severe eye irritation and irreversible eye damage in some cases, human experience suggests that 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 centre data demonstrating that accidental eye contact with AE 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.

The accidental ingestion of an AE 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 AE 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 alcohol ethoxylates.

5.3.3 Summary and conclusion

Consumers are exposed to alcohol ethoxylates through their presence in household laundry and cleaning products mainly via the dermal route, but to some extend 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 very minor extent also through AE residues in the fabric after the washing cycle and skin contact during hard surface cleaning. Consumers are orally exposed to AE through residues deposited on eating utensils and dishes after hand dishwashing. Since AE is also used in spray cleaners, the consumer can also be exposed to AE containing aerosols generated by the sprayer. The consumer aggregate exposure to AE has been estimated to be at maximum 6.48 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. No reproductive effects were observed in 2-generation studies with AEs in rats. Only at maternally toxic doses, developmental effects such as reduced pup weight were noted. The critical adverse effect identified after repeat long term dosing of alcohol ethoxylates were related to significantly elevated organ-to-body weight ratios for liver, kidney and heart. The lowest NOAEL for an individual alcohol ethoxylate (*i.e.*, $C_{12\text{-}13}AE_{6.5}$) was established to be 50 mg/kg bw/day. This NOAEL was subsequently considered as a NOAEL for the whole group of alcohol ethoxylates. Recognizing that the majority of available subchronic and chronic repeated dose toxicity studies revealed NOAELs higher than 100 mg/kg bw/d, this approach must be viewed as very conservative. Although no investigation was identified that thoroughly examined the systemic availability of AEs following oral exposure, several investigators considered AEs to be absorbed in the GI tract to at least 75%. Thus, a conservative approach was taken by considering a systemic availability of AE of 75% following gastrointestinal absorption. The respective systemic NOAEL was determined to 37.5 mg/kg bw/d.

The comparison of the aggregate exposure and the systemic NOAEL results in a MOE of 5,800. Taking into account the conservatism in the exposure calculations and the assigned systemic NOAEL for the group of alcohol ethoxylates, this margin of exposure is considered to be large enough to account for the inherent uncertainty and variability of the hazard database and inter and intra-species extrapolations.

Neat alcohol ethoxylates are irritant to eyes and skin. The irritation potential of aqueous solutions of AEs depends on concentrations. Local dermal effects due to direct or indirect skin contact with AE containing solutions in hand-washed laundry or hand dishwashing are not of concern because AEs are not contact sensitizers and not expected to be irritating to the skin at in-use concentrations. Occasional mild irritations due to short contacts with undiluted formulated products will easily be avoided by prompt rinsing of the hands in water. Potential irritation of the respiratory tract is not a concern given the very low levels of airborne AE generated as a consequence of spray cleaner aerosols or laundry powder detergent dust.

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

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7. Contributors to this risk assessment

The Human Health portion of this risk assessment and a substantial part of chapter 3 were developed by experts from the following companies: BASF, Cognis, Henkel, Huntsman, Procter & Gamble Eurocor, Sasol, Shell Chemicals Ltd (lead), Unilever, Uniqema, Zschimmer and Schwarz and the Weinberg Group (consultant).
Additional input was given by the HERA Human Health Task Force.

Contributions to the Environmental portion of this risk assessment were made by experts from the following companies: Akzo Nobel, BASF, Clariant, Cognis (lead), Degussa, Henkel, Huntsman, Kao, Lion, Procter & Gamble Eurocor, Sasol, Shell Chemicals Ltd, Unilever, Uniqema, Zschimmer & Schwarz and Katharine Fox (consultant).
Much input to the Environmental part was provided by the ERASM CSARA Task Force (a joint initiative of A.I.S.E. and CESIO), the Japanese Soap and Detergents Association (JSDA), the International Association for Soaps, Detergents and Maintenance Products (A.I.S.E.), the European Chemical Industry Council (Cefic) and its sector group CESIO, and the HERA Environmental Task Force.

Annex I - CAS Numbers

Description of Substance and/or Synonims	CAS Number
Poly(oxy-1,2-ethanediyl), .alpha.-dodecyl-.omega.-hydroxy-	9002-92-0
Poly(oxy-1,2-ethanediyl), .alpha.-hexadecyl-.omega.-hydroxy-	9004-95-9
Poly(oxy-1,2-ethanediyl), .alpha.-9-octadecenyl-.omega.-hydroxy-, (Z)-	9004-98-2
Polyethyleneglycol monooctadecyl ethe, (EO=2) octadecylether, Poly(oxy-1,2-ethanediyl), .alpha.-octadecyl-.omega.-hydroxy-	9005-00-9
Polyethyleneglycol Isotridecyl Ether	9043-30-5
Alcohols, C10-13, ethoxylated	9057-32-3
Poly(oxy-1,2-ethanediyl), .alpha.-tridecyl-.omega.-hydroxy-	24938-91-8
Poly(oxy-1,2-ethanediyl), .alpha.-tridecyl-.omega.-hydroxy-	24938-91-8
Poly(oxy-1,2-ethanediyl), .alpha.-decyl-.omega.-hydroxy-	26183-52-8
Poly(oxy-1,2-ethanediyl), .alpha.-(2-ethylhexyl)-.omega.-hydroxy-	26468-86-0
Poly(oxy-1,2-ethanediyl), .alpha.-docosyl-.omega.-hydroxy-	26636-40-8
Poly(oxy-1,2-ethanediyl), .alpha.-eicosyl-.omega.-hydroxy-	26636-39-5
Poly(oxy-1,2-ethanediyl), .alpha.-(3,5,5-trimethylhexyl)-.omega.-hydroxy-	26912-60-7
Poly(oxy-1,2-ethanediyl), .alpha.-(1,1,3,3-tetramethylbutyl)-.omega.-hydroxy-	26912-49-2
Poly(oxy-1,2-ethanediyl), .alpha.-octyl-.omega.-hydroxy-	27252-75-1
Poly(oxy-1,2-ethanediyl), .alpha.-tetradecyl-.omega.-hydroxy-	27306-79-2
Glycols, polyethylene, mono(1-propylpentyl) ether	31514-36-0
Glycols, polyethylene, mono(1-ethylhexyl) ether	31497-05-9
Poly(oxy-1,2-ethanediyl), .alpha.-(1-methylheptyl)-.omega.-hydroxy-	31497-04-8
Poly(oxy-1,2-ethanediyl), .alpha.-(2-octyldodecyl)-.omega.-hydroxy-	32128-65-7
Poly(oxy-1,2-ethanediyl), .alpha.-undecyl-.omega.-hydroxy- C11EO10, C12-15EO5.5	34398-01-1
Poly(oxy-1,2-ethanediyl), .alpha.-nonyl-.omega.-hydroxy-	39587-22-9
Poly(oxy-1,2-ethanediyl), .alpha.-hydro-.omega.-hydroxy-, ether with 1,14-tetradecanediol	52228-33-8
Poly(oxy-1,2-ethanediyl), .alpha.-(2-hexyldecyl)-.omega.-hydroxy-	52609-19-5
Poly(oxy-1,2-ethanediyl), .alpha.-isononyl-.omega.-hydroxy-	56619-62-6
Poly(oxy-1,2-ethanediyl), .alpha.-isooctyl-.omega.-hydroxy-	61723-78-2

