201-14869B

APPENDIX

Robust Summaries for Substances in the HPV Test Plan for the Sorbitan Esters Category of the Aliphatic Esters Chemicals

Part I. HPV Substances in the Sorbitan Esters Category
Part II. Surrogate Sorbitan Esters

November 26, 2003

Appendix -Robust Summaries for Aliphatic Esters - Sorbitan Esters HPV Test Plan

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Part I - Robust Summaries for HPV Substances in the Sorbitan Esters Category of Test Plan

HPV Sorbitan Esters Substances
identified by CAS Numbers and as organized in Table 1B of the HPV Test Plan

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Part II - Robust Summaries for Surrogate Sorbitan Ester

One Surrogate Sorbitan Esters Substance

- Sorbitan, Fatty Acid C6-10 Tetraester (CAS No. 228573-47-5)

PART I. HPV Substances in the Sorbitan Esters Category

Acute Oral Toxicity (CAS No. 1338-39-2)

Test Substance	Sorbitan, monolaurate
CAS Number	1338-39-2
Remarks	Purity was not indicated
Method/guideline	Not indicated
Test type	Acute oral toxicity in rats
GLP	No
Year	1985
Test system	Species (Strain) Rats (strain not specified Sex: Male and female No. of animals: 30 or 60/sex/treatment Vehicle: None, undiluted test material Route: Oral gavage
Test conditions	Remarks: Test material was administered by oral gavage to fasted rats. Mortality observed over 14 day period. Statistical methods were not specified.
Results/Remarks	Elder (1985) reported three acute oral toxicity studies carried out in fasted rats. One group of 30 male rats were orally gavaged with 15.1 to 39.8 g/kg/bw. Two of 10 rats died after administration of 39.8 gm/kg but none of the 20 rats given the lower dosages died during the 14 day observation period. The LD_{50} was estimated to be greater than 39.8 g/kg body weight. Another group of 30 female rats was given similar doses of sorbitan monolaurate. The LD_{50} for this group was 33.6 g/kg body weight with 95% confidence limits of 28.0 to 40.3 g/kg. A group of 60 male and 60 female rats also received similar doses of the test material and the LD_{50} was reported to be 41.25 g/kg body weight with 95% confidence limits of 35.3 to 48.3 g/kg b.w.
Conclusions	The acute oral LD_{50} for the test substance was reported to be > 39.8 g/kg , 33.6 g/kg and 41.25 g/kg in three different studies.
Data Quality	Not assignable [Klimisch reliability 4]. Secondary literature. Limited experimental details and information given.
References	Elder RL (1985). Final report on the safety assessment of sorbitan stearate, sorbitan laurate, sorbitan sesquioleate, sorbitan oleate, sorbitan tristearate, sorbitan palmitate, and sorbitan trioleate, J. Amer. Coll. Toxicol. **4 (3):** 65-121.
Other	Date last updated November 3, 2003.

Repeated Dose Toxicity / Reproductive Toxicity (CAS No. 1338-39-2)

Test Substance	Sorbitan, monolaurate
CAS Number	1338-39-2
Remarks	Purity was not indicated
Method/guideline	Similar to OECD 408 guidelines with exceptions noted below
Test type	13-week oral toxicity study
GLP	No
Year	1978
Species/strain	Rat / Wistar

Route of Administ.	Oral administration for 90 days at 0, 2.5, 5 and 10% in diet
Duration of test	13 weeks
No. of animals	15/sex/dose level
Dose/Conc. Levels	0, 2.5, 5 and 10% in the diet
Sex	Female and male / weight: 69-71 g (female); 84-86 g (male)
Frequency of treatment	Daily in the diet
Control Group	Yes
Post-exposure observat.	Toxicity was assessed by mortality, clinical observations, body weight, food and water consumption, hematology, clinical chemistry (serum collected at week 6 and 13), urinalysis, organ weights, histopathology. At necropsy, any macroscopic abnormality was noted and the brain, pituitary, thyroid, heart, liver, spleen, kidneys, adrenal glands, gonads, stomach, small intestine and cecum were weighed. Samples of these organs as well as of the lungs, salivary glands, aortic arch, thymus, various lymph glands, urinary bladder, colon, rectum, pancreas, uterus and skeletal muscle were fixed, prepared, sectioned and stained for histopathology examination.
Statist. Methods	Student's *t*-test, ranking method of White.
Remarks on Test Conditions	This 90-day oral toxicity study was essentially similar to the OECD 408 guideline except that no ophthalmoscopy, no behavioral effects, limited blood biochemistry, no blood clotting potential and limited histopathology (no parathyroid, esophagus, trachea, mammary gland, prostate, bone marrow, skin and eyes) were performed.
Results	Results are summarized in the table below

Dose (% in diet)	0		2.5% Diet		5% Diet		10% Diet		Dose-related	
Dose (mg/kg bw) estimated	0	0	2100	2300	4200	4500	8000	8400		
Sex	M	F	M	F	M	F	M	F	M	F
Mortality	None									
Clinical signs	No treatment related effects									
Body weight			dc	d	dc	dc	dc	dc	x	x
Food consumption			dc	d	dc	dc	dc	dc	x	x
Water consumption					ic			dc		
Hematology										
Hb/hematocrit					dc	dc	dc	dc	x	x
RBC[(a)]			dc		dc			ic		
Leukocytes			dc		dc		dc		x	
Clinical chemistry	Serum enzyme levels reported to be normal									
Urinalysis [(b)]	No treatment related effects									
Organ weight										
Brain			ic^r	ic^r	ic^r	ic^r	ic^r	ic^r	x	x
Kidney			ic^r	ic^r	ic^r	ic^r	ic^r	ic^r		x
Liver							ic^r	ic^r		
GI-tract					ic^r	ic^r	ic^r	ic^r	x	x
Heart							ic^r	ic^r		
Histopathology [(c)]										
Liver - periportal vacuolation							+	+		
- increased periportal fat						+	+	+		

Abbreviations:
ic = increase (significant) i = increase dc= decrease (significant) d = decrease
r= relative to body weight x = dose-related + = effect present

Footnotes:
(a) There was a tendency for higher reticulocytes counts.
(b) Among treated males less urinary production with higher specific gravity.
(c) Signs of early respiratory disease were reported among animals.

Remarks	Cater et al. (1978) reported dose-related reductions in the rate of body weight gain were associated with reduced intakes of diet containing test material. Rats fed 5% or 10% of the test material in diet had reduced hemoglobin concentrations and packed cell volumes but other hematological parameters and serum enzyme levels were normal. Variations in organ weight were mainly associated with the lower body weights. Those

	thought to be related to treatment were increases in relative liver and small intestine weights in the rats given 10% and 5 or 10% diets, respectively, and increases in kidney weight at all dose levels. Periportal fat-containing vacuoles were demonstrated in the livers of the rats fed 10% of the test material but the increased relative kidney weights were not accompanied by histological changes or impairment of renal function and no abnormal constituents were present in the urine.
Conclusions	LOAEL was 2.5% diet [estimated as ~ 2200 mg/kg/day (male and female, mean]. The 13-week toxicity study did not indicate that the test material adversely affect the reproductive organs in male and female rats. No histopathological or gross abnormalities were reported for male and female gonads.
Data quality	Reliable with restrictions [Klimisch reliability 2]. Study was similar to OECD 408 guidelines with some exceptions.
References	Cater BR, Butterworth KR, Gaunt IF, Hoosan J, Grasso P and Gangoli SD (1978). Short-term toxicity study of sorbitan monolaurate (Span 20) in rats. Food Cosmet. Toxicol. **16:** 519-526.
Other	Date last updated November 4, 2003.

Acute fish toxicity (CAS No. 1338-39-2)

Test Substance	Sorbitan, monolaurate
CAS Number	1338-39-2
Remarks	Purity was not indicated
Method/guideline	Not specified
Type (test type)	Static 96-hr acute fish toxicity
Test System	Fish, freshwater
GLP	No
Year	1987
Species/Strain	Fish, rainbow trout (*Salmo gairdneri*)
Analyt. Monitoring	Not specified
Exposure period	96 hours
Statist. Methods	Not indicated
Remarks on Test Conditions	Rainbow trout, mean weight 2.67 g, No. of fish: 10/treatment group Nominal concentrations: 10, 18, 32, 56 and 100 mg/L, untreated control Test Conditions: 96-hr static test, aerated, 15 ± 1 °C Observations: Mortality at 24, 48, 72 and 96 hrs.
Results	Nominal test conc. Loading Level (mg/L) — Mortality (96h) Control (untreated) — 0 10 — 0 18 — 0 32 — 0 56 — 0 100 — 100
Conclusions	96-hr LC_{50} was estimated to be 75 mg/L in the report. No mortality observed at 56 mg/L (nominal). Test material expected to be at water saturated limits in the test solution at the 56 mg/L nominal loading rate. Hence, data indicate that test substance would not be expected to cause acute toxicity in fish at its water saturation limit.

