CLH - Report

PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

Octadecylamine has already been prioritised under (EEC) No 793/93 in a substance group approach for 5 primary alkyl amines. This approach, risk assessment and classification and labelling have already been agreed within the Member States at a technical level (TCNES, TC C&L). For this reason, data and discussions in this dossier are also based on a group approach covering coco alkyl amines, tallow alkyl amines, hydrogenated tallow alkyl amines, octadecylamine, and (Z)-octadec-9-enylamine.

Substance Name: **Octadecylamine**

EC Number: **204-695-3**

CAS Number: **124-30-1**

Submitted by: **Germany**

Version/Date: **Revision, July 2010**

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TABLES

PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

Substance Name: Octadecylamine

EC Number: 204-695-3

CAS number: 124-30-1

Registration number (s):

Purity: > 90 % w/w

Impurities: 5 % tetradecylamine
0.4 % hexadecylamine
< 10 % unknowns

Proposed classification based on Directive 67/548/EEC criteria:

Octadecylamine has already been prioritised under ESR (Regulation No (EEC) 793/93). The group approach and risk assessment were also agreed at a technical level (TCNES). However, the risk evaluation work for this substance was not finalised by 1 June 2008, but reported in a Transitional Dossier to ECHA and the European Commission.[1]

With regard to human health, the following classification/labelling is proposed:

Xi,Xn,; R 37/38-41-48/22

The provisional classification with regard to the environment as outlined below has already been agreed at TC C&L 09 2005 and was confirmed at TC C&L 04 2006.[2]

Classification: N, R50-53, S60-61

Specific concentration limits: $C \geq 2.5\%$: N, R50-53, (S60-61)

$0.25\% \leq Cn < 2.5\%$: N, R51-53, (S61)

$0.025\% \leq Cn < 0.25\%$: R52-53, (S61)

Proposed classification based on GHS criteria:

- *With regard to human health:* Skin Irrit 2, H315; Eye Dam 1, H318; STOT SE 3, H335; STOT RE 2, H373 (Harmful if swallowed, causes severe skin burns and eye damage, may cause respiratory irritation, may cause damage to organs (gastro-intestinal tract, liver, immune system) through prolonged or repeated exposure)
- *With regard to the environment:* Aquatic Acute 1, H400; Aquatic Chronic 1, H410 (Hazardous to the aquatic environment)

[1]The work which has been done under the ESR (EEC) No 793/93 is documented in an ANNEX XV Transitional Report including the EU Risk Assessment Report R070_410_412_429_430_0811_ENV_HH.DOC.

[2] The results of the TC C&L 04/2006 meeting are documented in the ECBI/92/06 Rev. 1 report of the ECB.

Proposed labelling:

Acc. to Dir. 67/548/EEC:

Xi,Xn,N; R 37/38-41-48/22-50/53

S 60-61

Acc. to Reg. (EC) No. 1272/2008:

Danger; H315-318-335-373-400-410

Proposed specific concentration limits:

The rapporteur proposes a specific concentration limit with an M-factor of 10 for classification and labelling with regard to the environment, which has already been agreed by the TC C&L.

Proposed notes:

None

JUSTIFICATION

1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

1.1 Name and other identifiers of the substance

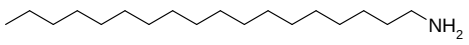
Chemical Name: Octadecylamine

EC Name: 1-Octadecanamine

CAS Number: 124-30-1

IUPAC Name: Octadecan-1-amine

1.2 Composition of the substance

Chemical Name:	Octadecylamine
EC Number:	204-695-3
CAS Number:	124-30-1
IUPAC Name:	Octadecan-1-amine
Molecular Formula:	$C_{18}H_{39}N$
Structural Formula:	
Molecular Weight:	269.5 g/mol
Typical concentration (% w/w):	> 90
Concentration range (% w/w):	90 - 99

1.3 Physico-chemical properties

Table 1: Summary of physico-chemical properties

Substance	Property	Value	Reference
	Physical State		
Amines, tallow alkyl		white waxy solid at 20 °C	
(Z)-Octadec-9-enylamine		liquid at 20 °C	
Octadecylamine		colourless solid	
Amines, hydrogenated tallow alkyl		white solid	
Amines, coco alkyl		Slightly yellow liquid	
	Melting point		
Amines, tallow alkyl		32 – 40 °C [1)]	Ullmann, 1985
(Z)-Octadec-9-enylamine		15 – 30 °C [2)]	Clariant, 2000
Octadecylamine		49 – 52 °C	Richardson et al., 1994
Amines, hydrogenated tallow alkyl		48-56 °C [1)]	Ullmann, 1985
Amines, coco alkyl		12 – 17 °C	Ullmann, 1985
	Boiling point		
Amines, tallow alkyl		200 – 230 °C at 36 hPa [1)]	Ullmann, 1985
(Z)-Octadec-9-enylamine		128 – 174 °C at 4 hPa 345.55 °C (calculated) 353 – 355 °C at 1013 Pa [3)]	Kao, 2001 Hoechst, 1996c Siemens Axiva, 2002b
Octadecylamine		348.8 °C at 1013 hPa [4)]	Ralston et al., 1959
Amines, hydrogenated tallow alkyl		348 - 351 °C at 1013 hPa [5)]	Siemens Axiva, 2003
Amines, coco alkyl		130 - 227 °C at 133 hPa	Ullmann, 1985
	Relative density		
Amines, tallow alkyl		0.79 g/cm3 at 60 °C 6)	Hoechst, 1980a
(Z)-Octadec-9-enylamine		0.8 g/cm3 at 60 °C 6)	Clariant, 2000
Octadecylamine		0.8618 at 20 °C 1)	Richardson et al., 1994
Amines, hydrogenated tallow alkyl		0.94 at 23.2 °C 7)	Siemens Axiva, 2003
Amines, coco alkyl		0.8 g/cm3 at 25 °C	Akzo, 2000

Substance	Property	Value	Reference
	Vapour pressure		
Amines, tallow alkyl		not conducted 8)	
(Z)-Octadec-9-enylamine		0.005 hPa at 20 °C (calculated)	Hoechst, 1996c
Octadecylamine		4.38 · 10-5 mm Hg at 25 °C (≡ 0.006 Pa at 25 °C) (calc.) 9)	Clariant, 2001a
Amines, hydrogenated tallow alkyl		not conducted 8)	
Amines, coco alkyl		not conducted 8)	
	Water solubility		
Amines, tallow alkyl		0.12 mg/l at 25 °C (calc.) 10)	Clariant, 1998
(Z)-Octadec-9-enylamine		insoluble at 25 °C 11) 0.07639 at 25 °C (calculated)	CECA, 2000 Hoechst, 1996c
Octadecylamine		insoluble at 25 °C 11) 0.04875 mg/l at 25 °C (calc.) 9)	Kao, 2000 Clariant, 2001a
Amines, hydrogenated tallow alkyl		insoluble at 25 °C 11)	Clariant, 2001b
Amines, coco alkyl		insoluble at 25 °C 11)	Clariant, 2001c
	Partition coefficient *n*-octanol/water (log value)		
Amines, tallow alkyl		log Pow 7.1 at 20 °C (calculated) 10)	APAG, 2003a
(Z)-Octadec-9-enylamine		log Pow 7.5 at 20 °C (calculated) 10)	Hoechst, 1996c
Octadecylamine		log Pow 7.71 (calc.) 9)	Clariant, 2001a
Amines, hydrogenated tallow alkyl		7.3 (calc.) 12)	Clariant, 2001a
Amines, coco alkyl		not conducted 13)	
	Flash point		
Amines, tallow alkyl		159 °C 14)	Hoechst, 1997
(Z)-Octadec-9-enylamine		156 °C 15)	Siemens Axiva, 2002b
Octadecylamine		not conducted (solid)	
Amines, hydrogenated tallow alkyl		not conducted (solid)	
Amines, coco alkyl		> 100 °C 16)	Akzo, 2000

Substance	Property	Value	Reference
	Autoflammability		
Amines, tallow alkyl		no selfignition up to the melting range	Chemsafe, 2001
(Z)-Octadec-9-enylamine		265 °C 17)	Siemens Axiva, 2002a
Octadecylamine		no selfignition up to the melting range	Chemsafe, 2001
Amines, hydrogenated tallow alkyl		no selfignition up to the melting range	Chemsafe, 2001
Amines, coco alkyl		255 °C 17)	Siemens Axiva, 2002a
	Flammability		
Amines, tallow alkyl		non flammable according to A.10 18)	Clariant, 1999
(Z)-Octadec-9-enylamine		non flammable according to A.9 19)	BAM, 2001
Octadecylamine		non flammable according to A.10 19)	Siemens Axiva, 2003
Amines, hydrogenated tallow alkyl		non flammable according to A.10 non flammable according to A.12 and A.13 for structural reasons	Siemens Axiva, 2003
Amines, coco alkyl		non flammable according to A.9 19)	BAM, 2001
	Explosive properties		
Amines, tallow alkyl		not explosive (structural reasons)	BAM, 2001
(Z)-Octadec-9-enylamine		not explosive (structural reasons)	BAM, 2001
Octadecylamine		not explosive (structural reasons)	BAM, 2001
Amines, hydrogenated tallow alkyl		not explosive (structural reasons)	BAM, 2001
Amines, coco alkyl		not explosive (structural reasons)	BAM, 2001
	Oxidizing properties		
Amines, tallow alkyl		no oxidising properties (structural reasons)	BAM, 2001
(Z)-Octadec-9-enylamine		no oxidising properties (structural reasons)	BAM, 2001
Octadecylamine		no oxidising properties (structural reasons)	BAM, 2001
Amines, hydrogenated tallow alkyl		no oxidising properties (structural reasons)	BAM, 2001
Amines, coco alkyl		non oxidizing properties (structural reasons)	BAM, 2001

Substance	Property	Value	Reference
	Surface tension		
Amines, tallow alkyl		not conducted (water solubility < 1 mg/l)	
(Z)-Octadec-9-enylamine		not conducted (water solubility < 1 mg/l)	
Octadecylamine		not conducted (water solubility < 1 mg/l)	
Amines, hydrogenated tallow alkyl		not conducted (water solubility < 1 mg/l)	
Amines, coco alkyl		not conducted (water solubility < 1 mg/l)	
	Dissociation constant (pKa) at 25 °C		
Amines, tallow alkyl		not conducted 20)	
(Z)-Octadec-9-enylamine		not conducted 20)	
Octadecylamine	10.60		Hoerr et al, 1943
Amines, hydrogenated tallow alkyl		not conducted 20)	
Amines, coco alkyl		not conducted 20)	

[1)] literature value; no information available about the method

[2)] pourpoint

[3)] DSC; the boiling range of 353 – 355 °C was used for the risk assessment because a valid test report was available

[4)] distillation method

[5)] DSC

[6)] DIN 51757

[7)] air comparison pycnometer

[8)] the vapour pressure of primary amines is very low and therefore not relevant with respect to the environmental risk assessment

[9)] calculated values using the program EPIWIN

[10)] weighted average from single compounds calculated with KowWin using an atom/fragment contribution method developed at SRC 1995

[11)] due to the characteristic sorption and the potential of forming aggregates the water solubility is expected to be very low

[12)] weighted average of the single compounds of hydrogenated tallow alkyl amine

[13)] the sorption characteristics and the potential to form aggregates prevent the measurement of the Kow for primary amines

[14)] the flash point refers on the molten substance

[15)] determination of the flash point according to Pensky-Martens apparatus EN 22719

[16)] Pensky-Martens, closed cup (ISO 2719)

[17)] the auto-flammability behaviour was determined using the apparatus described in IEC 79-4 (see also DIN 51794)

[18)] the tests according to A.12 and A.13 were not conducted. Due to the properties and the handling of the substance it has not to be assumed that the substance does not form flammable gases on contact with water and does not have pyrophoric properties.

[19)] tests according to A.12 and A.13 not conducted (for structural reasons)

[20)] pKa-values are known for C_8(10.65), C_{10} (10.64), C_{12} (10.63), C_{14} (C10.62) and C_{16} (10.61). They are nearly independent from the chain length.

2 MANUFACTURE AND USES

Not relevant for this dossier

3 CLASSIFICATION AND LABELLING

3.1 Classification in Annex I of Directive 67/548/EEC

The substance has not been listed in Annex I of Directive 67/548/EEC up to the 31st ATP.

3.2 Classification in Annex VI of Regulation (EC) 1272/2008

The substance is currently not listed in Annex VI of Regulation (EC) Nr. 1272/2008 up to the 1st ATP (Commission Regulation (EC) No 790/2009).

3.3 Self classification

Not applicable

4 ENVIRONMENTAL FATE PROPERTIES

4.1 Degradation

4.1.1 Stability

Hydrolytic degradation appears to be unlikely because of the absence of hydrolysable groups. There are no important releases into the atmosphere and volatilisation is expected to be negligible, thus degradation processes in the atmosphere are considered to be of low relevance.

4.1.2 Biodegradation

4.1.2.1 Biodegradation estimation

No relevant data available

4.1.2.2 Screening tests

The biodegradbility of primary alkyl amines was tested in a number of screening tests on ready biodegradability following the OECD 301 guidelines. An overview of the results is presented in Table 2.

Table 2: Screening tests on ready biodegradability

Substance	Test OECD	Endpoint	Conc. [mg/L]	lag Phase	Degradation %		Result	Relia-bility	Reference
					28 d	10-d wind.			
coco alkyl amine	301 B	CO_2	13	4 d	60%	45%	readily degradable failing 10-d window	1	Hoechst (1996a)
	301 D	O_2	2	n.d.	56%	n.d.	not readily degradable (see *)	1	Akzo (1992a)
dodecylamine	301 C	O_2	100 30	7 d	0% (12 d) ~65% (12 d)	ca. 50%	readily degradable failing 10-d window	1	Yoshimura (1980)
tallow alkyl amine	301 B	CO_2	13	2 d	61%	46%	readily degradable failing 10-d window	1	Hoechst (1996b)
	301 D	O_2	2	n.d.	55%	n.d.	not readily degradable (see *)	1	Akzo (1992b)
hydrogenated tallow alkyl amine	301 D	O_2	2	n.d.	75%	n.d.	readily degradable, no information on 10-d window	2	Akzo (1992c)
octadecyl-amine	301 C	O_2	100	1	~75% (12 d)	~ 60%	readily degradable	1	Yoshimura (1980)
	301 F	O_2	126.5	< 7	70%	< 50%	readily degradable failing 10-d window	1	Hoechst (1994)

Substance	Test OECD	Endpoint	Conc. [mg/L]	lag Phase	Degradation %		Result	Relia-bility	Reference
					28 d	10-d wind.			
(Z)-octadec-9-enylamine	301 B	CO_2	24.6	ca. 10 d	66%	52%	readily degradable failing 10-d window	1	CECA (1994)
	301 D	O_2	2	n.d.	44%	n.d.	not readily degradable (see *)	1	Akzo (1992d)

*) In some cases degradation after 28 d shortly failed the required 60% level thus the test results were classified as "not readily biodegradable". Nevertheless ready biodregadability was assigned because the slope of the exponential part of degradation curve is similar to other readily biodegradable substances.

In the majority of the tests the pass level for ready biodegradability was reached within the test period (generally 28 d), but not within the 10 d window. The prolonged lag phase may be attributed to the high sorption tendency of long-chain alkyl amines to glass surfaces or organic matter, leading to a reduced bioavailability. In many tests a similar shape of degradation curve was obtained: after a lag phase of a few days, a rapid increase of degradation up to 50 or 60% of total mineralisation was observed. Based on the results of all tests primary long-chain alkyl amines can be classified as "readily degradable, but failing the 10 d window".

Considering the problem of bioavailability, degradation was further studied with tallow alkyl amine (Akzo, 1998) and coco alkyl amine (Akzo, 2002). Slightly modified Closed Bottle Tests were conducted using different inocula: secondary activated sludge from a plant treating predominantly domestic wastewater, preadapted activated sludge from a continuous activated sludge (CAS) reactor, ditch, river and sea water sampled in the Netherlands. To increase bioavailability the medium was continuously mixed by a stirring rod. For the exponential part of the degradation curve, the average specific growth rate was determined (Table 3). Comparing the slope, calculated for the exponential part of the degradation curve, shows a congruence with values found for readily degradable standard compounds. Additionally it is apparent that the degradation rates obtained in tests with different inoculi are comparable. This is a strong indication that long-chain alkyl amines are degraded by micro-organisms ubiquitously distributed in the environment, rather than by "specialists".

Table 3: Growth rates for tallow alkyl amine and coco alkyl amine

	tallow alkyl amine (1.9 – 3.7 mg/L)		coco alkyl amine (2.5 – 10 mg/L)	
	growth rate μ [d^{-1}]	lag period [h]	growth rate μ [d^{-1}]	lag period [h]
adapted sludge	5.57	21.5		
unadapted sludge	5.49	43.8		
river water	8.97	33	8.4	30
sea water	4.63	46	6.0	35
ditch water	3.67	25	6.5	70

The dependence of biodegradability on chain length was examined by Yoshimura (1980). In an O_2 consumption test similar to MITI I, dispersions (100 mg/L) of a number of primary alkyl amines

(C4, C8, C12, C14, C16 and C18) were examined. All amines except of dodecylamine were degraded by more than 60% after 12 d. Dodecylamine showed no oxygen consumption at 100 mg/L, but at 30 mg/L about 65% were degraded. In high concentrations long-chain alkyl amines exhibit toxic effects to microorganisms (e.g. respiration inhibition) so the supressed degradation at 100 mg/L might be caused by such an inhibition.

Considering the similar molecular structures and experimentally determined degradation rates from screening tests (Table 2), an integrative degradation characteristic can be utilized for all primary long-chain alkyl amines.

4.1.2.3 Simulation tests

Not relevant for this dossier

4.1.3 Summary and discussion of persistence

As long-chain alkyl amines are unlikely to undergo hydrolytic degradation and releases to the atmosphere as well as volatilisation appears to be of low importance, abiotic degradation processes do not require special consideration.

In screening tests on ready biodegradability, the pass level criteria were shortly failed within the 10 d window, probably because of reduced bioavailability due to adsorption onto glass surfaces Further tests (Akzo, 1998) as well as CAS-tests demonstrated that the rates during the exponential part of the degradation curve are comparable with readily degradable substances. In screening tests full mineralisation is detected. As the molecular structure is similar for all members of the category, great differences in degradability are not expected.

Considering all experimental results, primary long-chain alkyl amines can be classified as readily degradable, but not fulfilling the requirements for the 10-days window.

4.2 Environmental distribution

4.2.1 Adsorption/desorption

Not relevant for this dossier

4.2.2 Volatilisation

Not relevant for this dossier

4.2.3 Distribution modelling

Not relevant for this dossier

4.3 Bioaccumulation

4.3.1 Aquatic bioaccumulation

4.3.1.1 Bioaccumulation estimation

For octadecylamine no experimentally determined log K_{OW} has been stated, but Clariant (2001) reported a calculated log K_{OW} of 7.7. Under environmental conditions a part of the primary amine proportion might be protonated yielding alkyl ammonium ions. Accounting for the protonation equilibrium of primary alkyl amines in environmental media the log K_{OW} might be adjusted to a lower level than 7, but an exact quantification is not possible.

4.3.1.2 Measured bioaccumulation data

At the moment there is only a single indicative study about bioaccumulation of long-chain alkyl amines available (Akzo, 2006). As this study was performed with several modifications within a PBT assessment and following neither GLP nor CA´s suggestions for alternative test procedures (e.g. using radio-labelled material), the test results are not robust and may be used as indicative values. In order to experimentally assess the bioaccumulation potential of long-chain alkyl amines in fish, hexadecylamine was chosen by Akzo as a representative. In all relevant properties (molecular weight, chain lenght, lipophilicity, adsorption) hexadecylamine sufficiently reflects physico-chemical parameters of all other compounds within the amine group (Table 4).

In the preliminary test on bioconcentration, uptake and bioconcentration of hexadecylamine in common carp (*Cyprinus carpio*) were determined. As the experimental setup and test prodecure were not conform with GLP the gained data show a some uncertainty. During the study using hexadecylamine as test substance, analytics were performed using GC/MS. The solution for the fish test was prepared from a stock solution (300 µg/L) by further dilution to a nominal concentration of 3 µg/L. Due to adsorption problems during the test as well as sampling and extraction procedure only 50 – 80 % of the nominal concentration in the aquaria could be recovered. After exposure period, the whole fish burden ranged from 1500 to 3600 µg/kg and from 8000 to 15000 µg/kg in mucous/scales, respectively. After removing mucous and scales and washing the fish with chloroform the residual concentration was 650 to 850 µg/kg. Repeating the rinsing procedure with acidified methanol, the concentration further dropped to 280-600 µg/kg. Hence, it can be concluded that physical adsorption as well as ionic binding to organism´s surfaces occured.

Considering these effects and using the nominal concentration of 3 µg/L and whole fish body burden as input, the BCF may reach 1200. Using only the analysed recovery concentration (50% of the nominal) in the test water, the BCF increases to 2400. Utilizing instead the concentrations measured in fish after removing and complete rinsing and assuming 50 % of the nominal concentration as input values, the calculated BCF ranges between 400 and 570.

Summarising all scenarios, a BCF for hexadecylamine might reach from 200 (nominal water concentration, concentration in fish after the removal and washing procedure) up to 2400 (lower reanalysed level in aquaria, concentration in whole fish without treatment), depending on the selected input values for this calculation.

Derivation of a BCF via group approach:

The derivation of one realistic worst case BCF for the 5 assessed primary alkyl amines based on the indicative bioaccumulation test using hexadecylamine is possible by respecting all physico-chemical properties influencing bioaccumulation (Table 4).

All of the 5 assessed fatty amines belong to the same group of chemical substances. They consist of a long chain aliphatic backbone and a primary amine as functional group. However, each fatty amine represents a mixture of constituents differing in chain length distribution and the degree of unsaturation. The physico-chemical properties are mostly influenced by the non-polar alkyl chain and the amino function which incorporates basicity and polarity. Therefore all long chain alkyl amines show surface activity and a propensity to adsorption and ionic binding to acidic surfaces/counterparts like humic acids. The chemical reactivity is mainly governed by the electron rich amine function as well as olefinic double bonds, which can be further functionalised.

The bioaccumulation potential of a substance is strongly correlated to its lipophilicity, expressed as log K_{OW}, the molecular dimensions as well as to its capability of being metabolised. In most cases, the bioconcentration of chemicals is determined mainly by their lipophility, which is reflected by the log K_{OW}. However, fatty amines are surfactants, consisting of a long, non-polar alkyl chain and a polar amino group. Common methods to determine the log K_{OW} do not fully account for the specific partitioning behavior of surface-active substances. As the experimental determination of log K_{OW} is hardly possible for surface active substances like fatty amines the calculated values (from IUCLID dataset) are given with the exception of coco alkyl amine. The calculation for hexadecylamine was carried out using the program KOWWIN v1.67, which is part of the EPISUITE (US EPA) software package. Note that, due to the surface-active properties of the fatty amines, the calculated values are considered to be outside the application domain of the model. Nonetheless, in absence of the possibility to generate realistic log K_{OW} values for surfactants, it is necessary to estimate lipophilicity and partitioning behaviour from structural considerations. The KOWWIN model is a fragment method developed by Meylan and Howard (Meylan, 1995) and reflects the influence of individual functional groups on the overall lipophilicity of a substance. It is based on sound scientific principles and thus, it is an appropriate tool to support the examination of lipophilicity and partitioning behaviour.

In accordance with qualitative expectations, the calculated log K_{OW} directly correlates with the length of the alkyl chain, and the influence of present olefinic double bonds on lipophilicity is negligible. The calculated log K_{OW} values for tallow alkyl amines, hydrogenated tallow alkyl amines, octadecanamine, (*Z*)-octadec-9-enylamine as well as the reference substance hexadecylamine are in the same range of 6.7-7.7, which corresponds to a high hydrophobicity. These results indicate that the long, non-polar alkyl chain dominates with respect to partitioning behaviour. Accounting for the greater amount of medium chain length constituents (8-14 carbons) the calculated log K_{OW} for coco alkyl amines may be lower than 6, but an exact quatification is not possible. So it can be stated that the 5 fatty amines including hexadecylamine show a similar lipophilicity with coco alkyl amines being slightly less lipophilic. The sorption behavior via ionic binding is solely caused by the amino function therefore a similar sorption propensity can be assumed for these 5 substances.

Growing molecular dimensions are also affecting bioaccumulation as they may prevent an uptake of substances by restricted gill permeation. The actual molecular weigth cut-off is supposed at about 700 g/mol, so all 5 discussed fatty amines as well as hexadecylamine are well below this threshold. The molecular cross section diameter is similarly restricting uptake when exceeding a certain value. However, the long aliphatic chain in fatty amines is highly flexible and thus the bulkyness is depending on the actual conformer. Though, as for each fatty amine the aliphatic chain is linear (not

ramified) the bulkiness of tallow alkyl amines, hydrogenated tallow alkyl amines, octadecamine and (*Z*)-octadec-9-enylamine is expected to be similar to hexadecylamine due to the similar chain length. The shorter alkyl chains of coco alkyl amines may slightly reduce the steric hindrance, but the exact effect on bioaccumulation can not be easily estimated.

Metabolisation of long chain alkyl amines normally proceeds via oxidation of the amino function to imines and further hydrolysis to aldehydes (oxidative deamination). The resulting aliphatic aldehydes are further oxidised to fatty acids which are common intermediates in lipid metabolism. Normally the chain length has no significant influence on the transformation of long chain amines to fatty acids so the metabolic rate is presumably the same. However, olefinic double bounds enable a second metabolic pathway to more polar products via epoxidation and dihydroxylation. Therefore fatty amines with unsaturated aliphatic chains may be converted slightly faster to more polar metabolites.

Summarising all, a similar bioaccumulation potential can be hypothesised for these 5 long chain alkyl amines with minor differences in rate of metabolism. Because all 5 fatty amines are considered as "readily biodegradable" these differences in metabolism can be disregarded and it is appropriate to assume the same realistic worst case BCF of 1200 as determined for hexadecylamine.

