9.0 Appendices

Robust Summaries

With reference to the SIDS Data Matrix, the reports have been evaluated and assessed according to the Klimisch criteria as described in previous sections.

1 = Reliable without restrictions
2 = Reliable with restrictions
3 = Not reliable
4 = Not assignable

This chapter will focus on each study specifically. The order of presentation will be physico-chemical data, environmental fate data, ecotoxicity and mammalian toxicity.

List of Abbreviations

^{a}	Absolute to body weight
-	Absent
+	Present
a.i.	Active ingredient
BP	Boiling point
d	Decrease
dc	Decrease (significant)
DR	Dose related
F	Female
Hb	Haemoglobin
i	Increase
ic	Increase (significant)
M	Male
N/A	Not applicable
^{r}	Relative to body weight
$THCO_2$	Theoretical amount of CO_2
TCO_2	Theoretical amount of CO_2
TS	Test substance
VP	Vapour pressure
WS	Water solubility

Appendix 9-1 - Physico-Chemical Data for LAS/ABS

9.1.01

Title	MSDS Rhodacal® 330
Date of report	May 14, 1999.
GLP	No data
Reference	22
Test substance	A (Benzenesulfonic acid, dodecyl-, compd. with isopropylamine (1:1)), purity 90%.
Guideline	Not specified.
Water solubility	Dispersible.
Boiling point	>149°C (at 1.0E5 Pa)
Vapor pressure	<3.1E3 Pa (25°C)
Klimisch criterium	4 - Secondary literature

9.1.02

Title	MSDS Rhodacal® CA/70
Date of report	August 17, 1999.
GLP	No data
Reference	21
Test substance	C (Benzenesulfonic acid, dodecyl-,calcium salt), purity 69-71%.
Guideline	Not specified.
Water solubility	Dispersible.
Rev. note	Secondary literature.
Klimisch criterium	4 – Secondary literature

9.1.03

Title	MSDS Casul 70 HF
Date of report	29 February 2000.
GLP	No data
Reference	3 and 4
Test substance	F (Benzenesulfonic acid, mono-C11-13-branched alkyl derivs., calcium salts), purity 69.5-71.5%.
Guideline	Not specified.
Boiling point	117°C (tested as a formulations containing 77% HPV substance plus organic solvent)
Vapor pressure	733 Pa (20°C)
Water solubility	Dispersible.
Rev. note	The second reference (#3) is an incomplete MSDS for a formulation containing 55% HPV substance plus organic solvent
Klimisch criterium	4 – Secondary literature

Appendix 9-2 - Environmental Fate Data and Pathways for LAS/ABS

9.2.02

Title The evaluation of the biodegradation of 910-92 using the OECD screening test method

Date of report July 2, 1986.

GLP Yes.

Reference 20

Test substance B (Benzenesulfonic acid, dodecyl-, compd. with 2,2',2"-nitrilotris(ethanol) (1:1)); Bio-Soft LD-190; Blend consists of 10% triethanolamine dodecyl benzene sulfonate (compound B), 59% nonylfenol ethoxylate, 17% ether sulfate, 10% TEA, <5% cocamide DEA and <5% ethanol.

Test method EPA TSCA test guidelines 40 CFR 796.3240, Modified OECD screening test (1985).

Test system

Treatments

- Inoculum: prepared from soil (supernatant of aqueous suspension), secondary effluent from a sewage treatment plant and surface water (1:1:1). Each flask was inoculated with 0.5 mL of the mixed composite inoculum.
- 2 flasks treated (medium + inoculum + Bio-Soft LD-190 (20 mg C/L));
- 2 flasks positive control (medium + inoculum + sodium benzoate (20 mg C/L));
- 2 flasks blank control (medium + inoculum).

Procedure Aliquots of a stock solution of the test substance (tested conc. 20 mg C/L), mixed composite inoculum (0.5 mL) and nutrient solution (1 L) were mixed. The test mixtures were incubated at 21-23°C for 35 days. Aliquots were removed from each flask on day 0, 7, 14, 21, 27, 28 and 35 for DOC analyses.

Results

	% degradation [% of day 0 values]	
day	***Bio-Soft LD-190***	***sodium benzoate (reference substance)***
0	0	0
7	58	99
14	63	100
21	68	100
27	73	98
28	71	100
35	72	100

Conclusion Test substance is biodegradable. 71% degraded after 28 days, but did not reach 60% in 10-day window.

Rev. note

1. Test substance is a blend containing 10% of substance B. Because (1) the resulting biodegradation (72%) is of the entire blend, (2) substance B is only 10% of the blend, and (3) the other components of the blend are known to be biodegradable, the biodegradation of substance B cannot be accurately estimated from this study.

Klimisch criterium 4 Test substance was a blend.

9.2.03

Title Biotic degradation (modified Sturm test) Evaluation, in an aqueous medium, of the "ultimate" biodegredability of substances: 1736-1A, 1736-1B, 1736-1C, 1736-1D, 1736-1E

Date of report Not indicated.

GLP No data

Reference 25

Test substance D, 1736-1E, purity 96%.

Test method OECD 301B.

Test system

Design Two control flasks (medium + inoculum 30 mL), 2 treated flasks (medium + inoculum 30 mL + test substance 10 and 20 mg C/L), 1 flask for positive control (medium + inoculum 30 mL + aniline 20 mg C/L).

Procedure Incubation was performed in 5 L flasks containing 3000 mL of mineral solution with test substance and/or inoculum from activated sludge from

a plant treating predominantly domestic sewage. The inoculum was treated and aerated for 28 days at 22±2°C with CO_2-free air in the dark. The outcoming air was passed through CO_2-traps containing $Ba(OH)_2$. CO_2 was determined in the traps by back titration of residual $Ba(OH)_2$ after 1, 4, 5, 7, 8, 11, 12, 13, 15, 18, 20, 22, 26, 27 and 28 days. Samples of the incubate were removed on day 0 and 28 for DOC analysis.

Results **Analysis** DOC analysis: 94-107% of nominal (day 0); after 28 days: 14.4-17.2% of nominal was left for 1736-1E (82-87% degraded) and 0.3% of nominal for aniline (100% degraded).
For further results see table below.

	% biodegradation [% of $ThCO_2$] on day:														
Treatment	**1**	**4**	**5**	**7**	**8**	**11**	**12**	**13**	**15**	**18**	**20**	**22**	**26**	**27**	**28**
1736-1E (10 mg C/L)	1.6	15	28	41	48	56	61	62	64	66	68	70	71	72	73
1736-1E (20 mg C/L)	0.3	3.9	16	32	40	49	51	53	56	59	61	62	64	65	64
Positive control	0.0	19	41	58	64	72	76	78	80	83	86	87	89	89	89

Conclusion Biodegradable. 64-73% after 28-days at 10 mg/L and 20 mg/L, respectively. Meets the 10-day window for readily biodegradable at 10 mg/L but not at 20 mg/L.

Klimisch Criterium 1

9.2.04

Title Bioconcentration of linear alkylbenzene sulfonate (LAS) in bluegill *(Lepomis macrochirus)*

Date of report 1981.

GLP No.

Reference 8

Test substance 2 (Benzenesulphonic acid, linear alkyl), 14C-ring-labeled LAS.

Test method Not specified.

Procedure Bluegill (*Lepomis macrochirus*), 4.0 g and 68 mm, were exposed to isotopically diluted ^{14}C-ring-labeled-LAS at mean measured concentration of 0.50 mg/L (SD 12%) for 21 days, followed by 14 days of depuration. The test included an untreated control and was conducted under flow-through (~20 changes/24 h) at 17±1°C, pH 7.1 in 60 L aquaria containing water of hardness 35 mg/L ($CaCO_3$). After equilibration of the test system (6 days), the control and the treatment were assigned to one tank each with initially 100 and 375 bluegills respectively (loading 12 and 3.2 L/fish/24 h). Fish were fed once daily and the O_2 was measured twice a week: O_2 >60%.
Four fish were removed for radiometric analysis on day 1, 3, 7, 11, 15 and 21 of uptake and on day 1, 2, 3, 5, 7, 9, 11 and 14 of depuration. On day 3, 16 and 21 of uptake and on day 1 and 3 of depuration 16 fish were removed for blood analysis. Water samples for radiometric analyses were taken at day 0, 1, 3, 7, 11, 15 and 21. The water samples were analysed by LSC. The fish for radiometric analysis were blotted dry, weighed and divided into gall bladder, liver, muscle with skin attached, visceral remains containing gills and esophagus and the remaining carcass with head, backbone, fins and tail and analysed by combustion/LSC.

Results The radioactivity (r.a.) concentration in the water was 100±12% (mean±SD). LOQ: 0.03 mg LAS/L.

Values for BCF, k_{uptake} and $k_{depuration}$, number of days to clear 50 and 90% of the steady state concentration (reached on day 7) were determined using the BIOFAC program.

Sample	K_{uptake} (L/mg·d)	$K_{depuration}$ (d^{-1})	BCF (L/mg)	Days to reach 90% of steady state	Days to reach 50% of steady state
Whole body	25 (8.0)[1]	0.24 (8.3)	104 (13)	9.7 (10)	2.9 (10)
Muscle (edible part)	9 (11)	0.24 (8.3)	36 (14)	9.4 (7.9)	2.8 (7.9)
Gall bladder	1461 (17)	0.28 (14)	5224 (22)	8.2 (13)	2.5 (14)
Liver	82 (26)	0.48 (8.3)	171 (29)	4.8 (85)	1.5 (8.3)
Gill and viscera	68 (12)	0.24 (13)	282 (17)	9.5 (12)	2.9 (12)
Blood	62 (1.6)	0.26 (3.8)	237 (2.5)	8.7 (1.4)	2.6 (0.4)
Remaining carcass	15 (13)	0.24 (8.3)	64 (14)	9.7 (9.4)	2.9 (9.3)

[1] ( ) Standard deviation (%)

Conclusion Whole fish: steady state uptake reached after 7 days; BCF (based on r.a.) 104; DT_{50} depuration r.a. 2.9 day, DT_{90} depuration r.a. 9.7 day.