Data Quality	Reliable with restrictions [Klimisch reliability 2]. No GLP. Limited information in report.
References	Unpublished confidential business information.
Other	Date last updated November 3, 2003.

Biodegradation (CAS 1338-39-2)

Test Substance **CAS Number** **Remarks**	Sorbitan, monolaurate 1338-39-2 Purity was not indicated
Method/guideline	OECD 301C (1981)
Test type **GLP** **Year**	Aerobic Biodegradation - respirometric method, oxygen uptake method No 1984
Test system	Exposure Period: 28 Days Inoculum: Activated sludge, 30 mg suspended solids per liter used in test Kinetics: Not Reported Biodegradation Products: Not Reported Analytical Monitoring: Oxygen uptake or biochemical oxygen demand (BOD)
Test Conditions/ Remarks	Treated Flasks [medium + inoculum + test material (100 mg/l)]; Positive Control Flasks [medium + inoculum + aniline]. Aniline conc was not given. Blank Control Flasks [medium + inoculum]. The test substance was stirred in an aqueous medium (100 mg/L) with activated sludge (30 mg/L) for a period of 28 days. During this period dissolved oxygen or BOD was measured and blank controls were used for background correction. Limited information available.
Results/Remarks	**Biodegradation Results:** % Biodegradation [% of ThOD] **Day 5 10 15 20 25 28** Test Material 51 56 59 60 59 60 Positive Control (aniline) 39 46 56 61 64 66 Test material did not meet "10-day window" criteria for ready biodegradability. Positive controls did not appear to meet the "readily biodegradable" criteria.
Conclusions	Biodegradation was 60% in 28 days based on oxygen uptake. The test substance did not meet "readily biodegradable" requirements.
Data Quality	Reliable with restrictions [Klimisch reliability 2]. Not GLP and limited information in report.
References	Unpublished confidential business information
Other	Date last updated November 3, 2003

Acute Oral Toxicity (CAS No. 1338-43-8)

Test Substance	Sorbitan, monooleate
CAS Number	1338-43-8
Remarks	Purity was not indicated
Method/guideline	Not indicated
Test type	Acute oral toxicity
GLP	No
Year	1966
Test system	Species (Strain) Rats (Wistar) Sex: Male and female, Weight: 144-154 g (male), 135-154 g (female) No. of animals: 10/sex/treatment Vehicle: Corn oil (test material at conc 90% v:v in corn oil) Route: Oral gavage Dosage: 50 ml/kg (90% conc v/v in corn oil) or 39.8 g/kg bw
Test conditions	Remarks: Single oral administration (gavage) of 39.8 g/kg bw; no controls; feeding *ad libitum* but food was withheld 16 hrs prior to dosing. Mortality observed several times on day 1 and daily thereafter until day 14. Clinical signs several times on day 1 and daily thereafter until day 14. Necropsy on day 14.
Results/Remarks	No deaths were observed in male or female rats during the 14-day observation period. Clinical observations included depression, decreased respiration, messy fur and diarrhea during the first 72 hours. Necropsy findings included minor focal hemorrhage and congestion in the lungs and hydronephrosis and congested medulla of the kidneys.
Conclusions	The acute oral LD_{50} for the test substance was reported to be > 39.8 g/kg.
Data Quality	Reliable with restrictions [Klimisch reliability 2]. Not GLP.
References	1) Confidential business information. 2) Findings have also been cited by Elder RL (1985). Final report on the safety assessment of sorbitan stearate, sorbitan laurate, sorbitan sesquioleate, sorbitan oleate, sorbitan tristearate, sorbitan palmitate, and sorbitan trioleate, J. Amer. Coll. Toxicol. **4(3)**: 65-121.
Other	Date last updated November 5, 2003.

Repeated Dose Toxicity / Reproductive Toxicity (CAS No. 1338-43-8)

Test Substance	Sorbitan, monooleate
CAS Number	1338-43-8
Remarks	Purity was not indicated
Method/guideline	Not indicated but method similar to the OECD 408 guidelines with some exceptions.
Test type	16-Week Oral Feeding Toxicity Study
GLP	No
Year	1978
Species/strain	Rat / Wistar
Route of Administ.	Oral administration for 16 weeks at 0, 2.5, 5 and 10% in the diet
Duration of test	16 weeks
No. of animals	15 sex/dose level and second group of 10/sex/dose level (see below)
Dose/Conc. Levels	0, 2.5, 5 and 10% in the diet
Sex / Weight	Female and male / weight: 90-91 g (female), 89-94 g (male)
Frequency of treatment	Daily in dietary feed
Control Group	Yes
Post-exposure observat.	Toxicity was assessed by mortality, clinical observations, body weight, food and water consumption, hematology, limited clinical chemistry (serum collected at week 2 and 6), urinalysis, organ weights, histopathology. At necropsy, any macroscopic abnormality was noted and the brain, heart, liver, stomach, small intestine, cecum, spleen, kidneys, adrenal glands, gonads, pituitary and thyroid were weighed. Samples of these organs and of the lungs, lymph nodes, salivary glands, trachaea, esophagus, aorta, thymus, urinary bladder, colon, rectum, pancreas, uterus and skeletal muscle were fixed, prepared, sectioned and stained for histopathology examination.
Statist. Methods	Student's *t*-test, ranking method of White, ranking test of Kruskal and Wallis.
Results	Results are summarized in the table below:

Dose (% in diet)	0		2.5% Diet		5% Diet		10% Diet		Dose-related	
Dose (mg/kg bw)	0	0	1700	2000	3100	3700	6300	6100		
Sex	M	F	M	F	M	F	M	F	M	F
Mortality	None									
Clinical signs	No treatment related effects									
Body weight (Day 105)							dc	dc		
Food consumption					d		dc	dc	x	
Water consumption					dc		dc	d	x	
Hematology										
Hb/RBC								dc		
Hematocrit						dc	dc	dc	x	x
Leukocytes							dc			
Clinical Chemistry										
Protein/albumin - week 2					dc					
- week 6					dc		dc			
Urea					dc wk 6			dc wk 16		
Urinalysis [(a)]	No treatment related effects									
Organ weight										
Brain							ic^r	i^r		
Heart					i^r	ic^r	ic^r	ic^r		
Liver / small intestine							ic^r	ic^r		
Kidney			ic^r	ic^r	ic^r	ic^r	ic^r	ic^r	x	x
Stomach/adrenals							ic^r	ic^r		
Pituitary / gonads										
Necropsy at Week 16										
Histopathology										
Kidney, liver [(b)]						+		+		

Abbreviations:
ic = increase (significant) i = increase dc= decrease (significant) d = decrease
r= relative to body weight x = dose-related + = effect present

	Footnotes: (a) Effects seen included increased gravity and decreased volume. (b) Renal tubular damage (dilation of proximal tubulus with vacuolation) and periportal fatty changes of the liver.
Remarks	This 16-week study was essentially similar to the OECD 408 guideline except for limited hematology and clinical chemistry, no ophthalmoscopy and no behavioral observation being carried out in the 16-week study. Ingram et al. (1978) reported that treatment with test material for 16 weeks resulted in significantly increased kidney weights, associated in the female groups given 5% or 10% in the diet and with renal tubular changes of uncertain pathological significance. In addition, liver enlargement was found in the males and females at 5% dietary levels. In females, the liver effect was associated with periportal fatty change. Reduced body weight gain was observed in groups given 5% or 10% dietary concentrations of the test material but this may be due largely to the unpalatability of the diet. Minor changes in the hematological findings could not definitively be attributed to treatment.
Conclusions	1) LOAEL was 2.5% diet [estimated ~ 1800 mg/kg/day (male and female, mean)] based on increased kidney weight changes. 2) In addition, the 16-week oral toxicity study did not indicate that the test material adversely affect the reproductive organs in male and female rats. No histopathological or gross abnormalities were reported for male and female gonads.
Data quality	Reliable with restrictions [Klimisch reliability 2]. Study was similar to OECD 408 guidelines with some exceptions. Not GLP.
References	Ingram AJ, Butterworth KR, Gaunt IF, Grasso P and Gangoli SD (1978). Short-term toxicity study of sorbitan mono-oleate (Span 80) in rats. Food Cosmet. Toxicol. **16:** 535-542.
Other	Date last updated November 5, 2003.

