AR201-13206B

Substance Group: **Group 3: Alkaryl Sulfonates**

Summary prepared by: **Petroleum Additives Panel**
Health & Environmental Research Task Group
October 9, 2001

2.0 Melting and Boiling Points

CAS #	Parameter	Value*
61789-86-4	^{o}C melting point	349.84
	^{o}C boiling point	935.88
61790-48-5	^{o}C melting point	349.84
	^{o}C boiling point	935.88
68608-26-4	^{o}C melting point	309.31
	^{o}C boiling point	707.03
68783-96-0	^{o}C melting point	349.84
	^{o}C boiling point	935.88
Analog of 70024-69-0	^{o}C melting point	349.84
	^{o}C boiling point	935.88
71549-79-6	^{o}C melting point	208.45
	^{o}C boiling point	506.34
71786-47-5	^{o}C melting point	349.84
	^{o}C boiling point	935.88
78330-12-8	^{o}C melting point	347.25
	^{o}C boiling point	788.26
115733-09-0	^{o}C melting point	349.84
	^{o}C boiling point	935.88
115829-36-2	^{o}C melting point	208.45
	^{o}C boiling point	506.34

*These data were modeled by an HERTG member company representative. The reliability code that should accompany the robust summaries prepared using these data is: (2) Valid with restrictions. The selection of this code is based on the data being modeled rather than measured. The use of these data should always be accompanied by the caveat that they were modeled using a structure based modeling program (see reference) and that the values selected are based on a structure that is representative of the CAS#.

The reference for the model is:
MPBPWIN (v1.31) In: Meylan W. and P. Howard. 1999. EPIWIN Modeling Program, Syracuse Research Corporation. Environmental Science Center, 6225 Running Ridge Road, North Syracuse, NY, 13212-2510, USA.

3.0 Biodegradation

Robust Summary 3-Biodeg-1

Test Substance	
CAS #	61789-86-4
Chemical Name	Sulfonic acids, petroleum, calcium salts
Remarks	This substance is referred to as petroleum derived calc ium salt in the HERTG's Test Plan for Alkaryl Sulfonates Category. For more information on the chemical, see Section 2.0 "Chemical Description of Alkaryl Sulfonates Category" in HERTG's Test Plan for Alkaryl Sulfonates Category.
Method	
Method/Guideline followed	OECD 301F
Test Type (aerobic/anaerobic)	Aerobic
GLP (Y/N)	Y
Year (Study Performed)	1996
Contact time (units)	28 days.
Inoculum	Activated sludge from domestic wastewater treatment plant.
Remarks for test conditions	Inoculum: The supernatant from the homogenized activated sludge was used as inoculum. The inoculum was pre-adapted to the test material for 14 days during which the test substance was added incrementally at concentrations equivalent to 4, 8, and 8 mg carbon / L on days 0, 7, and 12, respectively. The targeted microbial level in the test mixture was 10,000 to 1,000,000 cells / mL. Concentration of test chemical: Test substance concentration was approximately 100 mg / L, giving at least 50 to 100 mg ThOD per L test medium. No organic solvents were used to facilitate the dispersion of the test material. The test substance was weighed onto a teflon coupon and introduced into the medium. Temp of incubation: 23 ± 1ºC Dosing procedure: A measured volume of the inoculated mineral medium containing approximately 100 mg / L test substance was continuously stirred in a closed system for 28 days. Sampling frequency: The oxygen uptake was monitored continuously and recorded every 4 hours throughout the test.

	Controls: Yes (blank and positive controls per guideline); abiotic and toxicity checks were not included. Sodium benzoate was used as the positive control. Analytical method: Oxygen uptake was measured using a BI-1000 electrolytic respirometer system. Method of calculating measured concentrations: N / A Other: The inoculum was pre-adapted to the test substance for 14 days.
Results	
Degradation % after time	8.6% after 28 days
Kinetic (for sample, positive and negative controls)	Reference (sodium benzoate) – >60% (3d) Test substance – 9.0% (28d)
Breakdown Products (Y/N) If yes describe breakdown products	N
Remarks	
Conclusions	8.6% in 28 days. The reference substance, sodium benzoate, reached a level of 88.8% in the same test period.
Data Quality	(1) Reliable without restriction
References	This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information).
Other	Updated: 9-6-00

Test Substance	
CAS #	71486-79-8
Chemical Name	Benzensulfonic acid, mono-C15-30-branched alkl and di-C11-13-branched and linear alkylderivs., calcium salts, overbased
Method	
Method/Guideline followed	OECD 301F
Test Type (aerobic/anaerobic)	Aerobic
GLP (Y/N)	Y
Year (Study Performed)	1997
Contact time (units)	28 days
Inoculum	Activated sludge from domestic wastewater treatment plant.
Remarks for test conditions	Inoculum: The supernatant from the homogenized activated sludge was used as inoculum. The inoculum was pre-adapted to the test material for 14 days during which the test substance was added incrementally at concentrations equivalent to 4, 4, and 8 mg carbon / L on days 0, 7, and 12, respectively. The targeted

	microbial level in the test mixture was 10,000 to 1,000,000 cells / mL. Concentration of test chemical: Test substance concentration was approximately 100 mg / L, giving at least 50 to 100 mg ThOD per L test medium. No organic solvents were used to facilitate the dispersion of the test material. The test substance was weighed onto a teflon coupon and introduced into the medium. Temp of incubation: 23 + 1ºC Dosing procedure: A measured volume of the inoculated mineral medium containing approximately 100 mg / L test substance was continuously stirred in a closed system for 28 days. Sampling frequency: The oxygen uptake was monitored continuously and recorded every 4 hours throughout the test. Controls: Yes (blank and positive controls per guideline); abiotic and toxicity checks were not included. Sodium benzoate was used as the positive control. Analytical method: Oxygen uptake was measured using a BI-1000 electrolytic respirometer system. Method of calculating measured concentrations: N / A Other: The inoculum was pre-adapted to the test substance for 14 days.
Results	
Degradation % after time	8.6% after 28 days.
Kinetics (for sample, positive and negative controls)	Positive control substance (sodium benzoate): >60% (3d) Test substance: 8.6%% (28d)
Breakdown Products (Y/N) If yes describe breakdown products	N
Remarks	
Conclusions	8.6% in 28 days.
Data Quality	(1) Reliable without restriction
References	This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information).
Other	Updated: 9-6-00

Robust Summary 3- Biodeg-2

Test Substance	
CAS #	Analog of 71786-47-5
Chemical Name	Magnesium long chain alkaryl sulfonate
Remarks	This substance is an analog for the group of substances referred to as alkaryl magnesium salt derivative, in HERTG's Test Plan for the Alkaryl Sulfonates Category. It is chemically similar to members of this CAS number, but does not belong to this CAS number. For more information on these substances, see Section 2.0 "Chemical Description of Alkaryl Sulfonate Category" in HERTG's Test Plan for the Alkaryl Sulfonate Category.
Method	
Method/Guideline Followed	OECD 301B, Ready Biodegradability, Modified Sturm Test
Test Type (aerobic/anaerobic)	Aerobic
GLP (Y/N)	Y
Year (study performed)	1995
Contact time (units)	28 days
Inoculum (source)	Domestic activated sewage sludge
Remarks For Test Conditions	Inoculum: Activated sewage sludge from domestic WWTP prepared per test guideline. Inoculum was not acclimated. Replicates: Triplicates for test substance, positive control material, and control blank. Temperature of incubation: 20 – 23 °C Dosing procedure: Neat test chemical was gravametrically determined on glass cover slips, which were then added to culture medium in test vessels. Sampling: Days 2, 4, 7, 10, 14, 17, 21, 24, 29 (after acidification on day 28) Concentration of test substance: Loading into each of 3 test vessels were 19.9, 20.1, and 20.0 mg C/L. Controls: Blank and positive controls used per guideline; toxicity control not used. Positive control was benzoic acid (Na salt) added to each control vessel at a loading of 20.2 mg C/L. Analytical method: Titration of residual Ba(OH)2 (0.05 N initially) in trapping solution, using 0.1N HCl. Method of calculating biodegradation values: Percent biodegradation calculated as percent ratio of cumulative net carbon dioxide to theoretical carbon dioxide as determined from elemental analysis of test material.