Description of Substance and/or Synonims	CAS Number
Polyethyleneglycol ether of tallow fatty alcohol	61791-28-4
Alcohols, sperm-oil, ethoxylated	61791-21-7
Alcohols, coco, ethoxylated, C12-14EO20	61791-13-7
Poly(oxy-1,2-ethanediyl), .alpha.-isodecyl-.omega.-hydroxy-, C9-11EO8	61827-42-7
Alcohols, C13-15, ethoxylated, C12-14EO7, C13-15EO11, C13-15EO3, C13-15EO7, C13-15EO7	64425-86-1
Alcohols, C12-13, ethoxylated, C12-13EO6.5	66455-14-9
Alcohols, C10-14, ethoxylated	66455-15-0
Alcohols, C10-12, ethoxylated	67254-71-1
Alcohols, C10-16, ethoxylated, C10-16EO7, C12-14EO3, 6, C12-14EO7	68002-97-1
Poly(oxy-1,2-ethanediyl), .alpha.-tert-nonyl-.omega.-hydroxy-	68035-42-7
Alcohols, C14-18, ethoxylated, C14-16EO5, C14-16EO7, C14-C18EO4	68154-96-1
Alcohols, C12-15, ethoxylated, C12-15EO11, C12-15EO7, C12-15EO7, C12-15EO7, C9-11EO5	68131-39-5
Alcohols, C16 and C18-unsatd., ethoxylated	68155-01-1
Polyethyleneglycol ethers of C12-C18 alcohols, C12-18EO7	68213-23-0
Polyethyleneglycol ethers of C12-C14 alcohols, C12-14EO7	68439-50-9
Alcohols, C11-13-branched, ethoxylated, C11-13EO3	68439-54-3
Polyethyleneglycol Monoalkyl(C16-C18) Ether, C16-18EO25	68439-49-6
Alcohols, C9-11, ethoxylated, C9-11EO4, C9-11EO6, C9-11EO8	68439-46-3
C13-15EO3	68439-45-2
Alcohols, C12-20, ethoxylated	68526-94-3
Alcohols, C12-16, ethoxylated	68551-12-2
Alcohols, C12-19, ethoxylated	68603-20-3
Alcohols, C8-20, ethoxylated	68954-94-9
Alcohols, C16-18 and C18-unsatd., ethoxylated	68920-66-1
Alcohols, C14-15, ethoxylated, C14-15EO5, C14-15EO8	68951-67-7
Poly(oxy-1,2-ethanediyl), .alpha.-tridecyl-.omega.-hydroxy-, branched, C10E05-20, C10EO2-5	69011-36-5
Alcohols, C8-22, ethoxylated	69013-19-0
Alcohols, C16-22, ethoxylated	69227-20-9

Description of Substance and/or Synonims	CAS Number
Alcohols, C8-10, ethoxylated	71060-57-6
Alcohols, C8-16, ethoxylated	71243-46-4
Alcohols, C13-18, ethoxylated	72905-87-4
Alcohols, ethoxylated	74432-13-6
Poly(oxy-1,2-ethanediyl), .alpha.-(1-propylhexyl)-.omega.-hydroxy-	77492-52-5
Poly(oxy-1,2-ethanediyl), .alpha.-(1-methyloctyl)-.omega.-hydroxy-	77492-49-0
Alcohols, C11-14-iso-, C13-rich, ethoxylated, C11-14EO7	78330-21-9
Alcohols, C9-11-iso-, C10-rich, ethoxylated	78330-20-8
Alcohols, C10-18, ethoxylated	85422-93-1
Poly(oxy-1,2-ethanediyl), .alpha.-(2,5,5-trimethylhexyl)-.omega.-hydroxy-	86871-90-1
Alcohols, C9-16, ethoxylated	97043-91-9
Alcohols, C14-22 and C14-22-unsatd., ethoxylated	100843-23-0
Alcohols, C12-20 and C12-20-unsatd., ethoxylated	106232-81-9
Alcohols, C12-15-branched and linear, ethoxylated	106232-83-1
Alcohols, C16-20, ethoxylated	106232-82-0
Alcohols, C18-22, ethoxylated	116810-32-3
Alcohols, C16-18-unsatd., ethoxylated	119415-06-4
Alcohols, C14-15-branched and linear, ethoxylated	120944-68-5
C14-15EO5	120944-68-5
Alcohols, C14-18 and C16-18-unsatd., ethoxylated	126646-02-4
Poly(oxy-1,2-ethanediyl), .alpha.-(11-methylnonadecyl)-.omega.-hydroxy-	146598-25-6
Alcohols, C9-15, ethoxylated	157627-88-8
Alcohols, C13-15-branched and linear, ethoxylated	157627-86-6
Alcohols, C8-18, ethoxylated	157707-43-2
Alcohols, C14-16, C14-15-rich, ethoxylated	157707-41-0
Alcohols, C12-22, ethoxylated	160305-84-0
Alcohols, C12-13-branched and linear, ethoxylated	160901-19-9
Alcohols, C11-14-branched and linear, ethoxylated	160901-20-2
Alcohols, C9-11-branched and linear, ethoxylated	160901-09-7
Alcohols, C10-16, C12-13-rich, ethoxylated	161025-22-5
Alcohols, C12-16, C12-15-rich, ethoxylated	161025-21-4

