Appendix A

Available Measured Data

This appendix contains the robust summaries of the available data. Reference 9 was not summarised, because this reference consisted of a review containing summaries only.

The reports have been evaluated and assessed according to the Klimisch criteria (Klimisch et al., 1997). The following criteria can be distinguished, based on reliability, relevance and adequacy of the data:

1 = Reliable without restrictions,
2 = Reliable with restrictions,
3 = Not reliable,
4 = Not adequate.

List of Abbreviations

a	Absolute to body weight
-	Absent
+	Present
a.i.	Active ingredient
BOD	Biochemical Oxygen Demand
BOD_5	Biochemical Oxygen Demand on day 5
BUN	Blood Urea Nitrogen
COD	Chemical Oxygen Demand
d	Decrease
dc	Decrease (significant)
DOC	Dissolved Organic Carbon
F	Female
i	Increase
ic	Increase (significant)
M	Male
r	Relative to body weight
TS	Test Substance
WBC	White Blood Cells
x	yes

Physicochemical Properties

Title	Determination of the melting and boiling temperature of Lathanol LAL powder by differential scanning calorimetry.
Date of report	November 22, 2004.
GLP	Yes.
Reference	19.
Test substance	CAS 1847-58-1, Lathanol LAL powder, purity 70.2%.
Guideline	OECD 102 and 103.
Remarks	Complete melting or boiling of the test substance was not observed below 163-175 °C (436 K – 448 K) at which reaction or decomposition started. The observation of a small endothermic effect between about 65°C and 130°C (338 K and 403 K) and the coalescing of the powder particles indicate that possibly a very small part of the test substance melted in the given temperature range. Below 65°C (338 K) a small part of the test substance evaporated (possibly volatile impurities).
Conclusion	The test substance reacts/decomposes before complete melting/boiling.
Rev. note	-
Klimisch criterium	1

Title	Statement on the determination of the dissociation constant(s) of Lathanol LAL powder in water.
Date of report	June 13, 2003.
GLP	Yes.
Reference	10.
Test substance	CAS 1847-58-1, Sodium Lauryl Sulfoacetate.
Guideline	OECD 112
Remarks	For the sulphonate group of Lathanol LAL powder a pK_a of –0.51 was calculated with Pkalc version 5.0. Since this value is not within the range 2-11 the dissociation constant could not be determined experimentally.
Conclusions	pK_a =-0.5.
Rev. note	-
Klimisch criterium	1

Title	Final report on the safety assessment of sodium lauryl sulfoacetate.
Date of report	1987.
GLP	No.
Reference	9.
Test substance	CAS: 1847-58-1, Sodium Lauryl Sulfoacetate.
Guideline	Not indicated.
Remarks	Specific gravity = 0.55. Water solubility = 10, 000 mg/L at 25 °C. The pH of a 0.25% solution is 6.9-7.1.
Rev. note	-
Klimisch criterium	2

Title	EPISUITE v.3.10
Date of report	-
GLP	Not applicable.
Reference	18.

Test substance	CAS 1847-58-1, acetic acid, sulfo-, 1-dodecyl ester, sodium salt.
Guideline	Not applicable.
Remarks	Melting point: 271 °C. Boiling point: 425 °C. Vapor pressure: 3.0E-14 hPa at 25 °C. Partition coefficient o/w: 2.66. Water solubility: 3.83 mg/L at 25 °C.
Rev. note	Calculated.
Klimisch criterium	2

Environmental Fate

Title	Final report on the safety assessment of sodium lauryl sulfoacetate.
Date of report	1987.
GLP	No.
Reference	9.
Test substance	CAS: 1847-58-1, Sodium Lauryl Sulfoacetate.
Guideline	Not indicated.
Remarks	The test substance is stable in weakly acidic and weakly alkaline solutions in a pH range of 5.0-8.5.
Rev. note	-
Klimisch criterium	4

Title	EPISUITE v.3.10
Date of report	-
GLP	Not applicable.
Reference	18.
Test substance	CAS 1847-58-1, acetic acid, sulfo-, 1-dodecyl ester, sodium salt.
Guideline	Not applicable.
Remarks	**Photodegradation (calculated):** AOP Program (v1.90) Results: ========== SMILES : S(=O)(=O)(O[Na])CC(=O)OCCCCCCCCCCCC CHEM : Acetic acid, sulfo-, 1-dodecyl ester, sodium salt MOL FOR: C14 H27 O5 S1 Na1 MOL WT : 330.42 ---------- SUMMARY (AOP v1.90): HYDROXYL RADICALS ---------- **Hydrogen Abstraction = 16.1612 E-12 cm3/molecule-sec Reaction with N, S and -OH = 0.0000 E-12 cm3/molecule-sec Addition to Triple Bonds = 0.0000 E-12 cm3/molecule-sec Addition to Olefinic Bonds = 0.0000 E-12 cm3/molecule-sec Addition to Aromatic Rings = 0.0000 E-12 cm3/molecule-sec Addition to Fused Rings = 0.0000 E-12 cm3/molecule-sec OVERALL OH Rate Constant = 16.1612 E-12 cm3/molecule-sec HALF-LIFE = 0.662 Days (12-hr day; 1.5E6 OH/cm3) HALF-LIFE = 7.942 Hrs ..... ** Designates Estimation(s) Using ASSUMED Value(s) ---------- SUMMARY (AOP v1.90): OZONE REACTION ---------- ****** NO OZONE REACTION ESTIMATION ****** (ONLY Olefins and Acetylenes are Estimated)

Distribution (calculated):

Level III Fugacity Model (Full-Output):

==========

Chem Name : Acetic acid, sulfo-, 1-dodecyl ester, sodium salt
Molecular Wt: 330.42
Henry's LC : 6.79e-010 atm-m3/mole (Henrywin program)
Vapor Press : 3.02e-014 mm Hg (Mpbpwin program)
Liquid VP : 8.26e-012 mm Hg (super-cooled)
Melting Pt : 271 deg C (Mpbpwin program)
Log Kow : 2.66 (Kowwin program)
Soil Koc : 187 (calc by model)

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	8.25e-006	15.9	0
Water	99.3	360	1000
Soil	0.000113	360	0
Sediment	0.654	1.44e+003	0

	Fugacity (atm)	Reaction (kg/hr)	Advection (kg/hr)	Reaction (percent)	Advection (percent)
Air	1.92e-022	0.00124	0.000284	0.000124	2.84e-005
Water	3.51e-015	657	341	65.7	34.1
Soil	9.22e-021	0.000746	0	7.46e-005	0
Sediment	2.1e-015	1.08	0.045	0.108	0.0045

Persistence Time: 344 hr
Reaction Time: 522 hr
Advection Time: 1.01e+003 hr
Percent Reacted: 65.8
Percent Advected: 34.2

Half-Lives (hr), (based upon Biowin (Ultimate) and Aopwin):
Air: 15.88
Water: 360
Soil: 360
Sediment: 1440
Biowin estimate: 3.149 (weeks)

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

Conclusion Indirect photolysis in air results in a half-life of 7.9 hours for the test substance. Discharge into water results in the following distribution: water/air/soil/sediment = 99.3/0/0/0.65%

Rev. note -

Klimisch criterium 4

Title Determination of 'ready' biodegradability: carbon dioxide (CO_2) evolution test (modified Sturm test) with Lathanol LAL powder.