Results	
Degradation % After Time	Test substance: 1.5% in 28 days Positive control substance: 89.2% in 28 days
Kinetics (for sample, positive and negative controls)	Positive control $t_{1/2}$: <10 days
Breakdown Products (Y/N) (if yes describe breakdown products)	N
Conclusions	1.5% in 28 days
Data Quality	(1) Reliable without restriction
References	This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information).
Other	Updated: 9-6-00

Robust Summary 3- Biodeg-3

Test Substance	
CAS #	Analog of 70024-69-0
Chemical Name	Benzenesulfonic acid, mono-C16-C24-alkyl derivatives, calcium salts
Remarks	This substance is referred to as C16-C24 alkaryl calcium salt derivative in the HERTG's Test Plan for Alkaryl Sulfonates Category. For more information on the chemical, see Section 2.0 "Chemical Description of Alkaryl Sulfonates Category" in HERTG's Test Plan for Alkaryl Sulfonates Category.
Method	
Method/Guideline followed	Closed bottle test according to OECD Guideline No. 301D, EEC Directive 79/831 and EEC Directive 67/548 Annex V C.6 as published in 84/499/EEC.
Test Type (aerobic/anaerobic)	Aerobic
GLP (Y/N)	Y
Year (Study Performed)	1989
Contact time (units)	28 days
Inoculum	Domestic activated sewage sludge
Remarks for test conditions	Inoculum: Activated sludge bacteria from domestic sewage treatment plant used at about 1 drop sludge filtrate inoculum/L basal medium. Concentration of test chemical: 2 mg/L. Inoculum was not pre-acclimated to test substance. Two replicates run per treatment. Temperature of incubation: 20±1 °C Dosing procedure: Test chemical was added onto Whatman GFA filter paper that were then placed inside test vessels immediately before culture medium was added to the vessels. Sampling: Days 0, 5, 15, and 28 after inoculation. Controls: Yes (blank and positive controls used per guideline); toxicity control not used. Standard Control Substances: Sodium benzoate and Aniline tested at 2 mg/L. Analytical method: Chemical oxygen demand (COD) of the test substance and standard control substances determined using the Hach semi-micro sample digestion methods followed by direct reading of the CODs using a Hach DR/2 Spectrophotometer. During the biodegradability test dissolved oxygen concentrations for each test medium were determined in duplicate using a Yellow Springs BOD Probe.

Remarks for test conditions, cont'd	Inoculum: Sludge from domestic WWTP used at 10 mL/L basal medium Conc of test chemical: Test chemical added directly to test vessels at 13.3 mg C/L (28.6 mg/L CAS# 68511-50-2). No preacclimation was used. Temp of incubation: 23 – 24 °C Dosing procedure: Neat test chemical added by micropipettor to culture medium in vessels immediately prior to addition of sewage and soil inocula Sampling: Days 1, 3, 6, 10, 14, 21, 29 (after acidification on d 28) Controls: Yes (blank and positive controls used per guideline); toxicity control not used. Positive Control was Benzoic acid (Na salt) at 20 mg C/L Analytical method: Titration of residual Ba(OH)2 in trapping solution, using HCl Method of calculating measured concentrations: The oxygen depletion values for the test substance and standard substances at each sampling time are corrected by means of the blank values and expressed as a percentage of the theoretical oxygen demand or chemical oxygen demand determined by the Hach semi-micro sample digestion method. Other: • Biodegradation of the Standard Control Substances: Sodium benzoate and Aniline attained 97% and 61% degradation within 28 days. Because both standard substances achieved greater or equal to 60% degradation the test was deemed valid. • Two replicates were run per treatment; values are average of replicates. The % biodegradation value reported is slightly inflated by the use of zero titration volume rather than negative volume when corrected for blanks; however, comparison of titration volumes for the test chemical and blank show them to be very similar, so inhibition of inoculum is not suspected.
Results	
Degradation % after time	Test Substance degraded 8.0% by day 28.
Kinetic (for sample, positive and negative controls)	None given
Breakdown Products (Y/N) If yes describe breakdown products	NA

Conclusions	8.0% Not Readily biodegradable; biodegradation was essentially zero
Data Quality	(1) Reliable without restriction
References	Douglas, M.T. (1989) The Ready Biodegradability of Analog of CAS# 70024-69-0 in a Closed bottle Test System, Huntington Research Centre, Ltd., Study #30/891706.
Other	Updated: 9-6-00

Robust Summary 3- Biodeg 4

Test Substance	
CAS #	Analog for 68783-96-0
Chemical Name	Calcium alkaryl sulfonate
Remarks	This substance is an analog for the group of substances referred to as petroleum derived calcium salt, overbased, in HERTG's Test Plan for the Alkaryl Sulfonates Category. It is chemically similar to members of this CAS number, but does not belong to this CAS number. For more information on these substances, see Section 2.0 "Chemical Description of Alkaryl Sulfonate Category" in HERTG's Test Plan for the Alkaryl Sulfonate Category.
Method	
Method/Guideline Followed	OECD 301B, Ready Biodegradability, Modified Sturm Test
Test Type (aerobic/anaerobic)	Aerobic
GLP (Y/N)	Y
Year (study performed)	1995
Contact time (units)	28 days
Inoculum (source)	Domestic activated sewage sludge
Remarks For Test Conditions	Inoculum: Activated sewage sludge from domestic WWTP prepared per test guideline. Inoculum was not acclimated. Replicates: Triplicates for test substance, positive control material, and control blank. Temperature of incubation: 20 – 23 °C Dosing procedure: Neat test chemical was gravametrically determined on glass cover slips, which were then added to culture medium in test vessels. Sampling: Days 1, 3, 5, 7, 10, 13, 17, 20, 24, 29 (after acidification on day 28) Concentration of test substance: Loadings into 3 test vessels were 19.8, 20.1, and 19.4 mg C/L. Controls: Blank and positive controls used per guideline; toxicity control not used. Positive control was benzoic acid (Na salt) added to each control vessel at a loading of 21.2 mg C/L. Analytical method: Titration of residual Ba(OH)2 (0.05 N initially) in trapping solution, using 0.1N HCl. Method of calculating biodegradation values: Percent biodegradation calculated as percent ratio of cumulative net carbon dioxide to theoretical carbon dioxide as determined from elemental analysis of test material.

Results	
Degradation % After Time	Test substance: 9.1% in 28 days Positive control substance: 86.1% in 28 days
Kinetics (for sample, positive and negative controls)	Positive control $t_{1/2}$: <10 days
Breakdown Products (Y/N) (if yes describe breakdown products)	N
Conclusions	9.1% in 28 days
Data Quality	(1) Reliable without restriction
References	This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information).
Other	Updated: 9-6-00

AQUATIC ORGANISMS

4.1 Acute and Prolonged Toxicity to Fish

Robust Summary 3-Fish Tox -1

Test Substance	
CAS #	61789-86-4
Chemical Name	Sulfonic acids, petroleum, Calcium salts
Remarks	This substance is referred to as petroleum derived calcium salt in the HERTG's Test Plan for Alkaryl Sulfonates Category. For more information on the chemical, see Section 2.0 "Chemical Description of Alkaryl Sulfonates Category" in HERTG's Test Plan for Alkaryl Sulfonates Category.
Method	
Method/Guideline followed	Test protocol followed OECD Guideline for Testing of Chemicals #203, Fish Acute Toxicity Test (1984).
Test Type	Static renewal test; a one level screening test
GLP (Y/N)	Y
Year (Study Performed)	1986
Species/Strain	Sheepshead minnow (*Cyprinodon variegatus*)
Analytical Monitoring	Total organic carbon (TOC) measurements of each freshly prepared test solution and control and after 24-h on test just before daily renewal with fresh test solution.
Exposure Period (unit)	96 hours
Statistical methods	Statistical analysis of survival data not warranted because there was no mortality in the study.
Remarks field for test conditions (fill as applicable)	Test Organisms: source – a commercial supplier in New Hampshire, age – 14 days old, total length – 12 mm average (range 10 to 15 mm; n =30), wet weight – 0.040 g average (range 0.004 to 0.11 g; n = 30). Loading – 0.080 g biomass/L, Pretreatment – none, fish held for a minimum of 7 days before testing. No feeding during the test. Test System: Individual WAFs (individual water accommodated fractions) were prepared for each daily renewal of the 10,000 mg/L test level. A measured weight of test material was added to a measured volume of dilution water (15-L) in a glass vessel and stirred for 16 to 24 hours. Stirring accomplished using a Telfon coated magnetic stir bar. Mixing speed adjusted such that a vortex formed between 30 to 50% of the distance to the bottom. Following the

	mixing period, the test solution was allowed to stand for 2 hours before the water phase was removed. To avoid removing test material from the surface or bottom, a siphon was placed in the mixing vessel prior to addition of water and test substance with the lower end approximately midway between bottom and surface. The siphoned water phase, designated WAF, was used for the aquatic toxicity test. About 90% of the test solution in each test vessel was renewed daily after 24, 48, and 72 hours. Two 5-L replicates per treatment, 10 fish per replicate (20 per treatment). Test vessels were loosely covered to reduce entry of dust. Dilution Water: Natural seawater collected from Cape Cod Canal, Bourne, Massachusetts. The water was filtered through 0.5-micron polypropylene core filter and activated carbon, then stored for 1 to 4 days prior to use while being constantly aerated. During storage the water had a salinity of 32 to 34 ppt and pH of 7.7 to 7.8. During the test: dissolved oxygen – 6.3 mg/L to above 100% saturation (7.2 mg/L), pH – 7.8 to 8.1, salinity – 32 to 34, temperature – 22 to 23 C. Mean measured TOC levels in the control and 10,000 mg/L WAF test level were 4.1 mg/L (range 2.3 to 7.9) and 10.2 mg/L (range 6.4 to 15.0), respectively Test Levels: Control & 10,000 mg/L WAF loading rate. Test Findings: No mortality or signs of toxicity were noted in the 10,000 WAF test level and the control throughout the entire test. Calculation of LL_{50}s: Statistical analysis of survival data not warranted. Test Substance: No undissolved test material was report on the surface of the test vessels during the entire aquatic toxicity test. Reference Substance: Sodium lauryl sulfate (SLS). The 96-h LC_{50} was 1.2 mg/L. No information provided on method of calculation.
Results	Nominal concentrations: 96-h LL_{50} >10,000 mg/L. This is equivalent to 96-h LL_0 = 10,000 mg/L (no mortality or toxic signs noted).
Remarks	Measured concentration: n/a Unit: mg/L Statistical results: Statistical analysis of survival data not warranted. Other:

	• Test results reported in original study as "lethal concentrations" are reported in this summary as "lethal loading", because test results are based on WAF loading rates. • Control response was satisfactory.
Conclusions	No mortality or signs of toxicity were noted in the 10,000 WAF test level and the control throughout the entire test.
Data Quality	(1) Reliable without restriction
References	Nicholson, R.B. (1986) Acute toxicity of CMA Test Material Code 504 to Sheepshead Minnow, *Cyprinodon variegatus*. Springborn Bionomics Study #10823-0186-6100-500-504, Report #BW-86-04-1983.
Other	Updated: 9-6-00

Robust Summary 3-Fish Tox:2

Test Substance	
CAS #	analog for Analog of CAS # 70024-69-0 (material tested = CAS #70024-71-4)
Chemical Name	analog to benzenesulfonic acid, mono-C16–C24 alkyl derivatives, calcium salts, overbased
Remarks	The tested substance is an analog for the group of substances referred to as C16-C24 alkyl calcium salt overbased derivative in the HERTG's Test Plan for Alkaryl Sulfonates Category. It is chemically similar to members of this CAS number, but does not belong to this CAS number. For more information on the chemical, see Section 2.0 "Chemical Description of Alkaryl Sulfonates Category" in HERTG's Test Plan for Alkaryl Sulfonates Category.
Method	
Method/Guideline followed	Test protocol followed OECD Guideline for Testing of Chemicals #203, Fish Acute Toxicity Test (1984).
Test Type	Static renewal test; a one level screening test
GLP (Y/N)	Y
Year (Study Performed)	1986
Species/Strain	Sheepshead minnow (*Cyprinodon variegatus*)
Analytical Monitoring	Total organic carbon (TOC) measurements of each freshly prepared test solution and control and after 24-h on test just before daily renewal with fresh test solution.
Exposure Period (unit)	96 hours
Statistical methods	Statistical analysis of survival data not warranted because there was no mortality in the study.
Remarks field for test conditions (fill as applicable)	Test Organisms: source – a commercial supplier in New Hampshire, age – 10 to 15 days old, total length – 11 mm average (range 10 to 13 mm; n =30), wet weight – 0.028 g average (range 0.02 to 0.05 g; n = 30). Loading – 0.056 g biomass/L, Pretreatment – none, fish held for a minimum of 6 days before testing. No feeding during the test. Test System: Individual water accommodated fractions (WAFs) were prepared for each daily renewal of the 10,000 mg/L test level. A measured weight of test material was added to a measured volume of dilution water (15-L) in a glass vessel and stirred for 16 to 24 hours. Stirring accomplished using a Telfon coated magnetic stir bar. Mixing speed adjusted such that a vortex formed between 30 to 50% of the distance to the bottom. Following the mixing period, the test solution were allowed to stand for 2 hours before the water phase was removed. To avoid removing test material from the surface or bottom, a siphon was placed in the mixing vessel prior to addition of water and test substance with the lower end approximately midway between bottom

	and surface. The siphoned water phase, designated water accomodated fraction (WAF), was used for the aquatic toxicity test. About 90% of the test solution in each test vessel was renewed daily after 24, 48, and 72 hours. Two 5-L replicates per treatment, 10 fish per replicate (20 per treatment). Test vessels were loosely covered to reduce entry of dust. Dilution Water: Natural seawater collected from Cape Cod Canal, Bourne, Massachusetts. The water was filtered through 0.5-micron polypropylene core filter and activated carbon, then stored for 1 to 4 days prior to use while being constantly aerated. During storage the water had a salinity of 32 to 34 ppt and pH of 7.7 to 7.8. During the test: dissolved oxygen – 5.5 mg/L to above 100% saturation (7.2 mg/L), pH – 7.9 to 8.1, salinity – 32 to 34, temperature – 22 to 23 C. Mean measured TOC levels in the control and 10,000 mg/L WAF test level were 3.0 mg/L (range 1.2 to 4.4) and 6.4 mg/L (range 5.0 to 7.9), respectively. Test Levels: Control & 10,000 mg/L WAF loading rate. Test Findings: No mortality or signs of toxicity were noted in the 10,000 WAF test level and the control throughout the entire test. Calculation of LL_{50}s: Statistical analysis of survival data not warranted because there was no mortality in the study. Test Substance: No undissolved test material was report on the surface of the test vessels during the entire aquatic toxicity test. Reference Substance: Sodium lauryl sulfate (SLS). The 96-h LC_{50} was 1.2 mg/L. No information provided on method of calculation.
Results	Nominal concentrations: 96-h LL_{50} >10,000 mg/L. This is equivalent to 96-h LL_0 = 10,000 mg/L (no mortality or toxic signs noted).
Remarks	Measured concentration: n/a Unit: mg/L Statistical results: Statistical analysis of survival data not warranted. Other: • Test results reported in original study as “lethal concentrations” are reported in this summary as “lethal loading”, because test results are based on WAF loading rates. • Control response was satisfactory.

Conclusions	No mortality or signs of toxicity were noted in the 10,000 WAF test level and the control throughout the entire test.
Data Quality	(1) Reliable without restriction
References	Nicholson, R.B. (1986) Acute toxicity of CMA Test Material Code 514 to Sheepshead Minnow, *Cyprinodon variegatus*. Springborn Bionomics Study #10823-0186-6100-500-514, Report #BW-86-04-1993.
Other	Updated: 9-6-00

Robust Summary 3-Fish Tox:3

Test Substance	
CAS #	71486-79-8
Chemical Name	Benzenesulfonic acid, mono-C15-30-branched alkyl and di-C11-13-branched and linear alkyl derivatives, calcium salts, overbased
Remarks	This substance is referred to as mixed C15-C30 and C11-13 alkaryl calcium salt, overbased derivative in the HERTG's Test Plan for Alkaryl Sulfonates Category. For more information on the chemical, see Section 2.0 "Chemical Description of Alkaryl Sulfonates Category" in HERTG's Test Plan for Alkaryl Sulfonates Category.
Method	
Method/Guideline followed	Test protocol followed US EPA Toxic Substances Control Act Test Guideline #797.1400 (1985), OECD Guideline for Testing of Chemicals #203, Fish Acute Toxicity Test (1984).
Test Type	Static renewal test
GLP (Y/N)	Y
Year (Study Performed)	1983
Species/Strain	Fathead minnow (*Pimephales promelas*)
Analytical Monitoring	Total organic carbon (TOC) measurements of initial (0-h) test solutions and after one day on test (24-h) before renewal of fresh test solutions. Water samples were passed through 0.45-micron filter prior to TOC analysis using EPA Method 415.1
Exposure Period (unit)	96 hours
Statistical methods	Statistical analysis of survival data not warranted because there was no mortality in the study.
Remarks field for test conditions (fill as applicable)	Test Organisms: Acquired from Aquatic Research Organisms, Hampton, New Hampshire, age: juvenile, total length: 29 mm average (longest fish not more than twice the shortest fish), wet weight: 0.2 g average (no range reported). Loading: <0.5 g biomass/L, Pretreatment: none, fish held for a minimum of 14 days before testing. No feeding during the test. Test System: Individual water accommodated fractions (WAFs) were prepared for each test level and renewed daily. A measured weight of test material was added to a measured volume of dilution water (30-L) in a glass vessel and stirred for 24 hours. Stirring accomplished using a Telfon coated magnetic stir bar. Mixing speed adjusted such that a vortex formed between 30 to 50% of the distance to the bottom. Following the mixing period, the test solutions were allowed to stand for 1 hour before the water phase was removed. To avoid removing test material from the surface or bottom, a siphon was placed in the mixing vessel prior to addition of water and test substance with the lower end 1-2 inches off the bottom. The siphoned water phase (i.e., WAF) was used in the aquatic toxicity test. About 80% of the solution

	in each test level was renewed daily after 24, 48, and 72 hours. Two 15-L replicates per treatment, 10 fish per replicate (20 per treatment). Test vessels loosely covered to reduce entry of dust. Dilution Water: Filtered well water collected at Hampton, New Hampshire and adjusted to the appropriate hardness of 176 mg/L as $CaCO_3$. The water was passed through activated carbon, a particle filter, and then an ultraviolet sterilizer, and then it was stored in a polyethylene tank where it was aerated. The water was characterized as moderately hard water. Light: 16-h light per day using cool-white fluorescent lights with an intensity of 20 uEin/m^2. Test Temperature: 21.4 to 22.8 C. Water Chemistry: Dissolved oxygen: 7.3 – 8.6 mg/L, pH: 7.4 - 8.1, conductivity: 860 – 910 umhos/cm. Alkalinity not reported. Element: Mortality Test Levels: Control, 100, 300, & 1,000 mg/L WAF loading rates. No undissolved test material was seen on the surface of the test vessels during the entire aquatic toxicity test. Test Findings: No mortality or signs of toxicity was observed in all treatments and the control throughout the entire test. No undissolved test material was seen on the surface of the test vessels during the entire aquatic toxicity test. Analytical Monitoring: TOC levels were between 2.3 - 3.0 mg/L in the control, 2.8 - 3.2 mg/L at 100 mg/L loading, between 2.6 - 3.2 mg/L at 300 mg/L loading and 2.6 - 3.3 mg/L at the 1,000 mg/L loading. TOC levels were not considered to be indicative of actual test material concentrations and results are therefore based on nominal loading rates. Reference Substance: No
Results	Nominal concentrations: 96-h LL_{50} >1,000 mg/L. This is equivalent to 96-h LL_0 = 1,000 mg/L (no mortality or toxic signs noted).
Remarks	Measured concentration: n/a Unit: mg/L