Table 4: Comparison of physico-chemical properties influencing bioaccumulation

Substance	**Amines, coco alkyl**	**Amines, tallow alkyl**	**Amines, hydrogenated tallow alkyl**	***n*-Octadecan-amine**	**(*Z*)-Octadec-9-enylamine**	**Hexadecyl-amine** (test substance for BCF-test)
typical chain length distribution (major constituents)	8% C_8, 7% C_{10}, 50% C_{12}, 36% C_{14}, 8% C_{16}, 8% C_{18}	4% C_{14}, 31% C_{16}, 65% C_{18}	4% C_{14}, 30% C_{16}, 62% C_{18}	5% C_{14}, > 90% C_{18}	3% C_{14}, 13% C_{16}, 80% C_{18}	100% C_{16}
molecular weight	194-204 g/mol (averaged)	267g/mol (averaged)	263 g/mol (averaged)	269.5 g/mol	267.5 g/mol	241.5 g/mol
log K_{OW}	no data[1]	7.1 (calc)[1]	7.3 (calc)[1]	7.7 (calc)[1]	7.5 (calc)[1]	6.7 (calc)[2]
BCF	no experimental data available					200-2400, 1200 as realistic worst case

1) values were collected from IUCLID dataset
2) calculated with KOWWIN v1.67

4.3.2 Terrestrial bioaccumulation

No data available

4.3.3 Summary and discussion of bioaccumulation

Appraising the calculated log K_{OW} value of 7.1 for the non-protonated tallow alkyl amines significant bioconcentration can be assumed.

When judging the validity of the performed fish test, the question about the adequacy of scale dissection or any washing/rinsing procedure prior to analysis may provoke controversial opinions. The different analytical results after these 2 rinsing procedures indicate that one part of surface bound amine is physically adsorbed (removable with chloroform) and another part is ionically bound (only removable with acidified methanol). From the rapporteur´s point of view fish´s surface (mucous and scales) is an integral organ of this organism. In particular gill membrane surface is vitally important for fish and a strong adsorption of amine on gill surface or scales may induce adverse effects on viability of fish.

As the adsorbability of long-chain amines is very high and desorption rate is expected to be low, the rapporteur strongly advocates an incorporation of surface loading in determination of body burden respectively BCF. Thus, all available informations indicate for a high bioaccumulation potential, probably with BCF > 1000. Using the results of the indicative bioaccumulation study, the rapporteur proposes to use a realistic worst case BCF of 1200 (whole fish burden and nominal amine concentration) for C&L purposes. This fact is further supported by the high log K_{OW} of about 7.

4.4 Secondary poisoning

Not relevant for this dossier

5 HUMAN HEALTH HAZARD ASSESSMENT

In the toxicological section of the original EU Risk Assessment Report prepared by Germany, five different primary alkyl amines were evaluated together in a 'many-to-many read-across' approach based on similarity in terms of common functional groups and common metabolic breakdown products (for details cf. further below in this section). Although now separate CLH reports are submitted for each of the five amines, it was decided to present in each case the complete patchwork of toxicological data for the entire group, in order to ensure a consistent hazard assessment for all compounds under question.

Table 5: Overview of the primary alkyl amines/amine mixtures included in this CLH report *

Substance	**Amines, coco alkyl**	**Amines, tallow alkyl**	**Amines, hydrogenated tallow alkyl**	**n-Octadecan-amine**	**(Z)-Octadec-9-enylamine**
CAS	61788-46-3	61790-33-8	61788-45-2	124-30-1	112-90-3
% $C_{6,\ saturated}$	0.5	-	-	-	-
% $C_{8,\ saturated}$	8	-	-	-	-
% $C_{10,\ saturated}$	7	-	-	-	-
% $C_{12,\ saturated}$	50	1	1	-	1
% $C_{14,\ saturated}$	18	3	4	-	2-4
% $C_{14,\ single\ unsaturated}$	18	1	0.5	5	-
% $C_{15,\ saturated}$	-	0.5	0.5	-	-
% $C_{16,\ saturated}$	8	29	30	0.6	12-14
% $C_{16,\ single\ unsaturated}$	-	3	0.5	-	
% $C_{17,\ saturated}$	-	1	1.5	-	-
% $C_{18,\ saturated}$	1.5	23	60	> 90	8
% $C_{18,\ single\ unsaturated}$	6	37	2	-	67
% $C_{18,\ double\ unsaturated}$	1	1.5	-	-	5
Total content of primary amines	> 99 %	99 %	> 99 %	> 99 %	> 98 %
Possible impurities	non-reacted tallow nitriles, secondary and tertiary amines, water	fatty acid nitriles, secondary fatty acid amides and hydrocarbons	non-reacted tallow nitriles, secondary and tertiary fatty amines, bis(hydrogenated tallow alkyl amines), water	no data	fatty acid amides, fatty acid nitriles, secondary fatty amines and paraffins
Proposed C & L for human health acc. to Dir. 67/548/EEC	Xn, C; R22-35-37-48/22	Xn, C; R22-35-37-48/22	Xn, Xi; R37/38-41-48/22	Xn, Xi; R37/38-41-48/22	Xn, C; R22-34-37-48/22
Proposed C & L for human health acc. to Reg. (EC) 1272/2008	Acute Tox 4 Skin Corr 1A STOT SE 3 STOT RE 2 H302-314-335-373	Acute Tox 4 Skin Corr 1A STOT SE 3 STOT RE 2 H302-314-335-373	Skin Irrit 2 Eye Dam 1 STOT SE 3 STOT RE 2 H315-318-335-373	Skin Irrit 2 Eye Dam 1 STOT SE 3 STOT RE 2 H315-318-335-373	Acute Tox 4 Skin Corr 1B STOT SE 3 STOT RE 2 H302-314-335-373

* Percentages given should be understood as coarse grain averages

Primary amines of different chain length

Each of the primary alkyl amines considered in the RAR represents a mixture derived from fatty acids of varying carbon chain length. An overview is given in **Table 5**. It is important to note, that coco alkyl amines consist of shorter carbon chain lengths (mainly C_{12}) as compared to the other mixtures (which are composed mainly of C_{16} and C_{18} primary amines). Tallow alkyl amines (CAS 61790-33-8) differ from hydrogenated tallow alkyl amines (CAS 61788-45-2) by a higher content of unsaturated bonds in the hydrocarbon chain.

The most prominent toxic property of the primary alkyl amine mixtures assessed in this report is irritancy/corrosivity based on strong basicity, on which, in the range present in the mixtures assessed by this report, carbon chain length can be expected to be of only minor influence, if any.

Saturated vs. unsaturated primary amines

The presence of one or more double bonds might account for additional chemical reactivity – and, thus, different biological activity - in unsaturated vs. saturated fatty primary amines.

With respect to direct reactivity, in principle, the electron density of a double bond and, thus, its nucleophilicity (tendency to react with suitable electrophils) is increased by the positive inductive (+I-)effect of neighbouring alkyl substituents, which is generally bound to increase with the size of the latter. On the other hand, '[…]field effects always decrease with increasing distance and in most cases […] cause very little difference in a bond four bonds away or more' (March J, 1985). For this reason, at most slight differences, if any, in nucleophilic double bond reactivity, which in addition might as well be balanced by enhanced steric hindrance in the longer-chain amines, are expected between n-tetradec-9-enylamine, the major unsaturated constituent of the coco alkyl amines, n-hexadec-9-enylamine (strong in tallow and hydrogenated amines), or n-octadec-9-enylamine (tallow amines, (Z)-octadec-9-enylamine).

Regarding metabolisation, another characteristic of unsaturated fatty amines – or their corresponding fatty acids – is given by the fact that these compound classes are subject to metabolic epoxidation and subsequent hydrolysis to vicinal diols (cf. section 5.1.1.3). These products of metabolism reportedly can increase oxidative stress in exposed tissues and provoke inflammatory processes. As an example, the epoxidation products of linoleic acid (Z,Z-octadeca-9,12-dienoic acid), present at low percentages both in tallow and coconut oil (and thus a potential breakdown product of coco alkyl and tallow alkyl amines), and their corresponding diols, have been linked to inflammation, adverse effects on the cardiovascular system and to the Acquired Respiratory Distress System (ARDS), e. g. in Greene JF et al., 2000. On the other hand, linoleic acid is an essential ω-6-fatty acid, required as a precursor of arachidonic acid, a key compound in prostaglandin synthesis. A net beneficial effect on atherosclerosis has been postulated (a. o. by Sato M et al., 2005).

Summary of the rationale for the grouping approach

In summary, common biological activity of the primary alkyl amines evaluated in this report can be assumed based on a) the common primary amine group, b) the common metabolic degradation via deamination to the corresponding aldehydes, followed by oxidation to the respective carboxylic acids, and subsequent β-oxidation. Additional biological activity of unsaturated vs. saturated fatty amines will be dealt with, if and where applicable.

Given the spatial distance between the amine and double bond moieties, it appears reasonable to assume that amine and double-bond reactivity will be independent of each other, i. e. amine reactivity will be comparable for all amines under question, while double bond reactivity will basically be the same for an amine and its corresponding carboxylic acid. With respect to the latter,

it should be borne in mind that most of the unsaturated primary amines under investigation in this report derive from – and will be metabolised to – fatty acids, which are present in humans at high endogenous levels and are also consumed in the diet via a multitude of fats of plant and animal origin, e. g. palmitoic acid (cis-n-hexadec-9-enoic acid) in palm oil and oleic acid (Z-octadec-9-enoic acid) in olive oil (with contents exceeding 70 %).

5.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

5.1.1 Summary of toxicokinetics, metabolism and distribution

Data on toxicokinetics, metabolism, and distribution of any of the amine mixtures listed in **Table 5** above are not available. However, studies exist on the toxicokinetics, metabolism, and distribution of single definite constituents of the mixtures.

5.1.1.1 Absorption

Based on the physico-chemical properties of the respective alkyl amine mixtures, low inhalation and oral bioavailability can be anticipated. Two compounds, i.e. coco alkyl amines and (Z)-octadec-2-enamine are liquid under normal ambient conditions, whereas the other three substances are waxy solids. The average molecular weight ranges from 194 (coco alkyl amines) to 269.5 g/mol (octadecylamine). Apart from the calculated water solubility of 0.12 mg/L for tallow alkyl amines, all other alkyl amines are insoluble in water. Log P_{OW} has been calculated for all amines with the exception of coco alkyl amines and ranges from 7.1 to 7.71. The vapour pressure of all amines is very low.

Oral route

On the other hand, data from acute and repeated dose studies in animals performed with compounds summarised in this dossier demonstrate systemic effects after oral administration. Therefore, as a worst-case estimate, a value of 100 % oral absorption is assumed.

Inhalative route

Based on physico-chemical parameters including low volatility, exposure by inhalation to vapours is expected to be low. However, due to the severe irritant/corrosive effects observed with primary amines, uptake via inhalation - as far as inhalation exposure to vapours is possible - might be facilitated by tissue damage caused at the site of entry. On the other hand, exposure to aerosols might lead to a higher absorption rate and thus might constitute a more severe hazard. Again, 100 % inhalation absorption is used as a worst-case estimate.

Dermal route

Based on the basicity and corrosive properties of the primary alkyl amines, dermal absorption as a consequence of facilitated penetration through damaged skin can be anticipated. 1-dodecanamine, as a constituent of the alkyl amine mixtures, was absorbed from the skin of mice following 24-h occlusive application. Depending on solvent and concentration, up to 57 % were absorbed (Iwata Y et al. 1987). This value for dermal absorption may therefore be used as a worst-case estimate in risk characterisation.

5.1.1.2 Distribution

Experimental data in animals show that, following intravenous injection of single alkyl amines of varying chain lengths ([^{11}C]-octanamine hydrochloride, [^{13}N]-octanamine hydrochloride, [^{11}C]-octanamine and [^{11}C]-decanamine), bioavailable amounts of alkyl amines are rapidly distributed into the lung, brain, heart, spleen, kidneys, and liver (Blaschko H, 1952; Fowler JS et al., 1976a,b; Gallagher BM et al., 1977a; Tominaga T et al., 1987). In lung, brain, and heart, tissue concentrations of amines increased with increasing chain length, when chain lengths of C_8, C_{10}, and C_{13} were compared.

5.1.1.3 Metabolism

After intravenous application of 3 - 4 mCi [^{11}C]-octanamine to human volunteers, 65-70 % of the applied radioactivity was taken up by the lung; this rate decreased to 16-19 % after 30 min. Oxidation by flavin-containing monoaminooxidases (MAO) was rapid with 95 % of the blood radioactivity at 2 min present in the form of deaminated metabolites. The build-up of deaminated metabolites in blood was followed by accumulation of radioactivity in the liver (18-27 % of the injected dose after 30 min). Cumulative [^{11}C]-carbon dioxide excretion within 35 min was 10-13 % of the injected dose, and urine accounted for 1-2 % of the applied dose after 1 hour (Gallagher B et al. 1977b).

It has been shown that alkyl amines are oxidatively deaminated by MAO with concomitant formation of ammonia and the corresponding alkyl amine aldehyde (Tenne M et al., 1985; Tominaga T et al., 1987; Tynes RE et al., 1986). Oxidative deamination decreases with increasing carbon chain length. Subsequently, the aldehyde products are oxidised by aldehyde dehydrogenases to the corresponding carboxylic acids (Fowler JS et al., 1976a; Tominaga T et al., 1987), which, in turn, are further metabolised by β-oxidation, a process which is described in broad detail in standard biochemistry textbooks (e. g. Stryer L, 1994).

Monounsaturated fatty acids, such as those obtained as metabolic intermediates from the amines evaluated in this report, are subject to hydroxylation and epoxidation (e. g. Ruettinger RT & Fulco AJ, 1981). Epoxides can then be hydrolysed to vicinal diols by soluble epoxide hydrolases (sEH), as has been shown e. g. for methyl linoleate.

5.1.1.4 Excretion

Fatty amines are mainly oxidised to and excreted as CO_2 via the lungs. This has been shown a. o. for n-dodecylamine in mice (Iwata Y et al., 1987; Fowler JS et al., 1976a) and rabbits (Fowler JS et al., 1976b). Only a minor part of administered radioactivity was found in the urine following i. v. administration to human volunteers (Gallagher B et al., 1977, cited in GDCh, 1994).

5.2 Acute toxicity

5.2.1 Acute toxicity: oral

5.2.1.1 Amines, coco alkyl

In an unpublished OECD TG 401 study, coco alkyl amines ('Armeen C'), a clear light-yellow, oily liquid (purity not given), was orally applied to five Wistar rats per dose group and sex at dose levels of 500, 1000, 1500, and 2000 mg/kg bw. The substance was applied as a 20 % solution in peanut oil

(pH 8.1). No mortalities were observed during the first 24 h post-exposure. At 500 mg/kg bw, one male died within 24 and 48 h, while no further mortalities occurred (total mortality within 14 days p. a.: 20/0/10 % for M/F/M+F). At 1000 mg/kg bw, one male died between 24 and 48 h and one female between 48 h and five days (total mortality within 14 days p. a.: 20/20/20 % for M/F/M+F). At 1500 mg/kg bw, one male and one female died between 24 and 48 h, while another male and female died between 48 h and five days (total mortality within 14 days p. a.: 40/40/40 % for M/F/M+F). At 2000 mg/kg bw, all males died within 5 days, two between 24 and 48 h and the remaining three between 48 h and five days; one female died between 24 and 48 h, another three between 48 h and 5 days, and the fifth between days 5 and 7 (total mortality within 14 days p. a.: 100 % for M/F/M+F) days 5-14. **An LD_{50} of 1300 mg/kg bw (1240 for male and 1390 for female rats) was calculated** (Probit analysis according to Finney). Clinical signs observed in all treatment groups included apathy, slight to pronounced irregular posture, uncoordinated movements, reduced reflexes, cyanosis, salivation, piloerection, slightly reduced breathing rate and some cases of slight hypothermia. Symptoms started from 20 minutes after dosing and were present until death or up to 7 days in survivors. After dissection of dead animals, slight reddening and liquid congregations were detected in the gastrointestinal tract. There were no pathological findings in the surviving animals (IBR Forschungs GmbH, 1983a).

In another unpublished GLP-compliant study according to OECD TG 401 (limit test with 5 M + 5 F at 2000 mg/kg bw, another five females each used at 2500 and 3000 mg/kg bw), coco alkyl amines ('Genamin CC 100 D'), a nearly colourless to light-yellow liquid (purity 99-100 %), was orally applied to Wistar rats using sesame oil as a vehicle. After treatment with 2000 mg/kg bw, one male (between post-exposure days 4 and 5) and one female (days 2-3) died. Two females (one after 90 minutes the other between days 1-2) of the 2500 mg/kg bw group and three females (140 minutes/day 0-1/days 1-2) of the 3000 mg/kg bw group died, respectively. **The LD_{50} was established at > 2000 mg/kg for males and 2820 mg/kg bw for females.** Clinical signs observed in all treatment groups included retracted flanks, hunched posture, stilted gait, irregular breathing, diarrhoea, and reduced spontaneous activity. Some animals showed bloody encrusted edges of the palpebral fissures. Symptoms started between 10-60 minutes after onset of treatment and were present until death or up to 5-10 days in survivors. Partly darkened liver, brightened spleen and yellow-dark red filling of the gastrointestinal tract was observed in dissected bodies, while there were no pathological findings in survivors (Hoechst AG, 1988k).

In a further unpublished study according to OECD TG 401 (GLP-compliance not stated), five Sprague-Dawley rats of each sex were administered a 40 % w/v aqueous solution of coco alkyl amines ('Armeen CD', purity 99 %) as a single oral dose (dose levels 1800, 2560, 3620 or 5120 mg/kg bw). Additionally, five rats of each sex were treated with the test vehicle (distilled water) at a level equal in volume (12.8 mL/kg bw) to the highest dose level of the test substance solution administered. Animals were observed for overt signs of toxicity at 0.25, 0.5, 1, 2, and 4 hours after dosing and daily thereafter for a total of 14 days. Individual body weights were measured at study initiation and termination (day 14). Necropsy was performed on all animals to assess any treatment-related gross pathological changes. Mortalities within the first 6 days of the observation period were as follows: 0/5 males and 0/5 females at 1800, 1/5 males and 1/5 females at 2560, 1/5 males and 4/5 females at 3620, and 5/5 males and 4/5 females at 5120 mg/kg bw. One additional death of a male animal from the 1800 mg/kg bw treatment group occurred on day 8. Animals in the 3620 and 5120 mg/kg bw treatment groups appeared lethargic within 15 minutes of treatment and throughout day 1. **The acute oral LD_{50} of the test substance for male and female animals combined** (calculated by Finney's Probit Methods (1964)), **was 2040 mg/kg bw with 95 % confidence limits of 1510 to 2760 mg/kg bw.** Clinical observations through the first week of observation included lethargy, chromodacryorrhoea, epistaxis with closed eyes, and emaciation. All animals in the 2560 mg/kg bw treatment group appeared subdued and lethargic on day 1 beginning

1 hour after treatment. Additional clinical signs reported on day 2 were chromodacryorrhoea and epistaxis with half-closed eyes by day 5 for all animals in this group. One animal in the 1800 mg/kg bw treatment group appeared lethargic 1 hour after treatment. Lethargy was observed in all animals in the group at day 2, with epistaxis in two animals at day 3 and recovery of all animals in the group by day 4. All control animals appeared normal through the observation period. The one surviving animal in the 5120 mg/kg bw treatment group and 2 males and 1 female in the 2560 mg/kg bw treatment group lost weight by the end of the observation period. The majority of animals necropsied revealed no abnormalities. Abnormalities detected included gaseous distended stomach with fluid filled gastrointestinal tract, congestion of the lungs, intestinal and renal adhesions, and hard and discoloured spleen (Hazleton Laboratories Europe Ltd., 1979a, cited in: Toxicology Regulatory Services Inc., 2003).

From a corresponding unpublished study which used coco alkyl amines ('Armeen CD') in an aqueous dilution of 20 % w/v, an **LD_{50} of > 6000 mg/kg bw** was concluded (Hazleton Laboratories Europe Ltd., 1979b, cited in Toxicology Regulatory Services Inc., 2003).

5.2.1.2 Amines, tallow alkyl

In an unpublished OECD TG 401 study, tallow alkyl amines ('Genamin TA 100 D'), a whitish wax (purity 99-100 %), were orally applied to Wistar rats at doses of 2000 (5 males + 5 females) and 2500 mg/kg bw (5 males only). The test substance was suspended in sesame oil. In the 2000 mg/kg dose group, 1/5 males was found dead on day 6; all females survived. After administration of 2500 mg/kg bw, 1/5 males died within 24 hours. **Hence, the oral LD_{50} of tallow alkyl amines exceeded 2000 mg/kg bw (2500 mg for males)**. The following clinical signs were observed following dosing: hunched posture, flanks drawn in, piloerection, irregular breathing, abnormal gait, diarrhoea, crusted eyelids and snout (both males and females), decreased spontaneous activity, uncontrolled movements, crawling-like movements, emaciation, drowsiness, gasping (males). All symptoms disappeared by day 11 or 12. Decreased body weight gain was observed the first week post-dosing. Necropsy of animals that had died revealed stained liver, pale-coloured lungs and gastrointestinal tract swollen with gas. Animals sacrificed following termination of the experiment showed no gross changes (Hoechst AG, 1988i).

In another unpublished, non-GLP, but guideline-compliant oral toxicity study according to OECD TG 401, tallow alkyl amines ('Armeen T', purity not given), were applied to groups of 5 male and 5 female Wistar rats at 1.5, 2.5, 3.5, and 5.0 mL/kg bw (1.17, 1.95, 2.73, and 3.9 g/kg bw). Water was used as a vehicle. Animals were observed for signs of toxicity at approximately 30 minutes at 1, 2, 3, 6, 24, and 48 hours and on days 4, 6, 7 and 14 following dosing. Body weights were determined on day 0 and 14 for all surviving animals. The LD_{50} value was determined by Probit analysis. Treatment with tallow alkyl amines was fatal for 1/5 males and 1/5 females at 1.5 mL/kg bw, 3/5 males and 2/5 females at 2.5 mL/kg bw, 4/5 males and 3/5 females at 3.5 mL/kg bw, and 5/5 males and 5/5 females at 5.0 mL/kg bw, **resulting in an LD_{50} of 2.40 mL/kg bw or ca. 1.87 g/kg bw for both sexes**. Clinical observations included: incoordination, cyanosis, excessive salivation, piloerection, reduced respiration, and hypothermia. The surviving animals appeared normal at the end of the 14-day post dosing observation period (IBR Forschungs GmbH, 1983b).

5.2.1.3 Amines, hydrogenated tallow alkyl

In an unpublished OECD TG 401 study, hydrogenated tallow alkyl amines ('Lilamin AC-HBG-P'), a white granular solid (purity not given), were orally applied to Wistar rats at a dose of 5000 mg/kg bw. The substance was applied as a 50 % suspension in 1 % methyl cellulose (limit-test). There were no deaths; hence, **the LD_{50} exceeded 5000 mg/kg bw.** Clinical signs were diarrhoea,

piloerection, hunched posture, abnormal gait, and pallor of extremities. Complete recovery from these clinical signs was observed by day 5. Body weights were initially reduced but body weight gain was not different from controls by the end of the study (day 15). No treatment-related effects were observed at necropsy (Huntingdon Research Centre, 1995).

In an unpublished OECD TG 420 study, hydrogenated tallow alkyl amines ('Farmin TH'), a white solid (purity not given), were orally applied to female Sprague-Dawley rats at doses of 500 and 2000 mg/kg bw. The test substance was suspended in 5 % aqueous Tween 80. One female treated at 2000 mg/kg died in the course of the last day of the observation period. There were no mortalities in the 500 mg/kg bw treatment group. Clinical signs at both dose levels included hunched posture, rattling breath, piloerection, ataxia, decreased motor activity and muscle tone, pallor and traces of blood in the snout. Necropsy revealed no pathological findings. It was concluded that the test substance was **'free of any significant toxicity'**. (Centro de Investigacion Y Desarollo Aplicado, S.A.L., 1995a).

In a further well-conducted unpublished, non-GLP compliant study according to OECD TG 401 (6 animals/dose group), hydrogenated tallow alkyl amines ('Amin HBG'), a semi-solid beige-coloured paste (purity not given) were orally applied in water to Sprague-Dawley rats at doses of 3000, 3900, 5070, 6590, and 8560 mg/kg bw in a volume of 20 mL/kg bw. Deaths were observed from 3900 mg/kg bw on (one animal died on day 2) and **an LD_{50} of 4800 mg/kg bw was established** (method: Thompson Moving Average Interpolation). All deaths occurred within one to three days following dosing. According to the main study, no deaths were reported in a pre-study in the dose groups ≤ 3000 mg/kg bw. Clinical signs, present in all treatments, were piloerection and hyperkinesia. Necropsy findings were normal at 3000 mg/kg bw. At 3900 mg/kg bw, fluid gut contents and red stains in gut and, at doses higher than 5070 mg/kg bw, additionally test substance in stomach, patchy lungs, and pale-coloured kidneys were found. (Inveresk Research International, 1979).

5.2.1.4 Octadecylamine

In an unpublished OECD TG 401 study, octadecylamine ('Genamin 18 R 100 D'), a whitish waxy solid (purity approximately 100 %), was orally applied to Wistar rats at a dose of 2000 mg/kg bw (limit-test). The substance was solubilised in sesame oil under heating. One male died on day 4, hence, **the LD_{50} exceeded 2000 mg/kg bw**. Clinical signs: all animals showed reduced spontaneous activity, piloerection, hunched posture, abnormal gait, reduced activity, and irregular breathing. One male had a swollen abdomen. All animals recovered by day 9. The animal that died had a decrease in body weight on day 4. No other effects on body weight were noted. The following observations were noted at necropsy for the animal that died: stomach filled with test substance and gas, flanks drawn in, dark-coloured liver, red staining of lungs, intestine and pancreas, intestines filled with gas, and shrunken spleen. Surviving animals did not show any remarkable findings at necropsy (Hoechst AG, 1989a).

In another unpublished OECD TG 401-like study, approximately 100 % octadecylamine ('Genamin SH 100 D') in sesame oil were orally applied to Wistar rats at a dose of **2000 mg/kg bw (limit-test). The dose was non-lethal**; however, for up to 7 hours following treatment, reduced spontaneity, crouching and retracted flanks were observed in the animals (Hoechst AG, 1988l).