Description of Substance and/or Synonims	CAS Number
Alcohols, C16-20-branched, ethoxylated	161133-70-6
Alcohols, C12-16-branched, ethoxylated	161133-69-3
Alcohols, C9-11-branched, ethoxylated	169107-21-5
Alcohols, C11-15, ethoxylated	173244-48-0
Poly(oxy-1,2-ethanediyl), .alpha.-(dimethylhexyl)-.omega.-hydroxy-	183259-65-6

Annex II – Rationale for structure-based category

In the HERA human health risk assessment, alcohol ethoxylates (AEs) used in household cleaning products are evaluated as a single chemical category, based on:

- Structural similarities
- Similar toxicokinetics
- Similar toxicological profile

AEs are composed of variable length carbon chains and ethoxylate chains. For the purpose of this category based approach it should be highlighted that the toxicological and toxicokinetic differences of differing length ethoxy units and differences in length of the alkyl chain components of AEs have already been dealt with extensively in the main body of the human health (HH) section of this report. However, the characteristics of the alcohol part of AEs with respect to their degree of branching were not explored in the main body of the HH risk assessment. The main purpose, therefore, of this category justification is to illustrate that AEs with linear carbon chains were comparable in their toxicological profile to those AEs where the carbon chain was highly branched.

Structure of alcohol ethoxylates

Surfactants can be grouped into four different categories: non-ionic, cationic, anionic and amphoteric. AEs are classed as non-ionic surfactants. Non-ionic surface-active surfactants have a hydrophobic/hydrophilic balance wherein there is neither a negative nor a positive charge in either part of the molecule, thus giving it the non-ionic terminology. These surface-active agents have the advantage that they are not affected by water hardness or pH changes as the anionic and cationic surfactants are, and in many cases it is an advantage that they are considered medium to low foaming agents (Little, 1981). AEs are prepared commercially by the reaction of an alcohol and ethylene oxide. An example of the chemical structure of an alcohol ethoxylate is shown below:

$CH_3\ (CH_2)_{x\text{-}y}O\ (CH_2CH_2O)_nH$	x-y range of carbon units n average number of ethylene oxide units

Structurally, AEs can be abbreviated to $C_{x\text{-}y}AE_n$ where the subscript following the 'C' indicates the range of carbon chain units. AEs with carbon unit range between C_3 to C_{16} are most commonly used in household detergent products. Further AEs contain an ethylene oxide (E) chain attached to the alcohol. The degree of ethylene oxide polymerization is indicated by a subscript which indicates the average number of ethylene oxide units. In household products the ethylene oxide commonly ranges between 3 and 20 units. The fact that each product contains a mixture of molecules that covers a range of chain lengths (both in the alcohol and in the ethoxylate chain) has importance to the health and safety evaluation of AEs. The functional characteristics of two related products may be different, but their biological effects should be comparable (Shell Chemical Company).

Alcohols used in the manufacture of AEs can be derived from two different sources either from oleochemical or petrochemical origin. Oleochemical AEs are derived from plant oils such as palm, palm kernel or coconut oil or from animal fats such as tallow, lard or fish oil. Petrochemical AEs on the other hand are most commonly derived from petroleum-based feedstock such as olefins, ethylene, and propylene oligomers.

AEs manufactured by oleochemical processes have a linear structure and even-numbered carbon chain usually in the range C_6 to C_{22}, whereas AEs generated from petrochemical feedstock may have different, highly-branched structures depending on the manufacturing process of the alcohol. Petrochemical AEs can have a linear structure and an even-numbered carbon chain usually in the range C_6 to C_{22} if their alcohol was generated via the Ziegler process. Whereas, petrochemical alcohols produced by other processes fall in the range C_7 to C_{17} contain even and odd numbered carbon chains. These alcohols are typically more than 20% mono-branched.

AEs with different degree of branching were evaluated with regard to their toxicokinetic similarities in mammals, pathways of metabolism in mammals and toxicological profiles in mammals. For the purpose of this category justification, two classes of AEs were compared; those, that contain alcohols with a linear or essentially linear carbon chain and those that contain alkyl chains that are more than 20% branched. Linear or essentially linear AEs are subsequently referred to as AE (linear) and branched AEs are denominated by AE (branched).

Mammalian toxicokinetics

The mammalian adsorption, distribution and excretion of AEs containing linear and branched carbon chains were comparable. When rats were administered $C_{12}AE_6$ (linear), $C_{13}AE_6$ (branched) and $C_{15}AE_7$ (branched) the distribution in the rat was similar with the major portion of the radioactivity appearing in the urine (52-55%) and smaller amounts in the faeces (23-27%) and expired CO_2 (2-3%) for all three compounds (Talmage, 1994).

In another study with volunteers the adsorption, distribution and excretion of ^{14}C labelled $C_{12}AE_6$ (linear) and $C_{13}AE_6$ (branched) was examined in human males given a capsule with the surfactants. The behaviour of the two compounds in the males was comparable and most of the radioactivity was recovered after 24 hours in the urine, 75% for both compounds (see section 5.2.1.10.3).