Repeated Dose Toxicity / Reproductive Toxicity (CAS No. 1338-43-8)

Test Substance	Sorbitan, monooleate
CAS Number	1338-43-8
Remarks	Purity was not indicated
Method/guideline	Not indicated
Test type	Two-year oral feeding study
GLP	No
Year	1950
Species/strain	Rats / strain not specified
Route of Administ.	Oral administration for two years at 0 and 5% in the diet
Duration of test	Two years
No. of animals/sex	30 male rats, weight 54-63 g
Dose/Conc. Levels	0 and 5 % in the diet
Frequency of treatment	Daily in dietary feed
Control Group	Yes, control group of 50 male rats
Post-exposure observat.	Toxicity was assessed by mortality, clinical observations, growth rate, hematology, clinical chemistry, tissue weights, necropsy and histopathology findings.
Statist. Methods	Statistical methods not indicated.
Results/Remarks	Atlas Chemical Industries (1970) reported the following summary of toxicological data for this 2-year oral dietary feeding study: *Mortality.* No deaths were attributed to sorbitan monooleate treatment based on comparison of survival/mortality data with that for control untreated animals.

	Growth. Sorbitan monooleate had no adverse effect on growth rate at any period during the study. *Hematology*. No effects on hemoglobin concentration or on RBC and WBC counts were seen following determination in two controls and one test rat at 6 months, on one control and one test rat at 12 and 17 months and finally in 13/50 and 5/30 control and test survivors, respectively, at the end of 2 years. *Clinical chemistry*. Levels of blood urea at 6 months, blood urea and glucose at 12, 17 and 24 months and serum cholesterol at 24 months were within normal limits. The number of animals examined were the same as those used for the hematological studies. *Necropsy and histopathology findings*. The heart, lungs, spleen, liver, kidneys, adrenals and thyroid were judged to be of normal size in animals fed 5% of the test material for 2 years. Histopathological examination of the liver, kidneys and bone marrow of several and control rats (as specified under hematology) at 6, 12 or 17 moths, revealed no significant lesions. No gross and no histological changes were observed in the liver, kidneys, bone marrow, testes, stritated muscle, prostate, GI tract, adrenals, pancreas, urinary bladder, spleen, lymph nodes, heart, salivary glands, lungs, brain, parathyroid and pituitary of survivors of 2-year feeding of sorbitan monooleate.
Conclusions	1) NOAEL was 5% in the diet. A dietary level of 5% test material fed to male rats for 2 years had no adverse effect on the growth, hematology, clinical chemistry, survival , organ size or histopathology. 2) In addition, the 2-year study indicated that the test material did not adversely affect the reproductive organs in male rats. No histopathological or gross abnormalities were reported in the testes.
Data quality	Not assignable [Klimisch reliability 4]. Secondary literature. Toxicity data from 2-year study were reviewed and summarized by BIBRA.
References	Atlas Chemical Industries (ACI) (1970). Summaries of toxicological data. Lifespan feeding studies on sorbitan monolaurate and sorbitan mono-oleate. Food Cosmet. Toxicol. **8:** 339-340 (1970).
Other	Date last updated November 5, 2003.

Acute fish toxicity (CAS No. 1338-43-8)

Test Substance	Sorbitan, monooleate
CAS Number	1338-43-8
Remarks	Purity was not indicated
Method/guideline	Not specified
Type (test type)	Static 96-hr acute fish toxicity
Test System	Fish, freshwater
GLP	No
Year	1987
Species/Strain	Fish, rainbow trout (*Salmo gairdneri*)
Analyt. Monitoring	Not specified
Exposure period	96 hours
Statist. Methods	Not applicable
Remarks on Test Conditions	Rainbow trout, mean weight 0.91 g, No. of fish: 10/treatment Nominal concentrations: 0 (untreated control) and 1000 mg/L Test Conditions: Limit test, 96-hr static, aerated, 15 ± 1 °C Observations: Mortality at 24, 48, 72 and 96 hrs.

Results	Nominal test conc. Loading Level (mg/L) — Mortality (96h) 0 (untreated control) — 0 1000 — 0
Conclusions	96-hr LC_{50} was >1000 mg/L (nominal concentration). No mortality observed at the limit concentration of 1000 mg/L (nominal). Test material expected to be at water saturated limit (WSL) at this nominal loading rate. Hence, data suggest that test substance would not be expected to cause mortality in fish at its WSL.
Data Quality	Reliable with restrictions [Klimisch reliability 2]. No GLP. Limited information in summary report.
References	Unpublished confidential business information.
Other	Date last updated November 5, 2003.

Biodegradation (CAS 1338-43-8)

Test Substance	Sorbitan, monooleate
CAS Number	1338-43-8
Remarks	Purity was not indicated
Method/guideline	OECD 301C (1981)
Test type	Aerobic Biodegradation - respirometric method, oxygen uptake method
GLP	No
Year	1984
Test system	Exposure Period: 28 Days Inoculum: Activated sludge, 30 mg suspended solids per liter used in test Biodegradation Products: Not Reported Analytical Monitoring: Oxygen uptake or biochemical oxygen demand (BOD)
Test Conditions	Treated Flasks [medium + inoculum + test material (100 mg/l)]; Positive Control Flasks [medium + inoculum + aniline]. Aniline conc was not given. Blank Control Flasks [medium + inoculum]. The test substance was stirred in an aqueous medium (100 mg/L) with activated sludge (30 mg/L) for a period of 28 days. During this period dissolved oxygen or BOD was measured and blank controls were used for background correction. Limited information available.
Results	**Biodegradation Results:** % Biodegradation [% of ThOD] **Day 5 10 15 20 25 28** Test Material: 29 43 54 56 61 62 Positive Control (aniline): 39 46 56 61 64 66 Test material did not meet "10-day window" criteria for ready biodegradability. Positive controls did not appear to meet the "readily biodegradable" criteria.
Conclusions	Biodegradation was 62% in 28 days. The test substance was not readily biodegradable.
Data Quality	Reliable with restrictions [Klimisch reliability 2]. Not GLP and limited information.
References	Unpublished confidential business information
Other	Date last updated November 5, 2003

Acute Oral Toxicity (CAS No. 1338-41-6)

Test Substance	Sorbitan, monostearate
CAS Number	1338-41-6
Remarks	Purity was not indicated
Method/guideline	Not indicated
Test type	Acute oral toxicity
GLP	No
Year	1966
Test system	Species (Strain) Rats (Wistar) Sex: Male and female, weight 140-164 g No. of animals: 10/sex/treatment Route: Oral gavage Dosage: 15.9 g/kg bw
Test conditions	Remarks: Single oral (gavage) administration(in two equal portions) of 15.9 g/kg (dosing volume 50 ml/kg); no controls; feeding *ad libitum* but food was withheld 16 hrs prior to dosing. Mortality observed on day 1, 2 and 14. Clinical signs observed several times on day 1 and daily until day 14. Body weights on day 1. Necropsy on day 14.
Results/Remarks	No deaths were observed in male or female rats during the 14-day observation period. No abnormal clinical observations reported. Necropsy findings included soft heart, bladder distended with urine, hydronephrosis, irregularly shaped kidneys, pale medulla of the kidneys, areas of pale discoloration in the kidneys, slight focal hemorrhage in the lungs and slightly congested lungs. Report was limited; no measurement of body weight was performed on days 7 and 14.
Conclusions	The acute oral LD_{50} for the test substance was reported to be > 15.9 g/kg.
Data Quality	Reliable with restrictions [Klimisch reliability 2]. Limited report and not GLP.
References	Confidential business information. However, findings have been cited by Elder RL (1985). Final report on the safety assessment of sorbitan stearate, sorbitan laurate, sorbitan sesquioleate, sorbitan oleate, sorbitan tristearate, sorbitan palmitate, and sorbitan trioleate, J. Amer. Coll. Toxicol. **4(3)**: 65-121.
Other	Date last updated November 5, 2003.