5.2.1.5 (Z)-Octadec-9-enylamine

In an OECD TG 401 study, octadecenylamine ('Noram O', oleylamine), an opaque, non-homogenous liquid (purity 97.9 %), was orally applied to Sprague-Dawley rats at doses of 200, 500, 1000, and 2000 mg/kg bw. In a pre-test (limit test) with 2000 mg/kg bw, no vehicle was used. The

substance was heated to approximately 30 °C and mixed with corn oil for the lower dose groups in the main test. Mortalities were 10, 0, 20, and 70 % in the respective dose groups. **Males (LD_{50} approximately 1200 mg/kg bw) were more sensitive compared to females (LD_{50} approximately 2000 mg/kg bw); the LD_{50} for both sexes was established at 1689 mg/kg** (Probit analysis). Clinical signs included hypokinesia and/or sedation, piloerection and dyspnoea, abdominal swelling. A decrease in body weight gain was noted between day 1 to 5 at 200 and 500 mg/kg bw, persisting in one male and one female at 200 mg/kg bw and at 1000 mg/kg, and returning to normal by day 8. Body weight gain was also decreased at 2000 mg/kg bw from day 1 to 8, returning to normal by day 15. No abnormalities were noted at necropsy (Centre International Toxicologie, 1993).

5.2.2 Acute toxicity: inhalation

5.2.2.1 Amines, coco alkyl

In an unpublished range finding study, groups of ten male Sprague-Dawley rats were exposed to a vapour of coco alkyl amines ('Armeen C') at mean analytical concentrations of 0.063 and 0.099 mg/L for one hour by whole-body exposure. Chamber concentrations were monitored during the entire one-hour exposure period at a rate of 0.52 L/min. Rats were observed for mortality and signs of toxicity and/or abnormal behaviour throughout the exposure and afterwards daily for 14 days. Body weight was recorded prior to exposure and on day 14. All surviving rats were subjected to a gross necropsy, and the following tissues excised and preserved in 10 % neutral buffer formalin: brain, liver, kidney, heart, pancreas, stomach, lungs, spleen, and testes. Tissues from animals of the 0.099 mg/L group were examined under a light microscope. **There were no deaths, accordingly, the one-hour LC_{50} was found to exceed 0.099 mg/L.** After five minutes of exposure, several rats in the 0.063 mg/L dose group showed cleaning behaviour, but were inactive otherwise. All animals were hypoactive after ten minutes. After 40 minutes, several animals exhibited a slight irritation around the muzzle. This latter effect, as well as hypoactivity in all rats, continued for the remainder of the exposure period. After ten minutes of exposure all rats in the 0.099 mg/L dose group were hypoactive. After 30 minutes, several animals showed signs of irritation, were preening, and exhibited a nasal discharge. At the end of the one-hour exposure, all rats showed mild to severe irritation around the muzzle and had reddish areas on the fur. All rats in both groups exhibited normal appearance and behaviour throughout the 14-day post-exposure observation period. A mean body weight gain in both dose groups was noted at the end of the observation period. No necropsy findings were noted in any rats from both dose groups. Microscopic evaluation of selected tissues from the rats in the 0.099 mg/L dose group included minimal to slight peribronchial lymphoid hyperplasia present in the lung, as well as minimal focal interstitial nephritis in seven of the ten rats, but these findings were not rated as compound-related histomorphologic alterations. All other tissues were within normal histological limits (Hazleton Laboratories America Inc., 1975, cited in: Toxicology Regulatory Services Inc., 2003).

5.2.2.2 Amines, tallow alkyl

No data available

5.2.2.3 Amines, hydrogenated tallow alkyl

No data available

5.2.2.4 Octadecylamine

No data available

5.2.2.5 (Z)-Octadec-9-enylamine

No data available

5.2.3 Acute toxicity: dermal

5.2.3.1 Amines, coco alkyl

In an unpublished, GLP-compliant study according to OECD TG 402, coco alkyl amines ('Amine KK'), a yellow liquid (purity 100 %), was dermally applied under occlusion to Sprague-Dawley rats. At a dose level of 500 mg/kg body weight, coco alkyl amines were applied undiluted (application volume 0.63 mL/kg bw), at a dose level of 2000 mg/kg bw the substance was applied as a 40 % solution (w/v) in distilled water (application volume 5 mL/kg). Compared to a regular OECD TG 402 study, the number of animals used was reduced (2 instead of 5 animals/sex/dose). **There were no mortalities, hence, the LD_{50} was > 2000 mg/kg bw.** No clinical signs were observed at 500 mg/kg bw. At 2000 mg/kg, hunched posture, abnormal gait, lethargy and decreased respiratory rate were noted. Signs of dermal reactions at the application site of both treatments were well defined to moderate oedema until days 4-5. Hard scabs, persisting to the end of the observation period, frequently prevented the assessment of oedema (Huntingdon Research Centre, 1985).

In another unpublished, GLP-compliant study based on OECD TG 402, coco alkyl amines ('Armeen CD'), purity 99 %, was dermally applied to New Zealand White rabbits under semi-occlusive conditions for 24 hours. Three rabbits of each sex were administered a single dermal dose of the undiluted test substance at a volume of 2.0 mL (1600 mg)/kg bw. The hair on the backs of the rabbits was clipped 24 hours prior to test substance administration. Abraded areas of one male and two female rabbits in each treatment group were prepared by penetrating the horny layer of the epidermis without causing bleeding. The treatment was applied to the intact or abraded exposure site of each rabbit. Animals were observed daily for mortality and signs of toxicity for a period of 14 days. Individual body weights of surviving animals were measured at study initiation and termination (day 14). Necropsy was performed on all rabbits and all abnormalities were recorded. One male animal with intact exposure site died by day 3. Upon necropsy, cause of death was concluded to be not treatment-related. This animal was replaced and no further animals died during the study. All animals showed small to moderate body weight gains by the end of the observation period. Skin irritation reactions were produced in all animals following treatment. Necrosis and eschar formations were reported for all animals. Moderate to severe oedema during day 1 to 4 was replaced by hard dry skin with marked atonia. Some exfoliation was noted at the end of the observation period revealing the presence of beet red lesions. Necropsy revealed the presence of hard necrotic treated skin with thickened new skin or normal to beet red colour underneath. No marked abnormalities were noted in any other organs. **The dermal LD_{50} was ≥ 2 mL (1600 mg)/kg bw for both sexes** (Hazleton Laboratories Europe Ltd., 1979c, cited in: Toxicology Regulatory Services Inc., 2003).

5.2.3.2 Amines, tallow alkyl

No data available. Studies on skin irritation do not indicate significant systemic toxicity after dermal application.

5.2.3.3 Amines, hydrogenated tallow alkyl

No data available. Studies on skin irritation do not indicate significant systemic toxicity after dermal application.

5.2.3.4 Octadecylamine

No data available. Studies on skin irritation do not indicate significant systemic toxicity after dermal application.

5.2.3.5 (Z)-Octadec-9-enylamine

No data available. Studies on skin irritation do not indicate significant systemic toxicity after dermal application.

5.2.4 Acute toxicity: other routes

5.2.4.1 Aspiration hazard

Normally, aside from concrete evidence in humans, classification/labelling of a substance for aspiration hazard is triggered if it is a hydrocarbon with a kinematic viscosity $\nu < 7 \times 10^{-6}$ m^2/s at 40 °C. The latter can be obtained as the quotient of dynamic viscosity (η, in Ns/m^2 or Pas) and density (ρ, in kg/m^3). The following arguments pro/contra C & L for aspiration hazard have been identified:

Viscosity

While the density of chemicals is frequently provided in their Material Safety Data Sheets (MSDS), viscosity data are only rarely available. In the numerous materials for which MSDS were provided in the frame of this report, dynamic viscosity data were found only for the mixtures listed in **Table 6**.

Table 6: Viscosity of alkyl amine mixtures (Source: MSDS)

Trade name	Contains	η (Ns/m^2) [1]	ρ (kg/m^3) [1]	ν (m^2/s, calculated [2])
Farmin C	Coco alkyl	5-20 (at 30 °C)	780 (at 60 °C)	ca. 6.4×10^{-6}-2.56×10^{-5}
Farmin TH	Hydrogenated tallow alkyl	5-20 (at 50 °C)	790 (at 50 °C)	ca. 6.3×10^{-6}-2.53×10^{-5}
Farmin O	(Z)-Octadec-9-enylamine	5-20 (at 30 °C)	806 (at 50 °C)	ca. 6.2×10^{-6}-2.53×10^{-5}

[1] Source: MSDS of the manufacturer; [2] Calculated by using the formula $\nu = \eta/\rho$

As a general trend, it can be seen that kinematic viscosities are below or borderline to the critical value of 7×10^{-6} m^2/s. Density values could be expected to be slightly but not significantly higher at 40 °C as compared to the values given for higher temperatures in the table. It is noted that these data are associated with considerable uncertainty, as no details on their generation are known. Also, only a range of dynamic viscosities is given.

Hydrocarbons

Due to the presence of a nitrogen atom, the primary alkyl amines under investigation are not hydrocarbons in the narrow sense. However, they contain a long linear hydrocarbon moiety significantly influencing their physicochemical properties.

Reactivity

At physiological pH, the primary amines considered in this report are present in their protonated form. As such, they are cationic surfactants which can be predicted to cause severe damage to the respiratory system, in particular in the bronchoalveolar region.

Toxicity

Histopathological evaluation of lung tissue is not routinely performed in oral acute toxicity studies. The clinical symptoms observed (laboured breathing, rattling noises) in these studies (cf. section 5.2.1) are not necessarily a result of aspiration of test substance. In addition, LD_{50} studies are performed by gavage, and aspiration might occur accidentally during this procedure.

On the other hand, severe lung damage was frequently observed following repeated oral administration of primary alkyl amines to rats, both by gavage and in the diet. However, in none of the cases it was possible to attribute these findings with sufficient certainty to substance treatment and to rule out other, (micro)biological causes (cf. section 5.6).

Nevertheless, even considering that observations such as breathing impairment and corresponding lung noises or histopathological signs of acute or chronified pneumonia potentially can be traced back to a great variety of factors, it is quite striking, how many acute and repeat-dose study reports cited in the present report make reference to such symptoms following administration of primary alkyl amines.

Conclusion

For the primary alkyl amines addressed in this report, the database with respect to aspiration hazard is inconclusive and thus insufficient to demand corresponding classification/labelling.

5.2.5 Summary and discussion of acute toxicity

5.2.5.1 Oral

As a general trend in acute oral toxicity, those amine mixtures containing significant portions of unsaturated fatty amines (i. e. coco alkyl amines, tallow alkyl amines, and (z)-octadec-9-enylamine) proved to be more harmful than those consisting mainly of saturated fatty amines (i. e. hydrogenated tallow alkyl amines and octadecylamine). It is left to speculation whether this can be explained by an altered bioavailability, by direct reactivity of the double bond(s), or by metabolic toxification (cf. introduction to this chapter and section 5.1). However, the LD_{50} of all three "unsaturated" mixtures was found to be roughly in the same range (1000-2000 mg/kg bw), and this can be seen as a confirmation of the hypothesis that chain length in the range of C_{12} to C_{18} is at most a minor factor of influence on the acute toxicity of primary fatty alkyl amines.

As a consequence of the obtained results it is proposed to classify/label **coco alkyl amines, tallow alkyl amines, and (Z)-octadec-9-enylamine** as **Xn;R22 (harmful if swallowed)** following the criteria of Annex VI to Dir. 67/548/EEC. According to the GHS as adopted by the EU CLP Regulation (Reg. (EC) 1272/2008), these substances should be classified as **Acute Toxicity Class 4** and the hazard phrase **H302 (harmful if swallowed)** should be assigned.

For the two mixtures predominantly consisting of saturated primary amine mixtures, i. e. **hydrogenated tallow amines and octadecylamine, no classification/labelling for acute oral toxicity is required.**

5.2.5.2 Inhalation

The one available acute (range-finding) inhalation rat study with coco alkyl amines (1 hour whole body exposure) resulted in no lethality, but in irritancy at concentrations below 0.1 mg/L. This level most likely does not represent the maximum technically attainable concentration (MAC), and data at higher concentrations are not available. In addition, animals were only exposed for one hour, which is only a quarter of the period required by the corresponding OECD test guideline.

On the other hand, in the context of this report, it is proposed to classify/label coco alkyl amines for corrosivity. Therefore, while **the database is insufficient to arrive at a conclusion with respect to acute inhalation toxicity**, based on animal welfare considerations, no further testing for acute inhalation toxicity is proposed.

For the other amine mixtures, no experimental data on acute inhalation toxicity are available. Based on their physical state (waxy to solid) at ambient temperature and corresponding low vapour pressure, exposure to vapours or aerosols via inhalation is expected to be low or even unlikely. In addition, considering the strongly irritant or even corrosive properties of these substances, again, additional animal testing is not seen as justified from an ethical point of view. On the other hand, testing with non-irritant dose levels will most likely not contribute significantly to the database, as the available study with coco alkyl amines has demonstrated a low likelihood for lethality of such dose levels.

5.2.5.3 Dermal

In one acute dermal study with rats, an LC_{50} > 2000 mg/kg bw was demonstrated for coco alkyl amines ('Amine KK'). In rabbits, the highest concentration tested, at which no treatment-related mortality, but irritancy was observed, corresponded to a dose level of 1600 mg/kg bw, and an LD_{50} below 2000 mg/kg bw can be considered unlikely. No classification/labelling is therefore proposed for acute toxicity via the dermal route.

Taking into account the low dermal toxicity experimentally established for coco alkyl amines as well as the corrosive or irritant nature of the other amine mixtures (and thus animal welfare), no need is seen to perform additional testing with regard to classification/labelling for acute dermal toxicity.

5.2.5.4 Aspiration

There is some evidence, that primary alkyl amines might pose an aspiration hazard and **classification/labelling with R65/H304** might be warranted, but overall the **available data are insufficient to arrive at a conclusion** with sufficient certainty.

5.3 Irritation

5.3.1 Skin

5.3.1.1 Amines, coco alkyl

In an unpublished OECD TG 404 study (deviation: exposure was only up to 1 hour), 0.5 mL coco alkyl amines ('Farmin C'), a light-brown clear liquid (purity 100 %), were applied semi-occlusively to the shaved skin of two female and one male New Zealand White rabbits. The duration of initial treatment was three minutes, after which the skin was flushed with lukewarm tap water and

examined for reaction. Because no reaction was noted after three minutes, identical patches with fresh test article were applied for a total exposure time of approximately one hour. The dressing was then removed and the skin was again flushed with lukewarm tap water. Readings of erythema and oedema were performed at 1, 4, 24, 48, and 72 hours as well as at 7, 14, and 21 days after the removal of the dressing, gauze patch and test article. Coco alkyl amines produced grade 3.00 erythema and grade 3.00 oedema (mean values from 24-72 hours). These symptoms were reversible within 7-14 days. In the area of application, brown staining was observed up to 7 days. Eschar and scabbing were observed up to 14 days, and subsequent scarring was noted up to the end of the observation period of 21 days, which is seen as indicative of full-thickness destruction of the skin. Based on their findings, the authors recommended **classification/labelling with C;R34** (**'causes burns'**; Research and Consulting Co. Ltd., Pfister, 1994a).

In an unpublished OECD TG 404 study, 0.5 mL coco alkyl amines ('Noram C'), a light brown clear liquid (purity 98.9 %), were semi-occlusively applied to the shaved skin of two New Zealand White rabbits for 3 minutes and 4 hours, respectively. Since clear effects were observed in both treatments, no additional animals were used. After a 3-minute contact, a well-defined and moderate erythema (grade 2 and 3) was recorded 24 and 48 hours post-exposure. No cutaneous necrosis was observed. After a 4-hour contact, a well-defined, severe erythema and severe oedema were noted 24 and 48 hours post-exposure. At the 48-hour reading, necrosis of the skin was observed. Apparently, these findings led the study authors to their conclusion to propose classification/labelling for corrosion. While no specific hazard phrase is proposed, this recommendation would best be translated into **C;R34 ('causes burns')**, as necrosis was only observed following exposure for 4 hours, but not after 3 minutes (Centre International de Toxicologie, 1999a).

In another unpublished OECD TG 404 study, 0.5 mL coco alkyl amines ('Genamin CC 100 D'), a clear, pale yellow liquid (purity approx. 99 %), were semi-occlusively applied to the shaved skin of six New Zealand White rabbits for 3 and 60 minutes (three animals/exposure). Both treatments caused moderate to severe erythema (24-72 hours average scores for the three animals at 3 min contact were 1.7/2.0/2.0) and oedema (2.7/1.7/1.0). Eschar, scabbing, and necroses were observed up to 14 days and subsequent scarring was noted up to the end of the observation period of 21 days. Some animals in the 1-hour exposure-group displayed open wounds. The study authors concluded that based on their results classification/labelling with **C;R35 ('causes severe burns')** was warranted (Hoechst AG, 1984).

In a further unpublished OECD TG 404 study with minor modifications (decontamination procedure, cf. below), 0.5 mL coco alkyl amines ('Cesio 5'), a clear, straw-coloured liquid (purity not given), were semi-occlusively applied to the shaved skin of six New Zealand White Rabbits for 3 minutes. The rabbits were treated on their left and right flanks. After 3 minutes of contact, the left hand treatment site was washed by gentle swabbing with cotton wool soaked in sterile distilled water; the right hand treatment site was decontaminated using 3 % (w/v) aqueous acetic acid followed by distilled water. Slight haemorrhages prevented scoring of erythema between 24-72 hours (degree: moderate to well-defined). 24-72 hours average scores for oedema were 3.8/3.3/2.5. Scar tissue, indicative of dermal corrosion, was noted at three treated skin sites. Severe dermal responses were noted at all remaining skin sites. The study author concluded that **classification/labelling with C;R35 ('causes severe burns')** was appropriate (Safepharm Laboratories Ltd., 1989).

In another unpublished OECD TG 404 study with deviations (contact was 1 hour compared to 4 hours in a regular study), 0.5 mL coco alkyl amines ('Amine KK'), a clear, straw-coloured liquid (purity not given), were semi-occlusively applied to the shaved skin of three New Zealand White Rabbits for 3 minutes and 1 hour. The rabbits were treated at their left and right flanks. After 3 minutes of contact, the first treatment site was washed by gentle swabbing with cotton wool soaked

with 3 % aqueous acetic acid, followed by decontamination performed with sterile distilled water. After 1 hour of treatment, the same procedure was repeated at the second treatment site. 24, 48, and 72 hours later, evaluations were performed. After 1 hour of contact, blanching of the skin and areas of brown discolouration of the epidermis were noted at all treated skin sites. Eschar, sometimes surrounded by blanching and/or well-defined erythema, was noted at all treated skin sites at subsequent 24, 48, and 72-hour observations. On day 7, eschar persisted at all treated sites, but at one site appeared sunken and was becoming detached to underlying tissues. On day 14, two small areas of blood-stained tissue persisted at the treated skin site of the two remaining rabbits. Desquamation, glossy skin, and lack of fur growth were also noted at this time. Average scores for erythema were 4.0 between 1 hour and 7 days and for oedema, values for 1/24/48/72 hours/7 days were 3.0/4.0/3.7/2.0/0.7, respectively. Severe irritation, but not corrosivity, was noted after a 3-minute contact. Consequently, the study authors concluded that **classification/labelling with C;R34 ('causes burns')** should be assigned (Safepharm Laboratories Ltd., 1987).

In an unpublished non-GLP pre-OECD TG 404 study, 0.5 g coco alkyl amines ('Armeen C'), a 'partly solid and partly liquid' slightly brown-coloured substance (purity not given) were applied occlusively for 4 hours to the shaved skin of six New Zealand White Rabbits under a 1 inch x 1 inch surgical patch. Examination of the treated skin sites was performed 4 and 48 hours after patch removal. After 4 hours, severe ischaemia, moderate oedema and brown discolouration of the treated skin were observed. After 48 hours, severe necrosis (incrustation) was noted. Scores for erythema/oedema were 4.0/3.0 both after 4 and 48 hours. Comparable results were obtained for a different batch of the test substance. The study author concluded that the test substance has to be 'considered corrosive'. However, due to deficits in experimental design and reporting, this study report **does not allow for a clear distinction between irritancy and corrosivity with respect** to the criteria laid down in Annex VI to Dir. 67/548/EEC or Reg. (EC) No. 1272/2008 (TNO, 1979).

In a non-guideline-compliant study, concentrations of 0.1-50 % lauramine (the major C_{12} component of coco alkyl amines; no further information on the substances identity given) were applied to the back of three hairless mice under occlusive patches. In addition, rabbits were also investigated (an "FDA method" is referred to, but no details are given). Strong irritation and necrosis occurred at concentrations of 0.5 % and above, presumably in both species. Due to strong deficiencies in reporting on methodology and obtained results, the study is considered unsuitable as a basis for classification/labelling (Iwata et al., 1987).

5.3.1.2 Amines, tallow alkyl

In an unpublished OECD TG 404 study with deviations (regular exposure time shortened from 4 hours to 1 hour), 0.5 g tallow alkyl amines ('Farmin T'), a white to yellow 'hard paste' (purity 96 %), were applied semi-occlusively for 3 minutes and 1 hour to the shaved skin of one male and two female New Zealand White rabbits, respectively. Prior to application, a pH value in the range of 6 of the undiluted test article was determined with a test strip. The substance was applied as delivered by the sponsor to the animals' right flank. Since no effects were observed after 3 minutes, exposure was repeated. After approximately 1 hour, the dressing was removed and the skin was rinsed with lukewarm tap water to clean the application site. The skin was examined 1, 24, 48 and 72 hours as well as 7, 14, and 21 days after removal of the dressing. Average skin irritation scores for erythema/oedema after 1, 4, 24, 48, 72 hours were: 0.33/1.0, 0.67/1.33, 2.0/3.0, 1.33/1.33, 1.0/1.33; no erythema/oedema remained after 7 days. Corrosive effects, i. e. formation of eschar, scabbing and subsequent scarring, were noted at the application sites of all animals. The study author concluded that the test substance should be assigned **classification/labelling with C;R34 ('causes burns')** (Research and Consulting Co. Ltd., 1994c).

In yet another unpublished OECD TG 404 study, 0.5 g tallow alkyl amines ('Genamin TA 100 D'), a whitish wax (purity 99-100 %), were applied semi-occlusively for 3 minutes or 4 hours to the shaved skin of three or one New Zealand White rabbits, respectively. The substance as delivered by the sponsor was applied to the animals' right flank. The skin was examined 30 minutes, 1, 24, 48, and 72 hours after removal of the dressing. Since effects were still present after 72 hours, additional examinations were performed after 7 and 14 days. Results for a three-minute contact (3 animals): From 30 minutes to 14 days after patch removal, treated skin areas showed mild to moderate erythema and mild to strong oedema. Between 24 hours and 14 days, the treated skin areas were sclerotic, vaulted, brownish discoloured, cracked, dry-brittle, scaly, translucent and pink-coloured. Additionally scaling was observed. After 7 days, all animals showed scars. Due to the severity of effects, scoring of erythema and oedema was restricted. Results for a 4-hour contact (1 animal): From 30 minutes to 7 days after application of the test substance, the skin was temporarily sclerotic, vaulted, encrusted, scabby, white and brown-white discoloured. Additionally, exudations were observed. Full thickness destruction of the epidermis had occurred after 7 days and the animal was killed for humane reasons on day 7. Due to the severity of effects, scoring of erythema and oedema was restricted. The study authors concluded that based on the obtained results, **classification/labelling with C;R35 ('causes severe burns')** was warranted (Hoechst AG, 1988j).

In a further unpublished OECD TG 404 study with minor modifications (decontamination procedure, cf. below), 0.5 mL tallow alkyl amines ('Cesio 10'), a colourless liquid (purity not given), were applied semi-occlusively for 3 minutes to the shaved skin of six New Zealand White rabbits, as provided by the sponsor. The rabbits were treated at their left and right flanks. After 3 minutes of contact, the left hand treatment site was washed by gentle swabbing with cotton wool soaked in sterile distilled water; the right hand treatment site was decontaminated using 3 % (w/v) aqueous acetic acid followed by distilled water. The skin was examined 1, 24, 48, and 72 hours after removal of the dressing. Additional examinations were performed after 7 and 14 days. Corrosivity was noted in 3/6 animals after water decontamination. Due to the severity of effects, erythema and oedema could not be evaluated quantitatively. Between 24 and 72 hours, blanching of skin was noted. Moderate to well-defined erythema surrounded the treatment site. On day 7, light brown coloured scabs and dark-brown hard scabs appeared. On day 14, desquamation, keratinolysis, scattered scabs, reduced growth of fur and thickening of dermal tissues were noted. Effects were comparable for both decontamination procedures. The study author concluded that **classification/labelling with C;R35 ('causes severe burns')** was appropriate.

In the same study, 0.5 mL tallow alkyl amines of another alkyl amine mixture ('Cesio 11'), an off-white coloured liquid (purity not given), was applied semi-occlusively for 3 minutes to the shaved skin of six New Zealand White rabbits. Prior to application, the test substance was moistened with water to enhance skin contact. The rabbits were treated at their left and right flanks. After 3 minutes of contact, the left hand treatment site was washed by gentle swabbing with cotton wool soaked in sterile distilled water; the right hand treatment site was decontaminated using 3 % (w/v) aqueous acetic acid followed by distilled water. The skin was examined 1, 24, 48 and 72 hours after removal of the dressing. Additional examinations were performed after 7 and 14 days. Corrosivity was noted in 4/6 animals. Due to the severity of effects, erythema could not be evaluated. Between 24 and 72 hours, blanching of skin was noted. Moderate to well-defined erythema surrounded the treatment site. On day 7, dark brown, hard scabs appeared. On day 14, scattered scabs, reduced growth of fur and thickening of dermal tissues were noted. Corrosivity was noted in 4/6 animals. Average scores for oedema (decontamination with water) were 4.0 (24 hours), 3.5 (48 hours) and 3.2 (72 hours). Results of both decontamination procedures were comparable. Again, the study author concluded that **classification/labelling with C;R35 ('causes severe burns')** was appropriate (Safepharm Laboratories Ltd., 1989).