The biggest structural component determining the adsorption, distribution and excretion of AEs was therefore not the degree of branching of the alcohol but rather the length of the ethoxy chain unit with more of the AE being excreted via the faeces and expired in air as the ethoxy unit length increased (see section 5.2.1.9). Also, the length of the alkyl chain may have determined how AEs were distributed in the rat. An oral gavage study with ^{14}C labelled $C_{14\text{-}18}AE_{10}$ (linear) indicated that AEs with longer alkyl chains were excreted at a higher proportion into expired air and less into the urine and faeces (*i.e.*, about 40-50%) (see section 5.2.1.9).

According to Shell Chemicals Ltd. (2002), the major degradation pathway of AEs appeared to be the hydrolysis of ether linkage and subsequent oxidation of the alkyl chain to form lower molecular weight polyethylene glycol-like materials and ultimately carbon dioxide and water.

Oxidation of the alkyl chain and subsequent elimination via urine and expired air appeared to be a common excretion pathway in aliphatic alcohols and branched-chain fatty acids (Verhoeven *et al*., 1998; Kamil *et al.,* 1952). Verhoeven *et al.* found that aliphatic alcohols were eliminated in humans by three pathways: oxidation, conjugation and elimination of the unchanged alcohol into the expired air and urine. Which route constituted a major pathway was contingent on physical and chemical factors of the alcohol including the number of carbon atoms in the alcohol, the nature of the alcoholic hydroxyl group and the extent of branching of the hydrocarbon chain (Verhoeven *et al*., 1998). It was, however,

not clear to which extent the branching of the carbon chain determined metabolism and elimination of the alcohol.

In other studies with AE surfactants labelled either with ^{14}C in the first carbon of the alkyl group or the hydroxyl-bearing position of the ethoxylate moiety showed that distribution and excretion of ethoxylate groups of varying length was similar in rats but the metabolism of their alkyl chains was a function of chain length (Drotman, 1980). Metabolism of the alkyl chain seemed to change as the alkyl chain length increased with longer alkyl chains giving rise to a higher percentage of $^{14}CO_2$ into expired air, and a lower percentage in the rat's urine. Distribution of label following dermal application followed a similar but slower pattern.

As mentioned above the most likely pathway of AE metabolism was predicted to be the hydrolysis of the ether linkage and subsequent oxidation of the alkyl chain, however, no studies were found that looked at the route of metabolism of AEs in mammals (Talmage 1994). It was therefore hard to predict if the pathway of metabolism of branched versus linear AEs would be significantly different. However, the above presented studies on the absorption, distribution and excretion have served to illustrate that the behaviour of the metabolites of the two different types of AEs was very similar and that other factors such as degree of ethoxylation and carbon chain length were probably more important structural determinants than branching of the alkyl chain.

Toxicological profile

Similarities in toxicological profile among the linear and branched AEs further substantiated the validity of treating them as a single category of chemicals for the health effects assessment. This is illustrated below by exploring three different toxicological endpoints or responses (*i.e.*, acute oral toxicity, eye irritation and repeated dose toxicity) for unbranched and branched AEs. Other endpoints were outside the scope of detailed reporting in this justification, especially as AEs were not skin sensitizers nor considered to be mutagenic, genotoxic or carcinogenic.

Acute oral toxicity

AEs have been shown to have a low to moderate order of acute oral toxicity in the rat with values ranging between 0.6 to more than 10 g/kg (see section 5.2.1.1.1). Oral toxicity was found to be most likely influenced by the number of ethoxy units in the AE rather than the length of the carbon chain (see section 5.2.1.1.1). To illustrate that oral toxicity was similar for linear and branched AEs the available data for oral toxicity was plotted against AE ethylene oxide average unit length or alkyl chain length (Figure 1). Figure 1 shows that the available data for branched AEs exceeded that for linear AEs, however, the comparison illustrated that the oral toxicity was not related to AE alkyl chain branching. The distribution of linear and branched AEs showed no particular pattern with respect to oral LD50. If branching of the alkyl chain played a role in oral toxicity one would have expected that either the linear or branched AEs to always have consistently higher or lower LD_{50} values in the different ethylene oxide or alkyl chain length categories. Rather, the different AEs were distributed randomly with respect to their toxicity as illustrated in Figure 1B for the $C_{12\text{-}14}/C_{12\text{-}15}$ alkyl chain length units where the linear AEs were not clustered at either the high end or low end of the LD_{50} range.

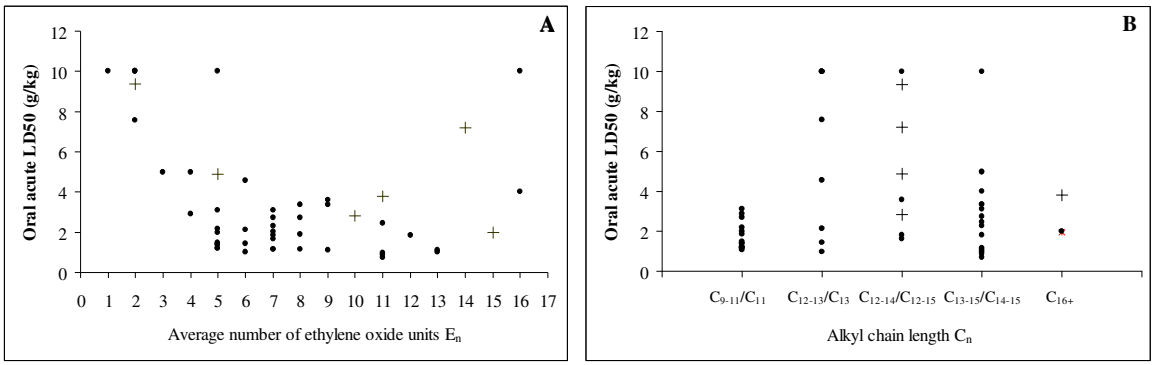

Figure 1: Acute oral LD_{50} values in the rat versus ethylene oxide chain length (A) and alkyl chain length (B)
+ linear AE; ♦ branched AE

Eye irritation

With regard to their potential to cause eye irritation, AEs generally range from mildly irritating to moderately irritating to rabbit eyes. Some tested AEs caused irreversible damage to the eyes. The degree of irritation was concentration dependant as dilutions in water cause proportionally lower irritation. No consistent structure activity relationship was established for the eye irritation potential of the tested AEs (see section 5.2.1.2.2).