Date of report June 17, 2003.

GLP Yes.

Reference 11.

Test substance CAS 1847-58-1, Lathanol LAL powder, purity 74.26%.

Guideline OECD 301B

Procedure

A stock solution of 1.00 g/L Lathanol LAL powder was prepared using ultrasonication. The stock was a light glassy solution. Since calculation of the theoretical TOC value was not possible, the TOC concentration of the stock was measured and determined to be 361.5 mg/L. The theoretical CO_2 production based on the TOC was calculated to be 1.33 mg CO_2/ml stock solution.
Duplicate test mixtures were incubated with activated sludge in 2 L brown-coloured glass bottles with three serial CO_2-absorbers ($(Ba(OH)_2)$) each and at a temperature of 21.4-23.5 °C for 28 days. Test mixtures contained test substance (33 mg/L) and filtrate of non-adapted inoculum (10 ml/L mineral medium) in mineral medium as prescribed in OECD 301B. The following controls were included:
- Inoculum blank: control without test substance but with inoculum (2 flasks).
- Positive control: reference substance (sodium acetate; 40 mg/L) with inoculum (1 flask).
- Toxicity control: Lathanol LAL powder (33 mg/L), sodium acetate (40 mg/L) and inoculum (1 flask).

Evolution of carbon dioxide was determined on day 0, 2, 5, 7, 9, 14, 14, 19, 23, 27 and 29 by titrating the remaining barium hydroxide with 0.05 M hydrogen chloride.

Results

Day	% degradation			
	TS with inoculum			Sodium acetate
	A (33 mg/L)	B (33 mg/L)	Mean	
2	0	0	0	6
5	5	20	12	40
9	18	33	26	65
19	42	51	47	85
23	45	56	50	88
29	51	62	56	94

Conclusions Lathanol LAL powder is not readily biodegradable under the above test conditions.
Rev. note 1. The test substance was not inhibitory on microbial activity.
Klimisch criterium 1

Title Lathanol® LAL – Determination of the Biodegradability of a Test Substance
Date of report 5 January, 2005 (revised draft version)
GLP Yes.
Reference 22.
Test substance CAS 1847-58-1, Lathanol LAL powder, purity 69.66%.
Guideline ASTM Guideline Number E 1720-95
ISO/DIS Guideline Number 14593
OPPTS Guideline Number 835.3120

Procedure

A stock solution of 0.50 mg C/ml Lathanol LAL powder was prepared. The TOC of this substance was calculated to be 50.9%. Inoculum consisted of activated sludge collected from wastewater treatment plants (primarily domestic) and fresh soil adjacent to the laboratory.
Triplicate test mixtures were incubated at 18-22°C with the inoculum in 20-ml serum vials containing 13.5 ml of medium in the dark for 28 days. Test mixtures contained test substance (10 mg C/L) and non-adapted inoculum (10 mg/L mineral medium) in mineral medium as described in OECD 310 (draft). The following controls were included:
- Inoculum blank: control without test substance but with inoculum (triplicate).
- Positive control: reference substance (sodium benzoate; 10 mg C/L) with inoculum (triplicate).
Vials were swirled on days 2, 7, 14, 21 and 26.
Evolution of carbon dioxide was determined on day 0, 2, 4, 7, 10, 14, 21, and 28 by determining the headspace CO_2 amount with a carbon analyzer after acidifying the medium.

Results Carbon analysis on day 0 showed that the inoculum blank contained 1.0 mg C/L, sodium benzoate 11.5 mg C/L and the test substance 9.3 mg C/L.

Day	% degradation	
	TS with inoculum	Sodium benzoate
	Mean of 3	(mean of 3)
2	18.0	63.6
4	42.5	79.3
7	49.8	81.9
10	57.7	85.1
14	66.5	90.8
21	73.1	89.5
28	70.2	93.5

Conclusions Lathanol LAL powder is readily biodegradable under the above test conditions.
Rev. note -
Klimisch criterium 1

Title Determination of ready biodegradability closed bottle test (Weston study 91-001),.
Date of report October 20, 1992.
GLP Yes.
Reference 3.
Test substance Lathanol LAL slurry (a.i. sodium lauryl sulfoacetate (CAS 1847-58-1)), purity 15.1% (carbon content 7.8% (w/w) in this formulation)
Guideline OECD 301 D
Procedure Duplicate test mixtures (7 flasks) were incubated in 300 mL glass BOD bottles at 20 +/- 0.2 ^{o}C for 28 days. Test mixtures (in completely filled flasks) contained test substance (2 mg/L or 5 mg/L), filtrate of (non-adapted) effluent from duplicate semi-continuous activated sludge units (40 µL) and mineral medium essentially as prescribed in OECD 301 D. The following controls were included:
- Inoculum blank: control without test substance but with inoculum (7 flasks).
- Positive control: reference substance (sodium benzoate; 2 mg/L) with inoculum (7 flasks).
- Complete blank: control without test substance and without inoculum (7 flasks).
Dissolved oxygen was determined on day 0, 5, 15 and 28. Degradation was calculated as BOD/COD. On day 0 single flasks were analysed, on the other time points duplicate flasks were analysed. Only BOD_5 was determined for glucose/glutamic acid control.

Results

Day	% degradation[1]		
	TS with inoculum[1]		Sodium benzoate[1]
	2 mg/L	5 mg/L	
5	31.6	30.9	68.4
15	43.2	34.9	74.7
28	65.9	>38.4[2]	>100[2]

1 mean of two replicates
2 dissolved oxygen value was below detection limit, therefore ">" value was reported

Conclusions Some components of the formulation are biodegradable.
Rev. note 1. Composition was not specified. Oxygen consumption observed may (partly) represent biodegradation of additives.
2. No abiotic control was included. Since the report does not indicatethat the test was performed in the dark, photodegradation cannot be excluded.
Klimisch criterium 3 Degradation may be related to other components (note 1).