Rev. note
1. Since no results of spiked water or spiked fish were included, the validity of the analytical methods cannot be checked.
2. The calculated BCF values are based on total radioactivity. The rapid elimination of LAS, suggests metabolic deactivation. The BCF based on radioactivity is presumably an overestimation of that based on parent.

Klimisch criterium 2 No QC analytic samples were included (note 1).

Appendix 9-3 - Ecotoxicity Data for LAS/ABS

Acute Toxicity to Fish:

9.3.01

Title Static acute toxicity of CASRN 26264-05-1 to the fathead minnow (*Pimephales promelas*)

Date of report April 27, 2000.

GLP No.

Reference 10

Test substance A (Benzenesulfonic acid, dodecyl-, compd. with isopropylamine (1:1)), purity 89.4%

Guideline OECD 203.

Stat. method None

Test system

Species Fathead minnow (*Pimephales promelas)*, mean length 17 mm.

No. of fish 10/replicate, 2 replicates/treatment.

Concentrations Nominal: 3.2, 5.6, 10, 18, 32 and 56 mg/L, water treated controls.

Test conditions Static; at 20±2°C in 21 L glass-silicone vessels containing 10 L reconstituted water (pH 8.3, hardness 168 mg/L $CaCO_3$); 16 h light; unfed, loading 0.04 g/l.

Phys. meas. Daily in all treatments: overall ranges for pH 8.2-8.4; O_2 87-100%; temperature 20-22°C.

Observations Mortality/symptoms at 24, 48, 72 and 96 h.

Results

		Nominal concentration [mg/L]						
Parameter	**Time [h]**	**0**	**3.2**	**5.6**	**10**	**18**	**32**	**56**
Mortality [%]	96	0	5	0	0	15	100	100
Symptoms*	24-96					+		

*Symptoms included twitching, quiescent, dark discolored, gulping air and/or labored.

Conclusion The 96-h LC_{50} calculated by the author using trimmed SPK was 22 mg/L (95% CI 20-24 mg/L) ⇔ 20 mg a.i./L (95% CI 18-22 mg/L).

Klimisch criterium 2 Static test with no chemical analyses performed; non-GLP study.

Acute Toxicity to Aquatic Invertebrates

Daphnia:

9.3.07

Title Static acute toxicity of CASRN 26264-05-1 to *Daphnia magna*

Date of report May 8, 2000.

GLP No.

Reference 24

Test substance A (Benzenesulfonic acid, dodecyl-, compd. with isopropylamine (1:1)), purity 89,4%.

Test method OECD 202.

Stat. method None.

Test system

Species *Daphnia magna*, <24 h old.

No. of daphnids 5/replicate, 4 replicates/treatment.

Concentrations Nominal: 1.56, 3.13, 6.25, 12.5, 25, 50 and 100 mg/L (no vehicle), untreated controls.

Test conditions Static; at 20±2°C in 225 mL crystallising dishes (covered), containing 100 mL of reconstituted water of hardness 168 mg/l ($CaCO_3$) and pH 8.3, 16 h light.

Phys. meas. At 0 and 48 h in one replicate for all concentrations; overall ranges for pH 8.4-8.5; O_2 91-97%; temperature (0, 24 and 48 h) 20-21°C.

Observations Immobility/mortality at 24 and 48 h.

Results

Parameter	Time [h]	Nominal concentration [mg/L]							
		0	**1.56**	**3.13**	**6.25**	**12.5**	**25**	**50**	**100**
Immobility [%]	48	0	0	85	100	100	100	100	100

Conclusion The 48-h EC_{50} calculated by the author using trimmed SPK was 2.5 mg/L (95% CI 2.2-2.7 mg/L) ⇔ 2.2 mg a.i./L (95% CI 2.0-2.4 mg a.i./L).

Klimisch criterium 2 Static test with no chemical analyses were performed; non-GLP study.

9.3.26

Title Toxicity of a linear alkylate sulfonate detergent to larvae of four species of freshwater fish

Date of report 1975.

GLP No data

Reference 14

Test substance 2 (Benzenesulphonic acid, linear alkyl); commercial detergent formulation containing 14% LAS; 2.3% alcoholethoxylate oxide condensate; 2.5% sodium soap; 48% sodium tripolyphosphate; 9.7% sodium silicate; 15.4% sodium sulphate; 8.1% moisture and miscellaneous.

Guideline Not indicated.

Stat. method One-way analysis of variance (Dunnett 1955).

Test system

Species Northern pike (*Esox lucius*); White sucker (*Catostomus commersoni*); Smallmouth bass (*Micropterus dolomieu*); Fathead minnow (*Pimephales promelas*): 2-3 days after hatching.

No. of fish 50/test vessel (2 vessel/treatment) for Northern pike and White sucker;
25/test vessel (2 vessel/treatment) for Smallmouth bass;
15/test vessel (2 vessel/treatment) for Fathead minnow.

Concentrations 0.2-0.3, 0.5, 1.1-1.2, 2.3-2.6 and 5.0-6.3 mg/L MBAS (apprx. equivalent to LAS); untreated controls.

Test conditions 30-day flow-through (no aeration) in tanks containing 12.5 L of lake water (hardness 36-48 mg/L as $CaCO_3$), 6 replacements/24 h, temperature 15±1°C, except for *Pimephales promelas* 23±1°C; feeding at least twice daily.

Analysis Once a week for all concentrations (composite of daily taken samples) using the MBAS-procedure after preservation with 1% formaldehyde (ref. standard 4.045% aqueous LAS).

Phys. meas. One tank per week: overall ranges for pH 7.2-7.9; overall ranges O_2 5.6-10 mg/L.

Observations Mortality; body weight (total = standing crop) on day 30.

Results For analytical results see 1st table below. Biological data are shown in the 2nd table. QCs were completely recoverable.

Analysis

Test chamber	Measured concentration ± standard error (mg/L)			
	E. lucius	***C. commersoni***	***M. dolomieu***	***P. promelas***
1	5.9 ± 0.2	5.0 ± 0.3	6.3 ± 0.05	5.8 ± 0.35
2	2.4 ± 0.1	2.6 ± 0.15	2.5 ± 0.05	2.3 ± 0.05
3	1.2 ± 0.05	1.1 ± 0.10	1.2 ± 0.05	1.2 ± 0.05
4	0.5 ± 0.05	0.5 ± 0.05	0.5 ± 0.05	0.5 ± 0.01
5	0.3 ± 0.01	0.2 ± 0.01	0.3 ± 0.01	0.2 ± 0.05
Control	0.02 ± 0.005	0.01 ± 0.005	0.02 ± 0.005	0.02 ± 0.01

Biological

Parameter	Time [d]	Mean measured concentration [mg/L]					
		0	**0.2-0.3**	**0.5**	**1.1-1.2**	**2.3-2.6**	**5.0-6.3**
Standing crop* *E. lucius*	30		I	i	dc	dc	-
Standing crop *C. commersoni*	30		Dc	dc	dc	dc	dc
Standing crop *M. dolomieu*	30		Ic	ic	ic	Ic	dc
Standing crop *P. promelas*	30		=	d	dc	dc	-

* Standing crop: the biomass of a particular area, ecosystem etc. at any specified time. d=decrease, I=increase, c=significant

Conclusions
Esox lucius : 96-h LC50 3.7 mg/L; 30-d NOEC 0.6 mg/L
Catostomus commersoni : 96-h LC50 4.0 mg/L; 30-d NOEC ~0.2 mg/L
Micropterus dolomieu : 96-h LC50 3.7 mg/L; 30-d NOEC 3 mg/L
Pimephales promelas : 96-h LC50 3.4 mg/L; 30-d NOEC 0.5 mg/L

30-day NOEC based on standing crop

Rev. note
1. Because the test substance is a formulation, the observed toxicity reflects exposure to LAS and the other components. Nominal concentrations were not reported, so mean measured concentrations have been used in this summary. From the report it is not completely clear whether concentrations are expressed in mg formulation or mg active ingredient. It is assumed that concentrations are expressed in mg active ingredient (i.e., LAS) and this is consistent with the MBAS analytical measurement.
2. Observations were made for the dead of juvenile fish, but mortality is not reported. The LC50 values included in the conclusions could not be checked with the original data.

Klimisch criterium 2 Incomplete description (notes 1 & 2).

9.3.27

Title Terrestrial safety assessment of linear alkylbenzene sulfonate
Date of report 1990.
GLP No.
Reference 16
Test substance 2 (Benzenesulphonic acid, linear alkyl), LAS $C_{10\text{-}13,\ mean\ 11.6}$.
Test method OECD 208 (1984).
Stat. method Not indicated.
Test system
Species Sorghum (*Sorghum bicolor*)
Sunflower (*Helianthus annuus*)
Mung bean (*Phaseolus aureus*)
No. of seeds 8 seeds/pot, 4 pots/treatment.
Procedure The test was performed in a greenhouse at 20°C with 14 h light in non-porous plastic plant pots (∅ 10 cm) containing 600 g soil (mixture of grit, loam and fertilizer). A premix was prepared from silver sand and a solution of LAS in water. The premix was blended with the soil (1:9). The treatment rates were 1, 10, 100 and 1000 mg a.i./kg dry soil. Untreated controls were included for sorghum.
Observations Emergence on day 7.
Growth on day 21.

Conclusion
Sorghum: Emergence [%] was 64-78% for 0-1000 mg/kg;
21-d $EC_{50,growth}$ 167 mg/kg.
Sunflower: Emergence [%] was >91% for 1-1000 mg/kg soil ;
21-d $EC_{50,growth}$ 289 mg/kg.
Mung bean: Emergence [%] was ≥75% for 1-1000 mg/kg soil;
21-d $EC_{50,growth}$ 316 mg/kg.
21-d $NOEC_{growth}$ 100 mg/kg for all species

Rev. note The information was essentially confined to what is included in the above summary. On the basis of the limited information provided, checking of compliance with guideline

requirements was only possible to a limited extent. The determination of the effect concentrations (NOEC and EC50) for growth cannot be checked by individual data.