	LC50, LC0, LL50 or LL0 at 48, 72, 96-hours: LL_{50} and LL_0 reported as LC_{50} and NOEC, respectively, although test results were based on WAF loading rate. Statistical results: Statistical analysis of survival data not warranted. Other: • Test results reported in original study as "lethal concentrations" are reported in this summary as "lethal loading", because test results are based on WAF loading rates. • Control response was satisfactory.
Conclusions	No mortality or signs of toxicity were observed in any of the treatments (100, 300, and 1,000 mg/L WAF loading rates) or in the control throughout the entire test.
Data Quality	(1) Reliable without restriction
References	Ward, T.J. (1993) Acute Toxicity of The Water Accommodated Fractions (WAFs) of CMA 605 to The Fathead Minnow, *Pimephales promelas.* T.R. Wilbury Study #9176-CMA/ESI-605.
Other	Updated: 9-6-00

Robust Summary 3-Fish Tox:4

Test Substance	
CAS #	71786-47-5
Chemical Name	Benzenesulfonic acid, mono and dialkyl derivatives, magnesium salts
Remarks	This substance is referred to as alkaryl magnesium salt derivative in the HERTG's Test Plan for Alkaryl Sulfonates Category. For more information on the chemical, see Section 2.0 "Chemical Description of Alkaryl Sulfonates Category" in HERTG's Test Plan for Alkaryl Sulfonates Category.
Method	
Method/Guideline followed	Test protocol followed US EPA Toxic Substances Control Act Test Guideline #797.1400 (1985), OECD Guideline for Testing of Chemicals #203, Fish Acute Toxicity Test (1984).
Test Type	Static renewal test
GLP (Y/N)	Y
Year (Study Performed)	1983
Species/Strain	Fathead minnow (*Pimephales promelas*)
Analytical Monitoring	Total organic carbon (TOC) measurements of initial (0-h) test solutions and after one day on test (24-h) before renewal of fresh test solutions. Water samples were passed through 0.45-micron filter prior to TOC analysis using EPA Method 415.1
Exposure Period (unit)	96 hours
Statistical methods	Statistical analysis of survival data not warranted because there was no mortality in this study.
Remarks field for test conditions (fill as applicable)	Test Organisms: Acquired from Aquatic Research Organisms, Hampton, New Hampshire, age: juvenile, total length: 38.4 mm average (longest fish not more than twice the shortest fish), wet weight: 0.5 g average (no range reported). Loading: <0.5 g biomass/L, Pretreatment: none, fish held for a minimum of 14 days before testing. No feeding during the test. Test System: Individual water accommodated fractions (WAFs) were prepared for each test level and renewed daily. A measured weight of test material was added to a measured volume of dilution water (30-L) in a glass vessel and stirred for 24 hours. Stirring accomplished using a Telfon coated magnetic stir bar. Mixing speed adjusted such that a vortex formed between 30 to 50% of the distance to the bottom. Following the mixing period, the test solutions were allowed to stand for 1 hour before the water phase was removed. To avoid removing test material from the surface or bottom, a siphon was placed in the mixing vessel prior to addition of water and test substance with the lower end 1-2 inches off the bottom. The siphoned water phase (i.e., WAF) was used in the aquatic toxicity test. About 80% of the solution in each test level was renewed daily after 24, 48, and 72 hours. Two

	15-L replicates per treatment, 10 fish per replicate (20 per treatment). Test vessels loosely covered to reduce entry of dust. Dilution Water: Filtered well water collected at Hampton, New Hampshire and adjusted to the appropriate hardness of 176 mg/L as $CaCO_3$. The water was passed through activated carbon, a particle filter, and then an ultraviolet sterilizer, and then it was stored in a polyethylene tank where it was aerated. The water was characterized as moderately hard water. Light: 16-h light per day using cool-white fluorescent lights with an intensity of 20 uEin/m^2. Test Temperature: 21.6 to 22.8 C. Water Chemistry: Dissolved oxygen: 6.9 – 8.3 mg/L, pH: 7.0 - 7.9, conductivity: 870 – 890 umhos/cm. Alkalinity not reported. Element: Mortality Test Levels: Control, 100, 300, & 1,000 mg/L WAF loading rates. No undissolved test material was seen on the surface of the test vessels during the entire aquatic toxicity test. Test Findings: No mortality or signs of toxicity was observed in all treatments and the control throughout the entire test. Calculation of LL_{50} s: Statistical analysis of survival data not warranted. Analytical Monitoring: TOC levels were between 2.8 - 3.2 mg/L in the control, 3.3 - 3.8 mg/L at 100 mg/L loading, between 3.1 - 4.0 mg/L at 300 mg/L loading and 3.2 - 4.4 mg/L at the 1,000 mg/L loading. TOC levels were not considered to be indicative of actual test material concentrations and results are therefore based on nominal loading rates. Reference Substance: No
Results	Nominal concentrations: 96-h LL_{50} >1,000 mg/L. This is equivalent to 96-h LL_0 = 1,000 mg/L (no mortality or toxic signs noted).
Remarks	Measured concentration: n/a Unit: mg/L

	Statistical results: Statistical analysis of survival data not warranted. Other: • Test results reported in original study as "lethal concentrations" are reported in this summary as "lethal loading", because test results are based on WAF loading rates. • Control response was satisfactory.
Conclusions	No mortality or signs of toxicity were observed in any of the treatments (100, 300, and 1,000 mg/L WAF loading rates) or in the control throughout the entire test.
Data Quality	(1) Reliable without restriction
References	Ward, T.J. (1993) Acute Toxicity of The Water Accommodated Fractions (WAFs) of CMA 609 to The Fathead Minnow, *Pimephales promelas.* T.R. Wilbury Study #9176-CMA/ESI-609.
Other	Updated: 9-6-00

Robust Summary 3-Fish Tox: 5

Test Substance	
CAS #	71786-47-5
Chemical Name	Benzenesulfonic acid, mono and dialkyl derivatives, Magnesium salts
Remarks	This substance is referred to as alkaryl magnesium salt derivative in the HERTG's Test Plan for Alkaryl Sulfonates Category. For more information on the chemical, see Section 2.0 "Chemical Description of Alkaryl Sulfonates Category" in HERTG's Test Plan for Alkaryl Sulfonates Category.
Method	
Method/Guideline followed	Test protocol followed OECD Guideline for Testing of Chemicals #203, Fish Acute Toxicity Test (1984).
Test Type	static renewal test; a one level screening test
GLP (Y/N)	Y
Year (Study Performed)	1986
Species/Strain	Sheepshead minnow (*Cyprinodon variegatus*)
Analytical Monitoring	Total organic carbon (TOC) measurements of each freshly prepared test solution and control and after 24-h on test just before daily renewal with fresh test solution.
Exposure Period (unit)	96 hours
Statistical methods	Statistical analysis of survival data not warranted because there was no mortality in this study.
Remarks field for test conditions (fill as applicable)	Test Organisms: source – a commercial supplier in New Hampshire, age – 17 to 22 days old, total length – 11 mm average (range 10 to 13 mm; n =30), wet weight – 0.028 g average (range 0.02 to 0.05 g; n = 30). Loading - 0.056 g biomass/L, Pretreatment – none, fish held for a minimum of 20 days before testing. No feeding during the test. Test System: Individual water accomodated fractions (WAFs) were prepared for each daily renewal of the 10,000 mg/L test level. A measured weight of test material was added to a measured volume of dilution water (15-L) in a glass vessel and stirred for 16 to 24 hours. Stirring accomplished using a Telfon coated magnetic stir bar. Mixing speed adjusted such that a vortex formed between 30 to 50% of the distance to the bottom. Following the mixing period, the test solution were allowed to stand for 2 hours before the water phase was removed. To avoid removing test material from the surface or bottom, a siphon was placed in the mixing vessel prior to addition of water and test substance with the lower end approximately midway between bottom and surface. The siphoned water phase, designated WAF was used for the aquatic toxicity test. About 90% of the test solution in each test vessel was renewed daily after 24, 48, and 72 hours. Two 5-L replicates per treatment, 10 fish per replicate (20 per treatment). Test vessels were loosely covered to reduce entry of dust.