Aquatic Toxicity

Title	96-hour acute toxicity study in zebra-fish with Lathanol LAL powder (semi-static).	
Date of report	November 5, 2004.	
GLP	Yes.	
Reference	20.	
Test substance	CAS 1847-58-1, Lathanol LAL powder , purity 70.2%.	
Test method	OECD 203.	
Test system	**Species**	Zebra-fish (*Danio rerio*, Teleostei, Cyprinidae): 3.2 ± 0.2 cm and 0.61 ± 0.19 g; loading: 0.43 g/L.
	No. of fish	7/vessel, 1 vessel/treatment.
	Concentrations	Nominal: 0, 1.0, 1.8, 3.2, 5.6 and 10 mg/L.
	Test conditions	Semi-static test with renewal each 24 hours and without aeration; 10.5 L glass vessels containing test medium (hardness 250 mg $CaCO_3$/L, pH 7.9-8.0); 16 h light, unfed (48 h prior to and during test).
	Exposure time	96 hours.
	Analysis	Analyses at the start of the test of freshly prepared and after 24 h of old solutions at 0, 1.0, 3.2 and 10 mg/L and at 72 h of freshly prepared and at 96 h of old solutions at 1.0 and 3.2 mg/L by HPLC-MS-MS.
	Phys. meas.	Daily for all vessels for pH (7.2-7.9) and O_2 >60% and temperature (21-22°C).
	Observations	Mortality/symptoms at 2, 24, 48, 72 and 96 h.
	Stat. method	Probit analysis for 24-h LC50.
Results	**Ref. product**	A test with the reference substance pentachlorophenol was performed in May 2004. The 96-h LC50 was 0.11 mg/L.
	Analysis	Mean measured concentration at 3.2 and 10 mg/L 73-98% of nominal and at 1.0 mg/L 52-68% of nominal.

Biological results

		Nominal concentration [mg/L]					
Parameter	**Time [h]**	**0**	**1.0**	**1.8**	**3.2**	**5.6**	**10**
Mortality [%]	2	0	0	0	0	0	0
	24	0	0	0	0	4	7
	48	1	0	0	0	7	7
	72	1	0	0	0	7	7
	96	1	0	0	0	7	7

Conclusion	24-h LC50 = 5.3 mg/L and 96-h LC50 = 4.2 mg/L.
Rev. note	-
Klimisch criterium	1

Title	Acute toxicity study in *Daphnia magna* with Lathanol LAL powder (semi-static).	
Date of report	18 December, 2003.	
GLP	Yes.	
Reference	13.	
Test substance	CAS 1847-58-1, Lathanol LAL powder, purity 74.26%.	
Test method	OECD 202.	
Test system	**Species**	*Daphnia magna*, <24 h old.
	No. of daphnids	5/replicate, 4 replicates/treatment.
	Concentrations	Nominal: 1.0, 2.2, 4.6, 10, 22 and 46 mg/L (no vehicle; prepared from stock solution 46 mg/L); blank control.
	Test conditions	Semi-static without aeration; in 100 mL glass beakers containing 80 mL of medium (hardness 201 mg/L as $CaCO_3$), 16 h light, no feeding.
	Exposure time	48 hours.
	Analyses	LC-MS. Samples taken at 0 and 24 h from freshly prepared solutions, and at 24 and 48 h from 24 h-old solutions.

	Phys. meas.	pH and dissolved oxygen: at 0, 24 and 48 h for all concentrations and control; pH = 7.8-7.9 and dissolved oxygen = 8.8-9.3 mg/L. Temperature: continuously; 20-21 °C Physical parameters remained within the required ranges during the test.
	Observations	Immobility at 24 and 48 h.
	Stat. method	Probit analysis.
Results	**Ref. product**	A test with the reference substance $K_2Cr_2O_7$ was performed in August 2003. The 48 h-EC_{50} of $K_2Cr_2O_7$ was 0.75 mg/L.
	Analysis	Measured concentrations were within 82-99% of the nominal concentrations.

Biological results

		Nominal concentration [mg/L]					
Parameter	**Time [h]**	**1.0**	**2.2**	**4.6**	**10**	**22**	**46**
Immobility [%]	24	0	0	0	40	85	100
	48	0	0	0	80	100	100

Conclusions 48-h EC_{50} = 7.9 mg/L (equivalent to 5.9 mg/L based on a.i.).
Rev. note 1. The two highest test concentrations still contained a very thin layer of foam. All final test solutions were clear and colourless.
Klimisch criterium 1

Title	Fresh water algal growth inhibition test with Lathanol LAL powder.	
Date of report	18 December, 2003.	
GLP	Yes.	
Reference	14.	
Test substance	CAS 1847-58-1, Lathanol LAL powder, purity 74.26%.	
Guideline	OECD 201.	
Test system	**Species**	*Selenastrum capricornutum*, strain: NIVA CHL 1.
	Initial cell conc.	$1*10^4$ cells/mL.
	No. of replicates	3 per treatment; 6 for blank control; 1 replicate of each test concentration without algae; 1 extra replicate of each test concentration and blank control for sampling purposes.
	Concentrations	Nominal 1.0, 2.2, 4.6, 10, 22, 46 and 100 mg/L, blank control.
	Test conditions	72-h static test in 100 ml glass vessels containing medium (in accordance with OECD 201) with continuous illumination (ca. 4900-6400 lux).
	Analysis	LC-MS. Samples were taken at 0, 24 and 72 h.
	Phys. meas.	pH: at 0 and 72 h; 7.8-9.0; in the blank control an increase of 1.5 was observed which correlated with a high rate of algal growth (7.9-9.4) Temperature: continuously; 23.0 – 24.1°C.
	Observations	Cell density at 0, 24, 48 and 72 h by spectrophotometry or microscope using a counting chamber.
Stat. method	ANOVA, Bonferroni t-test, Tukey test and Williams' test.	
Results	For biological data see table below. Growth factor control = 95.	

Biological results

		Measured concentration [mg/L]							
Parameter	**Time [h]**	**0**	**0.36**	**0.86**	**1.7**	**3.4**	**7.2**	**14**	**31**
Mean cell density [x10^4 cells/ml]	**0**	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	24	3.1	2.8	2.6	2.1	1.4	1.3	2.2	1.3
	48	24.3	20.3	19.4	15.0	8.1	1.9	2.0	1.3
	72	95.5	80.4	81.0	62.9	33.6	6.0	6.1	1.3
Inhibition [%] – AUC	**72-h**		16.3	17.5	36.7	67.2	94.9	93.5	99.1
Inhibition [%] – growth rate	**72-h**		4.0	3.7	9.2	24.2	60.7	60.4	95.9

Conclusions 72 h-E_bC_{50} = 1.9 mg/L.
72 h-E_rC_{50} = 6.8 mg/L.
$NOEC_r$ = 0.86 mg/L.