When examining available eye irritation data of linear and branched AEs of similar alkyl chain lengths and ethoxylation degree, branching did not appear to impact the eye irritation potential. For example, for a range of linear AEs with the structure $C_{12-14}E_n$, which were tested in a series of OECD compliant studies, the calculated eye irritation indexes (EII) ranged from 7.6 to 37.8. A similar pattern was observed for the branched variant $C_{12-15}AE_n$ were eye irritation indexes ranged from 1.5 to 48 (Figure 2B).

Similarly, when looking at the distribution of linear AEs with respect to ethylene oxide unit no emergent pattern was identified (Figure 2A).

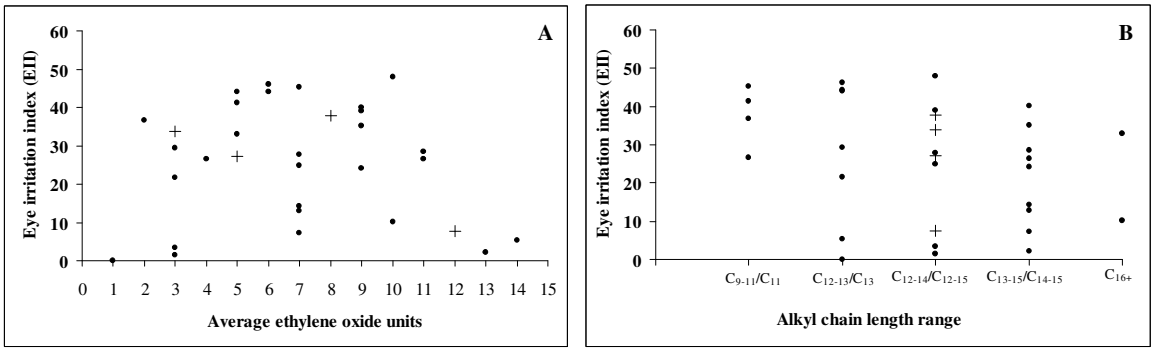

Figure 2: Eye irritation index (EII) ethylene oxide chain length (A) and versus alkyl chain length (B)
+ linear AE; ♦ branched AE

Repeated dose

A series of 21-day oral feeding studies on two linear (*i.e.*, $C_{12-14}AE_7$ and $C_{16-18}AE_{18}$) and three branched (*i.e.*, $C_{12-15}AE_3$, $C_{12-15}AE_7$ and $C_{12-15}AE_{11}$) derived AEs served to evaluate whether branching in the case of petrochemically derived AEs could have an impact on the repeated dose toxicity of AEs. These AEs were of comparable alkyl chain length and to some extent of similar ethoxylation degree and were tested side by side in the same laboratory according to the same protocol (Unilever, 1977).

The studies revealed that for all five AEs, the established NOAELs were in the same narrow range from 433 to 519 mg/kg bw. Hence, branching of the AEs did not appear to alter the overall repeated dose toxicity. Neither did the ethoxylation degree, nor the length of the alkyl chain. In all studies, the organ mostly affected was the liver, as was indicated by increased liver weight and hepatic hypertrophy at the higher doses (see section 5.2.1.4.4).

Conclusion

The review and comparison of data available on the toxicokinetics, metabolism and toxicology (i.e., acute oral toxicity, eye irritation and repeated dose toxicity) showed that AEs with comparable structure (i.e., AEs with similar alkyl chain length and ethoxylation degree) but different with respect to branching of the carbon chain behaved similarly. Linear and branched AEs were also similar with regard to their toxicokinetic profile. No differences between linear and branched AEs could be found in the reviewed presented data. It should be noted here that most of the assessed AEs in the branched category were 80% linear however some of assessed AEs were up to 50% branched. Based on the above available data it may be concluded that branching of alkyl chain of AEs did not change the toxicological profile justifying the grouping of theses AEs in household cleaning products in context of this human health risk assessment.

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Annex IIB – Rationale for structure-based category – Environmental Data supporting the Definition of the AE Category used in this HERA report.

The alcohol SIAR (2006) shows that the alcohols used to form AEs can be grouped into a single category. Significant points used to justify grouping of the selected alcohols into a single category include:

- The category can be justified on the basis of structural features of the alcohols

    - The long chain aliphatic alcohol family has at its centre a homologous series of increasing carbon chain length. In addition, certain branched and unsaturated structures are considered to have such similar properties that their inclusion in the category may be justified. Commercial products contain a range of alcohols, which in some products may include: unsaturated alcohol components; essentially linear (mono-alkyl branched) components as well as linear alcohols. All components of all commercial products relevant to this category are primary alcohol structures. (SIAR 2006).

    - The hydroxyl group in alcohols confers upon the hydrocarbon chain a considerable degree of polarity, and hence affinity for water. It is susceptible to oxidation by metabolic processes. Linear or essentially linear hydrocarbon chains are also readily oxidised metabolically. No highly branched structures are proposed for inclusion in the Category. Substances that contain a number of homologous components can be expected to behave in a way consistent with the carbon number distribution present (SIAR 2006).