	Dilution Water: Natural seawater collected from Cape Cod Canal, Bourne, Massachusetts. The water was filtered through 0.5-micron polypropylene core filter and activated carbon, then stored for 1 to 4 days prior to use while being constantly aerated. During storage the water had a salinity of 32 to 34 ppt and pH of 7.7 to 7.8. During the test: dissolved oxygen – 4.9 mg/L to above 100% saturation (7.4 mg/L), pH – 7.6 to 8.1, salinity – 32 ppt, temperature – 22 to 23 C. Mean measured TOC levels in the control and 1,000 mg/L WAF test level were 5.0 mg/L (range 2.6 to 7.0) and 3.6 mg/L (range 1.0 to 7.5), respectively. Test Levels: Control & 10,000 mg/L WAF loading rate. Test Findings: No mortality or signs of toxicity were noted in the 10,000 WAF test level and the control throughout the entire test. Analytical Monitoring:). Mean measured TOC in the 10,000 mg/L WAF test level was 5.0 mg/L compared to 3.6 mg/L in the control. Test Substance: No undissolved test material was seen on the surface of the test vessels during the entire aquatic toxicity test. Reference Substance: Sodium lauryl sulfate (SLS). The 96-h LC_{50} was 1.2 mg/L. No information provided on method of calculation.
Results	Nominal concentrations: 96-h LL_{50} >10,000 mg/L. This is equivalent to 96-h LL_0 = 10,000 mg/L (no mortality or toxic signs noted).
Remarks	Measured concentration: n/a Unit: mg/L Statistical results: Statistical analysis of survival data not warranted because there was no mortality in this study. Other: • Test results reported in original study as “lethal concentrations” are reported in this summary as “lethal loading”, because test results are based on WAF loading rates. • Control response was satisfactory.

Conclusions	No mortality or signs of toxicity were noted in the 10,000 WAF test level and the control throughout the entire test.
Data Quality	(1) Reliable without restriction
References	Nicholson, R.B. (1986) Acute toxicity of CMA Test Material Code 523 to Sheepshead Minnow, *Cyprinodon variegatus*. Springborn Bionomics Study #10823-0186-6100-500-523, Report #BW-86-04-1986.
Other	Updated: 9-6-00

4.2 Acute Toxicity to Aquatic Invertebrates (e.g. Daphnia)

Robust Summary 3-Aquatic Invertebrates Tox - 1

Test Substance	
CAS #	115733-09-0
Chemical Name	Benzenesulfonic acid, C14-C24 branched and linear alkyl derivatives, calcium salts
Remarks	This substance is referred to as C14-C24 alkyl calcium salt derivative in the HERTG's Test Plan for Alkaryl Sulfonates Category. For more information on the chemical, see Section 2.0 "Chemical Description of Alkaryl Sulfonates Category" in HERTG's Test Plan for Alkaryl Sulfonates Category.
Method	
Method/Guideline followed	Test protocol followed US EPA Toxic Substances Control Act Test Guideline #797.1300 (1985, 1987), OECD Guideline for Testing of Chemicals #202 *Daphnia* sp. Acute Immobilization Test and Reproduction Test (1984).
Test Type	Static acute toxicity test
GLP (Y/N)	Y
Year (Study Performed)	1993
Species/Strain	Cladoceran, *Daphnia magna*
Analytical Monitoring	Total organic carbon (TOC) measurements of initial (0-h) test solutions and at test termination (48-h). Water samples were passed through 0.45-micron filter prior to TOC analysis using EPA Method 415.1.
Exposure Period (unit)	48 hours
Statistical methods	Statistical analysis of data not warranted.
Remarks field for test conditions (fill as applicable)	Test species: Juvenile daphnids less than 24-hours old were produced from laboratory in-house culture. Test System: Individual WAFs were prepared for each test level and renewed daily. A measured weight of test material was added to a measured volume of dilution water (1-L) in a glass vessel and stirred for 24 hours. Stirring accomplished using a Telfon coated magnetic stir bar. Mixing speed adjusted such that a vortex formed between 30 to 50% of the distance to the bottom. Following the mixing period, the test solutions were allowed to stand for 1 hour before the water phase was removed. To avoid removing test material from the surface or bottom, a siphon was placed in the mixing vessel prior to addition of water and test substance with the lower end 1-2 inches off the bottom. The siphoned water phase (i.e., WAF) was used for the aquatic toxicity test.

	Test conditions: Two 250-mL glass beakers that contained 200 mL of test solution were used per treatment. The 250-mL test vessels were loosely covered to reduce entry of dust. Light: 16-hour light per day using cool-white fluorescent lights with an intensity of 20 uEin/m^2. Test temperature: 20.4 – 20.9 C Dilution water: Filtered well water collected at Hampton, New Hampshire and adjusted to the appropriate hardness 176 mg/L as $CaCO_3$. The water was passed through activated carbon, a particle filter, and then an ultraviolet sterilizer and stored in a polyethylene tank where it was aerated. TOC levels were 2 mg/L at the beginning and end of the test and <10 mg/L at the end of the test. Water chemistry: Dissolved oxygen: 7.9 - 8.7 mg/L; pH: 7.2 - 8.1; conductivity: 860 – 880 umhos/cm. Element: Immobilization/mortality Test Levels: Control, 100, 300, & 1,000 mg/L WAF loading rates: 10 daphnids per replicate (20 per treatment). No undissolved test material was seen on the surface of the test vessels during the entire test. Test Findings: At 24 hours, no immobilized or dead organisms were observed in the control or treatments. At 48-hours 5, 0, 20, and 5% immobilization were reported for control, 100, 300, and 1,000 mg/L, respectively. Calculation of EL_{50}s: Statistical analysis of survival data not warranted. Exposure period: 48 hours Analytical Monitoring: TOC levels were 2.8 – 3.5 mg/L in the control, 2.8 - 3.6 mg/l at 100, 3.0 - 3.7 mg/L at 300 mg/L, and 2.7 - 3.4 mg/L at 1,000 mg/L loading. TOC levels were not considered to be indicative of actual test material concentrations and results are therefore based on nominal loading rates.
Results	Nominal concentrations: 48-h EL_{50} >1,000 mg/L. This is equivalent to 48-h EL_0 = 1000 mg/L based on WAF loading rates.
Remarks	Measured concentration: n/a

	Unit: mg/L Statistical results: Not applicable. Other: • Test results reported in original study as "lethal concentrations" are reported in this summary as "lethal loading", because test results are based on WAF loading rates. • 20% immobilization/mortality seen at the middle test concentration of 300 mg/L (WAF) was not considered to be treatment related. This was based on findings of insignificant effects, or no effects, in both the highest and lowest test levels, respectively. • Control response was satisfactory.
Conclusions	The test material was not toxic to daphnids at loading rates tested. Percent survival/unaffected organisms was 95% in the control, 100% at 100, 80% at 300, and 95% at 1,000 mg/L.
Data Quality	(1) Reliable without restriction
References	Ward, T.J. (1993) Acute Toxicity of the Water Accommodated Fractions (WAFs) of CMA #604 to the Daphnid, *Daphnia magna.* T.R. Wilbury Study #9178-CMA/ESI-604.
Other	Updated: 9-6-00

Robust Summary 3-Aquatic Invertebrate Tox-2

Test Substance	
CAS #	71486-79-8
Chemical Name	Benzenesulfonic acid, mono-C15-30-branched alkyl and di-C11-13-branched and linear alkyl derivatives, calcium salts, overbased
Remarks	This substance is referred to as mixed C15-C30 and C11-13 alkaryl calcium salt, overbased derivative in the HERTG's Test Plan for Alkaryl Sulfonates Category. For more information on the chemical, see Section 2.0 "Chemical Description of Alkaryl Sulfonates Category" in HERTG's Test Plan for Alkaryl Sulfonates Category.
Method	
Method/Guideline followed	Test protocol followed US EPA Toxic Substances Control Act Test Guideline #797.1300 (1985, 1987), OECD Guideline for Testing of Chemicals #202 *Daphnia* sp. Acute Immobilization Test and Reproduction Test (1984).
Test Type	Static acute toxicity test
GLP (Y/N)	Y
Year (Study Performed)	1993
Species/Strain	Cladoceran, *Daphnia magna*
Analytical Monitoring	Total organic carbon (TOC) measurements of initial (0-h) test solutions and at test termination (48-h). Water samples were passed through 0.45-micron filter prior to TOC analysis using EPA Method 415.1.
Exposure Period (unit)	48 hours
Statistical methods	Statistical analysis of data not warranted because immobilization seen at high dose not significant.
Remarks field for test conditions (fill as applicable)	Test species: Juvenile daphnids less than 24-hours old were produced from laboratory in-house culture. Test System: Individual WAFs were prepared for each test level and renewed daily. A measured weight of test material was added to a measured volume of dilution water (1-L) in a glass vessel and stirred for 24 hours. Stirring accomplished using a Telfon coated magnetic stir bar. Mixing speed adjusted such that a vortex formed between 30 to 50% of the distance to the bottom. Following the mixing period, the test solutions were allowed to stand for 1 hour before the water phase was removed. To avoid removing test material from the surface or bottom, a siphon was placed in the mixing vessel prior to addition of water and test substance with the lower end 1-2 inches off the bottom. The siphoned water phase (i.e., WAF) was used for the aquatic toxicity test. Test conditions: Two 250-mL glass beakers that contained 200 mL of test solution were used per treatment. The 250-mL test vessels were