Repeated-Dose Toxicity / Reproductive Toxicity (CAS No. 1338-41-6)

Test Substance	Sorbitan, monostearate
CAS Number	1338-41-6
Remarks	Purity was not indicated.
Method/guideline	Not indicated
Test type	80-week oral feeding study
GLP	No
Year	1978
Species/strain	Mice (TO strain)
Route of Administ.	Oral administration for 80 weeks at 0, 0.5, 2 and 4% in the diet
Duration of test	80 weeks
No. of animals	48 /sex/dose level
Dose/Conc. Levels	0, 0.5, 2 and 4% in the diet
Sex / Weight	Female and male / weight 29-31 g
Frequency of treatment	Daily in dietary feed
Control Group	Yes
Post-exposure observat.	Toxicity was assessed by mortality, clinical observations, body weight, food and water consumption, hematology, clinical chemistry (blood collected at week 12 and 52), organ weights, gross morphology and histopathology. At necropsy, any macroscopic abnormality was noted and the brain, heart, liver, kidneys, spleen, stomach, and small intestine were weighed. Samples of these organs together with the cecum, salivary gland, thyroid, thymus, adrenal glands, lymph nodes, pancreas, pituitary, testes, seminal vesicles, prostate, ovaries, uterus, urinary bladder, lungs, trachea, esophagus, colon, rectum, spinal cord, skeletal muscle, eye and Harderian gland and any other tissue that appeared abnormal were fixed, prepared, sectioned and stained for histopathological examination.
Statist. Methods	Student's t-test, chi-square test.
Results/Remarks	Hendy et al. (1978) reported that there was no evidence of carcinogenic activity at any of the dietary dose levels. Treatment had no adverse effects on the mortality/survival or body weight gain. Both male and female mice receiving 4% sorbitan monostearate in the diet showed enlargement of the kidneys and a higher incidence of nephrosis compared with controls. Other organ weight changes appeared unlikely to be directly related to treatment as did a significant depression in the total leucocyte count in the blood of female but not of male mice receiving 4% sorbitan monostearate in the diet. Hendy et al. (1978) concluded that the NOAEL level in this study was 2% of the diet or approximately 2600 mg/kg/day in mice. Other remarks: Reproductive tissues (e.g., testes, ovaries) were collected at necropsy in this study and there were no reports of any macroscopic or histopathological abnormalities or adverse findings. Hendy et al. (1978) also cited unpublished data by J.C. Krantz in which "young rats fed on diets containing 1 or 4% sorbitan monostearate for 6 weeks showed no effect on weight gain, nor were there any significant histopathological changes in the liver, kidneys, intestine and bladder".
Conclusions	1) NOAEL was 2% diet [estimated ~ 2600 mg/kg/day] based on kidney organ weight and hematology (total leucocyte count). 2) In addition, this 80-week oral toxicity study did not indicate that the test material adversely affect the reproductive organs in male and female rats. No histopathological or gross abnormalities were reported for male and female reproductive organs.
Data quality	Reliable with restrictions [Klimisch reliability 2]. Not GLP.
References	Hendy RJ, Butterworth KR, Gaunt IF, Kiss IS, Grasso P (1978). Long-term toxicity study of sorbitan monostearate (Span 60) in mice. Food Cosmet Toxicol. **16:** 527-534.
Other	Date last updated November 6, 2003

Repeated-Dose Toxicity/Reproductive Toxicity (CAS No. 1338-41-6)

Test Substance	Sorbitan, monostearate
CAS Number	1338-41-6
Remarks	Purity was not indicated
Method/guideline	Not indicated
Test type	2-Year oral dietary feeding study
GLP	No
Year	1959
Species/strain	Rat (Osborne-Mendel), weight 40-50 g.
Route of Administ.	Oral administration for 2 years at 2, 5, 10 and 25% in diet
Duration of test	80 weeks
No. of animals	12/sex/dose level.
Dose/Conc. Levels	Dietary administration for 2 years at 0, 2, 5, 10 and 25%
Sex / Weight	Female and male
Frequency of treatment	Daily in dietary feed
Control Group	Yes
Post-exposure observat.	Toxicity was assessed by mortality, clinical observations, body weight, food consumption, hematology (Hb, RBC, WBC and differential counts), organ weight, gross morphology, abnormality at necropsy and histopathology on all animals.
Statist. Methods	Not specified.
Results	Results are summarized in the table below:

Dose (% in diet)	0		2% Diet		5% Diet		10% Diet		25% Diet	
Dose (mg/kg bw) estimate	0	0	1300	1500	3300	3800	6700	7500	25000	24000
Sex	M	F	M	F	M	F	M	F	M	F
Mortality	12 /24		12 /24		14 /24		18 /24		18 /24	
Clinical signs	No clinical adverse effects reported									
Body weight gain (wk 12)									dc	dc
Food consumption										d
Haematology	No treatment related effects									
Organ weight										
Liver										
Kidney									ic^r	ic^r
Necropsy	No gross macroscopic abnormality reported at necropsy									
Histopathology										
Liver [(a)]									+	+

Abbreviations:
ic = increase (significant) i = increase dc= decrease (significant) d = decrease
r= relative to body weight x = dose-related + = effect present
Footnotes:
a) Fatty changes of the liver (hepatic cell vacuolation) was reported among animals in the 25% diet level

Remarks	At highest dietary levels, growth depression was reported in both female and male rats.
Conclusions	1) NOAEL was 5% diet in rats based on mortality/survival. 2) In addition, this 2-year feeding study did not indicate that the test material adversely affect the reproductive organs in male and female rats. No histopathological or gross abnormalities were reported for testis, uterus and ovaries.
Data quality	Reliable with restrictions [Klimisch reliability 2]. Not GLP. This is an older 1959 study that contains useful data to support the low degree of toxicity for repeated-dose effects. Also no adverse effects on the reproductive organs in male and female rats were observed.
References	Fitzhugh OG, Bourke AR, Nelson AA, Frawley JP (1959). Chronic oral toxicities of four stearic acid emulsifiers. Toxicol. Appl. Pharmacol. **1:** 315-331..
Other	Date last updated November 6, 2003.

Reproductive /Developmental Toxicity (CAS No. 1338-41-6)

Test Substance	Sorbitan, monostearate
CAS Number	1338-41-6
Remarks	Purity not indicated; however, chemical analysis and stability conducted
Method/guideline	Other, not indicated (but methodology approved by FDA)
Test type	2-Year, 4-generation feeding study
GLP	No (prior to GLP regulations but appears to be a well conducted study)
Year	1952
Species/strain	Rat (Wistar strain), 28 days old, mean weight 50-70 g
Route of Administ.	Oral (Chronic feeding study)
Duration of test	2-Years
Sex, No. of animals	Study started with 25 groups consisting of 12 males and 20 females each (F_0 generation)
Dose/Conc. Levels	Diet consisted of the following percentages: 0, 5, 10 and 20%.
Frequency of treatment	Daily
Control Group	Yes, untreated controls
Statist. Methods	Not provided
Remarks on Test Conditions	Reproduction and lactation program was initiated shortly after 12th week on the test diet when rats were approximately 110 days old. Matings were set up with one male and two female rats per cage. Pregnant rats were transferred to individual cages. If pregnancy was not established by the 3rd week, the male was replaced. Following three unproductive trials with females of known fertility, males were considered sterile and retired. Females were continued for a minimum of 6 matings with fertile males, even though some failures may have intervened. Lactation was permitted for three weeks. Following weaning, death, or destruction of their litters, the females were allowed a 1-week rest period before remating. In successive matings, the males were rotated among the females within their respective test groups. Matings continued in the F_0 generation throughout the entire 2-year period. First litters were discarded at weaning. From the second litters of as many different mothers as possible, 10 rats of each sex were selected whose individual weights approximated the averages for their respective litters. These F_1 generation animals were raised to maturity and mated like the parent generation. The second litters of the F_2 generation were carried through the same breeding program. Similarly, representative rats of the F_3 generation were raised to maturity for growth observations but not mated because the entire study was terminated when the F_0 rats reached two years on test. During the reproduction phase of the experiment body weights were recorded biweekly; females were also weighed at the time of mating and at appropriate intervals to confirm pregnancy. Pups were weighed at 4, 12, and 21-days of age at which time they were weaned. The following indexes of reproductive and lactating efficiency were calculated: fertility index (FI), the percentages of matings resulting in pregnancy; gestation index (GI), the percentage of pregnancies resulting in the birth of live litters; viability index (VI), the percentages of rats born that survived 4-days or longer; and lactation index (LI), the percentage of rats alive at 4-days that survived the 21-day lactation period.
Results	The FI indicated that sorbitan, monostearate groups responded quite the same, on the average, as the control group, approximately 7 out of 10 matings resulting in pregnancy. Thus indicating no diminution in fertility with the increase of test material level from 10 to 20%. All pregnancies, once established, went to term, as indicated by the GI values that ranged from 94 to 98% for test material and 98% for control. No evidence was observed of impairment of the reproductive function of F_0 generation. Since the rats in the F_0 generation were permitted to mate as long as they were productive, additional data on reproductive efficiency were obtained, namely the age at which loss of fertility

occurred. The average age in days at loss of fertility in F_0 generation females as estimated from successive mating failures was >402 for controls and >462, >474, and 440 at dose levels of 5%, 10%, and 20%, respectively. From the relative magnitude of the VI and LI values, it is apparent that a drop in the litter size occurred principally during the first few days immediately after birth. Results are as follows:

Summary of Reproduction and Lactation Data of F_0 Generation Rats for 6-Matings

Dose	Number Mating	Litters Born Alive	Average Num. pups/litter Born	Weaned	Average wt. pups at Weaning	FI	GI	VI	LI
0%	119	76	8.6	6.5	43.3	66	98	83	91
5%	109	82	8.7	6.5	40.5	79	97	71	91
10%	112	84	8.8	5.3	37.5	79	97	78	89
20%	109	78	8.8	3.7	30.7	73	98	51	90

An indication of possible neglect of the litters was noted from the relatively greater drop in the VI values. A much larger proportion of deaths among the newborn occurred shortly after birth (i.e., up to 4-days of age) than during the remainder of the 21-day nursing period. The laxative effect and posterior ventral irritation at the high dose may have had an adverse influence on the interest of the dams in caring for their offspring.