- The category can be justified on the basis of physicochemical properties of the alcohols. Annexes I and II of the SIAR show that these properties are predicted well by various QSAR methods. Whilst QSAR techniques are always in part validated by the very measured data reported, they are validated across many other structural types. Their success in predicting properties for Category members for which measured data exist suggested that the members do not possess any particularly unusual features. Substances comprising of a range of carbon chain lengths can be dealt with by appropriate addition of their individual contributions to the whole.

- The category can be justified on the basis of the similar mechanism of toxicity shown by the alcohols.

    - Alcohols proposed for inclusion in this category act by non-polar narcosis (Lipnick et al., 1985, referred to in the Long Chain Alcohol SIAR). As chain length increases, hydrophobicity increases resulting in greater toxicity, and in parallel, solubility decreases. At a critical point, solubility becomes lower than expected toxicity and longer chain lengths show no acute toxicity.

    - Chronic effects for alcohols in the proposed category are also known; present data again indicate that effects are anticipated up to C15. For alcohols with carbon numbers higher than C15 there are significant experimental difficulties in producing, maintaining and quantifying exposures of the test substance. Even so it is unlikely that they would exhibit chronic toxicity because the relationship between carbon number and chronic toxicity, established from the test results that

are available, suggests that the solubility of the alcohol would limit the bioavailable dissolved fraction to sub-toxic concentrations (Long Chain Alcohol SIAR, 2006).

- In both the long chain alcohol SIAR and in the HERA AE Environmental assessment, trends between aquatic toxicity and carbon chain length are based on linear alcohols, since data do not exist on single carbon chain length essentially-linear alcohols. However, the comparability of the toxicity of straight-chain and essentially-linear alcohols is shown by a comparison of commercial products, whose properties are shown in the SIAR to be predicted well by QSAR models, in which alcohols comprising of a range of carbon chain lengths can be dealt with by appropriate addition of their individual contributions to the whole (Long Chain Alcohol SIAR, 2006).

- The category can be justified on the basis of the similar biodegradation shown by the alcohols, where the comparability of linear and essentially-linear alcohols is demonstrated from data on commercial products. The Long Chain Alcohol SIAR shows that these properties are predicted well by various QSAR methods. Whilst QSAR techniques are always in part validated by the very measured data reported, they are validated across many other structural types. Their success in predicting properties for Category members suggested that the members do not possess any particularly unusual features (Long Chain Alcohol SIAR).

- The category can be justified on the basis of the similar mammalian biotransformation of aliphatic alcohols shown by the alcohols, which involves an oxidation step of the alcohol function to the corresponding aliphatic carboxylic acid, with the aldehyde being a transient intermediate. These carboxylic acids (i.e. fatty acids) are subsequently broken down by stepwise removal of one or several C-2 units from the aliphatic carbon chain through the β-oxidation process. The stepwise breakdown of aliphatic alcohols results in common intermediate metabolites with shorter chain lengths, providing further justification that the aliphatic alcohols under consideration can be regarded as a single category and explains the similarity in toxicological profile for systemic effects. Aliphatic alcohols are generally highly efficiently metabolized and there is limited potential for retention or bioaccumulation for the parent alcohols and their biotransformation products (Long Chain Alcohol SIAR, 2006).

  The long chain alcohol SIAR concludes that, by considering the sponsored long chain aliphatic alcohols as a single family structure activity trends are more clearly illustrated; the assessment of any one member of the family is strengthened by reference to the other members.

AEs are formed from the linear and essentially linear alcohols, whose inclusion in one category is justified in the Long Chain Alcohol SIAR, by addition of varying numbers of ethylene oxide units (EO ranging from 0 to 22). Significant points used to justify grouping of these AEs into a single category include:

- Structural similarity. The ethylene oxide chains, of varying length, are all attached to the hydrocarbon chain of the linear or essentially linear alcohol by reaction with the primary alcohol group to form the resulting AE homologue. Thus the structures of the AEs used in formulated products within the scope of HERA differ progressively only in the number of CH_2 or EO units.

- Trend behaviour of physical chemical properties, which can be described by QSAR. As with the long chain alcohols, the use of QSARs to predict physico-chemical properties for the AE with one or more ethylene oxide units is generally validated by the available data. An example with a particularly good data set is given in section 4.1.1.1, which discusses the sorption data obtained and/or analysed by van Compernolle et al (2006), and the QSARs predicting the sorption parameters K_d and Koc which are derived from it. The good fit of the data to both the K_d and the Koc QSARs justifies the inclusion of the AE homologues in one group, for risk assessment purposes.

- Similar mechanism of eco-toxicity. As with the long chain alcohols, the toxicity mechanism for AE is accepted to be non-polar narcosis (Boeije et al 2006), in which the AE homologues with longer hydrocarbon chains and higher logKow are more efficient at penetration of the cell membrane, and thus more toxic. However, the AE homologues must be sufficiently soluble in water to allow a toxic concentration to reach the target organism. For the long chain alcohols, generally the least soluble and the most toxic of the AE homologues, the long chain alcohol SIAR (SIAR 2006) shows that the toxicity is restricted by solubility considerations for hydrocarbon chainlengths of 15 and above. Although the addition of ethylene oxide groups makes the other AE homologues more soluble, solubility considerations may reduce the toxicity of several higher hydrocarbon chainlength and lower EO chainlength AE. The available acute toxicity data, tabulated in the sub sections of section 4.2.1.1, follows this generally accepted pattern, and also indicates that the linear, essentially linear and branched AE are of similar toxicity. It is important to note that the toxicity of the branched and essentially linear AE is not greater than that of the linear AE, and thus that the use of QSARs which have been developed for linear AE is appropriate for use with the other AE which are included in this group for this risk assessment.