Rev. note 1. The nominal 72 h-E_rC_{50} of potassium dichromate was 0.89 mg/L.
2. Initial test solutions were all clear and colourless. Concentrations were not stable, especially over the last 48 hours. The concentrations in the vessels without algae decreased to the same extent. This is explained by possible biodegradation, since the test solutions became turbid during exposure, which may indicate bacterial growth.

Reliability 1.

Title EPISUITE v.3.10
Date of report -
GLP Not applicable.
Reference 18.
Test substance CAS 1847-58-1, acetic acid, sulfo-, 1-dodecyl ester, sodium salt.
Guideline Not applicable.
Remarks

ECOSAR Class		Organism	Duration	End Point	mg/L (ppm)
Esters	:	Fish	96-hr	LC50	22.180
Esters	:	Daphnid	48-hr	LC50	65.836
Esters	:	Green Algae	96-hr	EC50	1.809

Rev. note Calculated.
Klimisch criterium 4

Mammalian Toxicity

Acute Toxicity

Title Acute oral toxicity study (TM study 97-119-3A).
Date of report October 31, 1997.
GLP Yes.
Reference 1.
Test substance CAS: 1847-58-1, sodium lauryl sulfoacetate, purity 64-85%; impurities 5-18% sodium sulphate and 10-18% sodium chloride.
Guideline OECD 401.
Stat. method Not applicable.
Test system

Species	Rat (Sprague-Dawley), weight males 219-223 g, weight females 200-204 g; age 6-10 weeks; source: Harlan Sprague-Dawley, Indianapolis
No. of animals	5/sex/treatment.
Dosage	Single oral administration by gavage of 2000 mg/kg bw (vehicle distilled water, concentration 33% w/v); no controls; feeding *ad libitum* (food was withheld overnight prior to dosing).
Observations	Mortality and clinical signs several times on day 1 and daily thereafter until day 14. Bodyweight at study initiation, on days 7 and 14 and at death Necropsy on day 14.

Results

Effect\Dose [mg/kg bw]		2000	
Sex	**Day**	**M**	**F**
Mortality (day)	1-14	1 (2)	1 (2)
BW		- (animal that died)	- (animal that died)
Clinical signs[(A)]	1-14	+	+
Necropsy[(B)]	14	+	+

(A) Clinical observations included loose stool, hypoactivity and prostration (one animal) on day 1.
(B) Only in the animals that died stomach and intestines distended with gas and fluid were seen. The female also displayed a small intestine red in colour.

Conclusions Oral LD_{50} > 2000 mg/kg bw.

Klimisch criterium 1

Title Acute dermal toxicity (TM study 97-119-4),
Date of report September 29, 1997
GLP Yes
Reference 2
Test substance CAS: 1847-58-1, Sodium Lauryl Sulfoacetate, purity 64 – 85%; impurities 5-18% sodium sulphate and 10-18% sodium chloride.
Guideline OECD 402
Stat. method Not applicable
Test system
Species Rabbit (New Zealand White), weight 2.0-2.4 kg, age 8-12 weeks
No. of animals 5/sex/treatment.
Dosage Single administration of 2000 mg/kg bw (substance was slightly moistened before administration); area of application ca. 10% of total body surface (under occlusion); contact period of 24 hours; no controls;
Observations Mortality and clinical signs several times on day 1 and daily thereafter until day 14.
Body weight prior to dosing , on days 7 and 14, and at death.
Necropsy on day 14.

Results

Effect\Dose [mg/kg bw]		2000	
Sex	**Day**	**M**	**F**
Mortality (day)	1-14	3 (4 or 5)	1(4)
Clinical signs[(A)]	1-14	+	+
Necropsy[(B)]	14	+	-

(A) Clinical observations included erythema (until day 7-8), oedema (until day 9-11), eschar&coriaceousness (until day 11-14) and formation of scar tissue. Three males exhibited chemical burns until day 3-4 (death).
(B) Findings consisted of severe tissue damage & necrosis of the skin at the application site in all rats that died, a stomach devoid of contents in two males that died and scar tissue at the application site in some surviving animals.

Conclusions Dermal LD_{50} > 2000 mg/kg bw.

Klimisch criterium 1

Title Final report on the safety assessment of sodium lauryl sulfoacetate.
Date of report 1987.
GLP No.
Reference 9.
Test substance Bath additive.
Guideline Not indicated.

Remarks Groups of 10 female Harlan Wistar rats (115-135 g) were given single oral doses (5-14 g bath additive/kg) of a bath additive (containing 50% sodium lauryl sulfoacetate) as a 35% aqueous solution. Leg weakness, obtunded righting reflex, ataxia, diuresis and diarrhea were observed. Most deaths occurred 4-24 h after treatment.

Conclusion LD_{50} = 5.75 g/kg bath additive (= 0.7 g/kg sodium lauryl sulfoacetate). Recalculated by the reviewer as 5.75 x 0.5 x 0.35 = 1.0 g/kg sodium lauryl sulfoacetate.

Rev. note The information given was limited to the above mentioned. The other components of the bath additive are not known and thus the toxicity seen might be attributable to another component.

Klimisch criterium 3

Skin/eye irritation

Title OECD guideline 404 primary dermal irritation/corrosion study (TM 97-119-2).
Date of report September 29, 1997.
GLP Yes.
Reference 15.
Test substance Lathanol LAL, purity 64-85%; impurities: 5-18% sodium sulfate, 10-18% sodium chloride.
Guideline OECD 404.
Test system
Species Rabbit (New Zealand White), weight 1850-2320 g.
No. of animals 6 males.
Dosage Application of 0.5 g test substance, moistened with distilled water, on the clipped skin under semi-occlusion for 4 hours.
Observations Skin observations at ½, 24, 48 and 72 h and at 7 and 14 days after removal of the dressing.