In another unpublished, non-GLP pre-OECD TG 404 study, 0.5 g tallow alkyl amines ('Armeen TD'), a white waxy substance (purity not given), were applied occlusively for 4 hours to the shaved skin of six New Zealand White Rabbits under a 1 inch x 1 inch surgical patch. Examination of treated skin sites were performed 4 and 48 hours after patch removal. After 4 hours, distinct ischaemia and moderate oedema were observed. After 48 hours, severe necrosis (incrustation) was noted. The study author concluded that the test substance has to be 'considered corrosive'. However, due to deficits in experimental design and reporting, this study report **does not allow for a clear distinction between irritancy and corrosivity** with respect to the criteria laid down in Annex VI to Dir. 67/548/EEC or Reg. (EC) No. 1272/2008 (TNO, 1979).

In an unpublished, non-GLP, and non-guideline skin irritation study, 0.1 g tallow alkyl amine ('Genamin TA 100 D'), a white waxy substance (purity not given) were applied for 72 h to the shaved skin (3 x 3 cm, application on right flank, type of patch not reported, apparently non-occluded) of 2 x 6 Himalayan White Rabbits. Examination of treated skin sites was performed 24 and 72 hours after start of treatment. After 72 hours, corrosivity was observed (no detailed signs reported). Average scores for erythema/oedema in all 12 animals combined were 3.9/2.3 after 24 hours and 4.0/1.9 after 72 hours. The results were described as **'strong irritation'** without further details. Due to strong deficiencies in reporting on methodology and obtained results, the study is considered unsuitable as a basis for classification/labelling (Hoechst AG, 1977).

5.3.1.3 Amines, hydrogenated tallow alkyl

In an unpublished OECD TG 404 study, 0.5 g hydrogenated tallow alkyl amines ('Lilamin AC-HBG-P'), a white granular solid (purity not given), were applied semi-occlusively for 4 hours to the shaved skin of three New Zealand White Rabbits. The test substance was moistened with 0.5 mL water and applied to a 2.5 cm^2 gauze patch. Examination of treated skin was carried out approx. 30 minutes after patch removal and on days 2, 3, and 4. Additional observations were performed on days 5 through 11. Well-defined erythema and oedema were observed in all three animals. A slight increase in the level of reaction was noted in two animals during days 4 and 5. The reactions abated by the beginning of the second week and by day 11, all skins were normal. Hyperkeratinisation was seen in one animal on day 5. The average score for erythema was 2.0 each at 24, 48, and 72 hours. Scores for oedema were 2.0, 2.3, and 2.3 for the same time points. The observed results call for **classification/labelling with Xi;R38 'irritating to skin'** (Huntingdon Research Centre, 1984).

In another unpublished, non-GLP study similar to OECD TG 404, 0.5 g hydrogenated tallow alkyl amine ('Armeen HT'), a white granular solid (purity not given), was applied for 4 hours to the shaved skin of three New Zealand White Rabbits. The test substance was moistened with 0.5 mL water applied to a 2.5 cm^2 gauze patch. Following transfer to the shaved skin, the patch was covered with sleek plaster. After removal of the patch, the treated skin area was washed with water to remove excess sample. Average scores for erythema/oedema evaluated after 24, 48, and 72 hours were 1.0/1.0, 1.0/0.5 and 1.0/0.5, respectively. No signs of corrosivity were present 72 hours after patch removal (Huntingdon Research Centre, 1982).

In an unpublished, non-GLP study similar to OECD TG 404, 0.5 g hydrogenated tallow alkyl amine ('Fatty Amine 73/312', 'Armeen HT'), a beige solid (purity not given, pH-value of the liquefied substance = 8.5), was applied for 4 hours to the shaved skin of three New Zealand White Rabbits. The test substance, liquefied after warming, was applied to a 2.5 cm^2 gauze patch. Immediately after removing the patches, well-defined erythema and slight oedema were observed in all animals. Until 5 days after patch removal, these symptoms had increased, but eased off until day 14. Average scores for erythema/oedema were 2.0/2.0 (1 h), 2.0/2.7 (24 h) 2.3/3.3 (48 h), and 3.7/3.7 (72 h). Beginning 3 days after patch removal, a slight exfoliation and partly a decreased growth of hair

were recorded. By the end of the observation period of 14 days, partial exfoliation was still present, but the growth of hair became normal again. No necrosis was noted throughout this study. The observed results call for **classification/labelling with Xi;R38 'irritating to skin'** (IBR Forschungs GmbH, 1982).

In another unpublished OECD TG 404 study, hydrogenated tallow alkyl amines ('Farmin TH'), a white solid (pH=9.93, purity not given), was applied semi-occlusively for 4 hours to the shaved skin of three New Zealand White Rabbits. 0.5 g of the test substance, wetted with water, were applied on 2.5 cm^2 surgical gauze and applied to the shaved backs of the rabbits. The average score for erythema was 2.0 at all time-points (24, 48, and 72 hours). Scores for oedema were 2.3, 0.7 and 0.0 for the same time points. Erythema was still present after 7 days, but reversed within 14 days. As concluded by the study authors, the observed results call for **classification/labelling with Xi;R38 'irritating to skin'** (Centro de Investigacion Y Desarollo Aplicado, S.A.L., 1995b).

In a further unpublished GLP-compliant skin irritation study based on OECD TG 404, 0.5 mL of hydrogenated tallow alkyl amines ('Amin HBG'), a white granular solid, (purity not given), was applied semi-occlusively for 4 hours to the shaved skin of three New Zealand White Rabbits. In a pre-test, no corrosivity was observed after exposure for 3 minute or 1 hour, respectively. Well-defined erythema was observed at all treated skin sites one hour after patch removal. A slight haemorrhage of the dermal capillaries was also noted at one treated skin site at this time. Well-defined erythema and a brown discolouration of the skin were noted at all treated skin sites at the 24 and 48 hour observations, and at two sites after 72 hours. A slight haemorrhage of the dermal capillaries and loss of skin suppleness was noted at one of the treatment sites after 24 and 48 hours. Loss of skin suppleness was noted in one animal after 72 hours. On day 7, dry, thickened, straw-coloured skin (possible hyperkeratinisation) and loss of skin suppleness were observed in two animals. Glossy skin, reduced fur growth, and small scattered scabs were noted in the remaining animal. The average score for erythema was 2.0 at all time-points (24, 48, and 72 hours). Scores for oedema were 2.3, 1.7, and 1.7 for the same time points. Due to the severity of skin reactions, reversibility of erythema could not be assessed. Oedema reversed within 7 days. As concluded by the study authors, the observed results call for **classification/labelling with Xi;R38 'irritating to skin'** (Safepharm Laboratories Ltd., 1987).

In an unpublished OECD TG 404 study with minor modifications (decontamination procedure, cf. below), 0.5 mL hydrogenated tallow alkyl amines ('Cesio 12'), a white flaky solid (purity not given), was applied semi-occlusively for 3 minutes or 1 hour to the shaved skin of six New Zealand White rabbits. Prior to application, the test substance was moistened with water to enhance skin contact. The rabbits were treated at their left and right flanks. After 3 minutes or 1 hour of contact, the left hand treatment site was washed by gentle swabbing with cotton wool soaked in sterile distilled water; the right hand treatment site was decontaminated using 3 (w/v) aqueous acetic acid followed by distilled water. The skin was examined 1, 24, 48, and 72 hours after removal of the dressing. Additional examinations were performed after 7 and 14 days. No evidence of skin irritation was noted for the 3-minute contact (Draize Scores 0 for all time points, water decontamination). Very slight erythema responses were noted for the 1-hour contact. In addition, desquamation was observed in 5/6 animals after 7 days. The average score for erythema (decontamination with water) was 1.0 at 24, 48, 72 hours, respectively. The score for oedema was 0.0 for the same time points. Results of both decontamination procedures were comparable. The author of this study concluded that based on the obtained results, **no classification/labelling for skin irritation was necessary** (Safepharm Laboratories Ltd., 1989).

In an unpublished, non-GLP-compliant skin irritation study according to OECD TG 404, 0.5 mL of hydrogenated tallow alkyl amines ('Amin HBG'), a semi-solid beige-coloured paste (purity not given), was applied as supplied to the skin of six New Zealand White Rabbits (males and females).

The test item was applied to two test sites on each rabbit, one with shaven, the other with abraded skin. Readings were performed at 24 and 72 hours only: mean Draize scores at 24 hours were 1.0/2.0 for erythema and oedema were 1.2/2.0 for shaven/abraded skin, respectively. After 72 hours, scores for erythema (1.0/1.5) and oedema (0.9/1.7) were the same or only slightly less. The results are **not in disagreement with classification/labelling for skin irritation**, but no definite conclusions can be drawn, as no reading has been performed at 48 hours and no statement on the potential reversibility of the observed effects can be given. For these reasons, the study is considered unsuitable as a basis for classification/labelling (Inveresk Research International, 1979).

In an unpublished non-GLP, pre-OECD TG 404 study, 0.5 g hydrogenated tallow alkyl amines ('Armeen HT'), a brownish waxy substance (purity not given) was applied occlusively for 4 hours to the shaved skin of six New Zealand White Rabbits under a 1 inch x 1 inch surgical patch. Examination of treated skin sites were performed 4 and 48 hours after patch removal. After 4 hours, well-defined erythema and very slight to slight oedema were observed. After 48 hours, distinct ischaemia and slight to distinct necrosis were noted. The study author concluded that the test substance 'caused visible tissue destruction (necrosis) when brought into contact with the skin for four hours and are therefore considered to be corrosive'**.** However, due to deficits in experimental design and reporting, this study report **does not allow for a clear distinction between irritancy and corrosivity with respect** to the criteria laid down in Annex VI to Dir. 67/548/EEC or Reg. (EC) No. 1272/2008. For these reasons, the study is considered unsuitable as a basis for classification/labelling (TNO, 1979).

5.3.1.4 Octadecylamine

In an unpublished OECD TG 404 study, 0.5 g octadecylamine ('Genamin 18 R 100 D'), a whitish waxy solid (purity approximately 100 %), were applied semi-occlusively for 4 hours to the shaved skin of three New Zealand White Rabbits. The substance was moistened with 0.4 mL polyethylene glycol 400. Examination of the treated skin was started approx. 30 minutes after patch removal and continued on days 2, 3, and 4. Additional observations were performed on days 7, 14, 21, and 28. From 30-60 minutes up to 7 days after patch-removal, treated skin areas showed pronounced erythema and slight to moderate oedema. The average score for erythema was 3.0 at 24, 48, and 72 hours. Scores for oedema were 2.7, 1.3, and 1.3 for the same time-points. Over time, skin was frequently dried and brittle, crusty, and cracked. Additionally, indurations and desquamation were observed. Effects were reversible after 14 days in one rabbit and 28 days in two rabbits. The obtained results call for **classification/labelling with Xi;R38 ('irritant to skin'**; Hoechst AG, 1989b).

In another unpublished OECD TG 404 study, octadecylamine ('Noram SH'), a yellow-beige solid (purity not given), was semi-occlusively applied for 4 hours to the shaved skin of three New Zealand White Rabbits. The test substance was moistened with water to ensure good skin contact and test substance remaining after patch removal was removed with water. Average scores for erythema were 2.0 (24 hours), 1.7 (48 hours), and 1.7 (72 hours). Scores for oedema were 2.3, 1.7, and 1.3 for the same time points. Erythema cleared by day 10 and oedema by day 9. Correspondigly the **results demonstrate a moderate irritation potential, slightly below the threshold for classification/labelling** (Centre International Toxicologie, 1986).

In yet another unpublished OECD TG 404 study (short report without details, GLP-compliance not stated), 0.5 g octadecylamine ('Genamin SH 100 D', purity approximately 100 %) was applied for 4 hours to the shaved skin of New Zealand White rabbits. No further details are presented. As a result, the authors concluded that the test item should be **classified/labelled as 'R38' ('irritant to skin')**. Though supporting the classification proposal made in this CLH report, the study is considered

unsuitable as a basis for classification/labelling due to strong deficiencies in reporting (Hoechst AG, 1988p).

In an unpublished, pre-guideline study, octadecylamine ('stearylamine') was applied to the shaved ventral skin of rabbits (no further details provided). 'Vigorous' skin reactions in the form of erythema, oedema, and necrosis were noted. Treated skin areas were sloughed off after 14-21 days. Though supporting the classification proposal made in this CLH report, the study is considered unsuitable as a basis for classification/labelling due to strong deficiencies in reporting (Hoechst AG, 1932).

In a further (unpublished) pre-guideline study, 10 % octadecylamine ('stearylamine in olive oil') was repeatedly applied to shaved guinea pig skin. After the third treatment, the skin showed a brown colour and was thickened. Histology revealed full thickness destruction of the epidermis, but not of the skin as a whole. Though supporting the classification proposal made in this CLH report, the study is considered unsuitable as a basis for classification/labelling due to strong deficiencies in reporting (Gewerbehygienisches I.G. Laboratorium, 1934).

Finally, in an *in vitro* study according to OECD guideline 430 (Transcutaneous electrical resistance assay, TER), octadecylamine ('stearylamine') was classified as 'skin corrosive'. In this publication, octadecylamine is rated as a weaker corrosive (UNESCO packing group III) *in vivo* than coco and tallow alkyl amines (both UNESCO packing group I), but supporting experimental data are not provided. Though supporting the classification proposal made in this CLH report, the study is considered unsuitable as a basis for classification/labelling due to strong deficiencies in reporting and uncertainty associated with the/lack of transferability of the results to the *in vivo* setting (Oliver et al., 1988).

5.3.1.5 (Z)-Octadec-9-enylamine

In an unpublished OECD TG 404 study, 0.5 mL octadecenylamine ('Noram O', oleylamine), a light brown, translucent, and pasty liquid (purity 97.7 %), were applied semi-occlusively for 3 minutes or 4 hours, respectively, to the closely-clipped skin of one male New Zealand White Rabbit. The substance was applied as delivered by the sponsor onto a 6 cm^2 gauze pad, which was then applied to the right flank. The skin was examined 1 and 24 hours after application. Following the three minute-exposure, well-defined or moderate erythema, but no necrosis was noted on days 1 and 2, respectively. After exposure for four hours, average Draize scores at 24 hours were 4.0 for both erythema and oedema. At the same time necrosis was noted and the study was terminated for humane reasons. The author concluded that the test substance should be considered corrosive; the obtained results call for **classification/labelling with C;R34 ('causes burns';** Centre International Toxicologie, 1999b).

In another well-conducted, unpublished OECD TG 404 study, 0.5 mL octadecenylamine ('Farmin O', a pale, yellow liquid of 100 % purity), were applied semi-occlusively for 4 hours to the shaved skin of three New Zealand White Rabbits (1 male, 2 females). The substance was applied as delivered by the sponsor. Prior to application, the pH value was determined with a test-strip and was found to be 10. No systemic symptoms were observed. Average Draize scores after 24, 48, and 72 hours for erythema and oedema were 4.0, 3.7 and 3.7. Corrosive effects (eschar, scabbing, and scarring) were noted at the application sites of all animals, but not already at the 3 minute reading. Consequently, the study authors concluded on **classification/labelling with C;R34 ('causes burns'**; Research and Consulting Company Ltd., 1994b).

In a further unpublished, non-guideline-compliant study, octadecenylamine ('oleylamine') was applied to the shaved ventral skin of rabbits (no further details provided). 'Vigorous' skin reactions

in the form of erythema, oedema, and necrosis were noted, reportedly even more intense than those observed for otadecylamine in the same experiment. Treated skin areas were sloughed off after 14-21 days. Though supporting the classification proposal made in this CLH report, the study is considered unsuitable as a basis for classification/labelling due to strong deficiencies in reporting (Hoechst AG, 1932).

5.3.2 Eye

Valid, test guideline-conform data are only available for the amine mixtures predominantly consisting of unsaturated primary alkyl amines, i. e. hydrogenated tallow amines and octadecyl-amine. For coco alkyl amines, tallow alkyl amines and (Z)-octadec-9-enylamine, equivalent data have not been generated. However, this does not constitute a data gap, as classification/labelling for eye damage is implicit with that for corrosion proposed for these three compounds.

5.3.2.1 Amines, coco alkyl

No data available. Nevertheless, as it is proposed in the frame of the present report to classify/label coco alkyl amines for corrosivity (and thus implicitly for severe eye damage), no further testing is needed.

5.3.2.2 Amines, tallow alkyl

In a non-GLP, pre-OECD TG 405 eye irritation study, 0.1 mL of a 10 % solution of tallow alkyl amine ('Genamin TA 100 D'), a white waxy substance (purity not given) diluted in sesame oil, was applied for 20 seconds (left eye, rinsing with PEG 400 or borate buffer) and unlimited (right eye) to the eyes of six Himalayan White Rabbits. Examinations of the treated eyes were performed after 1, 7, 24, 72 hours and 7 days after start of treatment. After 72 hours, corrosivity was observed (no detailed signs reported). Average scores for erythema/chemosis were 2.4/2.0 between 1 and 72 hours and 0.2/0.0 after 7 days, and for cornea opacity 1.1 between 1 and 72 hours and 1.5 after 7 days. **Due to the limited observation period, no conclusions on corrosivity or other persistent effects can be drawn.** As a consequence of the deficiencies in methodology and reporting, the study is considered unsuitable as a basis for classification/labelling (Hoechst AG, 1977). Nevertheless, as it is proposed in the frame of the present report to classify/label tallow alkyl amines for corrosivity (and thus implicitly for severe eye damage), no further testing is needed.

5.3.2.3 Amines, hydrogenated tallow alkyl

In an OECD TG 405 study, 100 mg hydrogenated tallow alkyl amines ('Farmin TH'), a white solid (pH = 9.93, purity not given), were applied to the eyes of three New Zealand White rabbits. Pronounced conjunctiva redness and chemosis were observed throughout the entire observation period. Additionally, severe cornea opacity and moderate iris lesions were noted. The mean Draize score for conjunctiva redness was 3.0 at 24, 48, and 72 hours. Mean scores for conjunctiva chemosis were 3.0, 3.0 and 3.3 for the same time points. These effects were not reversible within the observation period of 21 days. Related scores for iris were 1.0, 1.0 and 1.0, reversible between 14 and 21 days. Scores for corneal opacity were 3.0, 3.3, and 3.7, respectively, and showed a value of 2.0 at the end of the observation period. Based on the obtained study results, the study authors concluded that **classification/labelling with Xi;R41 ('risk of serious damage to eyes')** should be assigned to the test substance (Centro de Investigacion Y Desarollo Aplicado, S.A.L., 1995c).

5.3.2.4 Octadecylamine

In an OECD TG 405 study, 0.1 mL octadecylamine ('Genamin 18 R 100 D'), a whitish waxy solid (purity approximately 100 %), were applied to the eye of one New Zealand White rabbit. 24 hours after treatment, the eye was washed with 0.9 % tepid NaCl solution. From 1 hour on, the conjunctiva showed red beefy discolouration and pronounced chemosis. Iris and cornea showed lesions. Additionally, a clear colourless to whitish, slimy discharge was noted. Due to progressed vascularisation of the eye, the study was terminated after 7 days. Mean Draize scores were 3.0/3.0/1.0/2.0 for conjunctiva redness/chemosis/iris lesions/corneal opacity, respectively, at each of the observation time points (i. e. 24, 48, and 72 hours). As a result of this test, the study authors concluded that **classification/labelling with Xi;R41 ('risk of serious damage to eyes')** is warranted for octadecylamine (Hoechst AG, 1989c).

In an OECD TG 405 study (short report, GLP-compliance not stated), 100 mg octadecylamine ("Genamin SH 100 D") was applied to the eye of one New Zealand White rabbit. No details were given, but as a result, the necessity of **classification/labelling with Xi;R41 ('risk of serious damage to eyes')** was concluded by the authors. Though supporting the classification proposal made in this CLH report, the study is considered unsuitable as a basis for classification/labelling due to strong deficiencies in reporting (Hoechst AG, 1988q).

5.3.2.5 (Z)-Octadec-9-enylamine

No data available. Nevertheless, as it is proposed in the frame of the present report to classify/label (Z)-octadec-9-enylamine for corrosivity (and thus implicitly for severe eye damage), no further testing is needed.

5.3.3 Respiratory tract

No human or specific animal data are available on respiratory tract irritation of the alkyl amines assessed in this report. It is noted that due to the low vapour pressure of the amine mixtures under investigation, exposure towards vapours is presumably low to negligible at room temperature. However, the situation might be different for scenarios in which exposure to aerosols or dust can be anticipated.

For the following reasons it is therefore proposed to **classify/label all of the amine mixtures covered by this report for respiratory irritation**:

- Corrosivity or strong irritancy towards skin and mucous membranes has been proven for all substances under investigation.
- In an acute inhalation toxicity study with coco alkyl amines, irritation of the airways was observed along with slight histological changes at a concentration of only 0.099 mg test substance/L ambient air (cf. section 5.2.2.1).
- A number of (shorter chain) aliphatic amines have been classified as respiratory irritants in Annex VI of Reg. (EC) 1272/2008: corrosive substances such as hexamethylenediamine as well as ‘only’ skin and/or eye irritants such as mono-, di-, and trimethylamine, ethylamine, and isopropylamine. This is seen as a proof that corrosivity is not a necessary requirement for respiratory irritation.
- Moreover, there is a plausible mode of action as regards cytotoxicity of the primary alkyl amines in this report: aside from their irritant/corrosive properties based on alkalinity, these substances constitute cationic surfactants under physiological conditions. Thus, it appears

plausible that they could also interfere with physiological processes of the respiratory system, e. g. alveolar gas exchange, due to their surface-active properties.

- Oral repeat-dose studies have frequently resulted in severe lung damage of unclear genesis, in particular of the bronchoalveolar region, for which a relationship to treatment could neither be confirmed nor ruled out. The weight of evidence for an aspiration hazard was evaluated and found to be inconclusive (cf. above). However, toxicokinetic investigations (cf. section 5.1.1) have demonstrated systemic distribution of aliphatic amines, with concentrations in lung tissue increasing with chain length. It might thus be speculated that these findings could be attributable to a surfactant effect of the administered amines following systemic uptake. This could then be seen as an additional argument that aliphatic amines are able to exert irritant effects on the respiratory system, if they reach the respective target tissue. The latter – as mentioned above – can be assumed for situations were humans might become exposed to aerosols containing these substances.

5.3.4 Summary and discussion of irritation

There are no human data available on skin, eye, and respiratory tract irritation of the alkyl amines assessed in this report.

5.3.4.1 Skin irritation

From the available animal tests, it is concluded that the three primary amine mixtures containing significant amounts of unsaturated amines have to be classified/labelled as **corrosive** (**coco alkyl. tallow alkyl: C;R35/Skin Corr 1A; H314**, **(Z)-octadec-9-enylamine; C;R34/Skin Corr 1B**), while for the other two amines (**hydrogenated tallow and octadecylamine**), classification/labelling as **Xi;R38/Skin Irrit. 2; H315** is warranted.

Again, it is left to speculation whether the difference in bioactivity of the 'saturated' vs. 'unsaturated' amines can be explained in terms of an altered bioavailability, by direct reactivity of the double bond(s), or by metabolic toxification (cf. introduction to this chapter and section 5.1).

5.3.4.2 Eye irritation

All available experimental data for eye irritation demonstrated the need for **classification/labelling with Xi:R41/Eye Dam 1**. This classification/labelling must be explicitly stated for hydrogenated tallow amines and octadecylamine, while for the other three amine mixtures, it is implicitly covered by the C&L for corrosivity.

5.3.4.3 Respiratory irritation

In contrast to eye irritation, C & L for respiratory irritation is not implicit with C & L for corrosivity both under Dir. 67/548/EEC and Reg. (EC) No. 1272/2008.

No human data and no specific animal tests for respiratory irritation of the primary amine mixtures are available. However, based on general knowledge as well as on a synopsis of data from acute and repeat-dose studies it is proposed to **classify/label all of the amine mixtures** covered by this report for respiratory irritation, i. e. as/with **Xi;R37 ('irritating to respiratory system')** or **STOT SE 3;H335 ('may cause respiratory irritation')**, respectively.

5.4 Corrosivity

Cf. previous section on irritation.

5.5 Sensitisation

5.5.1 Skin

5.5.1.1 Human data

In Ullmann´s Encyclopedia of Industrial Chemistry (2005), section on Fatty Amines, it is reported that ‘A small number of workers became sensitized to selected fatty amines. Symptoms included skin rash, dermatitis, eye swelling, and a sensation of the skin, described as “crawling”.’ On the request of the rapporteur to validate this information, no relevant data have been provided so far from the authors of this section and no other sources of information were found to substantiate this report. Therefore, this data is not considered further for hazard characterisation of the alkyl amine mixtures assessed in this report.

In a general study on the effects of anti-corrosives it is stated, that sensitisation is less frequent after contact to octadecylamine as compared to triethanolamine, monoethanolamine, and cyclohexyl-amine chromate (Selisskij et al., 1978). Since no details on method and results were provided, this statement cannot be used for hazard identification.

5.5.1.2 Animal data

Amines, coco alkyl

In a GLP-compliant OECD TG 406 skin sensitisation study according to Magnusson and Kligman (guinea pig maximisation test, GPMT), 10 female Dunkin Hartley guinea pigs + 5 control animals were treated with coco alkyl amines ("Genamin CC 100 D", technical grade, purity 99.9 %, liquid) using cotton seed oil as a vehicle. Based on the results of a pre-test, a test substance concentration of 0.1 % was used for intradermal induction (0.1 mL), followed by a 1 % concentration at epidermal induction. Challenge was performed using a 0.5 % substance concentration. For epidermal treatment, patches were loaded with 0.5 mL. In contrast to the results of the pre-test, no dermal irritation was observed after epidermal induction. After challenge treatment, 2/10 animals showed grade 1 erythema at the 24 h reading (positive rate = 20 %). One out of these positive animals showed scaling after 48 and 72 h. No second challenge treatment was performed (BSL, 2008).