Klimisch criterium 2 Incomplete description.

9.3.28

Title Terrestrial safety assessment of linear alkylbenzene sulfonate

Date of report 1990.

GLP No.

Reference 16

Test substance 2 (Benzenesulphonic acid, linear alkyl), LAS $C_{10\text{-}13,\ mean\ 11.6}$.

Test method OECD 207 (1984).

Stat. method Not indicated.

Test system

Species Earthworm (*Eisenia foetida*), mean weight 660 mg.

No. of worms 10 worms/jar, 4 jars/treatment.

Procedure The test was performed at 20±2°C under continuous illumination in 0.9 L glass jars, containing 900 g of wet artificial soil (peat/clay/sand: 10/20/70%). An aqueous solution of LAS was added to the soil. The treatment rates were 63, 125, 250, 500, and 1000 mg/kg soil. Untreated controls were included. Moisture level was maintained at 35±1%.

Observations Mortality, symptoms, body weight on day 7 and 14.

Analysis At 250 mg/kg by HPLC.

Results Reductions in body weights of respectively 14, 33 and 23% were observed at 0, 100 and 500 mg/kg.

Measured concentration was 94% of nominal.

		Nominal concentration [mg/kg soil]					
Parameter	**Time [d]**	**0**	**63**	**125**	**250**	**500**	**1000**
Mortality [%]	14	0	0	0	0	0	5

Conclusions 14-day LC_{50} >1000 mg/kg

Rev. note 1. No positive control included.

Klimisch criterium 2 No positive control; non-GLP study

Appendix 9-4 - Health Data for LAS/ABS

Acute Toxicity:

Oral:

9.4.01

Title	MSDS Rhodacal® 330
Date of report	May 14, 1999.
GLP	No data
Reference	22
Test substance	A (Benzenesulfonic acid, dodecyl-, compd. With isopropylamine (1:1)), purity 90%.
Guideline	Not specified.
Toxicity	LD50-rat 1836 mg/kg.
Klimisch criterium	4 – Secondary literature

9.4.02

Title	Defined oral LD_{50}	
Date of report	October 8, 1980.	
GLP	No data	
Reference	32	
Test substance	A (Benzenesulfonic acid, dodecyl-, compd. With isopropylamine (1:1)), purity 90.9%.	
Guideline	Defined oral LD50. Adapted from appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, by the Association of Food and Drug Officials of the United States, 1965.	
Stat. method	Litchfield-Wilcoxin (Probit analysis).	
Test system	**Species**	Rat (Sprague-Dawley), weight 200-300 g.
	No. of animals	5/sex/dose group.
	Dosage	Single dose by oral gavage of 1.0, 1.5, 2.0, 2.5 and 3.0 mL/kg bw.
	Observations	Mortality/clinical signs daily for 14 days. Body weight on day 0 and 14. Macroscopy on animals that died.

Results

Dose [mL kg bw] \ effect		**1.0**		**1.5**		**2.0**		**2.5**		**3.0**		**DR**	
Sex	**Day**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Mortality	0-14		1/5		4/5		4/5	3/5	5/5	5/5	5/5	x	x
Body weight gain survivors	0-14	No treatment related effects							N/A	N/A	N/A		
Clinical signs	0-14	No treatment related effects											
Necropsy(A)					+		+	+	+	+	+		

(A) Pulmonary haemorrhage among animals that died.

Conclusions	Oral LD_{50} 1.8 ml/kg bw. which is equivalent to 1300 mg/kg bw
Klimisch criterium	2 Only partial report available.

9.4.03

Title	Acute oral toxicity studies with ten samples in albino rats	
Date of report	May 29, 1973.	
GLP	No.	
Reference	9	
Test substance	B (Benzenesulfonic acid, dodecyl-, compd. With 2,2',2''-nitrilotris(ethanol)(1:1)), purity 25%.	
Guideline	Not indicated.	
Test system	**Species**	Rat.
	No. of animals	Not indicated.
	Dosage/observations	Not indicated.
Stat. method	Weil, Thompson.	

Results	Limited to LD_{50}-value.	
Conclusions	Oral LD_{50} 1653 (±238) mg a.i./kg bw.	
Rev. note	Only select pages of the report were available.	
Klimisch criterium	4 Incomplete report.	

9.4.04

Title	Final report on the safety assessment of sodium dodecylbenzenesulfonate/TEA-dodecylbenzenesulfonate/sodiumdecylbenzenesulfonate	
Date of report	1997.	
GLP	No data	
Reference	1	
Test substance	B, Benzenesulfonic acid, dodecyl-, compd. With 2,2',2''-nitrilotris(ethanol) (1:1).	
Guideline	Not indicated.	
Stat. method	Not applicable.	
Test system	**Species**	Rat (Sprague-Dawley).
	No. of animals	5/sex/dose group.
	Dosage	Single oral administration of 91, 195, 420, 906 and 1953 mg/kg bw (vehicle water, dosing volume 0.464-10 ml/kg).
	Observations	Mortality/clinical signs during 14 days. Necropsy on day 14.
Results	No deaths, diarrhea among animals.	
Conclusions	Oral LD_{50} >10 ml/kg bw ⇔ >1953 mg/kg bw.	
Rev. note	The report was limited to the above mentioned.	
Klimisch criterium	2 A CTFA Cosmetic Ingredient Review	

9.4.05

Title	MSDS Rhodacal® CA/70
Date of report	August 17, 1999.
GLP	No data
Reference	21
Test substance	C (Benzenesulfonic acid, dodecyl-,calcium salt), purity 69-71%.
Guideline	Not specified.
Toxicity	LD50-rat 1.8 mL/kg ⇔ 1.3 g a.i./kg = 1300 mg/kg bw
Klimisch criterium	4 - Secondary literature

9.4.06

Title	Akute orale Toxizität von Marlon A 386 für Ratten	
Date of report	February 15, 1984.	
GLP	No data	
Reference	15	
Test substance	D (Benzenesulfonic acid, dodecyl-, branched) or 1 (Benzenesulfonic acid, mono-C11-13-branched alkyl) or 4 (Benzenesulfonic acid, linear alkyl), purity 86%.	
Guideline	OECD 401.	
Stat. method	Lichtfield and Wilcoxon.	
Test system	**Species**	Rat (Bor: WISW), mean weight 123-146 g.
	No. of animals	5/sex/dose group.
	Dosage	Single oral administration of 1250, 1415, 1580 and 1990 mg/kg bw (vehicle water, dosing volume 10 ml/kg); no controls; feeding *ad libitum* (food was withheld ~16 h prior to dosing).
	Observations	Mortality/clinical signs several times during the first 6 h and daily until day 14. Body weights on day 0, 1, 7 and 14. Necropsy on day 14.

Results

Dose [mg a.i./kg bw] \ effect		1250		1415		1580		1990		DR	
Sex	**Day**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Mortality	1-14	0/5	4/5	5/5	3/5	4/5	5/5	5/5	5/5		
Clinical signs(A)	1-14	+	+	+	+	+	+	+	+		
Body weight gain	1-15	No treatment related effects									
Necropsy(B)	15	+		+		+		+			

(A) Clinical observations included piloerection, hunched posture, diarrhoea, difficult respiration, nasal bleedings, uncoordinated movements, ataxia and (minor) sedation during day 1-5.
(B) Findings consisted of redness of the mucous membrane of the stomach and intestine, hyperaemia of the stomach, adhesions in stomach, liver, spleen and kidneys with peritoneum.

Conclusions Oral LD_{50} 1260 mg/kg bw ⇔ 1080 mg a.i./kg bw (95% C.I. 970-1210 mg a.i./kg bw).
Klimisch criterium 2 non-GLP study

9.4.07

Title Toxicologic studies with branched and linear alkyl benzene sulfonates in rats
Date of report 1965.
GLP No data
Reference 17
Test substance E (Benzenesulfonic acid, mono-C11-13-branched alkyl derivs.) (C_{10}-C_{14}), purity 87.1% (sodium sulfate 10.5%, water 2.2 %, oil 0.9%).
Guideline Hagan (1959).
Stat. method Calculation by method of Miller and Tainter.
Test system
Species Rat (FDRL(Wistar)).
No. of animals 3/sex/dose group.
Procedure Single dose by oral gavage (10% dispersion in water).
Observations Mortality/clinical signs at least daily during 14 days after dosing; Body weights on day 0, 7 and 14; Necropsy on day 14 or on day of death.
Conclusions Oral LD_{50} 520 mg a.i./kg bw.
Klimisch criterium 2 Older study; published but no lab report

9.4.15

Title Toxicologic studies with branched and linear alkyl benzene sulfonates in rats
Date of report 1965.
GLP No data
Reference 17
Test substance 2 (Benzenesulphonic acid, linear alkyl) (C_9-C_{15}), purity 39.5% (sodium sulphate 8.8%, water 50.9 %, free alkali (NaOH) 0.05%, unidentified 0.64%).
Guideline Hagan (1959).
Stat. method Calculation by method of Miller and Tainter.
Test system
Species Rat (FDRL(Wistar)).
No. of animals 3/sex/dose group.
Procedure Single dose by oral gavage (10% dispersion in water).
Observations Mortality/clinical signs at least daily during 14 days after dosing; Body weights on day 0, 7 and 14; Necropsy on day 14 or on day of death.
Conclusions Oral LD_{50} 650 mg a.i./kg bw.
Rev. note
1. No individual data were presented.
2. Equivalent doses as 10 and 40% dispersion were given at 600 and 1580 mg/kg. Mortality was not affected by the use of a more concentrated suspension, but a higher incidence of diarrhoea was noted at the most concentrated suspension.

Klimisch criterium 2 Limited report, non-GLP study.