	loosely covered to reduce entry of dust, etc. Light: 16-hour light per day using cool-white fluorescent lights with an intensity of 20 uEin/m. Test temperature: 20.3 – 20.7 C Dilution water: Filtered well water collected at Hampton, New Hampshire and adjusted to the appropriate hardness 176 mg/L as $CaCO_3$. The water was passed through activated carbon, a particle filter, and then an ultraviolet sterilizer and stored in a polyethylene tank where it was aerated. TOC levels were 2 mg/L at the beginning and end of the test and <10 mg/L at the end of the test. Water chemistry: Dissolved oxygen: 7.8 - 8.8 mg/L; pH: 7.4 - 8.5; conductivity: 850 – 900 umhos/cm. Element: Immobilization/mortality Test Levels: Control, 100, 300, & 1,000 mg/L WAF loading rates: 10 daphnids per replicate (20 per treatment). No undissolved test material was seen on the surface of the test vessels during the entire test. Test Findings: At 24 hours, no immobilized or dead organisms were observed in the control or at 300 and 1,000 mg/L, but 5% immobilization /mortality was seen in the 100-mg/L treatment. At 48-hours 0, 10, 0, and 0% immobilization were reported for control, 100, 300, and 1,000 mg/L, respectively. Calculation of EL_{50} s: Statistical analysis of survival data not warranted. Exposure period: 48 hours Analytical Monitoring: TOC levels were 2.3 – 2.8 mg/L in the control, 2.8 - 2.9 mg/l at 100, 2.6 - 2.8 mg/L at 300 mg/L, and 2.6 - 3.0 mg/L at 1,000 mg/L loading. TOC levels were not considered to be indicative of actual test material concentrations and results are therefore based on nominal loading rates.
Results	Nominal concentrations: 48-h EL_{50} >1,000 mg/L. This is equivalent to 48-h EL_0 = 1000 mg/L based on WAF loading rates.
Remarks	Measured concentration: n/a

	Unit: mg/L Statistical results: Not applicable because immobilization seen at high dose was not significant. Other: • Test results reported in original study as "lethal concentrations" are reported in this summary as "lethal loading", because test results are based on WAF loading rates. • Control response was satisfactory.
Conclusions	The test material was not toxic to daphnids at loading rates tested. Percent survival/unaffected test organisms was 100% in the control, 90% at 100, 100% at 300, and 100% at 1,000 mg/L.
Data Quality	(1) Reliable without restriction
References	Ward, T.J. (1993) Acute Toxicity of the Water Accommodated Fractions (WAFs) of CMA #605 to the Daphnid, *Daphnia magna.* T.R. Wilbury Study #9178-CMA/ESI-605.
Other	Updated: 9-6-00

Robust Summary 3-Aquatic Invertebrate Tox: 3

Test Substance	
CAS #	71786-47-5
Chemical Name	Benzenesulfonic acid, mono and dialkyl derivatives, magnesium salts
Remarks	This substance is referred to as alkaryl magnesium salt derivative in the HERTG's Test Plan for Alkaryl Sulfonates Category. For more information on the chemical, see Section 2.0 "Chemical Description of Alkaryl Sulfonates Category" in HERTG's Test Plan for Alkaryl Sulfonates Category.
Method	
Method/Guideline followed	Test protocol followed US EPA Toxic Substances Control Act Test Guideline #797.1300 (1985, 1987), OECD Guideline for Testing of Chemicals #202 *Daphnia* sp. Acute Immobilization Test and Reproduction Test (1984).
Test Type	Static acute toxicity test
GLP (Y/N)	Y
Year (Study Performed)	1993
Species/Strain	Cladoceran, *Daphnia magna*
Analytical Monitoring	Total organic carbon (TOC) measurements of initial (0-h) test solutions and at test termination (48-h). Water samples were passed through 0.45-micron filter prior to TOC analysis using EPA Method 415.1.
Exposure Period (unit)	48 hours
Statistical methods	Statistical analysis of data not warranted because there was no immobilization occurred in this study.
Remarks field for test conditions (fill as applicable)	Test species: Juvenile daphnids less than 24-hours old were produced from laboratory in-house culture. Test System: Individual WAFs were prepared for each test level and renewed daily. A measured weight of test material was added to a measured volume of dilution water (1-L) in a glass vessel and stirred for 24 hours. Stirring accomplished using a Telfon coated magnetic stir bar. Mixing speed adjusted such that a vortex formed between 30 to 50% of the distance to the bottom. Following the mixing period, the test solutions were allowed to stand for 1 hour before the water phase was removed. To avoid removing test material from the surface or bottom, a siphon was placed in the mixing vessel prior to addition of water and test substance with the lower end 1-2 inches off the bottom. The siphoned water phase (i.e., WAF) was used for the aquatic toxicity test. Test conditions: Two 250-mL glass beakers that contained 200 mL of test solution were used per treatment. The 250-mL test vessels were

	loosely covered to reduce entry of dust. Light: 16-hour light per day using cool-white fluorescent lights with an intensity of 20 uEin/m^2. Test temperature: 19.7 – 20.9 C Dilution water: Filtered well water collected at Hampton, New Hampshire and adjusted to the appropriate hardness 176 mg/L as $CaCO_3$. The water was passed through activated carbon, a particle filter, and then an ultraviolet sterilizer and stored in a polyethylene tank where it was aerated. TOC levels were 2 mg/L at the beginning and end of the test and <10 mg/L at the end of the test. Water chemistry: Dissolved oxygen: 7.9 - 9.3 mg/L; pH: 7.0 - 8.4; conductivity: 870 – 910 umhos/cm. Element: Immobilization/mortality Test Levels: Control, 100, 300, and 1,000 mg/L WAF loading rates: 10 daphnids per replicate (20 per treatment). No undissolved test material was seen on the surface of the test vessels during the entire test. Calculation of EL_{50}s: Statistical analysis of survival data not warranted. Exposure period: 48 hours Analytical Monitoring: TOC levels were 1.8 – 2.8 mg/L in the control, 2.2 - 3.3 mg/l at 100, 2.1 - 3.2 mg/L at 300 mg/L, and 1.8 - 3.3 mg/L at 1,000 mg/L loading. TOC levels were not considered to be indicative of actual test material concentrations and results are therefore based on nominal loading rates.
Results	Nominal concentrations: 48-h EL_{50} >1,000 mg/L. This is equivalent to 48-h EL_0 = 1000 mg/L based on WAF loading rates (no immobilization noted).
Remarks	Measured concentration: n/a Unit: mg/L Statistical results: Not applicable because there was no immobilization.

	Other: • Test results reported in original study as "lethal concentrations" are reported in this summary as "lethal loading", because test results are based on WAF loading rates. • Control response was satisfactory.
Conclusions	The test material was not toxic to daphnids at loading rates tested. Percent survival/unaffected test organisms was 100% in the control, 100, 300, and 1,000 mg/L.
Data Quality	(1) Reliable without restriction
References	Ward, T.J. (1993) Acute Toxicity of the Water Accommodated Fractions (WAFs) of CMA #609 to the Daphnid, *Daphnia magna.* T.R. Wilbury Study #9178-CMA/ESI-609.
Other	Updated: 9-6-00

4.3 Toxicity to Aquatic Plants (e.g. Algae)

Robust Summary 3-Aquatic Plant Tox - 1

Test Substance	
CAS #	115733-09-0
Chemical Name	Benzenesulfonic acid, C14-C24 branched and linear alkyl derivatives, calcium salts
Remarks	This substance is referred to as C14-C24 alkaryl calcium salt derivative in the HERTG's Test Plan for Alkaryl Sulfonates Category. For more information on the chemical, see Section 2.0 "Chemical Description of Alkaryl Sulfonates Category" in HERTG's Test Plan for Alkaryl Sulfonates Category.
Method	
Method/Guideline followed	Test protocol followed US EPA Toxic Substances Control Act Test Guideline #797.1050 (1985, 1987), OECD Guideline for Testing of Chemicals #201 Alga, Growth Inhibition Test (1984).
Test Type	Static acute toxicity test
GLP (Y/N)	Y
Year (Study Performed)	1994
Species/Strain	Freshwater algae, *Pseudokirchneriella subcapitata* formerly called *Selenastrum capricornutum*
Element basis (# of cells/mL)	approximately 10,000 cells/mL
Exposure period/duration	96 hours
Analytical monitoring	Total organic carbon (TOC) measurements of initial (0-h) high, low and control test solutions and at test termination (96-h). Water samples were passed through 0.45-micron filter prior to TOC analysis using EPA Method 415.1
Statistical methods	A parametric one-way analysis of variance (ANOVA) and Dunnett's test were used to calculate the no-observed effect level.
Remarks field for test conditions (fill as applicable)	Test Species: Cells taken from a log-growth phase in-house culture of *Pseudokirchneriella subcapitata* that was originally purchased from University of Texas at Austin alga collection. Test System: Individual WAFs were prepared for each test level and renewed daily. A measured weight of test material was added to a measured volume of dilution water (1-L) in a glass vessel and stirred for 24 hours. Stirring accomplished using a Telfon coated magnetic stir bar. Mixing speed adjusted such that a vortex formed between 30 to 50% of the distance to the bottom. Following the mixing period, the test solutions were allowed to stand for 1 hour before the water phase was removed. To avoid removing test material from the surface or bottom, a siphon was placed in the mixing vessel prior to addition of