These results do not allow for a clear conclusion, since the number of positive animals was borderline. While the test guideline 'strongly recommends' to test additional animals, if the result of a test using only 5 control animals and 10 animals in the treatment group is equivocal, such additional testing has not been performed in this study. Furthermore, since epidermal induction treatment using a 1 % substance concentration did not cause any signs of irritation, the performance of this study is not fully compliant with ORCD TG 406. **In summary, due to methodological deficiencies of this study, it does not allow for a clear decision on the potential of primary alkyl amine mixtures to cause skin sensitisation.**

Amines, tallow alkyl

No data available

Amines, hydrogenated tallow alkyl

In a non-GLP-compliant skin sensitisation study according to OECD TG 406 (Magnusson- Kligman GPMT), 10 female guinea pigs (strain Dunkin Hartley; 5 control animals) were treated with hydrogenated tallow alkyl amines ('Amin HBG'), a semisolid beige-coloured paste (purity not given). At the intradermal induction stage, 0.5 mL test substance emulsified with 0.05 mL Freund's adjuvant were injected s. c. (additional injections: 0.1 mL Freund's adjuvant alone + 0.1 mL test agent). Prior to injection, the test substance was diluted with water to 1 % w/w. Six days after injections, the skin at the treatment site was shaved once again and treated with 10 % sodium lauryl sulfate to provoke an inflammatory reaction. Twenty-four hours later, treatment sites were occlusively covered with a 2 x 2 cm filter patch, saturated with 5 % aqueous hydrogenated tallow alkyl amines for 48 hours (topical induction, volume not given). A concentration of 2 % hydrogenated tallow alkyl amines was selected for the final challenge. Fourteen days after the first challenge, the second challenge was performed (24 h closed patch exposure). No positive responses were observed in the 10 induced and rechallenged animals (readings not given; Inveresk Research International, 1979).

Since the test substance is nearly insoluble in water, it appears doubtful that reported nominal test concentrations of up to 10 % could have been achieved. In consequence, these study results **are not valid and cannot be used as a basis for classification/labelling**.

Octadecylamine

No data available

(Z)-Octadec-9-enylamine

No data available

5.5.2 Respiratory system

There are no data from humans or from animal testing on respiratory tract sensitisation caused by the alkyl amines assessed in this dossier.

5.5.3 Summary and discussion of sensitisation

The available experimental data for coco and hydrogenated tallow alkyl amines are either inconclusive or of insufficient validity, and thus do not allow for a conclusion on the skin sensitisation potential of the alkyl amines assessed in this dossier. At least for coco alkyl amines, skin reactions have been observed at a level slightly below, but borderline to the classification threshold, but an insufficient number of animals has been used in the respective test.

In summary, **no data on respiratory sensitisation are available**, while **the database is inconclusive with respect to skin sensitisation**. It is noted, that if new data were to be generated, the test substance should be one of the mixtures containing a significant amount of unsaturated fatty alkyl amines, as these compounds might show higher reactivity than their saturated analogues. It could then be considered justified to read-across the results to those mixtures exclusively or predominantly containing unsaturated fatty alkyl amines.

5.6 Repeated dose toxicity

Valid data are available for the oral route of exposure for tallow alkyl amines and for (Z)-octadec-9-enylamine indicating a toxic potential after repeated exposure. No further testing is required, since it is assumed that due to their structural similarity the other compounds assessed in this report would

display a similar toxic profile for this endpoint. Thus, it is proposed that all compounds assessed in this report are labelled uniformly.

5.6.1 Repeated dose toxicity: oral

5.6.1.1 Amines, coco alkyl

No data available

5.6.1.2 Amines, tallow alkyl

In an oral 28-day study (gavage, SD rats) tallow alkyl amines (GENAMIN TA 100) were administered in sesame oil (vehicle) to Sprague Dawley Crl:CD rats at dosages of 0, 12.5, 50, and 150 mg/kg bw/d once a day for 4 consecutive weeks (Istituto di Ricerche Biomediche, 2000a). The subacute test procedure was in accordance with OECD TG 407 except for the fact that all specific investigations on neurofunction were lacking.

At 12.5 mg/kg bw/d, no toxicologically relevant clinical signs occurred. At 50 mg/kg bw/d, salivation and piloerection were observed. One female died on day 11 showing lung changes as described for the high dose animals (see below). At 150 mg/kg bw/d two males and three females of the high dose group died between days 8 and 27. Animals at this dose level showed salivation after treatment, piloerection, hunched posture, fur loss, and soft stools.

There was a dose-related body weight decrease. For males, this reduction involved the intermediate and high dose group, i. e. -5 and -25 % vs. controls, respectively, while in females all treated groups were affected, i. e. – 5 %, - 12 %, - 21 % vs. controls. Food consumption during the administration period of animals treated with 50 and 150 mg/kg bw/d was generally lower than in controls. At 12.5 mg/kg bw/d, food consumption was comparable to that of the controls.

Haematology revealed a slight increase in erythrocyte counts at 150 mg/kg bw/d, which was accompanied by reduced values for MCV. A dose-related moderate to marked increase in leukocyte counts and a slight to moderate increase in platelet counts were recorded at 50 and 150 mg/kg bw/d. Leukocyte enhancement was generally accompanied by variations in white blood cell differential count, consisting of an increased percentage of neutrophils (at 50 mg/kg bw/d in males and at 150 mg/kg bw/d in both sexes).

Clinical chemistry tests showed a number of alterations. Toxicologically relevant changes consisted of markedly increased activities of ALAT and ASAT at 150 mg/kg bw/d. GGT activity was slightly increased in females of this dose group. The serum concentrations of protein and albumin and the A/G ratios were dose-dependently reduced in the 50 and 150 mg/kg bw/d groups. Compared to control levels, a decrease in total cholesterol in high dose females and increases in the urea level were observed in both sexes of this group.

Organ weight of almost all organs of the surviving high dose group animals showed statistically significant absolute and/or relative changes (most often a decrease in absolute but increase in relative organ weight). This was considered to be related to the growth depression in this group. There was, however, a decrease in thymus weight and an increase in adrenal weight histopathologically associated with cell atrophy or hyperplasia, respectively, thus interpreted as a direct effect of treatment. At the intermediate dose, there was a significant decrease in absolute and an increase in relative liver and kidney weight of males, indicating again a relation to general body weight loss.

The main finding with respect to gross pathology consisted of slight to moderate dilation of the intestinal tract in intermediate and high dose animals.

Histopathologically, premature decedents of the high dose group showed erosions in the gastric and small intestinal mucosa with marked accumulation of vacuolated histiocytes in mesenteric lymph nodes and in the lamina propria of the small intestine as well as epithelial hyperplasia in the mucosa. Histiocytic effects were more pronounced in the jejunum and ileum than in the duodenum. In the lung, abnormalities were mainly localised to the bronchi/bronchioli and consisted of inflammation associated with fibrosis and bronchial oedema.

Similar histopathological findings were observed in surviving animals of the intermediate dose group together with single cell necrosis and focal inflammation in the liver and areas of hyperplasia in the small intestinal mucosa as well as atrophy of the thymus and splenic follicle. In one male and one female there was a slight cortical hypertrophy of the adrenal glands.

At 50 mg/kg bw/d, signs indicative of thymus atrophy were seen in one male and one female. Histiocytic involvement in small intestine and mesenteric lymph nodes was still present to a moderate degree and also mucosal hyperplasia occurred in the intestine. The lungs of animals from this group displayed similar changes as in high dose animals.

At the low dose, histopathological changes were limited to slight histiocytic vacuolation in mesenteric lymph nodes and small intestine, without abnormal accumulation of histiocytes.

In conclusion, the primary adverse effects following repeated gavage administration into the stomach were gastrointestinal dilatation and erosions of the gastric and small intestine mucosa. Repeated exposure over time appears to exacerbate the gastrointestinal damage and the bad general health status and is suggested to be a possible cause of unscheduled deaths. Reduced food consumption, emaciation, mucosal hyperplasia, and leukocytosis were related effects. Histiocytosis and histiocytic vacuolation indicated that the test substance was absorbed and accumulated intracellularly in the lymphoid tissues of the intestine. Liver cell necrosis associated with inflammatory response and increased activities of liver enzymes demonstrated that the test substance also has a hepatotoxic potential. Elevated urea concentrations might point to minor renal toxicity. Follicle atrophy in the spleen as well as thymus atrophy indicated an immunosuppressive effect (most likely T-cell targeted). Since thymus atrophy was observed not only at 150 mg/kg bw/d, but also 50 mg/kg bw/d, a dose level at which no mucosa erosion was seen, it is concluded that immunosuppression was an independent adverse effect.

An increase in adrenal weight is in accordance with the observed hyperplasia of this gland, which may be a non-specific effect. Bronchiolar and peribronchiolar inflammatory and fibrotic changes could have their cause in an accidental aspiration of some test material in the course of gavage application; while in females, these effects were limited to the mid and high dose groups, in males no clear dose-relationship could be established, and also control animals were affected. On the other hand, similar effects were observed in repeat-dose studies of longer duration also after dietary uptake of fatty alkyl amines. The case pro/contra a generic aspiration hazard posed by the amines assessed in this report has already been discussed above (cf. section 5.2.4.1). Whether the lung effects contributed to premature mortality could not be clearly identified, but it does not appear completely unlikely given the fact that in the one death at 50 mg/kg bw/d, lung lesions were the only effect observed. Histiocytic vacuolation of the mesenteric lymph nodes and small intestine, observed to a slight degree at 12.5 mg/kg bw/d, occurred at all dose levels, increasing dose-dependently to higher severity and progressing in accumulation. Therefore, as a NOAEL could not be derived, the LOAEL was 12.5 mg/kg bw/d.

5.6.1.3 Amines, hydrogenated tallow alkyl

No standard test on repeated dose toxicity was available for hydrogenated tallow amines. Only few data from studies with limited study design were reported for a metabolite or components of the tallow amine:

One group of 10 Sprague-Dawley rats (5/sex) received a diet containing 3000 ppm of stearic acid (a metabolite of hydrogenated tallow amine, ca. 200 mg/kg bw/d based on a food consumption of 7 % per kg bw) for 209 days (Deichmann et al., 1958 cf. also below, section 5.6.1.4). Another group was fed with diet containing octadecylamine dissolved in corn oil, while a control group was not carried along. Food consumption and the weekly change of body weight were recorded. Gross and microscopic examinations were performed on 20 major organs/tissues. Two out of five female rats died spontaneously (no further details). Average weight gain was 12 g in males and 58 g in females, the animals were reported to be anorexic. Tissues from rats fed with stearic acid showed severe pulmonary infection consisting of tracheobronchitis, lobular pneumonia, lipoid histiocytic response, and abscess formation. Similar findings indicative of pneumonia were reported also for octadecylamine at 3000 ppm under the same study design, as well as for treated and control groups in another experiment reported in the same publication (in which octadecylamine was fed to rats at dose levels of 20-500 ppm). For a discussion of these findings, the reader is referred to section 5.6.1.4. In conclusion, keeping in mind also the findings in other repeat-dose studies with primary alkyl amines, a relationship of the observed lung damage to treatment can neither be confirmed nor ruled out with sufficient certainty. No histomorphological abnormalities were observed in the other organs. The authors concluded anorexia and increased mortalities to be substance-related toxic effects of stearic acid.

Comparative investigations on hypolipidaemic activity were conducted in rats receiving isolated components of hydrogenated tallow alkyl amines, i. e. octadecyl-, hexadecyl-, and tetradecylamine (Griffin et al., 1991):

Six male Sprague-Dawley rats each received 0, 8, or 20 mg/kg bw/d of tetradecyl-, hexadecyl- or octadecylamine (purities not further characterised, no data on vehicle) by gavage for 14 days. Liver, small intestine, aorta, and faeces (24 h collection) were removed, extracted and analysed for cholesterol (CL) levels, triglyceride (TG) levels, neutral lipid and phospholipid content. Blood was collected from the abdominal vein and lipoprotein fractions were obtained and analysed for CL, TG, neutral lipids, and protein levels.

Daily food consumption was significantly reduced in rats fed with tetra- and hexadecylamine, but without any clear effect on growth. Administration of octadecylamine led to a significant reduction of both daily food consumption and body weight gain. Significantly reduced concentrations of CL and TG were observed in serum for all three amines. In faeces, cholesterol and triglyceride concentrations were lower in tetradecyl- and hexadecylamine-treated rats, with statistical significance attained for TG by both compounds, but for CL only by tetradecylamine. In contrast, octadecylamine administration resulted also in significant reduction of cholesterol- and triglyceride concentrations significantly increased concentrations of triglycerides in faeces. In several analysed compartments, phospholipid levels were statistically significantly changed by treatment in the following manner: in the liver they were decreased by tetra- and increased by octadecylamine, in the small intestine, an increase was noted for all three amines, in the aorta an increase by tetra- and hexadecylamine was observed, and in faeces levels were decreased by tetra- and hexa-, but increased by octadecylamine. For octadecylamine, no difference in organ weight, histopathology of the liver, kidney, and spleen, and in clinical chemistry was found between treated and untreated animals. No data on organ weights or (histo)morphology were available for tetradecyl- and hexadecylamine.

In the same publication, octadecylamine significantly reduced activities of several hepatic enzymes involved in the early de novo synthesis of fatty acid and cholesterol in the liver (these experiments were not performed for tetra- and hexadecylamine).

In conclusion, a consistent finding with all three alkyl amine constituents of hydrogenated tallow amine was the hypolipidaemic effect on serum triglycerides and cholesterol in rats.

5.6.1.4 Octadecylamine

No repeat-dose toxicity study compliant with present test protocol standards is available.

Rat

Two male Sprague-Dawley rats were fed with a diet containing 1 % octadecylamine sulfate (about 700 mg/kg bw/d) (Carroll, 1960). Within six days, body weight was reduced by up to 30 % and some animals died (no other information available). In preliminary studies, the effect of 1-octadecylamine sulfate on the growth of male and female rats was estimated at dietary concentrations of 0, 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, and 1.0 % 1-octadecylamine sulfate (Parenteau et al., 1991). All test concentrations of 0.01 % (ca. 7 mg/kg bw/d) and above produced concentration-related lower body weight gain as compared to that of the controls by day 16. At concentrations of 0.5 and 1.0 %, body weight was progressively reduced reaching maximal losses of about 40-60 % on days 8-11.

Six male Sprague-Dawley rats each were administered 0 or 8 mg/kg bw/d of octadecylamine (Griffin et al., 1991, cf. above, section 5.6.1.3).

Five male and five female Sprague-Dawley rats were fed 3000 ppm octadecylamine (‘stearamine’) in their diet for 209 days (Deichmann et al., 1958, cf. also above, section 5.6.1.3). Calculated based on average daily diet consumption, 3000 ppm corresponded to 88 mg/kg bw/d for males and 138 mg/kg bw/d for females. No untreated control animals were used in the experiment, but the results were compared with those for animals fed 3000 ppm stearic acid. Tissues examined by histopathology examination included brain, viscera, gonads, skeletal muscle, bone marrow, heart, lung, liver, spleen, kidneys, gastroenteric tract (3 levels), pancreas and lymph nodes. The two chemicals were equally toxic, causing anorexia, weight loss in male rats, reduced weight gain in female rats and increased mortality. The animals fed the stearamine diet had a lower daily feed intake (9.8/11.5 g for M/F) than those fed the stearic acid diet (15/13.5 g, respectively). The average weight change under the stearamine diet was –31 g for males and +30 g for females. One of five male rats and four of five female rats from the stearamine group survived to the end of the study. Average survival time was 87/199 d for M/F. When microscopic examinations were performed on the tissues from five rats in the stearamine group, accumulated histiocytes with pale or foamy cytoplasm were observed in the mucosa of the small intestine and mesenteric lymph nodes. The lymph nodes were enlarged and matted, and revealed a marked granulomatous inflammation with abundant histiocytes, nodule or tubercle formation, necrosis, and fibrosis. Sections of the livers of 3/5 animals showed nodular aggregates of histiocytes, with slight necrosis.

The lungs from rats fed stearamine showed varying degrees of pulmonary infection. It appears likely, that affected animals suffered from a generic type of pneumonia, which was not substance-related. On the other hand, Deichmann and co-workers mention in their report that ‘[...] numerous attempts were made to culture the organisms responsible for these deaths. However, only normal inhabitants of a rat’s respiratory tract were found. The impression was gained that these deaths were the result of a nonspecific endemic pulmonary infection occasionally observed in rat colonies’.

Hepatic focal granuloma was also found in three rats. Reduction in food consumption of male rats treated with stearamine was not as marked as compared to the females explaining the significant weight loss and high mortality rates in male rates. Whether (respiratory tract) infections or other effects had contributed to unscheduled deaths, remains open.

A 2-year toxicity study of octadecylamine ('stearamine') was conducted using Sprague-Dawley rats. Four groups of 24 rats (12/sex) were fed diets containing 20, 100, 200, and 500 ppm stearamine (Deichmann et al., 1958). Calculated from daily diet consumption, the mean dose was about 1.05, 5.79, 10.68, and 27.15 mg/kg bw/d for 350 g males and 1.20, 5.80, 10.08, and 28.20 mg/kg bw/d for 250 g females. A control group was fed the base diet alone. Organs examined microscopically included the heart, lung, liver, spleen, kidneys, gastroenteric tract (3 levels), pancreas, lymph nodes, bone marrow, brain, pituitary, adrenals, and ovary or testis. Feed consumption, growth rate, death rate, and blood cell counts for the treated animals were comparable to those of the controls. The survival rate for this study was 17-33 % including the control group (meaning that 2, 3, or 4 animals/dose only survived until the end of study). In both control and experimental rats mortality was higher than expected due to respiratory infections associated with chronic pneumonia and multiple organ inflammation. One rat receiving 500 ppm showed histiocytic hyperplasia of the mesenteric lymph node. At necropsy, no significant differences were found between experimental and control groups in the incidence and types of lesions observed. Having the histiocytic accumulations at 3000 ppm (seen in the 209 day-study in rats) and lesions of the same nature in the dog in mind, the single case of histiocytic hyperplasia in the mesenteric lymph node of the single rat treated with 500 ppm (25 mg/kg bw/d) for 2 years (in this study) could also be interpreted as a treatment-related adverse effect. Therefore the authors' conclusion is not followed and 200 ppm (10 mg/kg bw/d) are proposed as NOAEL for the 2-year rat studies.

Because of the high mortality rates primarily due to respiratory infections in the study of Deichmann and co-workers (1958), another 2-year study on groups of 10 male and 10 female Sprague-Dawley rats fed with diet containing 0, 200, or 500 ppm octadecylamine (0, 10 and 25 mg/kg bw/d) dissolved in corn oil was conducted (MacDonald et al., 1962). No data on group incidences and severity of microscopic findings available. Quite strikingly, respiratory tract infections and inflammatory lesions in multiple organs were again observed in control and treatment groups. There were no significant differences between experimental and control rats with regard to mean daily food consumption, mean weight gain, and mean survival time. No treatment-related pathological lesions could be attributed to the test substance.

Comparative investigations on hypolipidaemic activity were conducted in rats receiving isolated a. o. octadecyl-, hexadecyl-, and tetradecylamine (Griffin et al., 1991, cf. section 5.6.1.3).

Dog

In a 1-year toxicity study using dogs, three groups of three dogs (2 females and 1 male or 2 males and 1 female) each were fed 0.6, 3.0, and 15.0 mg/kg bw/d octadecylamine ("stearamine") in corn-oil solution that was administered by capsule once daily (5 d/wk) (Deichmann et al. 1958). A control group of dogs was given untreated feed. Dogs were housed in individual cages and fed once daily. Haematological examinations consisted of total red blood cell count, haemoglobin concentration, total and differential white cell counts and were performed on all dogs at the start of the experiment and at 1, 2, 3, 6, 8, 11, and 12 months thereafter. The organs examined microscopically included the heart, lung, liver, spleen, kidneys, gastroenteric tract (3 levels), pancreas, lymph nodes, bone marrow, brain, pituitary, adrenals, and ovary or testis. The high dose group gained less weight than the control and lower-dose animals. Weight gain for the two lower dose groups was similar to that of the controls. Blood cell counts were comparable in experimental and control animals. Only one dog from the high-dose group died before study termination (after

week 22) suffering from anorexia, bloody diarrhoea, and from what appeared to be gastroenteric irritation. At necropsy, two animals from the high-dose group had lesions of the mesenteric lymph nodes, which were filled with pale histiocytes. Also, pale staining of the tips of the villi of the small intestine mucosa was observed in these dogs. However, no definite lesions were identified in the intestinal tracts of these animals or in any of the other experimental animals.

Considering the mortality associated with anorexia and haemorrhagic diarrhoea in one dog, growth depression in two other dogs, and the lymph node histiocytosis observed at 15 mg/kg bw/d as a treatment-related toxic effect, the NOAEL in dogs fed with octadecylamine for 1 year was 3 mg/kg bw/d.

5.6.1.5 (Z)-Octadec-9-enylamine

Groups of five male and female SD rats received octadecenylamine (Genamin OL 100 D, vehicle sesame oil) by oral gavage at dose levels of 0, 3.25, 12.5, or 50 mg/kg bw/d for a period of 28 days (Aventis, 2003). On day 29, animals were necropsied. In the control and high dose groups, additional five male and females were examined and necropsied after a recovery period of 14 days. The study design and examinations conducted were in full accordance to the EU method B.7 (OECD 407) for subacute oral toxicity (including neurobehavioral observation and functional observation battery testing).

In a preliminary study on dose-range finding, three males and females received the test substance at dose levels of 25, 100, and 400 mg/kg bw/d over a period of 14 days; surviving animals were necropsied on day 15. After administration of 400 mg/kg bw/d, one male and one female died on days 4 and 7, respectively. The other animals of this dose group were killed for animal welfare reasons. The animals of the 100 mg/kg bw/d group showed clinical signs of impaired motility and respiration. Male animals were clearly more sensitive than female animals. After administration of 25 mg/kg bw/d, no symptoms were observed except for one female rat, which showed uncoordinated gait on study day 2. Body weight gain of animals exposed to dose levels of 25 and 100 mg/kg bw/d was impaired. Necropsy of decedent and prematurely killed animals showed changes in the stomach and intestinal mucosa. The animals of 100 mg/kg bw/d showed reddening of the stomach mucosa. No macroscopically visible changes were observed at 25 mg/kg bw/d.

Throughout the main study, treatment did not result in unscheduled deaths. Behaviour and state of health remained unaffected by the administration of the test compound in the low and mid dose groups. Clinical findings in the high dose group (2 males and 5 females out of 10) from the 2nd or 3rd week onwards comprised impairment of motility (stilted and/or uncoordinated gait) and lasted until the end of treatment, with subsequent recovery. In addition, respiratory sounds were noted in one high dose female (only on study day 13). No opacity of the refracting media of the eyes, changes of the oral mucosa, or impairment of dental growth were observed. No abnormal neurobehaviour was observed in any group.

Mean body weight was significantly lower for high dose males from study day 11 and for high dose females from day 22 until the end of treatment, and remained different from that of the controls at the end of recovery period. Also mean body weight for mid dose males was significantly lower from day 22 onwards to the end of treatment. At the end of treatment, body weight in males was statistically significantly reduced by 7.5 % at the mid and by 10% at the high dose level when compared to the body weight of controls. At the end of recovery, body weight in high dose males remained at the same, significantly lower level (-9.8 %). Body weight in high dose females was 10.4 % lower than the control values at the end of treatment, while a tendency to recover was seen at the end of 4-week recovery (difference was still -5.9 %, but statistical significance was no longer obtained).

Compared to control animals, mean body weight gain was significantly lower at the end of treatment for high dose males (-19 %), high dose females (-20 %) and for mid dose males (-15 %). A dose-dependent, small (-9 %, statistically non-significant) reduction of body weight gain was also reported for the low dose males. Weight gain was normalised at the end of the recovery for high dose males or even higher in the high dose female group indicating a tendency for recovery (no recovery period was for the mid dose group). Food consumption remained unaffected throughout the study in all dose groups.

Haematology findings in high dose group animals included significantly increased haematocrit and decreased reticulocyte counts (males only) and slightly increased white blood cell counts with a shift towards neutrophils (both genders), all findings being reversible. Clinical chemistry changes comprised significantly increased total bilirubin for the high dose group, slightly increased urea nitrogen for mid and high dose females and very slightly increased ASAT and ALAT activity in the liver of high dose males. Urinalysis remained unaffected in all dose groups. Likewise the urine sediment was unobtrusive for control and high dose group animals.

No anatomic pathology correlates (organ weight, macroscopy, microscopy) of toxicological significance were detected.

In conclusion, repeated administration of octadecenylamine (Genamin OL 100 D) at a dose of 50 mg/kg bw/d induced clinical signs such as gait abnormalities, reduction in body weight gain and clinical pathology findings indicating mild toxic effects on the liver and kidneys. The only treatment-related effects observed at the mid-dose level were reduction in growth and increased urinary concentration of urea nitrogen. By standard criteria of screening for neurotoxicology given for this type of study, stilted gait or uncoordinated gait were not associated with any other symptom of altered neurobehavior or neurotoxicity and may be discussed as being of an unspecific nature. In the preliminary study, macroscopic findings in the gastrointestinal mucosa were observed in early deaths and in animals receiving 100 mg/kg bw/d, but were absent in animals at a dosage of 25 mg/kg bw/d from the 14-d study and in dose groups receiving 50 mg/kg bw/d, where gait abnormalities were also present. Thus, in view of the absence of other severe clinical symptoms that may explain gait abnormalities and considering the screening nature of the 28-d study design for neurotoxicity, uncertainty on the cause of gait abnormality remains.

With the exception of growth reduction, full recovery from substance-related effects was seen at the end of the recovery period.

Based on the significantly reduced body weight gain at 12.5 mg/kg bw/d observed in this 28-d study, a NOAEL of 3.25 mg/kg bw/d was derived.

<u>In vitro studies</u>

No data available

5.6.2 Repeated dose toxicity: inhalation

No data available

5.6.3 Repeated dose toxicity: dermal

5.6.3.1 Amines, coco alkyl

No data available

5.6.3.2 Amines, tallow alkyl

No data available

5.6.3.3 Amines, hydrogenated tallow alkyl

No data available

5.6.3.4 Octadecylamine

0, 3, or 30 mg of octadecylamine ('n-octadecylamine', ca. 0, 1.5, or 15 g/kg bw/d for a 20 g mouse) were applied using ether as vehicle to the shaved back of male albino (Rockland) mice on days 1, 3, and 5 of the experiment (Brooks et al., 1957). On day 6, skin was analysed for cholesterol content, sterol and epidermal weight per cm^2. Three mg of the test substance produced substantial hyperplasia of the epidermis with increase in cholesterol. Severe hyperplasia with a 5fold increase of weight/cm^2 epidermis and absence of sebaceous glands and hair follicles was observed. No data on systemic toxic effects were generated.