Dermal:

9.4.16

Title	Final report on the safety assessment of sodium dodecylbenzenesulfonate/TEA-dodecylbenzenesulfonate/sodiumdecylbenzenesulfonate	
Date of report	1997.	
GLP	No data	
Reference	1	
Test substance	B, Benzenesulfonic acid, dodecyl-, compd. with 2,2',2''-nitrilotris(ethanol) (1:1).	
Guideline	Not indicated.	
Stat. method	Not applicable.	
Test system	**Species**	Rabbit (New Zealand White).
	No. of animals	8.
	Dosage	Single application of 4199 mg/kg bw (vehicle water) to the clipped skin under occlusion for 24 hours..
	Observations	Mortality/clinical signs during 14 days. Necropsy on day 14.
Results	No deaths, diarrhea and emaciation in two animals, erythema.	
Conclusions	Dermal LD_{50} >21.5 ml/kg bw ⇔ >4199 mg/kg bw.	
Klimisch criterium	2 - CTFA Cosmetic Ingredient Review	

Irritation and Sensitization:

9.4.21

Title	Primary skin irritation	
Date of report	September 18, 1980.	
GLP	No data	
Reference	31	
Test substance	A (Benzenesulfonic acid, dodecyl-, compd. with isopropylamine (1:1)), purity 90.9%.	
Guideline	FHSLA 16 CFR 1500.	
Stat. method	Not applicable.	
Test system	**Species**	Rabbit (New Zealand White).
	No. of animals	6 (sex not indicated)
	Dosage	Application of 0.5 ml test substance (no vehicle) on ~6.25 cm^2 of the clipped skin (intact and abraded) under semi-occlusion for 24 hours.
	Observations	Skin observations at 24 and 72 h after application.

Results

	Mean score	
Time	**Erythema**	**Oedema**
24 h	1.83	2.33
72 h	3.00	1.67

E=erythema O=oedema

Conclusions	Irritating.
Rev. note	1. The application time was 24 h, which is considered to be a worst case situation (OECD 404, 4 h application).
Klimisch criterium	4 - Only an incomplete lab report available

9.4.22

Title	D.O.T. corrosivity study (Modified)
Date of report	September 13, 1993.
GLP	No (Quality Assurance Statement included).
Reference	11
Test substance	*Test 1*: B (Benzenesulfonic acid, dodecyl-, compd. with 2,2',2''-nitrilotris(ethanol)(1:1)), purity 60% (40% water), *Test 2*: D (Benzenesulfonic acid, dodecyl-, branched) or 1 (Benzenesulfonic acid, mono-C11-13-branched alkyl), purity 93-95% (1% sulfuric acid, 0.7% water).

Guideline Not indicated.

Test system

Species	Rabbit (New Zealand White), 8-10 weeks old.
No. of animals	3 (sex not indicated).
Dosage	Application of 0.5 g test substance on the skin under occlusion for 4 hours.
Observations	Skin observations at 4, 24, 48 and 72 h after application.

Stat. Method Not applicable.

Results ***Test 1***

Animal	**1**		**2**		**3**	
Time	**E**	**O**	**E**	**O**	**E**	**O**
4 h	1	1	1	1	1	1
24 h	2	2	1	1	3	2
48 h	3	2	3	2	3	2
72 h	3	2	3	1	3	2

E=erythema O=oedema

Conclusion Irritating

Results ***Test 2***

Animal	**1**		**2**		**3**	
Time	**E**	**O**	**E**	**O**	**E**	**O**
4 h	1	1	1	2	1	1
24 h	2	2	2	2	2	2
48 h	2	1	3	2	3	2
72 h	2	1	2	2	3	2

E=erythema O=oedema

Conclusion Irritating.

Rev. note
1. The test was performed with occlusive dressing. This is considered to represent a worst case situation, since the occlusion is expected to increase penetration through the skin.

Klimisch criterium 1

9.4.23

Title MSDS Rhodacal® CA/70

Date of report August 17, 1999.

GLP No data

Reference 21

Test substance C (Benzenesulfonic acid, dodecyl-,calcium salt), purity 69-71%.

Guideline Not specified.

Skin irritation Moderately irritating in rabbit.

Klimisch criterium 4 - Secondary literature

9.4.24

Title D.O.T. corrosivity study (Modified)

Date of report April 21, 1993.

GLP No (Quality Assurance Statement included).

Reference 13

Test substance E (Benzenesulfonic acid, mono-C11-13-branched alkyl derivs.), purity 96% (2% sulfuric acid, 2% benzene (tetrapropenyl derivs)).

Guideline Not indicated.

Test system

Species	Rabbit (New Zealand White), 8-10 weeks old.
No. of animals	3 (sex not indicated).
Dosage	Application of 0.5 g test substance on the skin under occlusion for 4 hours.
Observations	Skin observations at 4, 24, 48 and 72 h after application.

Stat. Method Not applicable.

Results ***Test 1*** *(90% solution in distilled water)*

Animal	**1**		**2**		**3**	
Time	**E**	**O**	**E**	**O**	**E**	**O**
4 h	4	2	4	2	3	2
24 h	4	2	4	1	4	1
48 h	4	1	4	1	4	1
72 h	4	1	4	1	4	1

E=erythema O=oedema

Conclusion Irritating

Results ***Test 2*** *(60% solution in distilled water)*

Animal	**1**		**2**		**3**	
Time	**E**	**O**	**E**	**O**	**E**	**O**
4 h	3	1	4	1	2	1
24 h	4	1	4	1	3	1
48 h	4	1	4	1	3	1
72 h	4	1	4	1	4	1

E=erythema O=oedema

Conclusion Irritating.

Results ***Test 3*** *(30% solution in distilled water)*

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

E=erythema O=oedema

Conclusion Irritating.

Rev. note 1. The test was performed with occlusive dressing. This is considered to represent a worst case situation, since the occlusion is expected to increase penetration through the skin.

Klimisch criterium 1

9.4.25

Title D.O.T. corrosivity study (Modified)

Date of report March 13, 1993.

GLP No. (Quality Assurance Statement included)

Reference 12

Test substance E (Benzenesulfonic acid, mono-C11-13-branched alkyl), purity 96% (2% sulfuric acid, 2% benzene (tetrapropenyl derivs.)).

Guideline Not indicated.

Test system
- **Species** Rabbit (New Zealand White), 8-10 weeks old.
- **No. of animals** 3 (sex not indicated).
- **Dosage** Application of 0.5 g test substance on the skin under occlusion for 4 hours.
- **Observations** Skin observations at 4, 24, 48 and 72 h after application.

Stat. method Not applicable.

Results

Animal	**1**		**2**		**3**	
Time	**E**	**O**	**E**	**O**	**E**	**O**
4 h	2	2	2	2	1	1
24 h	2	2	3	3	2	2
48 h	2	2	3	3	2	2
72 h	3	3	3	3	3	3

E=erythema O=oedema

Conclusion Irritating.

Rev. note 1. The test was performed with occlusive dressing. This is considered to represent a worst case situation, since the occlusion is expected to increase penetration through the skin.

Klimisch criterium 1

9.4.30

Title Modified eye irritation
Date of report September 30, 1980.
GLP No data
Reference 23
Test substance A (Benzenesulfonic acid, dodecyl-, compd. with isopropylamine (1:1)), purity 90.9%.
Guideline EPA 40 CFR 163.81-4
Test system
Species Rabbit (New Zealand White).
No. of animals 3 (with rinsing) and 6 (without rinsing), sex not indicated.
Dosage Application of 0.1 ml test substance in the eye. For 3 animals eyes were rinsed with water 30 seconds after instillation.
Observations Observations at 24, 48 and 72 h and on day 4 and 7 after application.
Stat. method Not applicable.
Results

Test with rinsing

Animal	**1**				**2**				**3**			
Effect	C	I	Conj		C	I	Conj		C	I	Conj	
Time			Red	Ch			Red	Ch			Red	Ch
24 h	2	0	2	2	2	0	2	3	2	0	2	4
48 h	2	0	2	3	2	0	2	4	2	1	2	4
72 h	2	1	1	3	2	0	2	3	3	1	2	4
4 d	1	1	1	2	2	0	3	3	2	1	2	3
7 d	2	1	0	1	2	0	1	2	3	1	2	3

Test without rinsing

Animal	**1**				**2**				**3**				**4**				**5**				**6**			
Effect	C	I	Conj		C	I	Conj		C	I	Conj		C	I	Conj		C	I	Conj		C	I	Conj	
Time			Red	Ch			Red	Ch			Red	Ch			Red	Ch			Red	Ch			Red	Ch
24 h	3	1	2	3	2	0	2	4	2	1	2	4	2	0	2	4	2	0	2	3	3	0	0	4
48 h	3	1	2	3	2	0	1	4	3	0	1	4	2	0	1	3	2	0	2	3	2	0	1	4
72 h	3	1	2	4	2	0	1	4	4	2	1	4	2	1	1	2	2	0	2	4	2	1	1	3
4 d	3	0	2	1	2	0	1	2	2	1	1	3	1	0	1	0	1	1	2	3	2	1	1	1
7 d	3	0	1	3	3	1	1	3	3	1	0	3	2	0	0	0	2	1	1	2	3	1	1	2

C=corneal opacity I=Iris Conj=conjunctiva Red=redness Ch=chemosis

Conclusion Irritating
Klimisch criterium 2 Non-GLP study

9.4.31

Title MSDS Rhodacal® CA/70
Date of report August 17, 1999.
GLP No.
Reference 21
Test substance C (Benzenesulfonic acid, dodecyl-,calcium salt), purity 69-71%.
Guideline Not specified.
Eye irritation Severely irritating in rabbit.
Klimisch criterium 4 - Secondary literature

Genetic Toxicity *in vitro*:

9.4.38

Title	Studies of *in vitro* cell transformation and mutagenicity by surfactants and other compounds	
Date of report	1979.	
GLP	No.	
Reference	6	
Test substance	2 (Benzenesulphonic acid, linear alkyl), C_{10} -C_{14}, purity 22% active (0.033% alkylbenzene, 0.02% $NaSO_4$).	
Guideline	Not indicated.	
Stat. method	Not indicated.	
Test system	**Cell culture**	Syrian golden hamster embryo cells.
	Test concentration	5, 10, 20 and 50 μg/ml. 0.5, 1, 5 and 10 μg/ml.
	Controls	Negative: vehicle (DMSO). Positive: 3-methylcholanthrene
	Procedure	Pregnant Syrian golden hamsters were killed on day 13 or 14 of gestation. Embryos were minced and trypsinised and cells were cryopreserved. Unthawed cells were plated twice (as feeder-layer and target cells) on day 0 and 3 resp.. On day 4 feeder-layer cells were plated (after irridiation and trypsination) at $6x10^4$ cells/dish and on day 5 500 target cells/dish were added to the dishes. On day 6 the test substance was added. On day 14 cultures were fixed and stained and normal and transformed colonies were counted.