- Similar mechanism(s) of biodegradation, and general similarity in biodegradability across the category. All the AEs considered in this report are readily biodegradable under aerobic conditions, and are also anaerobically biodegradable. In ready biodegradation tests resembling OECD 301E, the main mechanism of primary biodegradation for the linear and essential linear AEs present in commercial blends has been shown to be the central cleavage of the AE molecule, leading to the formation of long chain alcohol and polyethylene glycol (PEG) (Marcomini, et al 2000a,b). Another mechanism for primary biodegradation involves omega oxidation of the terminal carbon of the alkyl chain, which is followed by alkyl chain degradation involving beta oxidation. Aternatively, the terminal end of the hydrophile can undergo hydrolytic or oxidative attack. Following central cleavage, ultimate biodegradation then proceeds via the oxidation processes for the alcohols and glycols described above, with alcohol oxidation being more rapid than glycol oxidation. Further tests using activated sludge at typical sewage treatment plant conditions (Itrich and Federle, 2004) showed that ethoxylate number had little effect on the first-order decay rates for primary biodegradation, which ranged from 61 to 78 h^{-1}. However, alkyl chain length had a larger effect, with the C16 chain-length homolog exhibiting a slower rate of parent decay (18 h^{-1}) compared to its corresponding C12 and C14 homologs (61–69 h^{-1}). This work showed that ethoxylate number affected the mechanism of biodegradation, with fission of the central ether bond to yield the corresponding fatty alcohol and (poly)ethylene glycol group increasing in dominance with increasing

ethoxylate number. Further work (Federle and Itrich, 2006) has shown that primary biodegradation of a significant amount of AE takes place by omega oxidation under sewage treatment conditions. However, in all cases AE degradation occurs utilising one or more of the three primary biodegradation mechanisms, with alkyl chain and PEG degradation occurring following central cleavage if they were not responsible for primary AE degradation. Thus AE biodegradation mechanisms are similar, with rates following structure-dependent trends, for the linear and essentially linear AEs considered in this report, which are all readily biodegradable.

The information given above concerning the structural similarity, the trend behaviour of the physical properties which can be described by QSARs, and the similar mechanisms for acute ecotoxicity and biodegradation show that, similarly to the long chain alcohols, the alcohol ethoxylates produced from them should be considered as a single category. This is justified for the linear, essentially linear, and branched AE which compose this category of AE used in detergent products.

Annex III. EUSES Calculations for the Environmental AE Assessments

A III.1 Data used as Input for EUSES Calculations.

A III.1.1. EPI Calculations

The calculated data from the US EPA (Syracuse Research) EPIWIN program which were used as inputs for EUSES calculations are given below. Melting Point, Boiling Point, Vapour pressure at 25C, and solubility in water are the data types which have been calculated.

Melting Point	**deg C**								
	C9	**C10**	**C11**	**C12**	**C13**	**C14**	**C15**	**C16**	**C17**	**C18**
EO-0		7.89 (6.9 exp)	Mp 18.7 (19 exp)	29.19 (24 exp)	31.7 (32.5 exp)	49 (exp 39.5)	59 (46 exp)	68.61 (49.3 exp)		86.85 (59.5 exp)
EO-1										
EO-2										
EO-3		112.71								
EO-4										
EO-5										
EO-6		181.96								
EO-7			215	223	230					
EO-8										
EO-9		249.59				271.3	277			
EO-10										
EO-11										
EO-12							326	332		342.4
EO-13										
EO-14		331.95		342.8	348					
EO-15										
EO-16										
EO-17										
EO-18		349.84	349.8	349.8	349.8	349.8	349.8	349.8		349.8
EO-19										

EO-20									
EO-21									
EO-22									

Boiling point	**deg C**								
	C9	**C10**	**C11**	**C12**	**C13**	**C14**	**C15**	**C16**	**C17**	**C18**
EO-0		238.62 (231.1 exp)	256(243exp)	272.96 (259 exp)	289	304 (exp 289)	318	331 (334 exp)		354.66 (no atm bp exp)
EO-1										
EO-2										
EO-3		367.62								
EO-4										
EO-5										
EO-6		473.4								
EO-7			520	532	543					
EO-8										
EO-9		579.19				625.6	637			
EO-10										
EO-11										
EO-12							743	755		777.8
EO-13										
EO-14		755.5		778.7	790					
EO-15										
EO-16										
EO-17										
EO-18		896.55	908	920	931.4	943	954.6	966		989
EO-19										
EO-20										
EO-21										
EO-22										

Vapour Pressure in mmHg at T = 25C

	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18
EO-0		0.0109 (0.0085 exp)	0.0051 (0.00297 exp)	0.00181 (0.00085exp)	0.000237 (4.36e-4 exp)	2.0E-04	0.0000284 (exp 3.85E-05)	8.9E-06		1.8E-06
EO-1										
EO-2										
EO-3		2.2E-07								
EO-4										
EO-5										
EO-6		2.8E-11								
EO-7			4.2E-13	1.5E-13	5.3E-14					
EO-8										
EO-9		2.4E-15				4.5E-17	1.6E-17			
EO-10										
EO-11										
EO-12							1.5E-21	5.3E-22		6.6E-23
120										
EO-14		4.9E-22		6.1E-23	2.2E-23					
EO-15										
EO-16										
EO-17										
EO-18		5.9E-27	2.4E-27	9.8E-28	4.0E-28	1.6E-28	6.5E-29	2.6E-29		4.2E-30
EO-19										
EO-20										
EO-21										
EO-22										

Water solubility, calculated from Kow					at 25 deg C				in mg/l
	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18
EO-0		28.21 (37 exp)	43	6.898 (4 mg/l exp)	4.5330	0.191	0.468 (0.103 exp)	0.0134 exp		0.015
EO-1										
EO-2										
EO-3		132.7								
EO-4										
EO-5										
EO-6		106.4								
EO-7			29.9	9.2	2.90					
EO-8										
EO-9		76.88				0.694	0.214			
EO-10										
EO-11										
EO-12							0.143	0.044		0.004
EO-13										
EO-14		39.73		3.729	1.14					
EO-15										
EO-16										
EO-17										
EO-18		22.01	6.7	2.05	0.627	0.191	0.058	0.02		0.002
EO-19										
EO-20										
EO-21										
EO-22										

AIII.1.2. LogKow, MW, and Koc information from the CSARA AE Workbook (ERASM 2005b)

The following log KOW, molecular weight, and Koc information have been taken from the CSARA AE Workbook (ERASM 2005b), and used as inputs to EUSES for the appropriate AE homologues.