5.6.3.5 (Z)-Octadec-9-enylamine

Based on the results of a pilot study, groups of young adult Sprague-Dawley rats (4 M and 4 F) were treated dermally with octadecenylamine (oleylamine) at concentrations of 0, 0.3, 1.5, and 3.0 % in mineral oil (corresponding to doses of 0, 12.5, 62.5 and 125 mg/kg bw/d). Animals of the control group received the vehicle only. Rats were treated for two five-day dosing periods with an intermediate two-day non-dosing period in order to more closely reproduce conditions of human exposure to the test substance. The first day of dosing was designated day 1. Due to excessive tissue destruction indicated by sloughing, scores of moderate to severe erythema, scabbing, hardening of the skin, and sensitivity to touch, the dosing at the intermediate and high dose levels (1.5 and 3.0 %) was discontinued on day 9. Affected animals were subsequently sacrificed on day 10. At initiation of dosing the rats' body weight ranged from 205.7 to 321.3 g. Rats were acclimated to the laboratory for seven days prior to test substance application. Water and food were provided ad libitum. Approximately 24 h prior to test substance application, the fur was clipped from the dorsal area of each animal. Shaving was repeated one week later. The test substance was applied at a dosage volume of 5 mL/kg. The application site was covered with a porous gauze dressing that was held in place with tape, and covered with a taped elastic bandage. Each day, wrappings were removed approximately 6 h after test substance application, and test sites were washed with warm water in order to remove excess test substance. Inspections for signs of toxicity were performed once each day. Body weight was recorded during acclimation, weekly during the study and at sacrifice. Food consumption was recorded weekly during the study. The skin was examined prior to test substance administration on days 2, 4, 6, 8, 10, 12, and 14 for signs of erythema and oedema. Twice daily, mortality/morbidity checks were performed. Animals were killed and discarded three days following the completion of dosing. No necropsies were performed, not even of those animals killed prematurely for humane reasons; the reason given by the study authors was that 'no animals died on study'.

All rats survived until scheduled sacrifice. Concentrations of 1.5 and 3.0 % produced moderate to severe irritation (erythema scores 2-4), which in some instances progressed to hardening and sloughing of the skin. A number of rats were sensitive to touch. In the 0.3 % group, erythema scores of 1 to 2, indicating mild to moderate irritation, and flaking of the outer layers of the epidermis were observed. An increased sensitivity in females to the irritant effects of the test substance as compared to males was observed. In the control group, one male showed an erythema score of 1 at one

observation. All rats in the 1.5 and 3 % groups were sacrificed on day 9 of the study due to the irritant/corrosive effects of the test substance. No other treatment-related irritant effects or clinical signs were observed. A significant treatment-related effect on body weight was observed for males on day 7. Individual group comparisons revealed that body weight in both the 1.5 and 3 % groups was significantly lower than that of the controls. Females in the 3.0 % group showed a mean weight loss during the first week of the study, but this finding was not statistically significant. Food consumption during the first week of study was reduced significantly in 1.5 % group males when expressed as total food consumed. No significant difference was noted when expressed on a per weight basis. The study provides additional data on the toxicity of repeated dermal dosing, including severe irritation, of this test substance at concentrations of 0.3, 1.5, and 3.0 %. A NOAEL for local dermal effects could not be derived, the LOAEL was 0.3 % (ca. 12.5 mg/kg bw/d). No picture of or threshold for systemic toxicity could be obtained from this study, due to the lack of macroscopic or microscopic necropsy data for any other organs and tissues (Intox Laboratories, 1985).

5.6.4 Other relevant information

No data available

5.6.5 Summary and discussion of repeated dose toxicity

Proposed classification

The outcome of the oral 28-day study on tallow alkyl amines indicated that fatty alkylamines may pose a risk for serious health effects after chronic oral exposure.

Leading health effects were delayed mortalities associated with precedent bad general health status and gait abnormalities, erosions of the mucosa of the gastrointestinal tract, accumulation of (material-) loaded histiocytes in the submucosa of the distal parts of the small intestine and in the mesenterial lymph nodes associated with inflammatory granuloma formation, liver toxicity, and indications of immunosuppression occurring at 150 mg/kg bw/d, which is within the critical dose range for R48/22 or STOT RE Cat 2, respectively.

The adverse lung effects were not considered for the classification proposal on the oral route, but the potential for damaging the surface epithelia of the respiratory tract should be considered elsewhere if the inhalation route is relevant. Data from the range-finding study on octadecenylamine indicated that bad general health condition (gait abnormalities) and stomach reddening was already seen in rats receiving 100 mg/kg bw/d for 14 days. Treatment-related reduction in food consumption and body growth started at $\geq$ 8 mg/kg bw/d in subacute studies on octadecylamine. Also a dose of 50 mg/kg bw/d in the valid 28-day study on octadecenylamine confirmed similar signs of impaired health condition (reduced body weight gain, impaired motility, stilted and uncoordinated gait) and hepatotoxicity. Although there was no histopathological evidence of mucosa damage in the gastrointestinal tract at this dose of octadecenylamine, 50 mg/kg bw/d of tallow alkyl amines induced mucosal hyperplasia and histiocyte accumulation. Since higher doses up to 150 mg/kg bw/d were not tested in this 28-day study, mortalities and other toxic effects that were observed at 150 mg/kg bw/d with tallow alkyl amines could not be ruled out for octadecenylamine.

Taking all data on fatty alkyl amines into consideration, the lowest dose with a depressive effect on growth was 0.01 % (about 7 mg/kg bw/d, estimated from repeated dose studies on octadecylamine), body weight loss was first seen at 50 mg/kg bw/d (also demonstrated for tallow alkyl amines (Istituto di Ricerche Biomediche, 2000a and for octadecylamine, see Parenteau et al., 1991). This is also the lowest dose associated with histopathological lesions of the gastrointestinal mucosa. With

respect to the classification of 'local' effects, the available data clearly demonstrated that fatty alkyl amines caused damage along the exposure route, the mucosa of the gastrointestinal tract. Although gavage administration is not a usual exposure condition for man, the data also demonstrated a cytotoxic potential at any site of contact (as intended in the studies via the oral or dermal route or as non-intended for the bronchial mucosa). Repeated exposure resulted in 'local cytotoxicity', i.e. erosions of the gastrointestinal mucosa, that was associated with secondary effects of systemic significance and progressed continuously with the duration of treatment leading to body weight loss, bad general health status and unscheduled deaths. Besides, the accumulation and migration of test substance loaded histiocytes along the intestinal passage into lymph nodes and other tissues (intestine submucosa and, putatively, liver), liver toxicity and immunosuppression are judged as adverse (systemic) effects.

Critical effects for R48/22:

- Delayed mortalities and erosion of gastrointestinal mucosa at 150 mg/kg bw/d (28-day study, tallow alkyl amines)
- Gait abnormalities at non-lethal, non-irritating concentrations (50 mg/kg bw/d, 28-day study, octadecenylamine)
- Treatment-related reduction in food consumption (≥ 7-8 mg/kg bw/d, subacute study, hydrogenated tallow alkyl amines) resulting in growth depression and anorexia. Effects could be interpreted as non-specific toxicity. However, intestine dysfunction such as malabsorption could also be a possible consequence of morphological damage of the intestine (through intramural substance accumulation and responsive inflammation and hyperplasia of intestinal wall).
- Accumulation of test material in the intestinal wall and in mesenteric lymph nodes (≥ 12 mg/kg bw/d, 28-day study, rat; 15 mg/kg bw/d, tallow alkyl amines; 1-year dog, octadecylamine). The effect is already present at non-irritating dosages. There is no excretion pathway for intracellular material, some redistribution among cells or among organs may be possible through re-phagocytosis or migration of loaden histiocytes. The effect is irreversible.
- Accumulation enteropathy is associated with inflammatory and hyperplastic responses of the intestine: Histiocytic granuloma in the intestinal wall and mesenteric lymph nodes, histiocytic hyperplasia in mesenteric lymph nodes, mucosal hyperplasia in the intestine. Related to the persistence of accumulated material, granuloma formation will also persist during life.
- Disturbance of lipid metabolism (8 mg/kg bw/d, 14-day study, octadecylamine): the significance/relevance of these findings cannot be assessed, but a lack of phospholipids, for example might affect central nervous function or lung function.
- Treatment-related liver toxicity (150 mg/kg bw/d, 28 day study, tallow alkylamines, 50 mg/kg bw/d, 28 day study, octadecenylamine). In addition, histiocytic granuloma formation in the liver is likely to be a secondary effect caused by accumulated (and/or migrated) material from intestinum.
- Thymus atrophy and atrophy of spleen follicles indicated immunosuppression (T-cell) (≥50 mg/kg bw/d, 28 day study).

In conclusion,

a) delayed mortalities occurred at 'irritant' concentrations/dose levels and

b) other serious health effects occurred at non-irritating concentrations/doses

Both, a) and b) were seen within the critical dose range for R48 or STOT RE 2, respectively, and corresponding **C & L with Xn;R48/22 or STOT RE 2; H373 is therefore proposed**.

Saturated vs. unsaturated fatty alkyl amines

The assumption has been raised that octadecylamine and hydrogenated tallow alkylamines might have lower potency in chronic toxicity that does not call for R48 classification.

From some studies, e.g. the chronic rat studies (Deichmann et al., 1958) it might appear that octadecylamine exerts its toxicity at concentrations, which are not critical for R48 classification, e. g. cumulative enteropathy observed at ≥ 500 ppm (25 mg/kg bw/d). However, read-across over the whole group of primary alkyl amines and subsequent classification as 'harmful' is suggested for the following reasons:

The data available for octadecylamine are clearly limited. There were high mortality rates of 67-83 % in limited dose groups (12 rats/sex/group) in the 2-year study (Deichmann et al. 1958) and respiratory infectious diseases associated to mortalities in all three long-term studies (two from the Deichmann publication and one from his colleague MacDonald and co-workers (1962). The test methods and results, gained in no GLP or guideline compliant studies, were roughly summarised and poorly documented in the publications.

The subacute study of Griffin et al. (1991) confirmed that already at low doses (8 mg/kg/day) disturbed lipid metabolism occurred. The repeated dose data, which were considered for hydrogenated tallow alkyl amines, included a limited study on a metabolite, i.e. stearic acid, and studies on hypolipidaemic activities of single components of hydrogenated tallow alkyl amines. No reliable repeated dose study on hydrogenated tallow alkyl amines themselves is available. Therefore, a lower potency in chronic toxicity of octadecylamine and hydrogenated tallow alkylamines could not be verified. It is concluded that there is no sufficient evidence to exclude these substances from read-across and classification as 'harmful', R48, is warranted.

Consequently, classification with R48/22 is proposed for all primary alkyl amines covered by this report. No further studies on repeated dose toxicity are required for an adequate hazard assessment of these substances.

Table 7: Overview of repeat-dose studies with primary alkyl amines

Alkyl amine (CAS No.)	**Repeated dose toxicity NOAEL**	**Information on carcinogenicity from repeated dose toxicity studies**
Coco alkyl amines (1788-46-3)	No data	No data
Tallow alkyl amines (61790-33-8)	EU method B.7-equivalent[2] oral 28 day study-rat LOAEL 12.5 mg/kg bw/d	No data

[2] Limited by lack of specific studies on neurobehaviour/function

Alkyl amine (CAS No.)	**Repeated dose toxicity NOAEL**	**Information on carcinogenicity from repeated dose toxicity studies**
Hydrogenated tallow alkyl amines (61788-45-2)	No guideline-compliant study: Limited data from chronic (206-day) oral study on a metabolite Additional data on hypolipidemic effects from oral 14 d studies in rats on structurally related components of hydrogenated tallow alkyl amines A reliable NOAEL could not be derived from the data available.	No data
Octadecylamine (124-30-1)	No guideline-compliant study: Limited dermal 5-day study - mouse Limited oral 6-14 day-studies –rat Limited oral chronic (209-day) study –rat NOAEL 200 ppm (10 mg/kg bw/d) Limited oral chronic (1-year) study - dog NOAEL 3 mg/kg bw/d	Limited data from oral chronic (2-year) studies in rats
Octadecenylamine (112-90-3)	One limited dermal 14-day study – rat $LOAEC_{local}$ 0.3% (12.5 mg/kg bw/d) EU method B.7 equivalent oral 28 day study – rat NOAEL 3.25 mg/kg bw/d	No data
Overall NOAEL	*Oral route:* *Overall NOAEL 3.25 mg/kg bw/d* *Dermal route:* *$LOAEC_{local}$ 0.3% (12.5 mg/kg bw/d)* *A $N(L)OAEL_{sys}$ could not be derived for this route.*	*No reliable data*

5.7 Mutagenicity

The data available for mutagenicity do not fully meet the requirements of Annex VII for any of the compounds assessed in this dossier. However, due to (i) structural similarity, (ii) availability of at least tests on bacterial mutagenicity for all compounds except hydrogenated tallow alkyl amines as well as (iii) the fact that not any of the available mutagenicity tests indicate concern for a mutagenic potential, the data base is considered sufficient to exclude a mutagenic potential for all of the compounds assessed in this report.

5.7.1 In vitro data

Generally, strong cytotoxicity of alkyl amines was observed *in vitro*. In most experiments, cytotoxic effects were induced at lower doses/concentrations in experiments without S-9 mix as compared to experiments with S-9 mix.

5.7.1.1 Amines, coco alkyl

An investigation into the induction of gene mutations in bacteria (OECD 471) was performed with coco alkyl amines ('Genamin CC 100 D') (Hoechst AG, 1988m). The test compound (purity approx. 100 %) was solved in acetone and tested in doses of 0.16 µg to 10 mg/plate with and without liver S-9 mix from Arochlor-induced male Sprague-Dawley rats. Only dose levels up to 100 µg/plate could be evaluated due to strain-specific strong cytotoxic effects at dose levels of 20 or 100 µg/plate and higher doses. In TA1537, the bacterial background lawn was reduced already at 4 µg/plate in the second experiment. Precipitation was seen at 500 µg/plate and higher doses. No increase in the number of revertants was induced in any of the tester strains, e. g. *S. typhimurium* TA100, TA1535, TA1537, TA1538, TA98 and *E. coli* WP2uvrA.

5.7.1.2 Amines, tallow alkyl

A bacterial gene mutation test (OECD TG 471) with tallow alkyl amines ('Genamin TA 100 D') was negative with and without Aroclor-induced rat liver S-9 mix in five *S. typhimurium* strains (TA98, TA100, TA1535, TA1537, TA1538) and *E. coli* WP2uvrA (Hoechst AG, 1988n). The test substance (purity 99-100 %) was solved in ethanol and tested in doses of 0.16 µg to 10 mg/plate. Due to strong and dose-dependent cytotoxicity in all tester strains only concentrations up to dose levels of 100 µg/plate without, and 500 µg/plate with S-9 mix were evaluable. Visible precipitation of the test compound was found at all dose levels from 20 µg/plate upwards with and without S-9 mix.

5.7.1.3 Amines, hydrogenated tallow alkyl

No genotoxicity tests with hydrogenated tallow alkyl amines are available.

5.7.1.4 Octadecylamine

Data on negative bacterial mutagenicity tests with octadecylamine were compiled by Zeiger et al. (1988). Negative results were reported for *S. typhimurium* strains TA100, TA1535, TA97, and TA98 with and without metabolic activation with 10 % and 30 % Arochlor-induced rat and hamster liver S-9 mix at doses of 100 to 6666 µg/plate. Water was used as solvent. Precipitation was found at 333 µg/plate and higher doses on plates with metabolic activation. No signs of toxicity were reported apart from a slight clearing of background lawn at the highest dose without metabolic activation in TA97. The absence of cytotoxic effects at lower doses was possibly caused by the use of water as solvent.

Further bacterial mutagenicity tests (OECD 471) with octadecylamine ('Genamin 18 R 100 D') were performed with tester strains *S. typhimurium* TA 100, TA1535, TA1537, TA1538, TA98 and *E. coli* WP2uvrA (Hoechst AG, 1988o). The test substance (purity approx. 100 %) was dissolved in ethanol and tested at dose levels of 4, 20, 100, 500, 2500 and 10000 µg/plate with and without Arochlor-induced liver S-9 mix from male Wistar rats. Strain-specific, dose-dependent cytotoxic effects (decrease in the number of spontaneous revertants and/or incomplete bacterial lawn) were seen at 100 or 500 µg/plate and higher doses. No increase in the number of revertant colonies was

induced in any of the tester strains. Precipitations were found at and above dose levels of 500 µg/plate.

Octadecylamine ('stearylamine', purity not stated) was tested as a component of multilamellar lipid vesicles (MLV) containing L-α-phosphatidyl choline (PC) and octadecylamine (PC/Octadec 7:2) in the human heteroploid EUE cell line (embryonic human explants) and in lymphocytes from a healthy human donor (Nuzzo et al. 1985). Cells were incubated with 0.14 to 4.44 mg lipids in buffer in the presence of 6 mmol/L $CaCl_2$ for 24 and 48 h. In EUE cells, the mitotic index (MI) was markedly reduced at 4.44 mg lipids after 48 h. When compared to controls, an increase in the percentage of mitoses with structural chromosomal aberrations (SCA) was seen in EUE cells after 24 h at 1.39 and 4.44 mg lipids and after 48 h over the whole dose range tested. In lymphocytes, the MI was markedly reduced at 1.39 mg lipids after 24 h, and no mitoses were found at both incubation times with 4.44 mg lipids. For these cells, an increase in mitoses with SCA under conditions that did not lead to extreme cytotoxicity was found at 1.39 mg lipids after 24 h. Since the number of mitoses with SCA in control cultures was extremely high in EUE cells (14 % after 24 h and 10 % after 48 h) and was also reported to be higher (by up to 5.4 %) than under standard culture conditions in lymphocytes, the test systems or test conditions do not seem to be adequate for cytogenetic investigations. Additionally, octadecylamine was not tested alone, but only in combination with PC. The study is therefore difficult to interpret and of low relevance for the classification/labelling of octadecylamine.

5.7.1.5 (Z)-Octadec-9-enylamine

Octadecenylamine was not mutagenic in *S. typhimurium* TA 98, TA 100, TA1535, TA1537, and TA1538 strains at dose levels up to 20 µg/plate without, and up to 200 µg/plate with metabolic activation (Arochlor-induced rat liver S-9 mix; Microbiological Associates Inc. 1985a, cited from IUCLID, original data not available for this report).

Negative results for mutagenicity of octadecenylamine (named 'oleylamine' in the test report) were obtained in a L5178Y TK+/- mouse lymphoma assay (OECD TG 476) (Microbiological Associates Inc., 1989a). Mutation frequency was determined after incubation with 0.13 to 1.8 nL octadecylamine/mL (purity not given) in the absence of metabolic activation and with 1.3 to 13 nL/mL in the presence of Arochlor-induced rat liver S-9 mix for 4 h (solvent: acetone). Concentrations were equivalent to ca. 0.1 to 1.5 µg/mL (0.39 nmol/L to 5.4 nmol/L) without S-9 mix and 1.0 to 10.6 µg/mL (3.9 nmol/L to 39.5 nmol/L) with S-9 mix. No increase in mutation frequency as compared to solvent controls was found. At 1.8 nL/mL without, and at 13 nL/mL with metabolic activation, a remarkable decrease in cell growth as compared to solvent controls was found. At higher concentrations, the cells were unable to form clones.

Octadecenylamine (ODA-FG-11-27-84) did not induce chromosomal aberrations in CHO cells in the absence or presence of Arochlor 1254-induced Sprague-Dawley rat liver S-9 mix (Microbiological Associates Inc., 1985b). Cells were treated with 0.05 to 5 nL/mL of the test substance for 16 h in the absence, and with 0.2 to 20 nL/mL for 2 h in the presence of S-9 mix, and cells were harvested after 18 h . Cell survival was reduced to 24 % with and without S-9 mix at the highest concentrations tested.

Mutagenicity at the hprt locus was investigated in Chinese hamster ovary cells at concentrations of 0.1 to 2.0 nL/mL without, and of 5.0 to 10 nL/mL with S-9 mix (Microbiological Associates Inc., 1985c). No relevant cytotoxicity (decrease in cloning efficiency) was found for the analysed concentrations; without S-9 mix, concentrations higher than 2.0 nL/mL led to strong cytotoxicity, so that cloning was not successful. In general, there was no increase in mutation frequencies after treatment, with the exception of the highest concentrations of 2.0 nL/mL (without S-9 mix) and

9.0 nL/mL (with S-9 mix) in the first experiment. Since no genetic effects were seen at lower concentrations and the second experiments were clearly negative with and without S-9 mix, these increased mutation frequencies can be interpreted as outliers (due to the low statistical power of this test system). Altogether, the test result is negative.

5.7.1.6 Other primary alkyl amines

Bacterial gene mutation tests with hexadecylamine in *S. typhimurium* TA100, TA1535, TA97, and TA98 strains revealed negative results (Zeiger et al., 1988). Dose levels of 300 to 33 000 µg/plate dissolved in DMSO were tested with 10 % and 30 % Arochlor-induced rat and hamster liver S-9 mix. No precipitation or signs of toxicity were found up to the highest dose level tested.

5.7.2 In vivo data

5.7.2.1 Amines, coco alkyl

No *in vivo* genotoxicity tests with coco alkyl amines are available.

5.7.2.2 Amines, tallow alkyl

A bone marrow micronucleus test (OECD TG 474) with tallow alkyl amines ('Genamin TA 100') in 50 Sprague-Dawley rats (25 male and 25 female) led to a negative result after a single oral dose of 2000 mg/kg bw (Istituto di Ricerche Biomediche, 2000c). The test substance was applied in sesame oil by intragastric gavage. Sampling times were 24 h and 48 h after treatment. The tested dose level induced clinical signs of toxicity (piloerection, hunched posture, hypoactivity and shallow breathing) in all animals and was lethal to one male rat from the 48 h sampling time group; no local cytotoxicity on bone marrow cells (PCE/NCE) was observed.

5.7.2.3 Amines, hydrogenated tallow alkyl

No *in vivo* genotoxicity tests with hydrogenated tallow alkyl amines are available.

5.7.2.4 Octadecylamine

No *in vivo* genotoxicity tests with octadecylamine are available.

5.7.2.5 (Z)-Octadec-9-enylamine

Negative results were reported from an chromosomal aberration test in mice bone marrow cells with octadecenylamine ('oleylamine') (Microbiological Associates Inc., 1989b). Groups of five male and five female mice were administered single doses of 500, 2500, or 5000 mg/kg bw in corn oil by oral gavage at a volume of 10 mL/kg bw (purity 90 %). Bone marrow cells were collected 6, 12, and 24 h after treatment. No increase in percentages of aberrant cells was observed in test substance-treated animals regardless of dose or harvest time. One female in the 2500 mg/kg bw group died prematurely. No significant reduction in the rate of body weight gain was observed. Clinical signs of toxicity were observed in test substance-treated mice indicating that the test substance was systemically available after oral application.

5.7.3 Human data

No data available.

5.7.4 Other relevant information

No data available.

5.7.5 Summary and discussion of mutagenicity

For all alkyl amines assessed in this report only negative results were obtained in mutagenicity tests.

There is no evidence for mutagenicity of **coco alkyl amines** from the available gene mutation test in bacteria. **Tallow alkyl amines** were tested negative in bacteria and in bone marrow cells *in vivo*. No tests on mutagenicity of **hydrogenated tallow alkyl amines** are available. From the available data on mutagenicity in bacteria there is no evidence for mutagenicity of **octadecylamine**. **Octadecenylamine** was tested negative *in vitro* in both bacteria and mammalian cells. A bone marrow chromosomal aberration test *in vivo* did not reveal any mutagenic effects of octadecenylamine up to systemically toxic doses.

For the group of alkyl amines mixtures with longer (i. e. mainly C_{16} and C_{18}) hydrocarbon chains (tallow alkyl amines, hydrogenated tallow alkyl amines, octadecenylamine, octadecylamine) negative results from tests on bacterial mutagenicity and data on gene mutations in mammalian cells *in vitro* and on chromosomal aberrations and micronuclei *in vivo* are available. The whole amount of data is judged as sufficient to exclude a mutagenic potential for the group of alkyl amines with longer chain lengths *in vivo*.

For coco alkyl amines, a mixture of alkyl amines featuring shorter hydrocarbon chains, only data on bacterial mutagenicity exist. No further genotoxicity tests in mammalian cells in vitro or in vivo are available. Altogether, the negative data from the bacterial tests together with negative data on structurally similar long-chain alkyl amines is considered as sufficient to exclude a mutagenic potential of coco alkyl amines in vivo.

No classification with respect to germ cell mutagenicity is proposed for the alkyl amines mixtures assessed in this report.