Results Positive control negative.

Doses tested [μg/ml]	Cytotoxicity [% of control survival] at highest dose	Test result(A)
5, 10, 20, 50	40%	-
0.5, 1, 5, 10	88%	-

(A) +/- : positive/negative result.

Conclusion Not mutagenic.

Rev. note
1. The results of a simultaneously performed test with the positive control (at 0.1, 0.5 and 1.0 μg/ml) were negative. This lowers the value of the assay

Klimisch criterium 4 Positive control was negative; secondary literature.

9.4.39

Title	Studies of *in vitro* cell transformation and mutagenicity by surfactants and other compounds	
Date of report	1979.	
GLP	No.	
Reference	6	
Test substance	2 (Benzenesulphonic acid, linear alkyl), C_{10} -C_{14}, purity 22% active (0.033% alkylbenzene, 0.02% $NaSO_4$).	
Guideline	Not indicated	
Stat. method	Not indicated.	
Test system	**Bacterial strains**	TA98, TA100.
	Metabolic activation	Rat liver S9 mix (polychlorinated biphenyl-induced).
	Test concentration	10, 25, 50, 100 and 200 μg/plate.
	Controls	Negative: vehicle (DMSO or water not specified). Positive: 4-nitroquinoline 1 oxide, *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine, benzo[a]pyrene, 2-acetylaminofluorene, *N*-nitrosomethylamine.
	Procedure	According to OECD 471.

Results

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

(A) +/- : positive/negative result; positive controls gave expected responses.

Conclusion Not mutagenic.
Rev. note Secondary literature.
Klimisch criterium 2 Secondary literature, non-GLP study.

Repeated Dose:

9.4.40

Title Final report on the safety assessment of sodium dodecylbenzenesulfonate/TEA-dodecylbenzenesulfonate/sodiumdecylbenzenesulfonate
Date of report 1997.
GLP No data
Reference 1
Test substance B, Benzenesulfonic acid, dodecyl-, compd. With 2,2',2''-nitrilotris(ethanol) (1:1), 0.5% a.i. in semipermanent hair dye.
Guideline Not indicated.
Stat. method Not applicable.
Test system
- **Species** Rabbit (New Zealand White).
- **No. of animals** 6/sex/dose group (3 control groups).
- **Dosage** 13 week-study with twice weekly dermal application of 1 ml/kg to the shaved skin (abraded in 3/sex/dose) with rinsing 1 hour after dosing.
- **Observations** Body weight weekly.
  Clinical chemistry, haematology and urinalysis at initiation and after 3, 7 and 13 weeks.
  Necropsy in week 13 (macro- and microscopy).

Results No treatment related effects. The significantly increased levels of BUN (all) and leukocyte count (males only) and decreased methaemaglobin level (females only) in treated animals were considered to be toxicologically irrelevant.
Conclusions NOAEL > 0.005 ml/kg bw (equivalent to 5 mg/kg bw); only dose tested
Klimisch criterium 2 A CTFA Cosmetic Ingredient Review

9.4.42

Title Toxicology Studies of Linear Alkylbenzene Sulphonate (LAS) in Rhesus Monkeys
I. Simultaneous Oral and Subcutaneous Administration for 28 Days
Date of report 1978.
GLP No.
Reference 5
Test substance 2 (Benzenesulphonic acid, linear alkyl), purity 20.5% (78.7% water).
Guideline Not indicated.
Stat. method Not indicated.
Test system
- **Species** Rhesus Monkey (*Macaca mulatta*), 2.0-4.4 kg, age 18-36 months.
- **No. of animals** 3/sex/treatment.
- **Dosage** Simultaneous oral (gavage) and subcutaneous administration of 30 p.o./0.1 s.c., 150 p.o./0.5 s.c. and 300 p.o./1.0 s.c. mg/kg during 28 days; dose volume 4 ml/kg (p.o.) and 0.17 ml/kg (s.c.); vehicle (water) controls.
- **Observations** As per OECD 407 with the exception of some clinical chemical parameters (cholesterol, albumine and creatinine).

Results

Dose (mg/kg bw)	0/0	30/0.1	150/0.5	300/1.0	DR
Mortality	None				
Clinical signs- systemic[(A)]			+	+	x
- local[(B)]		+	+	+	x
Body weight gain/food consumption	No treatment related effects				
Ophthalmoscopy	No treatment related effects				
Blood parameters/urine analysis	No treatment related effects				
Organ weights	No treatment related effects				
Macroscopy/histopathology	No treatment related effects				

(A) Vomiting (~3 h after application) and abnormal faeces.
(B) Chronic inflammatory cell infiltration (mainly fibroblasts) at the injection site associated with pseudocysts, haemorrhage and necrosis.

Conclusions NOAEL = 301 mg/kg ⇔ 60 mg a.i./kg.

Rev. note
1. Clinical signs were treatment related but not considered to be significantly adverse.
2. Most probably no statistical evaluation of the results was performed in view of the low number of animals in this study.

Klimisch criterium 2 Non-GLP study

9.4.47

Title Ultrastructural observations of the protective effect of glycyrrhizin for mouse liver injury caused by oral administration of detergent ingredient (LAS)

Date of report 1977.

GLP No.

Reference 30

Test substance 2 (Benzenesulphonic acid, linear alkyl).

Guideline Not indicated.

Stat. method Not indicated.

Test system
- **Species** Mouse (DDY-strain).
- **No. of animals** Not indicated.
- **Dosage** Administration for 6 months at 0 and 100 ppm in drinking water with 2 months recovery ⇔ males: 0 and 17 mg/kg bw, females: 0 and 20 mg/kg bw.
- **Observations** Microscopical examination (electron microscope) of liver tissues of animals sacrified at 1, 2, 3, 6 and 8 months after study initiation.

Results Hypofunctional and injured liver cells with disappeared nucleolonema, atrophic Golgi apparatus, degranulation of RER and mitochondria and increased number of lysosomes with autophagic vacuoles. After the recovery period mitochondria were still altered and in some hepatic cells fatty metamorphosis was observed.

Conclusions Liver effects at 17 mg/kg bw.

Rev. note
1. No information on accuracy of preparation, stability and homogeneity was provided. The actual test substance intake was calculated by the reviewer from estimated water intake of 5 ml/day and a mean bodyweight 30 g for males and 25 g for females.
2. The information in this journal article was limited to the above-mentioned.
3. The identity of the test substance could not be established (most probably #2).

Klimisch criterium 4 Limited report and no confirmation of test substance.

Reproductive Toxicity:

9.4.48

Title	Final report on the safety assessment of sodium dodecylbenzenesulfonate/TEA-dodecylbenzenesulfonate/sodiumdecylbenzenesulfonate	
Date of report	1997.	
GLP	No data	
Reference	1	
Test substance	B, Benzenesulfonic acid, dodecyl-, compd. with 2,2',2''-nitrilotris(ethanol) (1:1), 0.2-0.3% a.i. in semipermanent hair dye.	
Guideline	Not indicated.	
Stat. method	Not applicable.	
Test system	**Species**	Rat (CD).
	No. of animals	25 males/dose group in the P-group.
	Dosage	Twice weekly dermal application of 0.5 ml/kg to the shaved skin during 10 weeks.
	Procedure	After 10 weeks of dosing, males were mated with untreated females to produce 75 mated females/group. Females were allowed to deliver and 2 healthy 21-day-old F1-males were selected from each litter to mate after 12 weeks with untreated females to produce 300 mated females. These females were killed on day 4-16 of gestation.
	Observations	Number and sex of pups of the F1-generation (live and dead pups) Uteri and offspring of the females mated to F1-males.
Results	No treatment related effects.	
Conclusions	NOAEL > 0.0015 ml/kg bw (equivalent to 1.5 mg/kg bw); only dose tested	
Klimisch criterium	2 A CTFA Cosmetic Ingredient Review	

9.4.50

Title	Effect of alcohol sulfate, linear alkylbenzene sulfonate and natural soap on the development of fertilized eggs of the mouse in vitro	
Date of report	1990.	
GLP	No.	
Reference	7	
Test substance	2 (Benzenesulphonic acid, linear alkyl), purity not indicated.	
Guideline	Not applicable.	
Stat. method	Not indicated.	
Test system	**Cells**	Fertilised mouse embryo cells.
	Test concentration	0.015, 0.025, 0.03 and 0.05% during 1 h. 0.01, 0.025 and 0.05% for 5 days.
	Procedure	*In vitro f*ertilised eggs at the pronucleus stage were incubated in culture medium containing the test substance for 1 h and observed for 5 days, or incubated for all 5 days of development.
	Observations	Embryo development and blastocyst formation frequency
Results	1 hr test: no impairment of development at 0.015% or 0.025%; at ≥0.03% there was no development (1-cell stage). 5 day test: at ≥0.025% there was no development (1-cell stage).	
Conclusion	NOAEL 0.025% (1 hr) and 0.01% (5 day).	
Klimisch criterium	2 Secondary literature.	