Calculation of log Kow (Leo & Hansch; Roberts)

	C#								
EO#	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**18**
0	3.57	4.11	4.65	5.19	5.73	6.27	6.81	7.35	8.43
1	3.61	4.15	4.69	5.23	5.77	6.31	6.85	7.39	8.47
2	3.51	4.05	4.59	5.13	5.67	6.21	6.75	7.29	8.37
3	3.41	3.95	4.49	5.03	5.57	6.11	6.65	7.19	8.27
4	3.31	3.85	4.39	4.93	5.47	6.01	6.55	7.09	8.17
5	3.21	3.75	4.29	4.83	5.37	5.91	6.45	6.99	8.07
6	3.11	3.65	4.19	4.73	5.27	5.81	6.35	6.89	7.97
7	3.01	3.55	4.09	4.63	5.17	5.71	6.25	6.79	7.87
8	2.91	3.45	3.99	4.53	5.07	5.61	6.15	6.69	7.77
9	2.81	3.35	3.89	4.43	4.97	5.51	6.05	6.59	7.67
10	2.71	3.25	3.79	4.33	4.87	5.41	5.95	6.49	7.57
11	2.61	3.15	3.69	4.23	4.77	5.31	5.85	6.39	7.47
12	2.51	3.05	3.59	4.13	4.67	5.21	5.75	6.29	7.37
13	2.41	2.95	3.49	4.03	4.57	5.11	5.65	6.19	7.27
14	2.31	2.85	3.39	3.93	4.47	5.01	5.55	6.09	7.17
15	2.21	2.75	3.29	3.83	4.37	4.91	5.45	5.99	7.07
16	2.11	2.65	3.19	3.73	4.27	4.81	5.35	5.89	6.97
17	2.01	2.55	3.09	3.63	4.17	4.71	5.25	5.79	6.87
18	1.91	2.45	2.99	3.53	4.07	4.61	5.15	5.69	6.77
19	1.81	2.35	2.89	3.43	3.97	4.51	5.05	5.59	6.67
20	1.71	2.25	2.79	3.33	3.87	4.41	4.95	5.49	6.57

Calculation of MW

	C#								
EO#	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**18**
0	144	158	172	186	200	214	228	242	270
1	188	202	216	230	244	258	272	286	314
2	232	246	260	274	288	302	316	330	358
3	276	290	304	318	332	346	360	374	402
4	320	334	348	362	376	390	404	418	446
5	364	378	392	406	420	434	448	462	490
6	408	422	436	450	464	478	492	506	534
7	452	466	480	494	508	522	536	550	578
8	496	510	524	538	552	566	580	594	622
9	540	554	568	582	596	610	624	638	666
10	584	598	612	626	640	654	668	682	710
11	628	642	656	670	684	698	712	726	754
12	672	686	700	714	728	742	756	770	798
13	716	730	744	758	772	786	800	814	842
14	760	774	788	802	816	830	844	858	886
15	804	818	832	846	860	874	888	902	930
16	848	862	876	890	904	918	932	946	974
17	892	906	920	934	948	962	976	990	1018
18	936	950	964	978	992	1006	1020	1034	1062
19	980	994	1008	1022	1036	1050	1064	1078	1106
20	1024	1038	1052	1066	1080	1094	1108	1122	1150

Calculation of partition coefficients - Koc

Units: L/kg

EO# \ C#	9	10	11	12	13	14	15	16	18
0	504	1057	2218	4656	9772	20512	43053	90365	398107
1	561	1178	2472	5188	10889	22856	47973	100693	443609
2	625	1312	2754	5781	12134	25468	53456	112202	494311
3	697	1462	3069	6442	13521	28379	59566	125026	550808
4	776	1629	3420	7178	15066	31623	66374	139316	613762
5	865	1816	3811	7998	16788	35237	73961	155239	683912
6	964	2023	4246	8913	18707	39264	82414	172982	762079
7	1074	2254	4732	9931	20845	43752	91833	192752	849180
8	1197	2512	5272	11066	23227	48753	102329	214783	946237
9	1334	2799	5875	12331	25882	54325	114025	239332	1054387
10	1486	3119	6546	13740	28840	60534	127057	266686	1174898
11	1656	3475	7295	15311	32137	67453	141579	297167	1309182
12	1845	3873	8128	17061	35810	75162	157761	331131	1458814
13	2056	4315	9057	19011	39902	83753	175792	368978	1625549
14	2291	4808	10093	21184	44463	93325	195884	411150	1811340
15	2553	5358	11246	23605	49545	103992	218273	458142	2018366
16	2844	5970	12531	26303	55208	115878	243220	510505	2249055
17	3170	6653	13964	29309	61518	129122	271019	568853	2506109
18	3532	7413	15560	32659	68549	143880	301995	633870	2792544
19	3936	8260	17338	36392	76384	160325	336512	706318	3111716
20	4385	9204	19320	40551	85114	178649	374973	787046	3467369
21	4887	10257	21528	45186	94842	199067	417830	877001	3863670
22	5445	11429	23988	50350	105682	221820	465586	977237	4305266

AIII.1.3. Additional Information

In addition AE homologue tonnage information based on a total annual AE tonnage of 290 000 tpa and the homologue hydrocarbon chain and EO distributions given in Tables 3.1 and 3.3 of the main report have been used. The information about the maximum half-life of AE in river waters given in the main report, in Table 4.3.

AIII. 2. Outputs from EUSES Calculations

The 29 EUSES documents, with a total size of approximately 4.7 MB, have been deposited with HERA.