	water and test substance with the lower end 1-2 inches off the bottom. The siphoned water phase (i.e., WAF) was used for the aquatic toxicity test. Test Conditions: A static test was conducted; i.e., there was no daily renewal of test solution. Three 100-mL replicates per treatment, inoculum ~10,000 cells/mL. The 250-mL Erlenmeyer flasks were stoppered with foam plugs to reduce entry of dust, etc. During the test all treatment and control flasks were randomly placed on an orbital shaker adjusted to approximately 100 cycles per minute under constant light (24 hours/day). Daily cell counts were made visually by means of direct microscopic examination with a hemocytometer. Light: Cool-white fluorescent lights provided a light intensity of 47-50 uEin/m^2sec 24-h per day. Test temperature: 23.3 to 24.0 C. Dilution Water: Sterile enriched alga growth media (US EPA, 1978, T.R. Wilbury SOP #6) adjusted to pH 7.5. Measured TOC and total suspended solids in fresh untreated alga media were <1.0 and <10 mg/L, respectively. Test media pH was 7.5 - 9.8 at 0-hour and 8.3 - 10.1 after 96 hours. Test Levels: Control, 100, 300 & 1000 mg/L WAF loading rates. No undissolved test material was seen on the surface of the test vessels during the entire aquatic toxicity test. Calculation of EL_{50}s and NOELs: Binomial nonlinear interpolation methods (Stephan, 1983) were used to calculate EC_{50}s (i.e., EL_{50}s). A parametric one-way analysis of variance (ANOVA) and Dunnett's test were used to calculate the no-observed effect level. Method of calculating mean measured concentrations: not applicable Exposure period: 96 hours Analytical monitoring: At the beginning and end of the test, TOC measurements were between 2 - 8 mg/L in control, 2 – 4 mg/L at 100 mg/L, 2 – 3 mg/L at 300 mg/L and 1,000 mg/L. TOC levels were not considered to be indicative of actual test material concentrations and results are therefore based on nominal loading rates.

Results	Nominal concentrations: 72- & 96-h EL_{50} >1,000 mg/L and 72- & 96-h NOEL = 1,000 mg/L based on both growth rate and biomass measurements.
Remarks	Measured concentration: n/a Unit: mg/L Test Findings: At 72-hours biomass measurements in the treatments were 80%, 62%, and 70% of the control at 100, 300, and 1,000 mg/L, respectively. At 96-hours biomass measurements were 70, 66, and 88% of the control at 100, 300 and 1,000 mg/L, respectively. Other: • Test results reported in original study as "effect concentrations" and "no observed effect concentrations" are reported in this summary as "effect loading" and "no observed effect levels", respectively, because test results are based on WAF loading rates. • Control response was satisfactory.
Conclusions	The test material was not toxic to freshwater alga at loading rates up to and including 1,000 mg/L.
Data Quality	(1) Reliable without restriction
References	Ward, T.J. (1994) Acute Toxicity of the Water Accommodated Fractions (WAFs) of CMA #604 to the Freshwater Alga, *Selenastrum capricornutum.* T.R. Wilbury Study #73-CM-604. Stephan, C.E. (1983). Computer Program for the Calculation of LC50 Values. U.S. EPA. Duluth, MN. Personal Communication.
Other	Updated: 9-6-00

Robust Summary 3-Aquatic Plant Tox-2

Test Substance	
CAS #	71486-79-8
Chemical Name	Benzenesulfonic acid, mono-C15-30-branched alkyl and di-C11-13-branched and linear alkyl derivatives, calcium salts, overbased
Remarks	This substance is referred to as mixed C15-C30 and C11-13 alkaryl calcium salt, overbased derivative in the HERTG's Test Plan for Alkaryl Sulfonates Category. For more information on the chemical, see Section 2.0 "Chemical Description of Alkaryl Sulfonates Category" in HERTG's Test Plan for Alkaryl Sulfonates Category.
Method	
Method/Guideline followed	Test protocol followed US EPA Toxic Substances Control Act Test Guideline #797.1050 (1985, 1987), OECD Guideline for Testing of Chemicals #201 Alga, Growth Inhibition Test (1984).
Test Type	Static acute toxicity test
GLP (Y/N)	Y
Year (Study Performed)	1994
Species/Strain	Freshwater algae, *Pseudokirchneriella subcapitata* formerly called *Selenastrum capricornutum*
Element basis (# of cells/mL)	approximately 10,000 cells/mL
Exposure period/duration	96 hours
Analytical monitoring	Total organic carbon (TOC) measurements of initial (0-h) high, low and control test solutions and at test termination (96-h). Water samples were passed through 0.45-micron filter prior to TOC analysis using EPA Method 415.1
Statistical methods	The computer program of Stephan (1983) was used to calculate EL_{50}s. A parametric one-way analysis of variance (ANOVA) and Dunnett's test were used to calculate the no-observed effect concentration.
Remarks field for test conditions (fill as applicable)	Test Species: Cells taken from a log-growth phase in-house culture of *Pseudokirchneriella subcapitata* that was originally purchased from University of Texas at Austin alga collection. Test System: Individual WAFs were prepared for each test level and renewed daily. A measured weight of test material was added to a measured volume of dilution water (1-L) in a glass vessel and stirred for 24 hours. Stirring accomplished using a Telfon coated magnetic stir bar. Mixing speed adjusted such that a vortex formed between 30 to 50% of the distance to the bottom. Following the mixing period, the test solutions were allowed to stand for 1 hour before the water phase was removed. To avoid removing test material from the surface or bottom, a siphon was placed in the mixing vessel prior to addition of water and test substance with the lower end 1-2 inches off the bottom. The siphoned water phase (i.e., WAF) was used for the aquatic toxicity test.

	Test Conditions: A static test was conducted; i.e., there was no daily renewal of test solution. Three 100-mL replicates per treatment, inoculum ~10,000 cells/mL. The 250-mL Erlenmeyer flasks were stoppered with foam plugs to reduce entry of dust, etc. During the test all treatment and control flasks were randomly placed on an orbital shaker adjusted to approximately 100 cycles per minute under constant light (24 hours/day). Daily cell counts were made visually by means of direct microscopic examination with a hemocytometer. Light: Cool-white fluorescent lights provided a light intensity of 47-50 uEin/m^2sec 24-h per day. Test temperature: 24.0 to 24.2 C. Dilution Water: Sterile enriched alga growth media (US EPA, 1978, T.R. Wilbury SOP #6) adjusted to pH 7.5. Measured TOC and total suspended solids in fresh untreated alga media were <1.0 and <10 mg/L, respectively. Test media pH was 7.3 - 10.8 at 0-hour and 9.7 - 10.8 after 96 hours. Test Levels: Control, 100, 300, 1000 mg/L WAF loading rates. No undissolved test material was seen on the surface of the test vessels during the entire aquatic toxicity test. Calculation of EL_{50}s and NOELs: Binomial nonlinear interpolation methods (Stephan, 1983) were used to calculate EC_{50}s (i.e., EL_{50}s). A parametric one-way analysis of variance (ANOVA) and Dunnett's test were used to calculate the no-observed effect level. Method of calculating mean measured concentrations: not applicable Exposure period: 96 hours Analytical monitoring: At the beginning and end of the test, TOC measurements were between non-detect (<1) - 3 mg/L in control, 1 – 2 mg/L at 100 mg/L, 3 mg/L at 300 mg/L, and 5 – 6 mg/L at 1,000 mg/L. TOC levels were not considered to be indicative of actual test material concentrations and results are therefore based on nominal loading rates.
Results	Nominal concentrations: 72- & 96-h EL_{50} >1,000 mg/L, based on both growth rate and biomass measurements. 72- & 96-h NOEL = 1000 mg/L.
Remarks	

	Measured concentration: n/a Unit: mg/L Test Findings: At 72-hours biomass measurements in the treatments were 133%, 75%, and 64% of the control at 100, 300, and 1,000 mg/L. At 96-hours biomass measurements were 93, 77, and 52% of the control at 100, 100% at 300, and 100% at 1,000 mg/L. Statistical results: A parametric one-way analysis of variance (ANOVA) and Dunnett's test were used to calculate the no-observed effect level. Other: • Test results reported in original study as "effect concentrations" and "no observed effect concentrations" are reported in this summary as "effect loading" and "no observed effect levels", respectively, because test results are based on WAF loading rates. • Control response was satisfactory.
Conclusions	The test material was not toxic to freshwater alga at loading rates up to and including 1,000 mg/L.
Data Quality	(1) Reliable without restriction
References	Ward, T.J. (1994) Acute Toxicity of the Water Accommodated Fractions (WAFs) of CMA #605 to the Freshwater Alga, *Selenastrum capricornutum.* T.R. Wilbury Study #73-CM-605. Stephan, C.E. (1983). Computer Program for the Calculation of LC50 Values. U.S. EPA. Duluth, MN. Personal Communication.
Other	Updated: 9-6-00