9.4.52

Title	LAS-Mg : Effects upon the reproductive performance of rats treated continuously through two successive generations	
Date of report	April 19, 1982.	
GLP	No (QA statement included).	
Reference	29	
Test substance	3, Magnesium salt of LAS, purity 38% (slurry).	
Guideline	Not indicated.	
Stat. method	Multiple t-test, Mann-Whitney U-test, chi-square test, Fisher's test.	
Test system	**Species**	Rat (CD), 30-40 days old, weight 66-90 g (males) and 64-85 g (females).
	No. of animals	P0/F1/F2 12M + 24F/dose level.
	Dosage	Continuous dietary administration at 0, 1250, 2500 and 5000 ppm (nominal a.i.) ⇔ 0, 50, 103 and 222 mg a.i./kg bw (mean measured) during the entire study period.
	Procedures	Males and females were mated (1:2) starting on day 91 (maximum 21 days) to produce the $F1_A$. After ~ 55 days females were re-mated with fresh males to produce the $F1_B$. The detection of a vaginal plug and/or presence of spermatozoa in a vaginal smear was used to define day 1 of gestation. Selected $F1_B$ animals were mated after a maturation period of 91 days according to the same scheme used for the P0 to produce the $F2_A$ and $F2_B$ generation. Selected $F2_B$ animals were killed after a maturation period of 91 days.
	Analyses	In week 0, 26 and 52.
	Observations	Parents • Mortality/clinical signs P0/F1/F2. • Body weight males weekly, females weekly and on day 1,3,7,14 and 21 of gestation and on day 1,7, 14 and 21 after parturation (day 25 (after the second litter only)). • Food and water intake weekly. • Gestation duration/Oestrus cycle. • Macroscopy P0/F1/F2. • Macroscopy (related to neoplasms)/organ weights F2 • histopathology on 5/sex of F2 only (+ on animals with macroscopic findings). Offspring • Clinical signs. • Mortality (visceral examination of dead pups). • Litter size daily until day 21 or 25 (second litters). • Body weight (individually on day 1 and total litter weight on day 4, 10, 14 and 21 (day 25 for second litters)). • Startle response and pupil closure on day 21 or 25 • Macroscopy on pups not selected for the production of the next generation.

Results **Analyses** Measured concentration 73-103% of nominal.

Dose (ppm a.i. in diet)	**0**		**1250**		**2500**		**5000**		**DR**	
Dose (mean measured mg a.i./kg bw[(A)])	**0**		**50**		**103**		**222**			
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
P0										
Mortality				1/24	1/12					
Clinical signs			No treatment related effects							
Body weight - wk 13								dc		
- wk 28							d			
- weaning								d		
Food consumption			No treatment related effects							
Water consumption						d		d		
Mating success/fertility			No treatment related effects							
Gestation time/oestrus cycle			No treatment related effects							
Litter size			No treatment related effects							
Live pups (until weaning)			No treatment related effects							
Pup body weight (gain)($F1_A$)			No treatment related effects							
($F1_B$)								dc		
Pup clinical signs/behaviour			No treatment related effects							
Pup macroscopy			No treatment related effects							
Parent macroscopy			No treatment related effects							
F1 (selected animals**)**										
Mortality		1/24				1/24				
Clinical signs			No treatment related effects							
Body weight - wk 13								dc		
- weaning								d		
Food/water consumption			No treatment related effects							
Mating success/fertility			No treatment related effects							
Gestation time/oestrus cycle			No treatment related effects							
Litter size ($F2_A$)			No treatment related effects							
($F2_B$) day 0-25			d		d					
Live pups (until weaning) ($F2_A$)			No treatment related effects							
($F2_B$)			No treatment related effects							
Pup body weight (gain)($F2_A$)					dc (10%)		dc (21%)		X	
($F2_B$)			No treatment related effects							
Pup clinical signs/behaviour			No treatment related effects							
Pup macroscopy			No treatment related effects							
Parent macroscopy			No treatment related effects							
F2 (selected animals)										
Mortality								1/24		
Clinical signs			No treatment related effects							
Body weight							d	d		
Food consumption							d			
Water consumption						d	d	d		
Macroscopy			No treatment related effects							
Organ weights										
Heart/spleen							dc[a]			
Lungs/kidneys								dc[a]		
Adrenals								ic[r]		
Prostate							ic[r]			
Histopathology			No treatment related effects							

(A) Based on a mean food intake of 45 mg/kg bw (calculation by the reviewer)

Conclusions "Continuous administration of LAS-Mg to male and female rats, at dietary concentrations of 2500 and 5000 ppm, over two generations, was associated with slight retardation of somatic growth, but there were no adverse effects upon reproductive performance or fertility. The responses of animals receiving LAS-Mg at 1250 ppm were essentially similar to the controls."
NOAEL for reproductive effects is 222 mg/kg bw.
NOAEL based on growth of F2 pups up through lactation is 50 mg/kg bw.

Rev. note	1.	The reduced litter size and reduced mean number of live pups in the F2B group treated at 1250 ppm could be attributed to the loss of a single litter.
	2.	The effects on organ weights were related to the reduced body weights seen in the highest dose group. The increased weight of the adrenals in this group could be attributed to a single female (no macroscopic investigation of this animal was performed). The increased relative prostate weight could be attributed to a single male (macroscopic investigation did not confirm this).
Klimisch criterium	1	

Developmental Toxicity and Teratogenicity:

9.4.53

Title	Final report on the safety assessment of sodium dodecylbenzenesulfonate/TEA-dodecylbenzenesulfonate/sodiumdecylbenzenesulfonate	
Date of report	1997.	
GLP	No data	
Reference	1	
Test substance	B, Benzenesulfonic acid, dodecyl-, compd. with 2,2',2''-nitrilotris(ethanol) (1:1), 0.5% a.i. in semipermanent hair dye.	
Guideline	Not indicated.	
Stat. method	Not applicable.	
Test system	**Species**	Rat (CD).
	No. of animals	20 females/dose group (3 control groups).
	Dosage	Dermal application of 2 ml/kg to the shaved skin on day 1, 4, 7, 10, 13, 16 and 19 of gestation.
	Observations	Necropsy on day 20 and examination of foeteuses
Results	No treatment related effects.	
Conclusions	NOAEL > 0.01 ml/kg bw (equivalent to 10 mg/kg bw); only dose tested	
Klimisch criterium	2 A CTFA Cosmetic Ingredient Review.	

9.4.54

Title	A Teratology Study of Topically Applied Linear Alkylbenzene Sulphonate in Rats	
Date of report	1980.	
GLP	No data	
Reference	2	
Test substance	2 (Benzenesulphonic acid, linear alkyl), purity 20.5% (0.2% alkylbenzene, 0.6% ash, 78.7% water).	
Guideline	Not indicated.	
Stat. method	*F*-test, Student's *t*-test (when applicable chi-square). Test groups were compared with water treated controls.	
Test system	**Species**	Rat (Wistar), age 12-18 weeks.
	No. of animals	20-21 mated females/treatment.
	Dosage	Dermal application of 1, 2, 10, 20, 100 and 400 mg/kg bw (0.5 ml in tap water) on the clipped skin (24 cm^2,10% of body surface); unclipped, clipped but not treated and clipped water treated controls; at 20, 100 and 400 mg/kg the test substance was washed off with water after 30 min.
	Procedures	Female rats were mated with untreated males (1/1) from the same strain. The day of observation of sperm was defined as day 0 of gestation. Females were treated daily from day 0 to 20 of gestation inclusive. Body weight, food consumption and clinical signs were recorded daily. All females were subjected to macroscopic examination on day 21. The uteri were removed and examined for no. of corpora lutea, no. of implantation sites and no. and location of foetuses and resorptions. Foetuses were inspected on total number, viability, sex,

weight and external, visceral and neural (½ of foetuses) and skeletal (½ of foetuses) defects. The number of vertebrae and phalanges was recorded.

Results

Dose (mg/kg bw)	**0 (unclipped)**	**0 (clipped)**	**0 (veh.)**	**1**	**2**	**10**	**20**	**100**	**400**	**DR**
Maternal data										
Mortality	None									
Clinical signs [(A)]							+	+	+	x
Mean body weight day 12-21									d (5%)	
Food intake	No treatment related effects									
Necropsy	Not reported									
No. of pregnant females	20/20	20/20	20/20	19/20	20/20	20/20	20/20	20/20	20/21	
No. of corpora lutea and implantation sites /dam	No treatment related effects									
Implantation loss/ resorptions	No treatment related effects									
No. live foetuses/ dam	No treatment related effects									
Foetal data										
No. of litters included in evaluations	19	20	20	19	20	20	20	20	20	
Foetal weight / sex	No treatment related effects									
External,visceral/neural/ Skeletal examination	No treatment related effects									
No. vertebrae and phalanges	No treatment related effects									

(A)Discolouration ((light) brown), erythema, fissuring and slight thickening of the skin. Reported as “marked” at 400, “slight” at 100, and discolouration only at 20.

Conclusions “This study demonstrated that LAS is free of teratogenic and embrypathic effects when applied to the dermis of pregnant Wistar rats at concentrations that elicit marked skin changes and reductions in maternal body weight.
NOAEL for maternal toxicity: 100 mg/kg bw⇔ 20.1 mg a.i./kg bw (based on 5% weight loss).
NOAEL for reproductive effects: 400 mg/kg bw ⇔ 82 mg a.i./kg bw.

Rev. note
1. The test substance was only applied for 30 minutes daily for 20, 100 and 400 mg/kg bw dose groups).
2. Clinical signs were treatment related but not considered toxicologically significant.

Klimisch criterium 2 Non-GLP study

9.4.55

Title Assessment of the teratogenic potential of surfactants Part 1 – LAS, AS and CLD
Date of report 1975.
GLP No.
Reference 18
Test substance 2 (Benzenesulphonic acid, linear alkyl), purity not indicated.
Guideline Not indicated.
Stat. method Wilcoxon-test.
Test system
Species Rabbit (New Zealand White), rat (CD) and mouse (CD-1).
No. of animals 20 females/treatment (13 for rabbits).
Dosage Administration by oral gavage at 0.2, 2.0, 300 and 600 mg/kg bw (vehicle: water); vehicle treated controls; solutions were prepared daily.
Procedures Females were mated. The day of observation of a vaginal plug (rats and mice) or observation of coitus (rabbit) was defined as day 0 of gestation. Females were treated daily from day 6 to 15 (18 for rabbits) of gestation. Mortality/clinical symptoms of dams were noted daily. Body weight was recorded regularly. All females were subjected to

macroscopic examination day 17, 20 and 29 for mice, rats and rabbits, respectively, or on day of death. The uteri were removed and examined for no. of corpora lutea, no. of implantation sites and no. of foetuses and resorptions. Foetuses were inspected on total number, sex, weight and external, visceral (1/3 of foetuses in rats and mice,all in rabbits) and skeletal (2/3 of foetuses in rats and mice,all in rabbits) defects.