The effects of the test material on reproduction and lactation were made in rats of three generations descended from the first or parent generation (F_0). As previously described groups of 10 males and 10 females selected from the second litters of each generation constituted the progenitors. Since the breeding experiments after the F_0 generation were terminated when the second litters were weaned, the values shown in these tables represent not more than 2-litters from each female, i.e., the product of 20 matings per group.

Summary of Reproduction and Lactation Data of F_0 Generation Rats for 2-Matings

Dose	Number Mating	Litters Born Alive	Average Num. pups/litter Born	Weaned	Average wt. pups at Weaning	FI	GI	VI	LI
0%	40	31	8.9	6.9	41.1	83	94	82	95
5%	39	36	9.9	7.6	38.1	95	97	82	93
10%	40	35	9.4	6.2	37.5	90	97	67	97
20%	39	36	9.7	4.3	26.8	92	100	57	78

Summary of Reproduction and Lactation Data of F_1 Generation Rats for 2-Matings

Dose	Number Mating	Litters Born Alive	Average Num. pups/litter Born	Weaned	Average wt. pups at Weaning	FI	GI	VI	LI
0%	20	18	10.1	6.0	40.9	90	100	86	69
5%	20	19	10.5	7.3	37.8	95	100	79	73
10%	20	18	10.4	7.1	31.8	100	90	82	82
20%	20	17	8.5	4.4	31.5	85	100	60	86

	Summary of Reproduction and Lactation Data of F_2 Generation Rats for 2-Matings Dose / Number Mating / Litters Born Alive / Average Num. pups/litter Born / Average Num. pups/litter Weaned / Average wt. pups at Weaning / FI / GI / VI / LI 0% / 19 / 15 / 9.2 / 6.7 / 39.1 / 84 / 94 / 84 / 87 5% / 20 / 14 / 9.1 / 4.9 / 34.4 / 70 / 100 / 68 / 80 10% / 20 / 16 / 9.4 / 5.5 / 36.1 / 80 / 100 / 67 / 88 20% / 20 / 6 / 9.2 / 3.2 / 30.2 / 30 / 100 / 58 / 59 The proportion of matings resulting in pregnancy tended to be lower at the 20% dose level. The third generation was generally less productive than the first two may be seen in the lower FI values. Nearly all pregnancies went to term for all generations. However, there was a trend toward higher mortality during the 4-days post partum as the doses increased, was not as marked in the F_1 and F_2 as in the F_0 generation. The proportion of nurslings surviving the lactation period was reduced in some cases in the F_2 generation at the 20% level. Reproductive performance in general appeared to be inferior in the third generation rats compared to their progenitors. Further testing of the high dose F_2 generation rats after their second litters were weaned with the basal diet (containing 9% vegetable fat) resulted in improved viability of the newborn.
Conclusions	1) No effects were observed on reproduction, gestation, growth, lactation, and mortality at 5 and 10% in the diet in a 2-year, 4-generation feeding study in rats. At 20% in diet, slight effects on growth and impairment of lactation were reported. 2) There were no reports of developmental effects. It appears, by increasing the fat level in the basal diet from its original concentration of 4% by adding 9% vegetable fat (near normal levels of dietary fat) would diminish the effect of the ester on survival. By comparison of the corresponding values, it can be seen that no adverse or cumulative effect on mating instinct, fertility or gestation occurred through three successive generations. A frequent observation was a diminution in viability of newborn at the highest level in the diet, which could be associated with added stress of perianal irritation. The effects of the 20% dose on fertility and on viability of the young, appeared to be attributable in part to the fact that the basal diet was relatively low in fat since these responses were improved by the addition of neutral vegetable fat to diets.
Data Quality	Reliable with restrictions [Klimisch reliability 2]. *NOTE: Information used by FDA and WHO for assessing reproductive effects of sorbitan, monostearate for application in food.*
References	Oser BL, Oser M (1956). Nutritional studies on the diets containing high levels of partial ester emulsifiers. II. Reproduction and lactation. J. Nutrit. 60:489-505.
Other	Date: November 5, 2003

Genetic Toxicity In Vitro (CAS No. 1338-41-6)

Test Substance	Sorbitan, monostearate
CAS Number	1338-41-6
Remarks	Purity was not indicated
Method/guideline	Not indicated
Type of Study	Ames *Salmonella* Mutation Assay
Test System	Bacterial
GLP	No
Year	1980
Species/Strain	*Salmonella typhimurium* / TA98; TA100
Metab. Activation	Polychlorinated biphenyl induced rat liver S9 mixture
Concentrations	0, 10, 100, 200, 1000, and 2000 μg/plate.
Statist. Methods	Not specified but positive controls were run concurrently with test substance.
Test Conditions/ Remarks	Concurrent positive control materials were benzo(a)pyrene, N-methyl-N'-nitro-N-nitrosoguanidine (MNG), N-acetylaminofluorene.
Results	The test substance was negative in the TA100 and TA98 tester strains. No mutagenic activity was observed at a range of five concentrations, from 100 to 2000 μg/plate, with or without metabolic activation.
Conclusions	The test substance was not mutagenic, with or without metabolic activation.
Data Quality	Reliable with restrictions [Klimisch reliability 2]. Not GLP and evaluated with two tester strains TA98 and TA100.
References	Inoue K, Sunakawa T, Takayama S (1980). Studies of *in vitro* cell transformation and mutagenicity by surfactants and other compounds. Food Cosmet. Toxicol. **18:** 289-296.
Other	Date last updated November 6, 2003.

Genetic Toxicity In Vitro (CAS No. 1338-41-6)

Test Substance	Sorbitan, monostearate
CAS Number	1338-41-6
Remarks	Purity was not indicated
Method/guideline	Not indicated
Type of Study	In vitro cell transformation assay
Test System	Cryopreserved primary cells from hamster embryo
GLP	No
Year	1980
Species/Strain	Syrian golden hamster embryo cells
Concentrations	0, 1, 10, 50, 100 and 300 μg/ml (in dimethyl sulfoxide, DMSO, the vehicle control)
Statist. Methods	Not specified. Criteria for morphological transformation were randomly oriented 3-dimensional growth with extensive crossing-over of the cells at the periphery of the colony. The centers of the transformed cells usually exhibited dense piling up of cells.
Test Conditions	Positive control was 3-methylcholanthrene (graded doses at 0.1, 0.5 and 1 μg/ml in DMSO). DMSO was solvent control.

Results/Remarks	Sorbitan monostearate at concentrations of 1, 10, 50, 100 and 300 μg/ml did not induce in vitro transformation of hamster ovary cells. 3-Methylcholanthrene, the positive control, induced morphological transformation of cryopreserved hamster embryo cells as expected. DMSO vehicle control did not induce embryo cell transformation.
Conclusions	The test substance did not induce in vitro transformation of hamster embryo cells at concentrations ranging from 1 to 300 μg/ml.
Data Quality	Reliable with restrictions [Klimisch reliability 2]. Not GLP.
References	Inoue K, Sunakawa T, Takayama S (1980). Studies of *in vitro* cell transformation and mutagenicity by surfactants and other compounds. Food Cosmet. Toxicol. **18:** 289-296.
Other	Date last updated November 6, 2003.