Results

Animal	1		2		3		4		5		6	
Time	**E**	**O**	**E**	**O**	**E**	**O**	**E**	**O**	**E**	**O**	**E**	**O**
½ h	1	0	1	1	1	2	1	2	1	2	1	2
24 h	1	0	0	0	1	1	2	1	2	1	2	2
48 h	1	0	0	0	2	0	2	0	2	0	3	1
72 h	1	0	0	0	2	0	2	0	2	0	3	1
7 days	0	0	0	0	0	0	0	0	0	0	1	0
14 days	0	0	0	0	0	0	0	0	0	0	0	0

E=erythema O=oedema

Conclusions Moderately irritating.
Klimisch criterium 1

Title DOT test for corrosivity.
Date of report May 31, 1977.
GLP No.
Reference 16.
Test substance Lathanol LAL, purity 64-85%; impurities: 5-18% sodium sulfate, 10-18% sodium chloride.
Guideline Not specified.
Test system
Species Rabbit.
No. of animals 6 males.
Dosage Application of 0.5 ml test substance on the clipped skin under occlusion for 4 hours.
Observations Skin observations at 0, 24, and 72 after removal of the dressing.

Results

Animal	1		2		3		4		5		6	
Time	**E**	**O**	**E**	**O**	**E**	**O**	**E**	**O**	**E**	**O**	**E**	**O**
0 h	1	1	1	0	1	1	1	1	1	1	1	1
24 h	1	0	1	0	1	0	0	0	1	0	0	0
72h	0	0	0	0	0	0	0	0	0	0	0	0

E=erythema O=oedema

Rev. note 1. The scoring used is not known to the reviewer; the appendix was not included.
Conclusion The test substance is not corrosive.

Klimisch criterium	4 The information given is limited to the above mentioned.

Title	Final report on the safety assessment of sodium lauryl sulfoacetate.
Date of report	1987.
GLP	No.
Reference	9.
Test substance	CAS: 1847-58-1, Sodium Lauryl Sulfoacetate.
Guideline	Not indicated.
Remarks	Undiluted sodium lauryl sulfoacetate (0.5 g) moistened with 0.9% saline was applied to the skin of 6 New Zealand rabbits for 24 hours (semi-occlusion). Test sites were scored at 30 min and 24 hours after patch removal. The mean PII was 2.7. One animal had areas of possible necrosis within the test site at 24 hours.
Rev. note	The information given was limited to the above mentioned. Worst case exposure (24 h).
Klimisch criterium	4

Title	Final report on the safety assessment of sodium lauryl sulfoacetate.
Date of report	1987.
GLP	No.
Reference	9.
Test substance	Bath additive.
Guideline	Not indicated.
Remarks	Undiluted bath additive (powder; 500 mg) containing 35% of sodium lauryl sulfoacetate (175 mg) and a 1% solution of the bath additive were applied to the skin of 3 rabbits for 4 days. No irritation was observed at the sites treated with the powdered bath product. All sites treated with 1% solution had slight erythema on day 2 but were normal on day 7.
Rev. note	The information given was limited to the above mentioned. The other components of the bath additive are not known and thus the toxicity seen might be attributable to another component.
Klimisch criterium	3

Title	Final report on the safety assessment of sodium lauryl sulfoacetate.
Date of report	1987.
GLP	No.
Reference	9.
Test substance	Bath additive.
Guideline	Not indicated.
Remarks	Undiluted bath additive (powder; 500 mg) containing 35% of sodium lauryl sulfoacetate (175 mg) and a 1% solution of the bath additive were applied to separate sites on the skin of 3 rabbits for 4 days. No irritation was observed at the sites treated with the powdered bath product. All sites treated with 1% solution had slight erythema on day 2 but were normal at day 7.
Rev. note	The information given was limited to the above mentioned. The other components of the bath additive are not known and thus the toxicity seen might be attributable to another component.
Klimisch criterium	3

Title	OECD guideline 405 acute eye irritation/corrosion study (TM 97-119-1).
Date of report	October 2, 1997.
GLP	Yes.
Reference	17.

Test substance Lathanol LAL, purity 64-85%; impurities: 5-18% sodium sulfate, 10-18% sodium chloride.
Guideline OECD 405.
Test system
Species Rabbit (New Zealand White), weight 2010-2300 g.
No. of animals 6 females.
Dosage Instillation of 87-90 mg test substance (0.1 ml).
Observations At 1, 24, 48 and 72 h and at 7, 14 and 21 days after removal of the dressing. From 24 hours onwards also fluorescein and UV-light examination was used.

Results

Animal	1				2				3				4				5				6			
Time	C	I	Conj[(A)]		C	I	Conj[(A)]		C	I	Conj		C	I	Conj[(A)]		C	I	Conj[(A)]		C	I	Conj[(A)]	
			R	Ch			R	Ch			R	Ch			R	Ch			R	Ch			R	Ch
1 h	-	1	2	2	-	1	2	3	-	0	2	2	-	1	2	3	-	0	2	3	-	1	2	2
24 h	1	1	2	2	1	1	2	2	1	1	2	1	1	1	2	2	1	1	2	2	1	1	2	2
48 h	1	1	2	2	1	1	2	2	1	1	2	1	1	0	2	2	1	0	2	2	1	0	2	1
72 h	1	1	2	2	1	1	2	2	1	0	2	1	1	0	2	2	1	0	1	1	1	0	1	1
7 days	1	1	1	1	1	1	2	2	1	0	1	1	1	0	2	2	1	0	1	1	0	0	1	1
14 days	2	1	1	1	2	1	2	2	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0
21 days	2	0	1	0	3	1	2	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0

C=corneal opacity I=Iris Conj=conjunctiva Red=redness Ch=chemosis.
(A) Severe discharge was observed.

Conclusions Moderately irritating.
Klimisch criterium 1

Title Final report on the safety assessment of sodium lauryl sulfoacetate.
Date of report 1987.
GLP No.
Reference 9.
Test substance Bath additive/milk.
Guideline Not indicated.
Remarks One eye of six rabbits was treated with 0.1 ml 1% solution of bath additive containing 35% sodium lauryl sulfoacetate and observed for 4-7 days. Slight conjunctival redness was observed 1 h after treatment and had dissipated by 48 h. The cornea and iris appeared normal.

One eye of three female New Zealand rabbits was treated with a 10% aqueous solution of a milk bath containing 30% sodium lauryl sulfoacetate. All rabbits had minimal conjunctival irritation at 1 and 24 h and no irritation at 48 h.
Conclusion A 0.35% and 3% solution of sodium lauryl sulfoacetate are not irritating to the eye.
Rev. note The information given was limited to the above mentioned. The other components of the bath additive/milk bath are not known and thus the toxicity seen might be attributable to another component.
Klimisch criterium 3

Mutagenicity

Title Mutagenicity evaluation of 2800-00, Lot 28M024 in the Ames Salmonella/ Microsome plate test.
Date of report September 20, 1978.
GLP No.
Reference 6.
Test substance 2800-00, Lot 28M024, purity not indicated.
Guideline Not indicated.
Test system
Bacterial strains TA98, TA100, TA1535, TA1537, TA1538 and D4(Saccharomyces cerevisiae).
Initial bacteria conc. Ca. 10^8 cells from an overnight culture.
Metabolic activation Liver S9 mix (Aroclor 1254-induced).