Results Mice

Dose (mg/kg bw)	**0**	**0.2**	**2.0**	**300**	**600**	**DR**
Maternal data						
Mortality	0/20	0/20	0/20	7/20	18/20	x
Clinical signs (A)				+	+	
Body weight gain				d	d	x
Necropsy			Not reported			
No. of pregnant females	17/20	18/20	18/20	20/20	19/20	
No. of implantation sites /dam			No treatment related effects			
Pre-implantation loss			Not reported			
Post-implantation loss/ resorptions					i	
No. live foetuses/ dam				dc	N/A	x
Foetal data						
No. of litters included in evaluations	17	18	18	9	N/A	
Foetal weight		ic			N/A	
External examination / sex			No treatment related effects		N/A	
Anomalies: visceral/ skeletal (B)				i	N/A	

(A) Disturbance of the gastro-intestinal tract.
(B) No details provided.

Results Rats

Dose (mg/kg bw)	**0**	**0.2**	**2.0**	**300**	**600**	**DR**
Maternal data						
Mortality	0/20	0/20	0/20	0/20	1/20	
Clinical signs (A)					+	
Body weight gain					d	
Necropsy			Not reported			
No. of pregnant females	15/20	15/20	18/20	16/20	17/20	
No. of corpora lutea / implantation sites per dam			No treatment related effects			
Pre/post-implantation loss/ resorptions			No treatment related effects			
No. live foetuses/ dam			No treatment related effects			
Foetal data						
No. of litters included in evaluations	15	14	18	16	16	
Foetal weight		ic	ic			
External examination / sex			No treatment related effects			
Anomalies: visceral/ skeletal			No treatment related effects			

(A)Disturbance of the gastro-intestinal tract.

Results Rabbits

Dose (mg/kg bw)	**0**	**0.2**	**2.0**	**300**	**600**	**DR**
Maternal data						
Mortality	2/13	0/13	1/13	11/13	13/13	x
Clinical signs (A)				+	+	
Body weight gain				d	d	x
Necropsy			Not reported			
No. of pregnant females	12/13	13/13	12/13	2/13	0/13	
No. of corpora lutea /implantation sitesper dam			No treatment related effects		N/A	
Pre-implantation loss			No treatment related effects			
Post-implantation loss/ resorptions				i	N/A	
No. live foetuses/ dam				dc	N/A	x
Foetal data						

No. of litters included in evaluations	9	12	11	2	N/A
Foetal weight	No treatment related effects			N/A	N/A
External examination / sex	No treatment related effects			N/A	N/A
Anomalies: visceral/ skeletal [(B)]	No treatment related effects			N/A	N/A

(A)Diarrhoea, anorexia and cachexia were seen among animals.

Conclusions "Effects on litter parameters were generally restricted to dosages causing marked maternal toxicity, the principal effects being higher foetal loss (with consequent reduction in litter size) arising from the increased incidence of total litter loss. When dams showing total litter loss were excluded from the calculations, litter parameters were not unduly different from those of controls. At dosages that were either non-toxic or only slightly to moderately toxic to the dam, litter parameters were essentially unaffected."
NOAEL for maternal toxicity: > 2 but <300 mg/kg for mice and rabbits; 300 mg/kg for rats.
There were no teratogenic and embryotoxic effects observed at any dose level.

Rev. note
1. Limited information was available on the identity of the test substance. It was assumed by the reviewer that the test substance was the benzenesulphonic acid, linear alkyl.
2. Effects on reproduction were seen at doses exhibiting maternal toxicity.
3. Anomalies reported in foetuses and sex of the foetuses were not identified.
4. Large (>100 X) gap in doses between NOAEL and LOAEL for maternal toxicity for mice and rabbits makes it difficult to establish a true NOAEL.

Klimisch criterium 4 Question regarding identity of test substance. Non-GLP study.

9.4.56

Title Assessment of the teratogenic potential of surfactants Part III – Dermal application of LAS and Soap

Date of report 1975.

GLP No.

Reference 19

Test substance 2 (Benzenesulphonic acid, linear alkyl), purity not indicated.

Guideline Not indicated.

Stat. method Wilcoxon-test.

Test system

Species Rabbit (New Zealand White), rat (CD) and mouse (CD-1).

No. of animals 20 females/treatment (13 for rabbits).

Dosage Dermal application of 0.03, 0.30 and 3.00% solutions (vehicle: water) to 240, 16 and 6 cm^2 for rabbits, rats and mice resp.(dosing volume 0.5 (rat, mouse) or 10 ml (rabbit)); vehicle treated controls; solutions were prepared daily; application in two parts (with drying period, no occlusion).

Procedures Females were mated. The day of observation of a vaginal plug (rats and mice) or observation of coitus (rabbit) was defined as day 0 of gestation. Females were treated daily from day 2 to 15 (rats), 2 to 13 (mice) and 1-16 (rabbits) of gestation. Mortality/clinical symptoms of dams were noted daily. Body weight was recorded regularly. All females were subjected to macroscopic examination day 17, 20 and 29 for mice, rats and rabbits resp. or on day of death. The uteri were removed and examined for no. of corpora lutea, no. of implantation sites and no. of foetuses and resorptions. Foetuses were inspected on total number, sex, weight and external, visceral (1/3 of foetuses in rats and mice,all in rabbits) and skeletal (2/3 of foetuses in rats and mice,all in rabbits) defects.

Results Mice

Dose (%)	**0**	**0.03**	**0.3**	**3**	**DR**
Dose (mg/kg bw)	**0**	**5**	**50**	**500**	
Maternal data					
Mortality	1/20	1/20	0/20	0/20	
Clinical signs [(A)]			+	+	x
Body weight gain				d	
Necropsy			Not reported		
No. of pregnant females	17/20	16/20	18/20	6/20	
No. of implantation sites /dam			No treatment related effects		
Post-implantation loss/ resorptions			l	i	x
No. live foetuses/ dam				d	
Foetal data					
No. of litters included in evaluations	14	15	14	1	
Foetal weight			No treatment related effects		
External examination			No treatment related effects	N/A	
Anomalies: visceral/ skeletal [(B)]				i	

(A)Erythema, oedema (peak on day 6, dead skin), irritability and hypersensitivity were seen among animals. Effects were reversible
(B)No visceral examination of foetuses of high dosed females. Skeletal examinations revealed extra ribs (cervical).

Results Rats

Dose (%)	**0**	**0.03**	**0.3**	**3**	**DR**
Dose (mg/kg bw)	**0**	**0.6**	**6**	**60**	
Maternal data					
Mortality			None		
Clinical signs [(A)]				+	
Body weight gain			No treatment related effects		
Necropsy			Not reported		
No. of pregnant females	20/20	18/20	20/20	18/20	
No. of corpera lutea / implantation sites per dam			No treatment related effects		
Pre/post-implantation loss/ resorptions			No treatment related effects		
No. live foetuses/ dam			No treatment related effects		
Foetal data					
No. of litters included in evaluations	19	18	20	18	
Foetal weight				ic	
External examination			No treatment related effects		
Anomalies: visceral/ skeletal			No treatment related effects		

(A) Erythema, oedema (peak on day 4-5), irritability and hypersensitivity were seen among animals. Effects were reversible.

Results Rabbits

Dose (%)	**0**	**0.03**	**0.3**	**3**	**DR**
Dose (mg/kg bw)	**0**	**0.9**	**9**	**90**	
Maternal data					
Mortality	0/13	0/13	1/13	0/13	
Clinical signs [(A)]			+	+	
Body weight gain			D	d	
Necropsy			Not reported		
No. of pregnant females	12/13	12/13	13/13	11/13	
No. of corpera lutea / implantation sites per dam			No treatment related effects		
Pre/post-implantation loss/ resorptions				i	
No. live foetuses/ dam				d	
Foetal data					
No. of litters included in evaluations	11	12	12	9	
Foetal weight			No treatment related effects		
External examination			No treatment related effects		
Anomalies: visceral/ skeletal			No treatment related effects		

(A) Erythema, oedema (peak on day 6-7, cracking and bleeding skin), irritability and hypersensitivity were seen among animals.

Conclusions	"Effects on litter parameters were generally restricted to dosages causing marked maternal toxicity in mice, the principal effects being higher foetal loss (with consequent reduction in viable litter size) arising from an increased incidence of total litter losses. When dams showing total litter loss were excluded from the calculations, litter parameters were not unduly different from those of controls. Although LAS at 3% was considered to show marked maternal toxicity in the rabbit, the slightly higher foetal loss and lower litter size did not differ significantly from control values. The moderate maternal toxicity of LAS, 0.3% in the mouse correlated with a higher incidence of embryonic deaths and lower litter size but only the former differed significantly from the corresponding control value. At dosages that were non-toxic or only slightly toxic to the dam, litter parameters were not adversely affected… The incidence of major malformations, minor visceral or skeletal anomalies, and skeletal variants provided no conclusive evidence of specific teratogenicity even at maternally toxic dosages." NOAEL for maternal toxicity: 0.3% = 50 mg/kg bw (mice), 0.3% = 9 mg/kg bw (rabbits) and 3% = 60 mg/kg bw (rats) NOAEL for teratogenic and embryotoxic effects: no effects at any dose level
Rev. note	1. Effects on reproduction were seen at doses exhibiting maternal toxicity 2. Limited information was available on the identity of the test substance. It was assumed by the reviewer that the test substance was the benzenesulphonic acid, linear alkyl. 3. Clinical signs were treatment related but not considered toxicologically significant.
Klimisch criterium	4 Question regarding identify of test substance. Non-GLP study.