Acute Oral Toxicity (CAS No. 8007-43-0)

Test Substance	Sorbitan, sesquioleate
CAS Number	8007-43-0
Remarks	Purity was not indicated
Method/guideline	Not indicated
Test type	Acute oral toxicity
GLP	No
Year	1966
Statistical Method	Not indicated
Test system	Species (Strain) Rats (Wistar) Sex: Male and female, Weight: 133-148 g (male), 145-162 g (female) No. of animals: 10/sex/treatment Vehicle: Corn oil Route: Oral gavage Dosage: 39.8 g/kg bw (test material at conc. 90% v:v in corn oil)
Test conditions	Remarks: Single oral administration (gavage) of 39.8 g/kg bw; no controls; feeding *ad libitum* but food was withheld 16 hrs prior to dosing. Mortality observed several times on day 1 and daily thereafter until day 14. Clinical signs several times on day 1 and daily thereafter until day 14. Necropsy on day 14.
Results/Remarks	No deaths were observed in the dosed male or female rats during the 14-day observation period. Clinical observations included depression, decreased respiration, messy fur and diarrhea during the first 5 days. Necropsy findings consisted of edema of the lungs, congestion of the adrenals, pelvic dilation, bladder filled with fluid and slight congestion of the stomach mucosa, consolidation of lungs, congestion of the lungs and medullary congestion in the kidneys. No measurement of body weight was performed or reported .
Conclusions	The acute oral LD_{50} for the test substance was reported to be > 39.8 g/kg.
Data Quality	Reliable with restrictions [Klimisch reliability 2]. Limited report and not GLP.

References	1) Confidential business information. 2) Findings have been cited by Elder RL (1985). Final report on the safety assessment of sorbitan stearate, sorbitan laurate, sorbitan sesquioleate, sorbitan oleate, sorbitan tristearate, sorbitan palmitate, and sorbitan trioleate, J. Amer. Coll. Toxicol. 4 (3): 65-121.
Other	Date last updated November 5, 2003.

Acute Oral Toxicity (CAS No. 26266-58-0)

Test Substance	Sorbitan, trioleate
CAS Number	26266-58-0,
Remarks	Purity was not indicated
Method/guideline	Not indicated
Test type	Acute oral toxicity
GLP	No
Year	1966
Statistical Method	Not indicated
Test system	Species (Strain) Rats (Wistar) Sex: Male and female, weight: 140-164 g No. of animals: 10/sex/treatment Vehicle: Corn oil (test material at conc. 90% v:v in corn oil) Dosage: Single oral (gavage) administration (in two equal portions) of 39.8 g/kg (dosing volume 50 ml/kg); no controls; feeding *ad libitum* but food was withheld 16 hrs prior to dosing Route: Oral gavage
Test conditions/ Remarks	Single oral (gavage) administration (in two equal portions) of 39.8 g/kg (dosing volume 50 ml/kg). Mortality was observed on day 1, 2 and 14. Clinical signs were observed several times on day 1 and daily until day 14. Body weights on day 1. Necropsy on day 14.
Results/Remarks	No deaths were observed in the dosed male or female rats during the 14-day observation period. Clinical observations included mucoid diarrhea and wet perineal area. Necropsy findings reported were soft heart, hydronephrosis, congested medulla of the kidneys, mucosa of the stomach reddened, slight congested lungs, bladder distended with urine, slightly granular spleen, and areas of dark discoloration in the pancreas. Report was limited; no measurement of body weight was performed on days 7 and 14.
Conclusions	The acute oral LD_{50} for the test substance was reported to be > 39.8 g/kg.
Data Quality	Reliable with restrictions [Klimisch reliability 2]. Limited report and not GLP.
References	1) Confidential business information. 2) Findings have been cited by Elder RL (1985). Final report on the safety assessment of sorbitan stearate, sorbitan laurate, sorbitan sesquioleate, sorbitan oleate, sorbitan tristearate, sorbitan palmitate, and sorbitan trioleate, J. Amer. Coll. Toxicol. 4 (3): 65-121.
Other	Date last updated November 5, 2003.

Part II. Surrogate Sorbitan Ester

Melting Point, Boiling Point, Vapor Pressure, Partition Coefficient, Water Solubililty (CAS No. 228573-47-5) Sorbitan, Fatty Acids C6-10 Tetraester - Surrogate Sorbitan Ester

Test Substance **CAS Number** **Remarks**	Sorbitan, fatty acids C6-10 tetraester 228573-47-5 Purity was >98%		
	GLP (Yes/No)	**METHOD/ GUIDELINE**	**RESULTS / CONCLUSIONS**
Physicochemical Properties			
Melting Point	Yes	OECD 102	< -25 °C
Boiling Point	Yes	OECD 103	> 295 °C
Vapor Pressure	Yes	OECD 104	1.7×10^{-7} Pa at 25 °C
Partition Coeffic.	Yes	OECD 107/117	log P > 7.7 (HPLC method)
Water Solubility	Yes	OECD 105	< 0.02 mg/L
Year	1999		
Remarks	Determination of a complete battery of physicochemical properties for the test substance CAS No. 228573-47-5, including those designated above has been carried out under GLP and by methods which are in compliance with the OECD and EEC Commission Directive 92/69/EEC guidelines. These physicochemical properties determination studies were performed at Huntingdon Life Sciences Ltd., Suffolk, United Kingdom.		
Data Quality	Reliable without restrictions [Klimisch reliability 1].		
References	Unpublished confidential business information.		
Other	Date: November 6, 2003		

Acute Oral Toxicity (CAS No. 228573-47-5)

Test Substance	Sorbitan, fatty acids C6-10 tetraester
CAS Number	228573-47-5
Remarks	Purity was >98%
Method/guideline	OECD 420 (1992)
Test type	Acute oral toxicity test – fixed dose method
GLP	Yes
Year	1999
Test system/ Test Conditions	Species/Strain: Rats / Sprague Dawley [Crl:CD (SD) IGS BR] Sex: Male and Female, weight: 281-307 g (males), 205-225 g (female) No. of animals: 5/sex Vehicle: None, administered undiluted Route: Oral gavage
Test Conditions	Group of five female and five male rats (fasted) were dosed by oral gavage at dose of 2.0 g/kg of body weight. The animals were observed daily for a period of 14 days for mortality and signs of systemic toxicity. The animals were necropsied at the end of the observation period.
Results	Oral LD_{50} > 2.0 g/kg
Remarks	No mortality was observed. All animals were free of clinical signs of toxicity throughout the study. No significant macroscopic postmortem abnormalities were noted at necropsy.
Conclusions	The acute oral LD_{50} of the test substance was > 2.0 g/kg.
Data Quality	Reliable without restrictions [Klimisch reliability 1].
References	Unpublished confidential business information.
Other	Date: November 6, 2003

Repeated-Dose Toxicity/Reproductive Toxicity (CAS No. 228573-47-5)

Test Substance	Sorbitan, fatty acids C6-10 tetraester
CAS Number	228573-47-5
Remarks	Purity was >98%
Method/guideline	OECD 407 Repeated 28 day Oral Toxicity
Test type	28-Day Oral Toxicity Study
GLP	Yes
Year	1999
Species/strain	Rats / Sprague Dawley [Crl:CD (SD) IGS BR]
Route of Administ.	Oral gavage
Duration of test	28 days
No. of animals	30 /sex (see below for sex/dose group)
Dose/Conc. Levels	0, 15, 150, and 1000 mg/kg/day
Sex	Male and female

Frequency of treatment **Control Group**	Daily oral gavage for 28 days 10/sex
Post-exposure observat.	Set of five female and five male rats from control group (Group 1) and from high dose group (Group 4)(1000 mg/kg) will be retained after the 28 day treatment phase for 14 day recovery period (see below).
Statist. Methods	ANOVA, Blom's normalized rank test, Shapiro-Wilk analysis test, Bartlett's test for variance.
Remarks on Test Conditions	The test substance was administered orally (gavage) to CD Sprague-Dawley rats for 28 consecutive days at dose levels of 0, 15, 150 and 1000 mg/kg body weight. Group 1 consisted of 10 males and 10 females that were treated with vehicle alone (corn oil) and served as the control group. A set of 5 males and 5 females from Group 1 were retained after the 28-day treatment phase for a 14-day recovery period (i.e., treatment-free period). Group 2 (low dose) and Group 3 (intermediate dose) consisted of 5 males and 5 females per group, were administered 15 and 150 mg/kg/dose, respectively. Group 4 (high dose) consisted of 10 males and 10 females that were administered 1000 mg/kg/dose of the test substance. A set of 5 males and 5 females from Group 4 were also retained after the 28-day treatment phase for a 14-day recovery period. After 28-days of treatment, 40 animals (5/sex/group) were euthanized and subjected to necropsy. After a recovery period of 2 weeks, the remaining 20 animals (5/sex from the control and high-dose groups) were euthanized. Histopathological evaluation of selected tissues from the 4-week high dose (1000 mg/kg/day) and control animals was performed.
Remarks/Test Conditions	Mortality, clinical observations, physical/neurobehavioral examination, body weights and food consumption were carried out. Hematology, coagulation, clinical chemistry, and urinalysis were performed. A complete necropsy was performed after 28 days for designated groups or at the end of the 14 day recovery period as described above. Organ weights were recorded and tissues collected, fixed, prepared, sectioned and stained for histopathology examination. Tissues examined microscopically also included testes, epididymides, and ovaries.
Results/Remarks	Oral (gavage) administration of the test substance to Sprague-Dawley rats at dose levels up to 1000 mg/kg/day for 28 days, produced no systemic toxicity. No treatment-related effects on mortality, clincal observations, body weights or feed consumption were observed. In addition, no treatment-related effects on neurobehavioral evaluations, hematology, coagulation, clinical chemistry, urinalysis, or macroscopic and microscopic postmortem findings were observed.
Conclusions	1) The no-observed effect level (NOEL) was 1000 mg/kg/day. 2) Oral gavage of the test substance to Sprague-Dawley rats at dose levels up to 1000 mg/kg/day over 28 days resulted in no systemic toxicity. 3) After 28 days of repeated dose exposure to up to 1000 mg/kg/day, there was no indication that the test material adversely affected the reproductive organs in male and female rats. No histopathological or gross abnormalities were reported for testes, epididymides and ovaries.
Data Quality	Reliable without restrictions [Klimisch reliability 1]
References	Unpublished confidential business information. Sudy was GLP and met requirements of the OECD 407 guidelines.
Other	Date: November 6, 2003.