Test concentrations	1, 10, 100, 500 and 1000 μg /plate.
Controls	Negative: solvent (DMSO). Positive: N-methyl,N-nitro,N-nitrosoguanidine (TA1535, TA100, D4), 9-aminoacridine (TA1537), 2-nitrofluorene (TA98 and TA1538) all without S9; 2-anthramine for all strains with S9.
Test type	Plate incorporation; incubation for 48 h at 37 ^{o}C. D4-yeast plates were incubated at 30 ^{o}C for 3-5 days.
No. of replicates	1.
Criteria for evaluating results	The result was considered positive, if a positive dose response was observed over three concentrations.

Results

	Test result [(A)]	
Tester strain	**Without activation**	**With activation**
TA98	-	-
TA100	-	-
TA1535	-	-
TA1537	-	-
TA1538	-	-
D4	-	-

+/- : positive/negative result; positive controls gave expected responses.
Cytotoxicity was observed at 1000 μg/plate.

Conclusion	Not mutagenic.
Rev. note	1. According to handwritten text on the front page of the report, substance 2800-00, Lot 28M024 corresponds to sodium lauryl sulfoacetate, 3% in shampoo. 2. For each test concentration only single experiments were performed.
Klimisch criterium	2 Single experiments (see note 2); non GLP.

Title	Mutagenicity evaluation of 2300-00, Lot 23N056 in the Ames Salmonella/microsome plate test.	
Date of report	September 21, 1978.	
GLP	No.	
Reference	7.	
Test substance	2300-00, Lot 23N056, purity not indicated.	
Guideline	Not indicated.	
Test system	**Bacterial strains**	TA98, TA100, TA1535, TA1537, TA1538 and D4(Saccharomyces cerevisiae).
	Initial bacteria conc.	Ca. 10^{8} cells from an overnight culture.
	Metabolic activation	Liver S9 mix (Aroclor 1254-induced).
	Test concentrations	1, 10, 100, 500 and 1000 μg /plate.
	Controls	Negative: solvent (water). Positive: N-methyl,N-nitro,N-nitrosoguanidine (TA1535, TA100, D4), 9-aminoacridine (TA1537), 2-nitrofluorene (TA98 and TA1538) all without S9; 2-anthramine for all strains with S9.
	Test type	Plate incorporation; incubation for 48 h at 37 ^{o}C. D4-yeast plates were incubated at 30 ^{o}C for 3-5 days.
	No. of replicates	1.
	Criteria for evaluating results	The result was considered positive, if a positive dose response was observed over three concentrations.

Results

	Test result [A]	
Tester strain	**Without activation**	**With activation**
TA98	-	-
TA100	-	-
TA1535	-	-
TA1537	-	-
TA1538	-	-
D4	-	-

+/- : positive/negative result; positive controls gave expected responses.
Slight cytotoxicity was observed at 1000 µg/plate.

Conclusion Not mutagenic.

Rev. note
3. According to handwritten text on the front page of the report, substance 2300-00, Lot 23N056 corresponds to sodium lauryl sulfoacetate, 23% in a cleansing bar.
4. For each test concentration only single experiments were performed. A repeat test was conducted for TA1535 and TA1537 and the result was also negative.

Klimisch criterium 2 Single experiments (see note 2); non GLP.

Title Mutagenicity evaluation of 3000-00, Lot 30M366 in the Ames Salmonella/microsome plate test.

Date of report September 13, 1978.

GLP No.

Reference 8.

Test substance 3000-00, Lot 30M366, purity not indicated.

Guideline Not indicated.

Test system

Bacterial strains	TA98, TA100, TA1535, TA1537, TA1538 and D4(Saccharomyces cerevisiae).
Initial bacteria conc.	Ca. 10^8 cells from an overnight culture.
Metabolic activation	Liver S9 mix (Aroclor 1254-induced).
Test concentrations	1, 10, 100, 500 and 1000 µg /plate.
Controls	Negative: solvent (water). Positive: N-methyl,N-nitro,N-nitrosoguanidine (TA1535, TA100, D4), 9-aminoacridine (TA1537), 2-nitrofluorene (TA98 and TA1538) all without S9; 2-anthramine for all strains with S9.
Test type	Plate incorporation; incubation for 48 h at 37 °C. D4-yeast plates were incubated at 30 °C for 3-5 days.
No. of replicates	1.
Criteria for evaluating results	The result was considered positive, if a positive dose response was observed over three concentrations.

Results

	Test result [A]	
Tester strain	**Without activation**	**With activation**
TA98	-	-
TA100	-	-
TA1535	-	-
TA1537	-	-
TA1538	-	-
D4	-	-

+/- : positive/negative result; positive controls gave expected responses.
Cytotoxicity was observed at 1000 µg/plate.

Conclusion Not mutagenic.

Rev. note
5. According to handwritten text on the front page of the report, substance 3000-00, Lot 30M366 corresponds to sodium lauryl sulfoacetate, 19% in a cleansing bar.
6. For each test concentration only single experiments were performed.

Klimisch criterium 2 Single experiments (see note 2); non GLP.

Title Evaluation of the ability of Lathanol LAL powder to induce chromosome aberrations in cultured peripheral human lymphocytes.
Date of report July 22, 2003.
GLP Yes.
Reference 12.
Test substance CAS 1847-58-1, Lathanol LAL powder, purity 74.26%.
Guideline OECD 473.
Stat. method Chi-square test.
Test system
Cell line Human lymphocytes.
Metabolic activation Rat S9 mix (Aroclor 1254-induced).
Test concentrations 50 - 500 μg/ml (based on cytotoxicity).
Controls Negative: vehicle control (DMSO).
Positive: mitomycin-C (-S9), cyclophosphamide (+S9).
Procedure -S9: 3 h exposure + 24 h fixation.
24 h exposure + 24 h fixation.
48 h exposure + 48 h fixation.
+S9: 3 h exposure + 24 h fixation.
3 h exposure + 48 h fixation.
Colchicine was added for the last 3 hours.