Table 8: Overview of mutagenicity studies with primary alkyl amines

Alkyl amine	Test system	Concentration range	Result	Toxicity	Solvent	Reference
Coco alkyl amines	Bacterial gene mutation test	0.16 10000 µg/plate with and without S-9 mix	Negative	Strain-specific at 20 µg/plate and higher doses	Acetone	Hoechst AG, 1988m
Tallow alkyl amines	Bacterial gene mutation test	0.16 to 10000 µg/plate without S-9 mix and 0.16 to 500 µg/plate with S-9 mix	Negative	Strain-specific at 20 µg/plate without S-9 mix and at 100 µg/plate with S-9 mix	Ethanol	Hoechst AG, 1988n

Alkyl amine	Test system	Concentration range	Result	Toxicity	Solvent	Reference
Tallow alkyl amines	Micronucleus test in rat bone marrow cells	2000 mg/kg bw, single oral dose	Negative	Systemic toxicity: yes, local toxicity : no	Sesame oil	Istituto di Ricerche Biomedice, 2000c
Octadecyl-amine	Bacterial gene mutation test	100 to 6666 µg/plate with and without S-9 mix	Negative	No	Water	Zeiger et al., 1988
Octadecyl-amine	Bacterial gene mutation test	4 to 10000 µg/plate with and without S-9 mix	Negative	Strain-specific, generally at 100 µg/plate and higher doses	Ethanol	Hoechst AG, 1988o
Octadecenyl-amine	Bacterial gene mutation test	Up to 20 µg/plate without S-9 mix and up to 200 µg/plate with S-9 mix	Negative	No data		Microbiological Associates Inc. ,1985a
Octadecenyl-amine	Chromosomal aberrations in CHO cells	0.05 to 5 nL/mL without S-9 mix and 0.2 to 20 nL/mL with S-9 mix	Negative	At the highest concentrations tested	Acetone	Microbiological Associates Inc., 1985b
Octadecenyl-amine	Mouse lymphoma assay	0.13 to 1.8 nL/mL without S-9 mix and 1.3 to 13 nL/mL with S-9 mix.	Negative	At 1.4 ng/mL without S-9 mix and 10.4 ng/mL with S-9 mix and higher concentrations	Acetone	Microbiological Associates Inc., 1989a
Octadecenyl-amine	Hprt gene mutation test in mammalian cells	0.1 to 2.0 nL/mL without S-9 mix and 5.0 to 10 nL/mL with S-9 mix	Negative	No strong toxicity at the analysed concentration; without S-9 mix higher concentration could not be tested due to toxicity	Acetone	Microbiological Associates Inc., 1985c
Octadecenyl-amine	Chromosomal aberrations in mouse bone marrow cells	500 to 5000 mg/kg bw , single oral doses	Negative	Systemic toxicity: yes, no local cytotoxicity (mitotic index)	Corn oil	Microbiological Associates Inc., 1989b

5.8 Carcinogenicity

5.8.1 Carcinogenicity: oral

5.8.1.1 Amines, coco alkyl

Neither animal nor human data on the carcinogenicity of coco alkyl amines are available.

5.8.1.2 Amines, tallow alkyl

Neither animal nor human data on the carcinogenicity of tallow alkyl amines are available.

5.8.1.3 Amines, hydrogenated tallow alkyl

Neither animal nor human data on the carcinogenicity of hydrogenated tallow alkyl amines are available. From a limited rat study with chronic administration of stearic acid, a metabolite, no reliable information on carcinogenic potential of hydrogenated tallow alkyl amines could be derived (Deichmann et al., 1958).

5.8.1.4 Octadecylamine

A 2-year toxicity study of octadecylamine (‘stearamine’) was conducted using Sprague-Dawley rats. Four groups of 24 rats (12/sex) were fed diets containing 20, 100, 200, and 500 ppm stearamine (Deichmann et al., 1958). A control group of animals was fed the base diet alone. Feed consumption, growth rate, death rate, and blood cell counts for treated animals were comparable to those of the control. The survival rate for this study was 17-33 % for both the control and treatment groups. At necropsy, no significant differences in the incidence and types of lesions observed were found between treated and control groups.

5.8.1.5 (Z)-Octadec-9-enylamine

Neither animal nor human data on the carcinogenicity of tallow alkyl amines are available.

5.8.2 Carcinogenicity: inhalation

No data available

5.8.3 Carcinogenicity: dermal

No data available

5.8.4 Carcinogenicity: human data

No data available

5.8.5 Other relevant information

The effects of octadecylamine (‘stearamine’) on mammary carcinogenesis induced by 7,12-dimethyl-benz(a)anthrazene (DMBA) was investigated using female Sprague-Dawley rats on high-fat diets (Parenteau et al., 1991). Four groups of 21 rats each were given a single 5 mg dose of

DMBA intragastrically at 47 to 51 days of age. One week later, the rats were fed diets containing 5 % corn oil, 20 % corn oil, 20 % corn oil and 0.01 % stearamine, or 20 % corn oil and 0.1 % stearamine. Stearamine was converted to the corresponding sulfate by mixing with a solution of sulfuric acid in ethanol. At 2-week intervals, the rats were weighed, examined, and palpated for neoplasms. All rats were killed 16 to 17 weeks following DMBA administration and necropsies were performed.

As expected, a greater number of neoplasms with a shorter latency period was observed among rats fed the 20 % than among those fed the 5 % corn oil diet. The addition of 0.01 % stearamine to the 20 % corn oil diet slightly reduced body weight gain and potentiated the effect of the high-fat diet, increasing the number of neoplasms that developed and shortening the latency period beyond that observed with the high-fat diet alone. However, 0.1 % stearamine appeared to inhibit tumor growth as well as to markedly reduce body weight gain. Significantly fewer neoplasms were found in rats fed the 0.1 % stearamine and 20 % corn oil diet as compared with the other three groups. All neoplasms were mammary adenocarcinomas. Authors discussed the reduced tumour response in the high (0.1 %) stearamine group as being related to growth inhibition or to the inhibitory effect on protein kinase C.

5.8.6 Summary and discussion of carcinogenicity

Neither human data nor conventional carcinogenicity studies in rodents with compliance to current test guideline standards are available with regard to the carcinogenicity of the alkyl amines assessed in this report. Earlier, limited studies on the chronic toxicity of octadecylamine, the main C_{18}-component of hydrogenated tallow alkyl amines with administration of up to 25 mg/kg bw/d for 2 years provided no indication of a carcinogenic effect.

Due to the lack of reliable data, no conclusion can be drawn on the carcinogenic potential of any of the fatty alkyl amines under consideration. However, negative data from mutagenicity and sparse data on chronic toxicity do not trigger a specific concern for a carcinogenic potential of the primary alkyl amines.

5.9 Toxicity for reproduction

Valid data for the oral route of exposure with regard to fertility/reproduction toxicity are available only for tallow alkyl amines, while with regard to developmental toxicity, such data only are available for octadecenylamine. Additional information is recruited from 28-day studies available for tallow alkyl amines and for octadecylamine, in which no adverse effects to reproductive organs were observed.

5.9.1 Effects on fertility

5.9.1.1 Amines, coco alkyl

No data available

5.9.1.2 Amines, tallow alkyl

For tallow alkyl amines, a GLP-conform reproduction/developmental toxicity screening test according to OECD TG 421 with ‘GENAMIN TA 100’ (> 96 % active compound) is available (Istituto di Ricerche Biomediche, 2000b). Groups of 10 rats (Crl:CD (SD) BR) per sex were treated with dosages of 0, 12.5, 50, and 150 mg/kg bw/d by gavage (administration volume 10 mL/kg

bw/d) using sesame oil as a vehicle. Males were treated daily from 14 days prior to mating until the end of the mating period for a maximum of 28 days. Females were treated daily for 14 days before the start of the mating period, throughout mating, during pregnancy, and until day 3 of lactation. The animals were mated one male with one female.

No haematological or clinical chemistry parameters were examined. Preservation and histopathology was exclusively conducted on organs of the reproductive system.

At 150 mg/kg bw/d, 6/10 males and 5/10 females died between day 9 and day 25 of treatment (during the premating and mating period). At 50 mg/kg bw/d, 1/10 males and 1/10 females died on treatment days 13 and 24, respectively. No animals died at 12.5 mg/kg bw/d, and in the control group 1 female died by accident.

Clinical observations at 150 mg/kg bw/d revealed salivation after treatment, hunched posture, and in some cases soft stool and piloerection. The only clinical sign present at 50 mg/kg bw/d was salivation. No changes were seen at 12.5 mg/kg bw/d.

Body weight loss of about 22 g in the 150 mg/kg bw/d group and statistically significantly ($p > 0.01$) lower body weight gain in the 50 mg/kg bw dose group during the premating period together with a lower mean food consumption was observed in the males. Also in the females body weight loss of about 17 g in the 150 mg/kg bw dose group and statistically significantly lower body weight gain in the 50 mg/kg bw dose group together with a lower mean food consumption during the premating period were observed.

At sacrifice of the parental animals, organ weight of ovaries, testes, and epididymides was determined for all experimental groups. Histopathology was carried out on testes, epididymides, and ovaries of the controls and of the animals of the 150 mg/kg bw/d group. A statistically significantly ($p > 0.01$) lower absolute and relative weight of the epididymides and a statistically significantly ($p > 0.01$) higher relative testis weight were observed at 150 mg/kg bw/d. No significant differences were noted in ovary weight among the various experimental groups. Histopathology of testes and epididymides of high dose group animals did not show any compound-related changes. In particular, no changes were seen in the testicular staging performed in PAS-haematoxylin stained sections of terminally sacrificed animals of the control and high dose groups. In the ovaries, compared to controls, a moderately increased frequency of atrophic corpora lutea was seen in animals of the high dose group which died or were sacrificed; this finding was considered a secondary effect to the decrease in body weight growth induced by treatment in this group.

At 150 mg/kg bw/d only 3 out of 7 mated females had positive vaginal smears and of these only one was pregnant, but only with implantation sites and no live pups. At this dosage level also the mean pre-coital interval was longer (13.4 days) than that of the control group (2.3 days). With regard to the mating index in the control, the 12.5 and the 50 mg/kg bw dose level groups 9/9 (100 %), 9/10 (90 %) and 9/9 (100 %) mated females were sperm-positive. With regard to the fertility index 9/9 (100 %), 8/10 (80 %) and 7/9 (78 %) of mated females became pregnant in the control, the 12.5 and the 50 mg/kg bw/d groups. Mean pre-coital time and parturition were unaffected in the low and mid dose groups.

In the control, the 12.5, and the 50 mg/kg bw dose level groups 9/9 (100 %), 8/9 (89 %) and 7/9 (78 %) sperm positive females became pregnant and delivered live pups. Numbers of corpora lutea were not determined in this study. Thus, pre-implantation loss was not evaluated. Staining for the presence of implantation sites was only performed with the uteri of apparently non-pregnant females. The mean number of visible implantation sites per dam in the control, the 12.5 and the 50 mg/kg bw/d groups were 17.6, 13.9 and 15.0. There were no stillborns or litters with only

implantations in the control, low, and mid dose groups. The mean number of total pups born per litter was 16.9, 12.3 and 14.3 in the control, the 12.5 and the 50 mg/kg bw/d dose groups. Thus, the mean litter index for post-implantation loss was calculated to 3.4, 9.9 and 4.5 % in the control, the low and the mid dose groups.

No effects were noted on pup sex ratio. No abnormalities were observed in any pup from the 12.5 or 50 mg/kg bw/d groups either at birth or at autopsy on day 4 of lactation. Lower mean values of live-born pups/litter and of pups/litter alive at day 4 were found for both the low and the intermediate dose groups in comparison to those of the control group, however, the decrease was not dose-dependent and differences were statistically significant for the 12.5 mg/kg bw dose group only. Thus, these findings were considered unlikely to be related to the compound. A slightly lower pup body weight was observed in the 50 mg/kg bw/d group in comparison with the control group at birth and at day 4 of lactation but not for the pups of the 12.5 mg/kg bw/d treated group.

Furthermore, in an oral 28-d study (OECD TG 407) with tallow alkyl amines (‘Genamin TA 100’) in rats (cf. section 5.6.1), also weight (uterus, ovaries, testes, epididymides) and histopathology (testes, epididymides) of reproductive organs have been investigated. No substance-related adverse effects on these organs were observed at oral doses up to and including 150 mg/kg bw/d (Istituto di Ricerche Biomediche, 2000a).

5.9.1.3 Amines, hydrogenated tallow alkyl

No data available

5.9.1.4 Octadecylamine

Experimental studies on the effects of octadecylamine on fertility or reproductive function are not available.

In several chronic studies (cf. section 5.6) with commercial octadecylamine (containing 20 % hexadecylamine), the original study reports of which were not available for this report, reportedly also an investigation of the gonads has been performed:

In a 2-year feeding study with rats tissues taken for histopathological examination included ovary or testis (Deichmann et al., 1958). In a further 2-year feeding study with rats (MacDonald et al., 1962) organ weight has been determined for testes, and histopathological examinations have been conducted on the gonads of seven rats from each group. Since results for the reproductive organs are not explicitly reported in these two studies, it is assumed that toxicologically relevant effects have not been observed. It is therefore deduced from these studies, that chronic treatment up to and including the highest tested concentration of 500 ppm (equivalent to oral dosages of ca. 25 mg/kg bw/d) did not adversely affect organs of the reproductive system. Higher dosages, however, have not been tested in these studies.

In a 1-year feeding study with young mongrel dogs (Deichmann et al., 1958) gross pathological organ changes in gonads (ovary and testis) were not observed after administration of daily oral dosages up to and including the highest tested dose level of 15 mg/kg bw/d. Higher dosages, however, have not been tested in this study.

5.9.1.5 Octadecenylamine

Experimental studies on the effects of octadecenylamine on fertility or reproductive functions are not available.

In an oral 28-d study with octadecenylamine (‘Genamin OL 100 D’) in rats (cf. section 5.6.1), also testes and epididymides weight was determined and histopathological investigations of testes, epididymides, prostate, seminal vesicles, ovaries including oviducts, and uteri were performed. No substance-related findings have been observed in these organs at oral dose levels up to and including 50 mg/kg bw/d (Aventis, 2003).

5.9.2 Developmental toxicity

5.9.2.1 Amines, coco alkyl

No data available

5.9.2.2 Amines, tallow alkyl

No data available

5.9.2.3 Amines, hydrogenated tallow alkyl

No data available

5.9.2.4 Octadecylamine

No data available

5.9.2.5 (Z)-Octadec-9-enylamine

For octadecenylamine (‘oleylamine’, no further substance identification provided) a guideline-conform teratology study in Sprague-Dawley rats has been performed (Springborn Laboratories Inc., 1989a). Prior to initiation of the main study, a range-finding study was conducted, for which no separate report was available for the present report. During the range finding-study, treatment-related deaths reportedly had occurred in the 100, 150, and 250 mg/kg bw/d groups. Outward clinical signs of toxicity and body weight loss or reduced weight gain occurred at 50, 100, 150 and 250 mg/kg bw/d. A dose level of 100 mg/kg bw/d was considered to be excessive for a high dose level of the main study due to the induced mortality. Conversely, 50 mg/kg bw/d did not produce sufficient maternal toxicity to be considered suitable as a high dose level. Thus, 80 mg/kg bw/d was selected as the high dose level for the main study in anticipation of producing sufficient maternal toxicity.

In the main study, groups of 28 pregnant females were treated orally (gavage) with dosages of 10, 40, or 80 mg/kg bw/d or with the vehicle (corn oil) during gestation days 6 to 15. During the study, animals were examined daily. Any clinical signs of toxicity including physical or behavioural abnormalities were recorded. Individual body weight and food consumption were recorded on gestation days 0, 6, 9, 12, 16, and 20. Two animals in each group were selected to be sacrificed and necropsied after treatment on gestation day 15 to determine the appearance and severity of gastrointestinal tract irritation. On gestation day 20, caesarean section was performed on all surviving animals. The number of viable foetuses, early and late resorptions, and corpora lutea was recorded. Foetuses were examined for external, visceral, and skeletal abnormalities.

All animals survived to scheduled sacrifice. Outward clinical signs of toxicity were observed at the 40 and 80 mg/kg bw/d dose levels. These observations most likely indicated a generalised irritant

effect of the test substance as characterised by rales, salivation, unkempt appearance and changes in the amount, colour, and consistency of the faeces. However, no other signs of treatment-related gastrointestinal irritation or other internal changes were observed at the gestation day 15 and 20 necropsies. More pronounced signs of toxicity were apparent only in the 80 mg/kg bw/d dose group and included emaciation, rough coat, and dark red material around the eyes, nose and/or mouth. Similar clinical signs were noted infrequently during post-dosage observations. Dose-dependent body weight loss (during gestation days 6-9) or reduced weight gain (during gestation days 12-16), along with a corresponding reduction in food consumption occurred during the treatment period in the 40 and 80 mg/kg bw/d groups. Net body weight gain (adjusted for gravid uterine weight) was also lower at these levels. Following cessation of treatment (days 16-20), increase in weight gain and food consumption were noted at both dose levels. No such effects were observed in the dose group treated with 10 mg/kg bw/d. Caesarean section data obtained from the treated groups did not reveal any meaningful differences (concerning number of corpora lutea, implantation sites, viable foetuses, foetal sex and foetal weight) when compared with the controls. Foetal evaluations of type and frequency of malformations and variations did not reveal any indications for a treatment related teratogenic effect.

In summary, oral administration of octadecenylamine to pregnant rats produced dose-dependent maternal toxicity in the 40 and 80 mg/kg bw/d dose groups. No indication of an embryotoxic, foetotoxic, or teratogenic effect was observed at any tested level. NOAELs of 10 mg/kg bw/d for maternal toxicity and $\geq$ 80 mg/kg bw/d for developmental toxicity can be derived from this study.

Furthermore, with octadecenylamine (‘oleylamine’, no further substance identification provided) a guideline-conform teratology study in New Zealand rabbits has been performed (Springborn Laboratories Inc., 1989b). Prior to initiation of the main study, a range-finding study at dose levels of 5, 25, 50, 100, and 150 mg/kg bw/d was conducted, for which no separate report was available for the present report. During the range finding-study, treatment-related deaths occurred in the 50, 100, and 150 mg/kg bw/d groups. Outward clinical signs of toxicity were observed at the 5 mg/kg bw/d dose level and above. Body weight loss occurred in the 25, 50, 100, and 150 mg/kg bw/d dose groups. A dose level of 50 mg/kg bw/d was considered to be excessive for a high dose level for the main study. On the other hand, 25 mg/kg bw/d did not produce sufficient maternal toxicity to be considered suitable as a high dose level. Thus 30 mg/kg bw/d were selected as the high dose level for the main study in anticipation of producing sufficient maternal toxicity.

In the main study groups of 22 inseminated females were treated orally (gavage) with 3, 10, or 30 mg/kg bw/d or with the vehicle (corn oil) during gestation days 6 to 18. During the study, animals were examined daily. Any clinical signs of toxicity including physical or behavioural abnormalities were recorded. Individual body weights were recorded on gestation days 0, 6, 9, 12, 1, 19, 24, and 29. Individual food consumption was measured daily. Two animals in each group were selected to be sacrificed and necropsied after treatment on gestation day 18 in order to determine the appearance and severity of gastrointestinal tract irritation. On gestation day 29 caesarean section was performed on all surviving animals. The number of viable foetuses, early and late resorptions, and corpora lutea was recorded. Foetuses were examined for external, visceral, and skeletal abnormalities.

As a result of the study, two females died in the 30 mg/kg bw/d group, one on gestation day 9 and the other on gestation day 25. In addition, one female each at the 3, 10, and 30 mg/kg bw/d levels aborted prior to scheduled sacrifice. Outward clinical signs of toxicity were observed at the 10 and 30 mg/kg bw/d levels. In the 10 mg/kg bw/d dose group, rales and laboured breathing were noted. Additional findings at the 30 mg/kg bw/d level included few or no faeces and emaciation. Irritation of the snout area also developed in females in this group. The irritation was characterised by swollen raised white areas, scab-like lesions and/or sloughing of the skin of the lips and the chin.

No other signs of treatment-related gastrointestinal irritation or internal changes were observed at gross necropsy at gestation days 18 and 29. Dose-dependent body weight loss (both during gestation days 6-9 and 6-19) or reduced weight gain, along with a corresponding reduction in food consumption occurred during treatment in the 10 and 30 mg/kg bw/d groups. Net body weight gain (adjusted for gravid uterine weight) was also lower. Following cessation of treatment, weight gain increased in the 30 mg/kg bw/d group. No such effects were observed in the dose group treated with 3 mg/kg bw/d. Caesarean section data obtained from treated groups did not reveal any meaningful differences (concerning number of corpora lutea, implantation sites, viable foetuses, implantation loss, foetal sex and foetal weight) when compared with the controls. Foetal evaluations of type and frequency of malformations and variations did not reveal any indications for a treatment-related teratogenic effect.

In summary, oral administration of octadecenylamine to pregnant rabbits produced dose-dependent maternal toxicity in the 10 and 30 mg/kg bw/d dose groups. No indications of an embryotoxic, foetotoxic or teratogenic effect were observed at any tested level. NOAELs of 3 mg/kg bw/d for maternal toxicity and $\geq$ 30 mg/kg bw/d for developmental toxicity can be derived from this study.

5.9.3 Human data

No data on fertility or developmental toxicity are available on any of the primary alkyl amines assessed in this report.

5.9.4 Other relevant information

No data available

5.9.5 Summary and discussion of reproductive toxicity

Reproduction toxicity studies according or similar to pertinent OECD test guidelines are only available for tallow alkyl amines (fertility, 1-generation screening test acc. to OECD TG 421) and (Z)-octadec-9-enylamine (developmental toxicity in rats/rabbits, sim. to OECD TG 414). In the one-generation test with tallow alkyl amines, an adverse impact of test substance administration on fertility was only seen at the highest dose level of 150 mg/kg bw/d, which was lethal for more than half of the treated dams.

No adverse effects on rat or rabbit offspring up to and including dose levels evoking marked maternal toxicity were reported in the two developmental toxicity studies performed with (Z)-octadec-9-enylamine.

Based on the notion, that the above test substances cover both saturated and unsaturated, primary alkyl amines, as well as those with hydrocarbon chains of medium- and greater length, read-across to the remaining primary alkyl amines covered by this report is considered justified.

In conclusion, the available data on reproduction toxicity do not call for specific classific-ation/labelling for this endpoint. This result is supported by several repeat-dose studies in which no adverse effects on reproductive organs were seen after treatment with primary alkyl amines.

Table 9: Overview of reproduction toxicity studies with primary alkyl amines

Alkyl amines mixture	Data availability	NOAEL	LOAEL	Reference
Coco alkyl amines	Fertility: no data			
	Dev. Tox.: no data			
Tallow alkyl amines	Fertility: OECD 421, rat, oral (gavage) GENAMIN TA 100 12.5/50/150 mg/kg/d	$NOAEL_{syst.\ tox}$: 12.5 mg/kg/d $NOAEL_{reprotox}$: 12.5 mg/kg/d	$LOAEL_{syst.\ tox}$: 50 mg/kg/d (based on 10% death, clin. signs, ↓ body wt gain) $LOAEL_{reprotox}$: 50 mg/kg/d (based on dose-dependently ↓ fertility index)	Istituto di Ricerche Biomediche, 2000b
	OECD 407, rat, oral (gavage) GENAMIN TA 100; 12.5/50/150 mg/kg/d	no effects on reproductive organ weights and on reproductive organ histopathology at doses up to and including 150 mg/kg/d → Results support data/results from OECD 421		Instituto di Ricerche Biomediche, 2000a
	Dev. Tox.: no data, limited information from OECD 421			
Hydrogenated tallow alkyl amines	Fertility: no data			
	Dev. Tox.: no data			

Alkyl amines mixture	**Data availability**	**NOAEL**	**LOAEL**	**Reference**
Octadecylamine	Fertility: chronic study (1year) dog, oral	$NOAEL_{reprod\ organ\ tox}$: > 15 mg/kg/d		Deichmann et al., 1958
	2 chronic studies (2 years), rat, oral	$NOAEL_{reprod\ organ\ tox}$: > 25 mg/kg/d		Deichmann et al., 1958 MacDonald et al., 1962
	Dev. Tox.: no data			
Octadecenylamine	Fertility: OECD 407, rat, oral (gavage) GENAMIN OL 100;3.25/12.5/50 mg/kg/d	No effects on reproductive organ weights and on reproductive organ histopathology at doses up to and including 50 mg/kg/d		Aventis, 2003
	Dev. Tox.: OECD 414-like, rat, oral (gavage), Oleylamine, 10/40/80 mg/kg/d	$NOAEL_{mat.\ tox}$: 10 mg/kg/d $NOAEL_{dev.\ tox}$: ≥ 80 mg/kg/d	$LOAEL_{mat.\ tox}$: 40 mg/kg/d (based on clin. signs, ↓ body wt gain)	Springborn Laboratories Inc., 1989a
	OECD 414-like, rabbit, oral (gavage), Oleylamine, 3/10/30 mg/kg/d	$NOAEL_{mat.\ tox}$: 3 mg/kg/d $NOAEL_{dev.\ tox}$: ≥ 30 mg/kg/d	$LOAEL_{mat.\ tox}$: 10 mg/kg/d (based on clin. signs, ↓ body wt gain)	Springborn Laboratories Inc., 1989b

5.10 Other effects

No data available

5.11 Derivation of DNEL(s) or other quantitative or qualitative measure for dose response

Not relevant for this type of dossier

6 HUMAN HEALTH HAZARD ASSESSMENT OF PHYSICO-CHEMICAL PROPERTIES

Not relevant for this dossier

7 ENVIRONMENTAL HAZARD ASSESSMENT

7.1 Aquatic compartment (including sediment)

7.1.1 Toxicity test results

7.1.1.1 Fish

Short-term toxicity to fish

Table 10 shows the most relevant test results for fish exposed to long-chain alkyl amines. The majority of tests were conducted in the end of the 1980s. Since the actual concentrations have not been analytically verified, the reliability of the results is limited. Hence, most of the tests are classified as valid with restrictions. The available data propose an increasing toxicity with raising chain length. The only exception is octadecylamine where only a summary test report is available which indicates a slight drop of toxicity.

The lowest well documented 96 h-LC50 reported for fish is 0.11 mg/L (nominal) for (*Z*)-octadec-9-enylamine. In this study the short-term toxicity to *Pimephales promelas* was examined by Akzo (1995b) using a static water test system according to OECD Guideline 203 (1984). Five test concentrations of (*Z*)-octadec-9-enylamine (94% purity) in the nominal concentration range between 0.05 and 0.49 mg/L were administered. During all tests the amine concentrations decreased rapidly showing a rather wide spread of recovery rates. This drop in concentration can be explained by adsorption onto walls of test vessels and organism´s surface as well as strong adsorptive or ionic binding to dissolved organic matter.

However, based on nominal concentrations a 96 h-LC50 of 0.11 mg/L resulted from this study. If the mean recovery rate (about 51%) as an indicator for the actual test concentration is considered, the 96 h-LC50 can be calculated for 0.06 mg/L.

Long-term toxicity to fish

Long-term test results for fish are not available.