Genetic Toxicity In Vitro (CAS No. 228573-47-5)

Test Substance	Sorbitan, fatty acids C6-10 tetraester
CAS Number	228573-47-5
Remarks	Purity was >98%
Method/guideline	OECD 471 (1997 Guideline)
Type of Study	Bacterial Reverse Mutation Assay
Test System	Bacterial (*Salmonella - Escherichia coli*)
GLP	Yes
Year	1999
Species/Strain	*Salmonella typhimurium* / TA1535, TA1537, TA98; TA100 and *Escherichia coli* / WP2uvrA/pKM101 (CM891)
Metab. Activation	Aroclor 1254-induced rat liver preparations (S9 mixture)
Concentrations	5, 15, 50, 150, 500, 1500, 5000 µg/plate of the test material (solubilized in DMSO)
Statist. Methods	Not specified but positive controls were run concurrently with test substance.
Test Conditions/ Remarks	Concurrent positive control materials were benzo(a)pyrene, 2-nitrofluorene, sodium azide, 2-aminoanthracene, 9-aminoacridine for the *Salmonella* tester strains; and 2-aminonthracene and AF-2 (2-furyl)-3-(5-nitro-furyl)acrylamide for the *E. coli* strain. DMSO was used a vehicle (negative) control.
Results	The test substance was negative for mutagenic activity in the four *Salmonella* tester strains and in the *E. coli* strain, with or without metabolic activation. No mutagenic activity was observed at concentrations ranging from 5 µg/plate to the highest concentration of 5000 µg/plate. The bacterial strains tested included *Salmonella typhimurium* strains TA1535, TA1537, TA98; TA100 and *Escherichia coli* strain WP2uvrA/pKM101 (CM891). The positive controls gave the appropriate responses as expected.
Conclusions	The test substance was not mutagenic, with or without metabolic activation in the *Salmonella-Escherichia coli* / Mammalian Microsome Reverse Mutation assay.
Data Quality	Reliable without restrictions [Klimisch reliability 1].
References	Unpublished confidential business information.
Other	Date November 6, 2003.

Genetic Toxicity In Vitro (CAS No. 228573-47-5)

Test Substance	Sorbitan, fatty acids C6-10 tetraester
CAS Number	228573-47-5
Remarks	Purity was > 98%
Method/guideline	OECD 473
Type of Study	In Vitro Mammalian Chromosomal Aberration Test
Test System	In vitro human lymphocyte cytogenetic system
GLP	Yes
Year	1999
Species/ cell type	Human lymphocyte in whole blood culture
Metab. activation	Arochlor 1254-induced rat liver S9 mixture (Sprague Dawley rat)
Concentrations	500, 1000 and 2000 µg/ml (vehicle used was DMSO)

Statist. Methods	Fisher's test.
Test Conditions /Remarks	Study was carried out to assess the ability of test substance to induce chromosomal aberrations in human lymphocytes cultured in vitro. Human lymphocytes in whole blood culture were stimulated to divide by addition of phytohemagglutinin and exposed to the test substance both in the presence and absence of S-9 mix derived from rat liver (Aroclor 1254-induced S-D rat). Solvent (DMSO) and positive control cultures were also prepared. Two hours before the end of the incubation period, cell division was arrested with Colemid, the cells harvested and slides prepared so that the metaphase cells could be examined for chromosomal damage. The mitotic index was calculated for all cultures treated with the test substance and the solvent control. On the basis of these data, the following concentrations were selected for metaphase analysis: • 500, 1000 and 2000 μg/ml - with and without S9 - 3 hr treatment, 18 hr recovery • 500, 1000 and 2000 μg/ml - without S9 - 21 hr continuous treatment • 500, 1000 and 2000 μg/ml - with S9 - 3 hr treatment, 18 hr recovery Positive controls used were mitomycin C (in absence of S9 mix) and cyclophosphamide (in presence of S9 mix).
Results/Remarks	1) In both the absence and presence of S9 mix, the test substance caused no statistically significant increase in the proportion of metaphase figures containing chromosomal aberrations, at any dose level, when compared with the solvent control, in either test. 2) A quantitative analysis for polypoidy was made in all cultures used in the analysis of chromosomal aberrations. No statistically significant increases in the proportion of polyploid cells were seen. 3) All positive control compounds caused large, statistically significant increases in the proportion of aberrant cells, demonstrating the sensitivity of the test system and the efficacy of the S9 mix.
Conclusions	The test material is <u>not</u> clastogenic in the *in vitro* human lymphocyte cytogenetics test system, with or without metabolic activation. Regardless of dose level (as high as 2000 μg/ml) and dosing regimen, the test substance did not cause a reduction in mitotic index, did not induce any increase in polyploid metaphase cells and did not cause increases in the proportion of cells with chromosome aberrations compared with solvent controls.
Data Quality	Reliable without restrictions [Klimisch reliability 1].
References	Unpublished confidential business information.
Other	Date: November 6, 2003.

Acute fish toxicity (CAS No. 228573-47-5)

Test Substance	Sorbitan, fatty acids C6-10 tetraester
CAS Number	228573-47-5
Remarks	Purity was 100%
Method/guideline	OECD 203
Type (test type)	96-hr Acute Fish Toxicity, Semi-static (renewal)
Test System	Fish, freshwater
GLP	Yes
Year	1999

Species/Strain	Fish: Rainbow trout *(Oncorhynchus mykiss)*, length 48 mm (mean)
Analyt. Monitoring	GC-FID analysis of test substance in control and WAF solutions, new or old at 0, 24, 72 or 96 hr samples.
Exposure period	96 hours
Statist. Methods	Not specified
Remarks on Test Conditions	96-hr semi-static test (renewals at 24, 48 and 72 hr) at limited concentration. Test performed in 15L glass aquarium vessels, dechlorinated tap water (hardness 172-180 mg/L $CaCO_3$); 14.3-15.7°C; aerated; loading 0.8 g/L. No. of fish: 10/treatment, 10 for control Concentrations: Water accommodated fraction (WAF) generated at 1000 mg/L nominal loading rate and control (0 mg/L , untreated). Observations for mortality, abnormal behavior, and treatment-related effects were performed at 0.25, 2, 4, 24, 48, 72 and 96 hrs. Daily physical measurement of pH, dissolved oxygen and temperature was carried out. The pH was 7.2 to 8.2, dissolved oxygen was 81-98% of saturation, temperature was 14.3 - 15.7 °C, total hardness was 172-180 mg/L as $CaCO_3$. Light 16 hr/dark 8 hr cycle was maintained.
Results/Remarks	Nominal test conc. (mg/L) — Mortality (96h) 0 (Untreated controls) — 0 % 1000 mg/L WAF — 0 %
Remarks	WAF was prepared by 20 hr stirring test material (1000 mg/L loading rate) and allowing for settling and equilibration for 3 hr before the WAF solution was taken for study. The analytical results (GC-FID) indicated that concentrations of the test substance in the test WAF solutions ranged from 0.160 mg/L to 0.274 mg/L.
Conclusions	96-hr LC_{50} >1000 mg/L (WAF, nominal loading rate). Analytical data indicated very low measured concentrations of test material (<0.3 mg/L) in the WAF solutions sampled. No mortality was observed with the 1000 mg/L nominal WAF solutions tested. Hence, the test substance did not cause mortality at or above its water solubility or water saturated level (reported to be 0.02 mg/L).
Data Quality	Reliable without restrictions [Klimisch reliability 1].
References	Unpublished confidential business information.
Other	Date: November 6, 2003.