Results

Exposure/fixation (h)	Metabolic activation	Doses evaluated [μg/ml]	Aberrations [%]	Test result(A)
3/24	Without	0, 100, 300, 500	3, 7, 4, 5	-
3/24	With	0, 100, 300, 500	2, 3, 6, 7	-
3/48	With	0, 300, 400, 500, 600	0, 0, 3, 5, 9	+/-
24/24	Without	0, 100, 125, 200	2, 5, 0, 1	-
48/48	Without	0, 56, 100, 130	1, 4, 1, 5	-

(A)+/- : positive/negative result; positive controls gave expected responses.
Cytoxicity was observed at ≥200 without metabolic activation and ≥500 μg/ml with metabolic activation.

Conclusion Not clastogenic.
Rev. note All values remained within historical control values. The statistically significant increase in the number of cells with chromosome aberrations at 600 μg/ml was ascribed to the strongly cytotoxic effect of this concentration (MI 36%).
Klimisch criterium 1.

Repeated Dose Toxicity

Title 28 day oral range finding study in the rat.
Date of report August 1985.
GLP Yes.
Reference 4.
Test substance CAS 1847-58-1 (sodium lauryl sulfoacetate), purity 73.80%; impurities: 13.09% sodium chloride, 11.39% sodium sulfate, 1.95% free oil and 0.28% water.
Guideline Not indicated.
Stat. method Student's t-test.
Test system
Species CD rat.
Source Charles River UK.
Bodyweight Males 123-149 g g, females 114-142 g.
No. of animals 5/sex/teatment.
Dosage 0, 50, 200 and 800 mg/kg/day by gavage (dosing volume 10 ml/kg/day).
Vehicle Distilled water.
Exposure period 28 days.

Investigations	**General**	Clinical signs, mortality (daily), food consumption (group mean weekly), bodyweight (daily).
	Clinical pathology	Haematology: haematocrit, haemoglobin, erythrocyte count, mean cell volume, mean cell haemoglobin concentration and total leucocyte count. Biochemistry: blood urea nitrogen, glucose, alkaline phosphatase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase and total protein.
	Necropsy	Gross examination, macroscopy of cranial, thoracic and visceral cavities, organ weights (brain, kidneys, liver).
	Analysis	Concentration analysis on day 1.

Results

Dose	*0 mg/kg*		*50 mg/kg*		*200 mg/kg*		*800 mg/kg*		*Dose related*	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Mortality	No deaths occurred									
Clinical Signs (A)						+	+	+		
Body weight (gain)										
Food consumption						d		d		
Haematology	No changes of toxicological significance.									
Clinical chemistry	No changes of toxicological significance.									
Necropsy										
Macroscopy (B)							+			
Liver weight							i[a,r]			
Kidney weight								ic[r]		
Brain weight								ic[r]		

Where i=increase; d=decrease; ic=significant increase; dc=significant decrease; [a]=absolute; [r]=relative.

(A) Poor coat condition; was already present in all animals before treatment but had disappeared by day 11 except for females at 800 mg/kg. Males at 800 mg/kg and females at 200 mg/kg showed the condition again from day 22 and 15 resp. Post dose salivation in all animals at 800 mg/kg.

(B) Raised black foci on the non glandular mucose of the stomach in one male.

Actual concentrations	Concentrations as measured were 98-108% of nominal concentrations.
Conclusions	NOAEL = 200 mg/kg/day and LOAEL = 800 mg/kg/day based on <10% effect on body weight.
Rev. note	1. No histological examinations were performed. 2. Only limited biochemical parameters were investigated. 3. Only a limited number of organs were weighed.
Klimisch criterium	2.

Title	90 day oral toxicity study in the rat.	
Date of report	March 1986.	
GLP	Yes.	
Reference	5.	
Test substance	CAS 1847-58-1 (sodium lauryl sulfoacetate), purity 73.80%; impurities: 13.09% sodium chloride, 11.39% sodium sulfate, 1.95% free oil and 0.28% water.	
Guideline	Not indicated.	
Stat. method	ANOVA, Student's t-test.	
Test system	**Species**	Rat, CD (SD) BR strain.
	Source	Charles River UK.
	Bodyweight	Males 114-153 g g, females 106-135 g.
	No. of animals	20/sex/treatment; satellite group of 10/sex for to provide pre-exposure clinical pathology.
	Dosage	0, 75, 250 and 750 mg/k/day by gavage (dosing volume 10 ml/kg).
	Vehicle	Distilled water.
	Exposure period	91 days.

Investigations

General Clinical signs and mortality (daily), food consumption (group mean weekly), bodyweight (daily), ophtalmoscopy (before study initiation in all animals and after 4 and 12 weeks of treatment in high dose and control animals).

Clinical pathology Haematology (pre-exposure, wk 4 and wk 12): haematocrit, haemoglobin, erythrocyte count, mean cell volume, mean cell haemoglobin concentration, total and differential leucocyte count prothrombin time and partial thromboplastin time.
Biochemistry (pre-exposure, wk 4 and wk 12) blood urea nitrogen, glucose, alkaline phosphatase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase, total protein and albumin/globulin, sodium and potassium.
Urinalysis (pre-exposure, wk 4 and wk 12): samples were collected overnight to measure following parameters: volume, appearance and colour, specific gravity, pH, glucose, protein, ketones, bilirubin, blood pigments and deposit.

Necropsy Gross examination;
Macroscopy of cranial, thoracic and visceral cavities;
Organ weights of brain, kidneys, liver, adrenals, heart, lungs, ovaries, pituitary, spleen, testes, thyroids and uterus;
Microscopy of following organs for animals in the high dose and control group: adrenals, aortic arch, brain, caecum, cervical lymph nodes, colon, duodenum, epididymes, eyes, heart, ileum, jejunum, kidneys, liver, lungs, caudal and cranial mammary gland, mesenteric lymph nodes, optic nerve, ovaries, pancreas, pituitary, prostate, spleen, stomach, testes, thymus, thyroids, urinary bladder and uterus. Stomachs of middle and low dose animals were also examined.

Analysis Concentration analysis on day 1 by comparing absorbances with those of standard solutions.

Results

Dose	*0 mg/kg*		*75 mg/kg*		*250 mg/kg*		*750 mg/kg*		*Dose related*	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Mortality	No test substance related deaths occurred*									
Clinical Signs (A)					+	+	+	+		
Body weight gain	No treatment related changes.									
Food consumption	No treatment related changes.									
Ophtalmoscopy	No treatment related changes.									
Haematology										
WBC, wk 4 (wk 12)**							dc (d)			
Clinical chemistry	No changes of toxicological significance.									
BUN, wk 4 (wk12)**				(ic)		Ic (ic)		Ic (ic)		
Urinalysis										
Urinary volume (B)								i		
Specific gravity								d		
Necropsy	No test substance related abnormalities.									
Macroscopy										
Liver weight								ic[a,r]		
Microscopy (C)					+	+	+	+	x	x

Where i=increase; d=decrease; ic=significant increase; dc=significant decrease; [a]=absolute; [r]=relative.