9.4.57

Title	LAS-Mg : Effects of oral administration upon the progress and outcome of pregnancy in the rabbit	
Date of report	December 21, 1978.	
GLP	No.	
Reference	26	
Test substance	3, Magnesium salt of LAS, purity not indicated.	
Guideline	Not indicated.	
Stat. method	Not indicated.	
Test system	**Species**	Rabbit (New Zealand White), body weight 2730-5200 g.
	No. of animals	14 females/treatment.
	Dosage	Oral administration of 0, 60, 125 and 250 mg/kg bw (vehicle water) during day 6 to 18 of gestation; dosage volume 5 mL.
	Procedures	Females were mated with fertile males and injected with luteinising hormone on day 0 of gestation. Mortality and clinical signs of dams were noted daily. Body weights were recorded on day 0, 6, 8, 10, 12, 14, 16, 18, 23 and 28 of gestation. Food/water consumption was recorded on day 0, 5, 11, 17, 22 and 28. All females were killed on day 29 of gestation and subjected to macroscopic examination. The reproductive tract (incl. Ovaries) was dissected and examined for number of corpora lutea, implantations, early and late resorptions and foetuses. Foetuses were weighed, sexed and examined for external and skeletal abnormalities. Placenta weights were determined.

Results

Dose (mg/kg bw)	0	60	125	250	DR
Maternal					
Mortality	1/14	1/14	2/14	0/14	
Clinical signs		Not reported			
Body weight gain		d	d	d	X
Food/water consumption (day 6-17)		d	d	d	X
Macroscopy		No treatment related effects			
Number of pregnancies	10	14	12	13	
Corpora lutea/implantation sites		No treatment related effects			
Post implantation loss			i	i	X
Resorptions early		No treatment related effects			
Late				i	X
Placental weight				i	X
Foetal					
Number of litters evaluated	10	13	12	11	
Number of live foetuses			d	d	X
Weight/sex		No treatment related effects			
External/Skeletal abnormalities		No treatment related effects			

Conclusions "It was concluded from this investigation that LAS-Mg, administered to pregnant rabbits at dosages up to 250 mg/kg/day, had no adverse effects upon foetal morphology, although at dosages of 125 or 250 mg/kg/day survival in-utero was impaired. At dosages of 60 mg/kg day or above, there was some impairment of maternal economy, but no effects upon the foetus."
NOAEL for maternal effects = 60 mg/kg bw based on post implantation loss.
Effects on reproduction were observed at doses exhibiting maternal toxicity.
No teratogenicity or embryotoxicity were observed at any dose level.

Rev. note
1. The purity of the test substance is not indicated; therefore, a.i. dose could not be calculated.
2. No visceral examination of foetuses was performed.

Klimisch criterium 2 Non-GLP study; also Notes 1 and 2

9.4.58

Title LAS-Mg : Effects upon the progress and outcome of pregnancy in the rabbit
Date of report August 31, 1978.
GLP No.
Reference 27
Test substance 3, Magnesium salt of LAS, purity not indicated.
Guideline Not indicated.
Stat. method Not indicated.
Test system
Species Rabbit (New Zealand White), mean body weight 3800-4100 g.
No. of animals 14 females/treatment.
Dosage Topical application of 0, 0.75, 1.5 and 3.0% in PEG (3% aqueous) during day 6 to 18 of gestation; application volume 5 mL, application area 100 cm^2.
Procedures Females were mated with fertile males and injected with luteinising hormone on day 0 of gestation. Body weights were recorded on day 0, 6, 8, 10, 12, 14, 16, 18, 23 and 28 of gestation. Food/water consumption was recorded on day 0, 5, 11, 17, 22 and 28. All females were killed on day 29 of gestation and subjected to macroscopic examination. The reproductive tract (incl. ovaries) was dissected and examined for number of corpora lutea, implantations, early and late resorptions and foetuses. Foetuses were weighed, sexed and examined for external and skeletal abnormalities. Placenta weights were determined.

Results

Dose (%)	0	0.75	1.5	3.0	DR
Maternal					
Mortality			1/14	1/14	
Clinical signs (A)	+	+	+	+	X
Body weight (gain)	No treatment related effects				
Food/water consumption	No treatment related effects				
Macroscopy	No treatment related effects				
Number of pregnancies	14	13	12	11	
Corpora lutea/implantation sites	No treatment related effects				
Resorptions	No treatment related effects				
Placental weight	No treatment related effects				
Foetal					
Number of litters evaluated	14	11	11	11	
Number of live foetuses	No treatment related effects				
Weight/sex	No treatment related effects				
External/Skeletal abnormalities	No treatment related effects				

(A) Erythema and hyperkeratinisation.

Conclusions NOAEL for maternal effects 3%. Clinical signs were observed but not considered toxicologically significant.
NOAEL for reproductive effects 3%.

Rev. note
1. The application area was less than 10% of the body surface.
2. No information on the use of a (semi)occlusive dressing was available. If no dressing is used, some oral intake of the test substance can not be fully excluded.
3. The purity of the test substance is not indicated. Therefore the actual amount (a.i.) applied can not be calculated.
4. No visceral examination of foetuses was performed.

Klimisch criterium 2 Non-GLP study; also Notes 3 and 4.

9.4.59

Title LAS-Mg : The effects of topical application upon reproduction : Segment II study
Date of report January 9, 1979.
GLP No.
Reference 28
Test substance 3, Magnesium salt of LAS, purity not indicated.
Guideline Guidelines of Japanese Ministry of Health and Welfare.
Stat. method ANOVA.
Test system
Species Rat (CD), 12 weeks old, weight 242-298 g.
No. of animals 32 females/dose level.
Dosage Application of 0, 1.75, 3.5 and 7.0% test substance in 3%PEG to the clipped dorsal skin (area 32 cm^2) of F0 females; vehicle treated controls.
Procedures F0:
Female rats were mated with untreated males (1/1) from the same strain. The day of observation of sperm or a copulatory plug was defined as day 0 of gestation. Females were treated daily from day 7 to 17 of gestation inclusive.
Two-thirds of the females were sacrified on day 20 of gestation, the remaining females were allowed to deliver and their off-spring was observed for at least 8 weeks after parturition.
F1:
Selected off-spring from dams of the same treatment group was allowed to mate (22/sex/group, 1/1) at the age of ten weeks. The day of observation of sperm or a copulatory plug was defined as day 0 of gestation. On day 20 of gestation the females were sacrified.
Observations **Maternal (F0)**
- Mortality/clinical signs.
- Body weight on day 0, 2, 7, 9, 11, 13, 15, 17 and 20 of gestation.

- Food and water intake twice weekly.

Teratology (F0)
- No. of corpora lutea.
- No. of implantation sites.
- No. and location of foetuses and resorptions.

Foetuses (F1)
- Total number.
- Sex, weight.
- External, visceral (½ of foetuses) and skeletal (½ of foetuses) defects.

Post-natal (F0)
- Body weight twice weekly until weaning.
- Gestation duration, parturation.
- Macroscopy.

Young (F1)
- No., sex, weight.
- Viability/abnormalities.
- Postnatal development (physical/behavioural).
- Macroscopy.

Reproduction (F1)
- No. of corpora lutea.
- No. of implantation sites.
- No. and location of foetuses and resorptions.
- Macroscopy (males and females).

Foetuses (F2)
- Total number.
- Sex, weight.
- External defects.

Results

Dose (%)	**0**	**1.75**	**3.5**	**7.0**	**DR**
F0 (prenatal)					
Mortality	None				
Clinical signs [A]		+	+	+	X
Body weight (gestation)				d	
Food/water consumption [B]	No treatment related effects				
Macroscopy[C]	No treatment related effects				
Non-pregnant females	0/32	1/32	0/32	0/32	
Corpora lutea/implantation sites	No treatment related effects				
Implantation loss/resorptions	No treatment related effects				
Foetal evaluation (F1)					
Number of litters evaluated	11	11	11	11	
Number of live foetuses	No treatment related effects				
Weight/sex	No treatment related effects				
External[D]/Skeletal/visceral abnormalities	No treatment related effects				
F0 (postnatal)					
Mortality/clinical signs	No treatment related effects				
Body weight (lactation)	No treatment related effects				
Gestation time/parturation	No treatment related effects				
Evaluation of offspring (F1)					
Number of viable young	No treatment related effects				
Body weight[E]				d	
Sex	No treatment related effects				
Postnatal development	No treatment related effects				
Macroscopy	No treatment related effects				

F1 (prenatal)	0	1.75	3.5	7.0	DR
Mortality			1 male		
Clinical signs		No treatment related effects			
Body weight		No treatment related effects			
Mating success		No treatment related effects			
Non-pregnant females	2/22	1/22	0/22	0/22	
Corpora lutea			d	d	
Implantation sites				d	
Pre-implantation loss		ic			
Post-implantation loss		ic	ic		
Resorptions		No treatment related effects			
Foetal evaluation (F2)					
Number of litters evaluated	20	20	19	22	
Number of live foetuses		No treatment related effects			
Weight/sex/external abnormalities		No treatment related effects			

(A) Erythema was seen during the treatment period, but turned out to be completely reversible.
(B) Incidental significant increases of water consumption were seen at the highest dose groups.
(C) Slight keratinisation of the skin in females treated with 3.5 and 7.0%.
(D) An increased incidence of hydroureter and hydronephrosis in the 3.5% group was considered to be unrelated to treatment.
(E) The decrease in mean foetal weight was caused by very low weights of the pups in one litter only.

Conclusions NOAEL for maternal toxicity and reproductive effects = 7%.

Rev. note
1. The slightly decreased number of copora lutea and/or implantation sites in the F1 of females treated at 3.5 and/or 7% remained within historical control values.
2. The significant post-implantation loss in the F1 of the lower dosed females was not considered to be related to treatment, but due to total litter loss from one female at 1.75% and 3 females at 3.5%.
3. No information on the use of a (semi)occlusive dressing was available. If no dressing is used, some oral intake of the test substance can not be fully excluded.
4. The purity of the test substance is not indicated. Therefore, the actual amount (a.i.) applied can not be calculated.

Klimisch criterium 2 Non-GLP study; also Notes 3 and 4.