Acute toxicity to aquatic invertebrate (CAS No. 228573-47-5)

Test Substance	Sorbitan, fatty acids C6-10 tetraester
CAS Number	228573-47-5
Remarks	Purity was 100%
Method/guideline	OECD 202
Type (test type)	Daphnia sp. Acute immobilization test .
Test System	Freshwater invertebrate
GLP	Yes
Year	1999
Species/Strain	Freshwater invertebrate, *Daphnia magna*, <24 hr-old
Analyt. Monitoring	GC-FID analysis of test substance in control and WAF solutions (generated from 1000 mg/L loading rate).
Exposure period	48 hours

Statist. Methods	Not specified
Remarks on Test Conditions	48-hr static test at limited concentration. Test performed in containers with 100 ml of the WAF solutions or Elendt M4 medium solution (controls) at 19.6- 20.4 °C. No. of daphnids: 20/treatment, 20/control. Concentrations: Water accommodated fraction (WAF) generated at 1000 mg/L (nominal loading rate) and control (0 mg/L, untreated). Observations for immobilized daphnids were performed after 24 and 48 hr. Daily physical measurements of pH, dissolved oxygen and temperature were carried out. The pH was 7.6 to 7.8, dissolved oxygen was 90-95% of saturation and temperature was 19.6- 20.4 °C during the study. A light 16 hr/dark 8 hr cycle was maintained.
Results	Nominal test conc. (mg/L) — % Immobilized (48h) 0 (Untreated controls) — 0 % 1000 mg/L WAF — 0 %
Remarks	No immobilization and no adverse effects on the daphnids were observed during this study. WAF solution was prepared by stirring test material (1000 mg/L loading rate) overnight in Elendt M4 medium and allowing for settling and equilibration for 3 hr before WAF solution was taken for study. The analytical results (GC-FID) indicated that concentrations of the test substance in the test WAF solutions ranged between 5 and 7 µg/L. The measured concentrations were close to limit of GC-FID detection and suggested that levels of test substance in the water samples were detectable but there was uncertainty in the measurements at the limit of detection.
Conclusions	48-hr EC_{50} >1000 mg/L (WAF, nominal loading rate). Analytical data (GC-FID) indicated presence of test material in WAF solutions, albeit detection limitations and may be at its water solubility limit (WSL) or water saturated levels. Data would suggest that test substance did not cause immobilization at or close to its water saturation limit.
Data Quality	Reliable without restrictions [Klimisch reliability 1]. No immobilization of daphnids was observed at the tested 1000 mg/L WAF solution (nominal loading rate). GC FID analysis indicated the presence of the test material in the WAF and may be close to its water saturated level of < 20 µg/L.
References	Unpublished confidential business information.
Other	Date: November 6, 2003.

Acute toxicity to aquatic plants (e.g., algae) (CAS No. 228573-47-5)

Test Substance	Sorbitan, fatty acids C6-10 tetraester
CAS Number	228573-47-5
Remarks	Purity was 100%
Method/guideline	OECD 201
Type (test type)	Algae, growth inhibition test
Test System	Aquatic plant (e.g., algae)
GLP	Yes
Year	1999
Species/Strain	Green algae (*Selenastrum capricornutum*).
Analyt. Monitoring	GC-FID analysis of test substance in test medium and in algal culture samples.
Exposure period	72 hours
Statist. Methods	Dunnett's test

Remarks on Test Conditions	72-hr static limited concentration test in 250 mL loosely cotton-plugged flasks with 100 mL of algal medium (pH 7.4-7.7); temperature: 21.5-22.6°C; continuous illumination (~8863 lux); continuously shaken at 150 rpm. Initial cell conc: 1 x 10^4 cells/ml in controls No. of replicates: 6 replicates/treatment, 6 replicates/control. 3 replicate flask for water quality measurements Concentrations: Water accommodated fraction (WAF) generated at 1000 mg/L nominal loading rate and untreated controls (0 mg/L). Observations: Cell density determined at 0, 24, 48 and 72 hr by counting with a haemacytometer. Measurement of pH: pH 7.4 at 0 hr and pH 7.4-7.7 at 72 hr
Results	In this study, an initial WAF solution with a loading rate of 1800 mg/L was prepared in sterile culture medium (1.8 L) and stirred for 23 hrs overnight and allow to standing for 3 hr. Then 1200 ml of the WAF was taken and sufficient algal inoculum are added to give an initial cell density of 1 x 10^4 cells/ml. GC-FID analysis of samples indicated that mean measured conc of test material in samples of the test algal culture decreased from 0.835 mg/L at the start of the test to 0.0716 mg/L after 72 hrs. In test culture medium with no algal cells, the measured level found at the start was maintained (0.925 mg/L at 72 hr). These results indicated that the test substance was stable in the dilution medium under conditions of the test but in the presence of algal cells, loss from solution probably occurred as a result of adsorption to the algae.
Conclusions	72-hr EC_{50} >1000 mg/L (WAF, nominal loading rate). Compared to controlled cultures, neither the area under the growth curve nor the growth rate were significantly reduced by the test substance. The 72-hr median effect loading rates were not identified. If a median effect exist for the test substance, it would be >1000 mg/L. The NOEL (no-observed effect loading rate) was >1000 mg/L. No inhibition of algae growth was observed with WAF solution of the test material. Data suggest that test substance would not be expected to inhibit algal growth at its water saturation limit.
Data Quality	Reliable without restrictions [Klimisch reliability 1].
References	Unpublished confidential business information.
Other	Date: November 6, 2003.

Biodegradation (CAS No. 228573-47-5)

Test Substance	Sorbitan, fatty acids C6-10 tetraester
CAS Number	228573-47-5
Remarks	Purity was >98%
Method/guideline	OECD 301B Modified Sturm
Test type	Aerobic Ready Biodegradability test (CO_2 evolution method)
GLP	Yes
Year	1997
Test system	Exposure Period: 28 Days Inoculum: Activated sludge from municipal sewage treatment plant Kinetics: Not Reported
Test Conditions	Inoculum: activated sludge from domestic wastewater treatment plant. Sufficient inoculum (29 ml) to provide final 30 mg suspended solids/L medium.

	Blank control [medium + inoculum] (n=2) Positive control [medium + inoculum + sodium benzoate (19.98 mg C/L)] (n=2 Treated [medium + inoculum + test material (19.82 mg C/L)]. (n=2) Medium was buffered mineral medium solution (initial pH 7.4) as outline in OECD 301B guidelines. Biodegradation experiments were performed in the dark under continuous stirring in 4 L glass vessels. The inoculum and medium (3 L) were pre-acclimated during 24 hours, and subsequently treated and aerated for 28 days at 23-24°C with CO_2-free air. The outcoming air was passed through 3 consecutive CO_2-traps containing 100 ml 0.0125 M $Ba(OH)_2$. The amount of CO_2 was determined in the traps by back-titrating with standardized 0.05M HCl at various time intervals (duplicate determinations). The pH was measured on day 28 in the individual vessels and were found to be 7.4-7.5. Concentrations for Test Substance was 19.82 mg C /L for test substance. Concentration for sodium benzoate (positive control) was 19.98 mg C/L.
Results	Biodegradation occurred to the extent of 83.1% in 28 days for the test substance. The test substance met the "10-day window" criterion for "readily biodegradable". Positive controls (sodium benzoate) achieved 86.3% biodegradation in 28 days and met the readily biodegradable classification. Biodegradation values were corrected for background CO_2 with blank controls. **Biodegradation Results:** % Biodegradation [% of ThCO2] **Day 1 3 6 10 14 21 28** Test Substance 0.6 15.0 34.0 53.4 67.7 76.8 83.1 Positive Control (sodium benzoate) 8.0 28.2 48.2 67.8 78.4 82.8 86.3 From the biodegradation time plot, the test material was estimated to reach the 10% biodegradation mark on Day 2.3 and 10 days later, on Day 12.3, the biodegradation was 61.7% ,
Conclusions	The test substance was readily biodegradable (i.e., met the 10-day window criterion) and was biodegraded to the extent of 83.1% in 28 days. The test substance met the "10-day window" criterion for ready biodegradability. Sodium benzoate (positive control) also was readily biodegradable.
Data Quality	Reliable without restrictions [Klimisch reliability 1].
References	Unpublished confidential business information
Other	Date: November 6, 2003