*One control female died during blood sampling.

**The changes were not of toxicological significance.

(A) Post dose salivation from week 3 (750 mg/kg) of week 8 (250 mg/kg) on.

(B) Increased volume after both 4 and 12 weeks, reduced specific gravity after 12 weeks.

(C) Changes were restricted to the stomach and consisted of hyperplasia of the non-glandular squamous epithelium, with associated focal epithelial erosion and varying degrees of gastritis in some rats. In females at high dose, epithelial whorls were seen in the thyroid.

Actual concentrations Concentrations as measured were 99% of nominal concentrations.

Conclusions NOAEL = 75 mg/kg/day and LOAEL = 250 mg/kg/day, as based on histological changes in stomach mucosa in both sexes.

Rev. note	Study is in accordance with the old OECD guideline 408 (1981). The new guideline requires a more extensive biochemical and histological examination, recording of more organ weights and measurement of sensory reactivity.
Klimisch criterium	1

Reproduction/developmental toxicity

Title	Reproduction/developmental toxicity screenings test with Lathanol LAL powder administered by oral gavage in Wistar rats.	
Date of report	September 2004.	
GLP	Yes.	
Reference	21.	
Test substance	CAS 1847-58-1, Lathanol LAL powder, purity 72.43% (dosing period: 29 March – 08 May) CAS 1847-58-1, Lathanol LAL powder, purity 74.26% (dosing period: 07 May – 13 May)	
Guideline	OECD 421	
Stat. method	Dunnett-test, Steel-test, Fisher Exact-test.	
Test system	**Species**	Crl: (WI) BR (outbred, SPF-Quality)
	Source	Charles River, Sulzfeld, Germany.
	Bodyweight	Males 285-350 g, females 206-242 g.
	No. of animals	10/sex/teatment.
	Dosage	0, 40, 200 and 1000 mg/kg/day by gavage (dosing volume 5 ml/kg/day).
	Vehicle	propylene glycol
	Exposure period	Two weeks prior to mating, during mating until necropsy (39 days for males) or until at least 3 days of lactation (42 to 46 days for females).
Mating procedure		Females were paired on a one-to-one-basis with males from the same treatment group. Each morning the cages were checked for copulation plugs (day 0 of gestation).
Investigations	**General**	
	Mortality	at least twice daily.
	Clinical signs	at least once daily.
	Body weights	weekly (males/females) and for mated females on days 0, 7, 14 and 21 of gestation and days 1 and 4 of lactation.
	Food consumption	weekly (males/females). During mating analysis of food consumption was suspended. For mated females on days 0, 7, 14 and 21 of gestation and days 1 and 4 of lactation.
	Water consumption	Subjective appraisal during the study period.
	Reproduction processes	Male number paired with, mating date, confirmation of pregnancy, delivery day, number of corpora lutea, number of implantations.
	Litter data	The number of live/dead pups (day 1 of lactation and daily thereafter), body weights of pups (days 1 and 4 of lactation), sex of each pup, the number of pups with physical/behavioural abnormalities (daily), external examinations of all pups if practically possible (the stomach was examined for the presence of milk).
	Necropsy	
	Termination	Males after mating and minimal 28 days of dosing, females on/shortly after day 4 post-partum.
	Macroscopy	Macroscopy of cranial, thoracic and visceral cavities. Samples of the following tissues were fixed: cervix, clitoral gland, coagulation gland, epididymides, ovaries, pituitary gland, preputial gland, prostate gland, seminal vesicles, stomach, testes, uterus, vagina and all gross lesions.
	Organ weights	Epididymides and testes of males
	Microscopy	Stomach, ovaries, epididymides and testes (including additional slides for staging spermatogenesis) from animals of groups 1 and 4, preserved organs and tissues of 1 female that was killed in extremis, all gross lesions of all animals and the reproductive organs of 1 female of group 2 (not pregnant) and 1 male of group 2 (suspected of infertility).

Analysis Analysis of accuracy (all groups) and homogeneity (groups 2 and 4) during weeks 1, 2 and 6, and after the study. Stability (groups 2 and 4) during weeks 1 and 2. Method by HPLC/MS

Results

Dose	*0 mg/kg*		*40 mg/kg*		*200 mg/kg*		*1000 mg/kg*		*Dose related*	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
General										
Mortality	0/10	0/10	0/10	1/10[1]	0/10	0/10	0/10	1/10[2]		
Clinical Signs (A)							+	+		
Body weight (gain)							dc[3]			
Food consumption							dc[3]	dc[3]		
Water consumption	No treatment-related findings									
Reproduction processes	No treatment-related findings									
Litter data	No treatment-related findings									
Necropsy										
Macroscopy										
-Stomach focus/foci							1			
-Stomach thickened							7*			
-Stomach irregular surface							3	2		
-Mandibular lymph node enlarged							2			
Organ weights	No treatment-related findings									
Microscopy										
-Forestomach hyperplasia of squamous epithelium							10	10		
-Forestomach lymphogranulocytic inflammation							4	2		
-Forestomach erosion							2	0		

Where i=increase; d=decrease; ic=significant increase; dc=significant decrease; [a]=absolute; [r]=relative.

1 Abortion, premature kill
2 During the first week of treatment.
3 Non-pregnant, killed 21 days post-coitum.
(A) Salivation in all males and females during almost the complete study period; rales incidentally in a few animals.
* Statistically significant Fisher's Exact test (1%)

Actual concentrations Quantitative analyses were based on two test substance peaks.
Accuracy of formulations was 77-123% (first week), 90-109% (week 2) and 91-115% (after study period) of nominal concentrations. The spread during the first week was considered to be related to analytical procedures.
Repeated analysis during week 2 revealed homogeneous and stable (at least 5 hours) formulations.

Conclusions The parental NOAEL = 200 mg/kg/day and LOAEL = 1000 mg/kg/day based on clinical signs, <10% effect on body weight and food consumption and on pathological findings in the forestomach. The NOAEL for reproduction and developmental toxicity was 1000 mg/kg/day.

Rev. note
1. Impurities not indicated. Purity approximately 73%.
2. Histopathology of the stomach of groups 2 and 3 was not performed in absence of any gross findings in these groups.

Klimisch criterium 1.