Table 10: Most relevant results of toxicity tests using fish

Species	Substance	Temp. [°C]	pH	Experimental conditions, type of test	Concen-trations*) [mg/L]	Endpoint	Concentration*) (95%-CI) [mg/L]	Reference	Validity
Pimephales promelas	octylamine	24.5	7.9+/- 0.02	flow through, water hardness 44.5 mg/L $CaCO_3$, O_2 content 7.1 mg/L	3.7 – 20	96h-LC50	5.2 (measured)	Geiger *et al.* (1988)	n. p.
Brachydanio rerio	octylamine "Genamin 8R 100D" purity 99%	22 +/-1	8-8.2	OECD 203, static, complete test report, GLP, O_2-saturation > 80 %, dispersion treatment: Ultra-Turrax, no analytics	1.0, 10, 100, 500	96h-LC0 96h-LC50 96h-LC100	1 10-100 100 (nominal)	Hoechst (1988a)	w. rest.
Pimephales promelas	decylamine	25.1	7.45	Flow through, water hardness 44.5 mg/L $CaCO_3$, O_2 content 6.2 mg/L, concentrations analytically verified	1.0 – 250	96h -LC50	1.04 (measured) (0.99-1.09)	Brooke *et al.* (1984)	n. p.
Brachydanio rerio	decylamine			OECD 203, static, no further information (summary test report)	1, 10	48,96h-LC0 48,96h-LC50 48,96h-LC100	1 > 1 and < 10 10	Hoechst (1988b)	n. p.
Pimephales promelas	dodecylamine					96h-LC50	0.1	Newsome *et al.* (1993)	n. p.
Brachydanio rerio	dodecylamine "Genamin 12 R 100 D" purity 99.9%	22 +/- 1	7.5-8.3	OECD 203, static, complete test report, GLP, O_2-saturation > 80 %, solubilizer: 0.1 ml/L ethanol, dispersion treatment: Ultra-Turrax/ Ultrasound, no pre-treatment of test vessels, no analytical verification	0.25-3.5	48h-LC0 48h-LC50 48h-LC100 96h-LC0 96h-LC50 96h-LC100	0.35 0.42 0.71 0.35 **0.42** 0.5	Hoechst (1988c)	w. rest.
Brachydanio rerio	coco alkyl amine "Genamin CC100D" purity >99%	21.6-22.9	7.1-8.3	OECD 203, static, complete test report, GLP O_2: 5.7-9.7 mg/L, solubilizer: 0.1 ml/L ethanol, dispersion treatment: Ultra-Turrax/ Ultrasound, no pre-treatment of test vessels, no analytical data	0.01-10	48h-LC0 48h-LC50 48h-LC100 96h-LC0 96h-LC50 96h-LC100	0.12 0.30 0.50 0.12 **0.24** (0.20-0.30) 0.35	Hoechst (1988e)	w. rest.

Tab. 10 continued overleaf: *) nominal; w. rest.) with restrictions; n. p.) statement about validity not possible due to missing data

Table 10 continued: Most relevant results of toxicity tests with aquatic vertebrates

Species	Substance	Temp. [°C]	pH	Experimental conditions, type of test	Concen-trations*) [mg/L]	Endpoint	Concentration*) (95%-CI) [mg/L]	Reference	Validity
Oncorhynchus mykiss	coco alkyl amines "Amine KK" purity >94%	14	7.3-7.6	OECD 203, semi-static (daily renewal), complete test report, GLP, dissolved O_2 9.9-10.1 mg/L, solubilizer: Tween 80-acetone, **test vessels were soaked with test substance overnight**, no analytical data	0.1-1	96h-NOEC 96h-LC50 96h-LC100	0.1 **0.16** (0.13-0.19) 0.32	Berol Nobel (1991a)	w. rest.
Brachydanio rerio	hydrogenated tallow alkyl amine "Armeen HT" purity 99%	22.7-23.8	8.0-8.3	OECD 203, semi-static (renewal after 48 hours), complete test report, GLP, lowest measured O_2 concentration 79%, solubilizer: 1 mg/L Tween 80, dispersion treatment: ultrasonic treatment, heating up to 50°C, no pre-treatment of test vessels, no analytical data	0.1-1.05	96h-$NOEC_{mort}$ 96h-$NOEC_{beh}$ 96h-LC50	0.58 0.1 **0.88** (0.72-1.1)	Akzo (1991)	w. rest.
Brachydanio rerio	tallow alkyl amine "Genamin TA 100D" purity 100%	21.0-22.9	7.2-8.2	OECD 203, static, complete test report, GLP, O_2 content 5.4-9.3 mg/L, solubilizer: 0.1 mL/L Tween 80, dispersion treatment: Ultra-Turrax/ Ultrasound, no pre-treatment of test vessels, no analytical data	0.01-10	48h-LC0 48h-LC50 48h-LC100 96h-LC0 96h-LC50 96h-LC100	0.18 0.32 0.50 0.18 **0.18-0.25** 0.35	Hoechst (1988g)	w. rest.
Brachydanio rerio	octadecylamine "Genamin 18 R 100D" purity: 100%			OECD 203, static, no further information (summary test report)	1, 10	48,96h-LC0 48,96h-LC50 48,96h-LC100	1 > 1 and < 10 10	Hoechst (1988d)	n. p.
Brachydanio rerio	octadecylamine "Genamin SH 100D" purity: 100%			OECD 203, static, no further information (summary test report)	1, 10	48,96h-LC0 48,96h-LC50 48,96h-LC100	1 > 1 and < 10 10	Hoechst (1988f)	n. p.

Tab. 10 continued overleaf: *) nominal; w. rest.) with restrictions; n. p.) statement about validity not possible due to missing data

Table 10 continued: Most relevant results of toxicity tests with aquatic vertebrates

Species	Substance	Temp. [°C]	pH	Experimental conditions, type of test	Concen-trations*) [mg/L]	Endpoint	Concentration*) (95%-CI) [mg/L]	Reference	Validity
Brachydanio rerio	(*Z*)-octadec-9-enylamine "Genamin OL 100D" purity: 100%			OECD 203, static, no further information (summary test report)	0.1, 1	48,96h-LC0 48,96h-LC50 48,96h-LC100	0.1 > 0.1 and < 1 1	Hoechst (1988h)	n. p.
Pimephales promelas	(*Z*)-octadec-9-enylamine "Armeen OD" purity 94%	20.4-21.4	7.7-8.3	OECD 203, static, GLP, complete test report, dispersion treatment: ultrasound at RT, no pre-treatment of test vessels, measured concentrations (HPLC) decreased during the test period, results based on nominal concentrations	0.05-0.49 recovery 13-82% → mean 51%	96h-LC50 96h-LC0	**0.11** (0.09-0.15) **0.06** (51% mean recovery rate) 0.085	Akzo (1995b)	w. rest.

*) nominal; w. rest.) with restrictions; n. p.) statement about validity not possible due to missing data

7.1.1.2 Aquatic invertebrates

Short-term toxicity to aquatic invertebrates

Regarding the acute toxicity data towards *Daphnia magna* a dependence on chain length of primary alkyl amines cannot be hypothesised (Table 11). The lowest short-term result for *Daphnia magna* was found for (*Z*)-octadec-9-enylamine (Akzo, 1995a). This study was conducted according to OECD Guideline 202 (1984) administering (*Z*)-octadec-9-enylamine (purity 94%) as test substance. Again, during all experiments test substance concentration (measured at 0 h and 48 h) decreased strongly showing a wide spread of recovery rates (recovery 48-118%, mean value 81%). Due to this uncertainty, no calculations based on measured concentrations were performed. Based on nominal concentrations the 48 h-EC50 values were calculated for 0.011 mg/L.

Long-term toxicity to aquatic invertebrates

Assessment of chronic toxicity to aquatic organisms is not necessary for the proposed classification as “Aquatic Chronic 1”.

7.1.1.3 Algae and aquatic plants

Algae

The lowest EC values concerning algae *Scenedesmus subspicatus* (OECD 201) have been found for coco alkyl amine (96 h-E_BC50 = 0.8 µg/L), hydrogenated tallow alkyl amine (96 h-E_BC50 = 12 µg/L) and tallow alkyl amine (96 h-E_BC50 = 7 µg/L) (Berol Nobel 1991c-e). Unfortunately the actual cell density after 72 h for the control group was not stated in the test report. Interpolation for 72 h based on datasets at the beginning and after 96 h is feasible, but then in general the growth within the control group is too slow (below required factor 16). Therefore these tests (Berol Nobel 1991c-e) need to be classified as invalid (Table 12).

Other EC50-values reported for long-chain alkyl amines are in the range between 0.04 mg/L (synthetic medium) and 0.46 mg/L (natural river water) both tested for (*Z*)-octadec-9-enylamine. For coco alkyl amine, tallow alkyl amine and (*Z*)-octadec-9-enylamine test results are available, determined for *Scenedesmus subspicatus* in natural, unfiltered river water (Noack 2002a-c). Studies were conducted according to OECD Guideline 201 (1984) in a static test system. Exposure concentrations were analytically verified for the highest tested concentration at the beginning and after 72 h. Again, due to variations in proportion of suspended matter and adsorbing properties of the amines, decreasing concentrations associated with strongly varying recovery rates were observed (coco alkyl amine: 0-120%, mean 60%; tallow alkyl amine: 73-23%, mean 48%; (*Z*)-octadec-9-enylamine: 72-0%, mean 36%). Therefore all calculations were based on nominal concentrations. As outlined in Table 12 the calculated 72 h-E_RC50 as well as 72h-NOEC values decline in the sequence coco alkyl amine, tallow alkyl amine and (*Z*)-octadec-9-enylamine as the mean recovery rate declines in the same order. For this reason an enhancing level of toxicity with increasing chain length can be proposed.

Table 11: Most relevant results of acute toxicity tests with aquatic invertebrates

Species	Substance	Temp. [°C]	pH	Experimental conditions, type of test	Concen-trations*) [mg/L]	Endpoint	Concentration*) (95%-CI) [mg/L]	Reference	Validity
Daphnia magna	coco alkyl amine ”Genamin CC100D“ purity 99.1%	21.0-21.8	7.46-7.62	OECD 202, static, GLP, complete test report, dispersion treatment: 30 min ultrasound at 40°C, no pre-treatment of test vessels, no analytical data	0.018-0.58	48h-EC0 48h-EC50 48h-EC100	0.058 **0.11-0.15** 0.18	Noack (1994a)	w. rest.
Daphnia magna	coco alkyl amine ”Amine KK“ purity 94%	21	7.5-7.7	OECD 202, static, GLP, complete test report, solubilizer: Tween 80-acetone, **test vessels were soaked with test substance overnight**, no analytical data	0.010-1	24h-EC0 24h-EC50 48h-EC0 48h-EC50	0.032 0.057 0.032 **0.045** (0.042-0.049)	Berol Nobel (1991b)	w. rest.
Daphnia magna	hydrogenated tallow alkyl amine ”FARMIN TH”	21.8-22.4	7.3-8.1	OECD 202, static, GLP, complete test report, solubilizer: Tween 80 (100 mg/L), no pre-treatment of test vessels, no analytical data	0.05-0.78	48h-EC0 48h-EC50 48h-EC100	0.05 **0.16** (0,116-0.21) 0.78	Kao (1995)	w. rest.
Daphnia magna	hydrogenated tallow alkyl amine ”NORAM SH”	19.5-20	7.47-8.39	OECD 202, static, complete test report, test substance was dissolved in reconstituted water and heated to 60-70°C. Before dilution, the stock solution was **centrifugated** for one hour and the supernatant collected (undissolved particles had a density <1 g/m³), all glassware was silanised to prevent the test substance adhering to the surface, 100% immobilization at concentrations below the limit of quantification (1 mg/L, GLC / FID)	1-50	48h-EC50 48h-EC100	< 1 (limit of quantification) < 1 (limit of quantification)	CECA (1995)	w. rest.

Tab. 11 continued overleaf: *) nominal; w. rest.) with restrictions; n. p.) statement about validity not possible due to missing data

Table 11 continued: Most relevant results of acute toxicity tests with aquatic invertebrates

Species	Substance	Temp. [°C]	pH	Experimental conditions, type of test	Concen-trations*) [mg/L]	Endpoint	Concentration*) (95%-CI) [mg/L]	Reference	Validity
Daphnia magna	tallow alkyl amine "Adogen 170"	20	7.7	acute toxicity test, static, dilution water: Town River water ($CaCO_3$ 50 mg/L, suspended solids 7.5 mg/L), solubilzer: acetone, no pre-treatment of test vessels, no analytical data	0.04-0.5	24h-LC50 48h-LC50	0.23 **0.093** (0.076-0.11	Witco (1986)	w. rest.
Daphnia magna	octadecylamine "Genamin SH 100D"	20.9-21.8	7.4-7.6	OECD 202, static, GLP, complete test report, dispersion treatment: 30 min ultrasound at 40°C, no pre-treatment of test vessels, no analytical data	0.018-0.58	48h-EC0 48h-EC50 48h-EC100	0.032 **0.13** (0.10-0.18) 0.58	Noack (1994c)	w. rest.
Daphnia magna	(*Z*)-octadec-9-enylamine "Armeen OD" purity 94%	19.1-19.7	8.0-8.2	OECD 202, static, GLP, complete test report, dispersion treatment: ultrasound at RT, no pre-treatment of test vessels, measured concentrations (HPLC) de-creased strongly during the test period, results therefore based on nominal concentrations	0.006-0.09 recovery 48-118% → mean 81%	48h-EC0 48h-EC50 48h-EC100	0.0056 **0.011** (0.01-0.013) 0.045	Akzo (1995a)	w. rest.

*) nominal; w. rest.) with restrictions; n. p.) statement about validity not possible due to missing data

Table 12: Most relevant results of toxicity tests with algae

Species	Substance	Temp. [°C]	pH	Experimental conditions, type of test	Concen-trations*) [mg/L]	Endpoint	Concentration*) (95%-CI) [mg/L]	Reference	Validity
Scenedesmus subspicatus	hydrogenated tallow alkyl amine "Amine HBG" purity 95%	24	8.0-9.0	OECD 201, static, GLP, complete test report, solubilizer: isopropyl alcohol, **test vessels were soaked with test substance overnight**, no analytical data	0.001-0.016	48h-E_RC50 96h-E_BC50 96h-NOEC	0.010 0.012 0.008	Berol Nobel (1991e)	invalid **)
Scenedesmus subspicatus	coco alkyl amine "Amine KK" purity 94%	24	7.8-8.7	OECD 201, static, GLP, complete test report, solubilizer: 1%-Tween 80-acetone, **test vessels were soaked with test substance overnight**, no analytical data	0.0001-0.0016	48h-E_RC50 96h-E_BC50 96h-NOEC	0.0014 0.0008 0.0002	Berol Nobel (1991c)	invalid **)
Scenedesmus subspicatus	tallow alkyl amine "Amine BG" purity 95%	24	7.8-9.9	OECD 201, static, GLP, complete test report, solubilizer: isopropyl alcohol, **test vessels were soaked with test substance overnight**, no analytical data	0.001-0.016	24h-E_RC50 96h-E_BC50 96h-NOEC	0.008 0.007 0.002	Berol Nobel (1991d)	invalid ***)
Scenedesmus subspicatus	coco alkyl amine "Armeen CD" purity 100%	22.5-24.0	8.06-8.16	OECD 201, static, GLP, complete test report, test medium: **unfiltered river water**, dispersion treatment: 30 min ultrasound at 40°C, no pre-treatment of test vessels, due to strongly varying recovery rates (GC / MS) test results were based on nominal concentrations (only highest test concentration analytically verified)	0.03-1 recovery rates: 0-120% mean: 60%	72h-E_RC50 72h-LOEC 72h-NOEC 72h-E_BC50 72h-LOEC 72h-NOEC	**0.16** (0.15-0.18) 0.13 0.06 **0.08** (0.07-0.09) 0.06 0.03	Noack (2002a)	w. rest.

Tab. 12 continued overleaf: *) nominal ; **) increase in cell concentration in the control too low, no analytical data at very low effect level
***) no analytical data at very low effect level; w. rest.) with restrictions

Table 12 continued: Most relevant results of toxicity tests with algae

Species	Substance	Temp. [°C]	pH	Experimental conditions, type of test	Concen-trations*) [mg/L]	Endpoint	Concentration*) (95%-CI) [mg/L]	Reference	Validity
Selenastrum capricornutum	octylamine	not given	not given	cell multiplication inhibition test (based on a standard test from the US Federal Register: Vol. 50, No. 188, Part 797, Sec. 797.1050, Algal Acute Toxicity Test)		96h-EC50	0.22 (0.03-0.42)	Bollmann *et al*. (1989)	n.p.
Scenedesmus subspicatus	coco alkyl amine "Genamin CC100D" purity 99.1%	23+/-2	7.90-8.14	OECD 201, static, GLP, complete test report, dispersion treatment: 30 min ultrasound at 40°C, no pre-treatment of test vessels, no analytical data	0.0032-1	72h-E_RC0 72h-E_RC10 72h-E_RC50 72h-E_BC0 72h-E_BC10 72h-E_BC50	0.032 0.071 **0.17** 0.032 0.041 **0.14**	Noack (1994b)	w. rest.
Scenedesmus subspicatus	tallow alkyl amine" Genamin TA 100D"	23+/-2	7.6-8.3	OECD 201, static, GLP, complete test report, dispersion treatment: 30 min ultrasound at 40°C, no pre-treatment of test vessels, no analytical data	0.001-0.32	72h-E_RC0 72h-E_RC10 72h-E_RC50 72h-E_BC0 72h-E_BC10 72h-E_BC50	0.032 0.045 **0.083** 0.032 0.036 **0.068**	Noack (1996)	w. rest.
Scenedesmus subspicatus	tallow alkyl amine "Armeen TD" purity 101.0%	21.8-23.2	8.06-8.23	OECD 201, static, GLP, complete test report, test medium: **unfiltered river water**, dispersion treatment: 30 min ultrasound at 40°C, no pre-treatment of test vessels, due to strongly varying recovery rates (GC / MS) test results were based on nominal concentrations (only highest test concentration analytically verified)	0.125-4 recovery rates: 72.5-22.5% mean: 47.5%	72h-E_RC50 72h-LOEC 72h-NOEC 72h-E_BC50 72h-LOEC 72h-NOEC	**0.39** (0.38-0.41) 0.25 0.125 **0.31** (0.30-0.32) 0.25 0.125	Noack (2002b)	w. rest.

Tab. 12 continued overleaf: *) nominal; w. rest.) with restrictions; n.p.) statement about validity not possible due to missing data

Table 12 continued: Most relevant results of toxicity tests with algae

Species	Substance	Temp. [°C]	pH	Experimental conditions, type of test	Concen-trations*) [mg/L]	Endpoint	Concentration*) (95%-CI) [mg/L]	Reference	Validity
Scenedesmus subspicatus	octadecylamine "Genamin SH 100D"	23+/-2	7.93-8.15	OECD 201, static, GLP, complete test report, dispersion treatment: 30 min ultrasound at 40°C, no pre-treatment of test vessels, no analytical data	0.001-0.32	72h-E_RC0 72h-E_RC10 72h-E_RC50 72h-E_BC0 72h-E_BC10 72h-E_BC50	0.01 0.029 **0.12** 0.01 0.018 0.062	Noack (1994d)	w. rest.
Scenedesmus subspicatus	(*Z*)-octadec-9-enylamine "Armeen OD" purity 99.1%	21.8-23.2	8.04-8.23	OECD 201, static, GLP, complete test report, test medium: **unfiltered river water**, dispersion treatment: 30 min ultrasound at 40°C, no pre-treatment of test vessels, due to strongly varying recovery rates (GC / MS) test results were based on nominal concentrations (only highest test concentration analytically verified)	0.15-2.5 recovery rates: 72-0% mean: 36%	72h-E_RC50 72h-LOEC 72h-NOEC 72h-E_BC50 72h-LOEC 72h-NOEC	**0.46** (0.44-0.49) 0.3 0.15 **0.38** (0.36-0.39) 0.3 0.15	Noack (2002c)	w. rest.
Selenastrum capricornutum	(*Z*)-octadec-9-enylamine "Armeen OD" purity 94%		7.6-9.4	OECD 201, static, GLP, complete test report, dispersion treatment: 5 min ultrasound at RT, no pre-treatment of test vessels, measured concentrations (HPLC) decreased strongly during the test period, results therefore based on nominal concentrations	0.01-0.15 recovery 28-100% → mean 64%	96h-E_RC50 96h-E_BC50 96h-NOEC	0.04 (0.04-0.04) 0.03 (0.03-0.03) 0.01	Akzo (1995c)	w. rest.

*) nominal; w. rest.) with restrictions

7.1.1.4 Sediment organisms

No data available

7.1.1.5 Other aquatic organisms

No data available

7.1.2 Calculation of Predicted No Effect Concentration (PNEC)

Not relevant for this dossier

7.2 Terrestrial compartment

Not relevant for this dossier

7.3 Atmospheric compartment

Not relevant for this dossier

7.4 Microbiological activity in sewage treatment systems

Not relevant for this dossier

7.5 Calculation of Predicted No Effect Concentration for secondary poisoning (PNEC_oral)

Not relevant for this dossier

7.6 Conclusion on the environmental classification and labelling

A comparison of decisive criteria for an environmental classification according to GHS and the 67/548/EEC Directive is given in Table 13.

For all relevant endpoints of acute toxicity (96 h-LC_{50} fish, 48 h-EC_{50} *Daphnia* and 72 h-E_RC_{50} algae) sufficient data are available to evaluate the toxicity of long-chain alkyl amines. LC_{50} for fish are generally calculated for below 1 mg/L (0.06 - 0.88 mg/L). *Daphniae* show a higher sensitivity for long-chain alkyl amines as the 48 h-EC_{50} values range from 0.011 - 0.16 mg/L. Effective concentrations entailing 50% growth reduction for algae (72 h-E_RC_{50}) are in a similar level (0.08 - 0.17 mg/L). Summarising all test results the toxicity criterion $L(E)C_{50} < 1$ mg/L in accordance with GHS and 67/548/EEC, required for a classification as H400 an R50, respectively, is met for each of these 3 endpoints.

The bioaccumulation potential of octadecylamine can be quantified by a calculated log K_{OW} of about 7. Although the performed bioaccumulation study with hexadecylamine was not conform to GLP the results allow to assume a realistic worst case BCF of 1200 for long-chain alkyl amines.

In the actual GHS the criteria for chronic aquatic toxicity will be met if the a substance elicits acute toxicity and is not readily biodegradable and/or the experimental BCF is ≥ 500. If no BCF value is available, a log $K_{OW} \geq 4$ will alternatively provide an appropriate criterion for the bioaccumulation potential required for a classification as H410. Under the former Directive 67/548/EEC chronic

aquatic toxicity is linked to the same criteria for acute toxicity. Additionally it is required that the substance is not readily biodegradable and/or the measured log K_{OW} is ≥ 3, unless the BCF is ≤ 100.

Although long-chain alkyl amines can be regarded as readily biodegradable, the log K_{OW} values as well as the realistic worst case BCF of 1200 are ranging beyond these required thresholds.

Table 13: Decisive criteria for environmental classification

Environmental classification acc. to		**CLP critera**	**DSD critera**	**Determined values**
CLP	**DSD**			
H400	R50	96 h-LC_{50} (fish) or 48 h-EC_{50} (crustacean) or 72 h-E_RC_{50} (algae) ≤ 1 mg/L	96 h-LC_{50} (fish) or 48 h-EC_{50} (crustacean) or 72 h-E_RC_{50} (algae) ≤ 1 mg/L	LC_{50} < 1 mg/L (0.06 - 0.88 mg/L) EC_{50} < 1 mg/L (0.011 - 0.16 mg/L) E_RC_{50} < 1 mg/L (0.08 -0.17 mg/L)
H410	R53	96 h-LC_{50} (fish) or 48 h-EC_{50} (crustacean) or 72 h-E_RC_{50} (algae) ≤ 1 mg/L and: 1) not readily biodegradable and/or 2) BCF ≥ 500 or, if no BCF available, log K_{OW} ≥ 4	96 h-LC_{50} (fish) or 48 h-EC_{50} (crustacean) or 72 h-E_RC_{50} (algae) ≤ 1 mg/L and: 1) not readily biodegradable and/or 2) log K_{OW} ≥ 3, unless determined BCF ≤ 100	criteria for acute toxicity are met (see above) long chain alkyl amines are considered as “readily biodegradable” but: BCF ≥ 500 (realistic worst case BCF = 1200) as well as log KOW ≥ 4 (calc. > 7)

For these reasons it is proposed to classify long-chain alkyl amines as:

- Aquatic Acute 1, H400 (very toxic to aquatic life)
  Aquatic Chronic 1, H410 (very toxic for aquatic life with long lasting effects)
- R50/53, very toxic for aquatic organisms, may cause long-term adverse effects in the aquatic environment

JUSTIFICATION THAT ACTION IS REQUIRED ON A COMMUNITY-WIDE BASIS

Octadecylamine has already been prioritised under the Existing Substance Regulation (ESR) (EEC) No 793/93. The group approach used for risk assessment and classification and labelling was already agreed within the Member States at a technical level (TCNES,TC C&L), but the work was not finished by 1 June 2008. Octadecylamine was classified at TC C&L 09/2005 based on the data summarised in the EU Risk Assessment Report. This classification was further confirmed at TC C&L 04/2006, but unfortunately this decision was not included in an ATP to Directive 67/548/EEC. Hence, action on a community-wide basis is required to finalise the harmonised classification and labelling under Regulation (EC) No 1272/2008, and include the C&L proposal in an ATP of the Regulation.

Additionally, remarkable work has been done to gather and evaluate information. The effort already done to propose harmonised C&L even for issues other than CMR and RS should not be dismissed in order to avoid wasting of resources.

Moreover, it is pointed out that a grouping approach is followed in the current CLH report. Each registrant for any of the substances in this report will most likely only have access to a limited subset of the data presented here. In such a scenario, contradictory entries in the inventory (which would THEN trigger the need for CLH) can be expected with high probability. The current CLH proposal therefore constitutes an efficient way of assuring a high quality standard by proactively evading conflicting C & L and - as a consequence - avoiding time-consuming follow-up work.

OTHER INFORMATION

The EU Risk Assessment Report R070_410_412_429_430_0811_ ENV_HH.DOC is publicly available via:

http://echa.europa.eu/doc/trd_substances/amines_tallow%20alkyl/rar/trd_rar_germany_amines_tallow_alkyl.pdf

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