Robust Summary 3-Aquatic Plant Tox-3

Test Substance	
CAS #	71786-47-5
Chemical Name	Benzenesulfonic acid, mono and dialkyl derivatives, magnesium salts
Remarks	This substance is referred to as alkaryl magnesium salt derivative in the HERTG's Test Plan for Alkaryl Sulfonates Category. For more information on the chemical, see Section 2.0 "Chemical Description of Alkaryl Sulfonates Category" in HERTG's Test Plan for Alkaryl Sulfonates Category.
Method	
Method/Guideline followed	Test protocol followed US EPA Toxic Substances Control Act Test Guideline #797.1050 (1985, 1987), OECD Guideline for Testing of Chemicals #201 Alga, Growth Inhibition Test (1984).
Test Type	Static acute toxicity test
GLP (Y/N)	Y
Year (Study Performed)	1994
Species/Strain	Freshwater algae, *Pseudokirchneriella subcapitata* formerly called *Selenastrum capricornutum*
Element basis (# of cells/mL)	approximately 10,000 cells/mL
Exposure period/duration	96 hours
Analytical monitoring	Total organic carbon (TOC) measurements of initial (0-h) high, low and control test solutions and at test termination (96-h). Water samples were passed through 0.45-micron filter prior to TOC analysis using EPA Method 415.1
Statistical methods	The computer program of Stephan (1983) was used to calculate EL_{50}s. A parametric one-way analysis of variance (ANOVA) and Dunnett's test were used to calculate the no-observed effect level.
Remarks field for test conditions (fill as applicable)	Test Species: Cells taken from a log-growth phase in-house culture of *Pseudokirchneriella subcapitata* that was originally purchased from University of Texas at Austin alga collection. Test System: Individual WAFs were prepared for each test level and renewed daily. A measured weight of test material was added to a measured volume of dilution water (1-L) in a glass vessel and stirred for 24 hours. Stirring accomplished using a Telfon coated magnetic stir bar. Mixing speed adjusted such that a vortex formed between 30 to 50% of the distance to the bottom. Following the mixing period, the test solutions were allowed to stand for 1 hour before the water phase was removed. To avoid removing test material from the surface or bottom, a siphon was placed in the mixing vessel prior to addition of water and test substance with the lower end 1-2 inches off the bottom. The siphoned water phase (i.e., WAF) was used for the aquatic toxicity test.

	Test Conditions: A static test was conducted; i.e., there was no daily renewal of test solution. Three 100-mL replicates per treatment, inoculum ~10,000 cells/mL. The 250-mL Erlenmeyer flasks were stoppered with foam plugs to reduce entry of dust, etc. During the test all treatment and control flasks were randomly placed on an orbital shaker adjusted to approximately 100 cycles per minute under constant light (24 hours/day). Daily cell counts were made visually by means of direct microscopic examination with a hemocytometer. Light: Cool-white fluorescent lights provided a light intensity of 47-50 uEin/m^2sec 24-h per day. Test temperature: 24.0 C. Dilution Water: Sterile enriched alga growth media (US EPA, 1978, T.R. Wilbury SOP #6) adjusted to pH 7.5. Measured TOC and total suspended solids in fresh untreated alga media were <1.0 and <10 mg/L, respectively. Test media pH was 7.5 – 9.9 at 0-hour and 8.3 - 10.8 after 96 hours. Test Levels: Control, 125, 250, 500, 1,000 and 1,500 mg/L WAF loading rates. No undissolved test material was seen on the surface of the test vessels during the entire aquatic toxicity test. Calculation of EL_{50}s and NOELs: Binomial nonlinear interpolation methods (Stephan, 1983) were used to calculate EC_{50}s (i.e., EL_{50}s). A parametric one-way analysis of variance (ANOVA) and Dunnett's test were used to calculate the no-observed effect level. Method of calculating mean measured concentrations: not applicable Exposure period: 96 hours Analytical monitoring: At the beginning and end of the test, TOC measurements were non-detect (<1) - 1 mg/L in control, 2 – 3 mg/L at 125 mg/L and between 5 – 6 mg/L at 1,000 mg/L. TOC levels were not considered to be indicative of actual test material concentrations and results are therefore based on nominal loading rates.

Results	Nominal concentrations: 72-h EL_{50}s = 1,400 mg/L and >1,500 mg/L based on biomass and growth rate, respectively. 96-h EL_{50}s = 1,100 mg/L and >1,500 mg/L, based on biomass and growth rate, respectively. The 72-h and 96-hr NOEL = 1,000 mg/L.
Remarks	Measured concentration: n/a Unit: mg/L Test Findings: At 72-hours biomass measurements in the treatments were 97, 100, 101, 95, and 35% of the control at 125, 250, 500, 1,000, and 1,500 mg/L, respectively. At 96-hours biomass measurements were 78, 61, 62, 58, and 31% of the control at 125, 250, 500, 1,000. and 1,500 mg/L, respectively. Logarithmic growth was observed at all treatments up to and including 1,000 mg/L; i.e., average biomass measurements 1,383 – 1,860 cells/mL $x10^3$. Therefore, the 96-h NOELs were determined to be 1,000 mg/L although statistical analysis determined the 96-h NOELs to be 125 mg/L. But, the hypothesis test was biased towards the unusually high control growth (2,383 cells/mL $x10^3$) between 72 – 96 hours upon which test concentration growth was compared. This produced an erroneous measurement of NOEL. Other: • Test results reported in original study as "effect concentrations" and "no observed effect concentrations" are reported in this summary as "effect loading" and "no observed effect levels", respectively, because test results are based on WAF loading rates. • Effects were determined to be algistatic based on the rapid re-growth of an aliquot of cells taken from 1,500 mg/L cultured in fresh control media. • Control response was satisfactory.
Conclusions	The test material was not toxic to freshwater alga at loading rates up to and including 1,000 mg/L.
Data Quality	(1) Reliable without restriction
References	Ward, T.J. (1994) Acute Toxicity of the Water Accommodated Fractions (WAFs) of CMA #609 to the Freshwater Alga, *Selenastrum capricornutum.* T.R. Wilbury Study #73-CM-609. Stephan, C.E. (1983). Computer Program for the Calculation of LC50 Values. U.S. EPA. Duluth, MN. Personal Communication.
Other	Updated: 9-6-00

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Robust Summary 3-Acute Oral -1

Test Substance	
CAS #	CAS# Analog of 70024-69-0
Chemical Name	C20-24 alkaryl calcium salt derivative
Remarks	Test material dosed as received, purity not provided.
Method	
Method/Guideline followed	OECD Guideline 401
Test Type	Acute oral toxicity
GLP (Y/N)	Y
Year (Study Performed)	1989
Species/Strain	Rats/Sprague-Dawley strain
Sex	Male and female
No. of animals/sex/dose	5
Vehicle	None
Route of administration	Oral (intragastric)
Dose level	5 g/kg
Dose volume	Not provided
Control group included	Yes
Remarks field for test conditions	A single dose of 5.0 g/kg of the undiluted test material was administered intragastrically to five fasted (over night) male and female rats. An untreated control group of 5/sex was included. The animals were observed for signs of physiological or behavioral changes frequently on the day of treatment. Thereafter all animals were examined for signs of toxicity once per day. Individual body weights were recorded on the day of dosing and at 2, 7 and 14 days after dosing. The surviving animals were euthanized at the conclusion of the observation period. Gross autopsies were performed on all animals after 14 days.
Results	LD50 > 5.0 g/kg (males and females)
Remarks	No mortality was observed. Diarrhea and reduced food intake were observed in one treated female on Day 1. No other signs of toxicity were observed. Body weights were unremarkable.

Conclusions	The test article, when administered as received to 5 male and 5 female Sprague-Dawley rats, had an acute oral LD50 of greater than 5.0 g/kg.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 2/10/00 (RTA-004)

Robust Summary 3-Acute Oral-2

Test Substance	
CAS #	CAS# 61789-86-4
Chemical Name	Petroleum derived calcium salt
Remarks	100% purity
Method	
Method/Guideline followed	OECD Guideline 401
Test Type	Acute oral toxicity (Limit Test)
GLP (Y/N)	Y
Year (Study Performed)	1985
Species/Strain	Rats/Sprague-Dawley Crl:CD® (SD)BR
Sex	Male and female
No. of animals/sex/dose	5
Vehicle	Peanut oil
Route of administration	Oral (intragastric)
Dose level	5 g/kg
Dose volume	7 ml/kg
Vehicle control group	Yes
Chemical analysis of dosing solution	Yes
Remarks field for test conditions	A single dose of 5.0 g/kg of the test material diluted in peanut oil at a concentration of 714 mg/ml was administered intragastrically to five fasted (over night) male and female rats. The concentration of the test material in the vehicle was analyzed for homogeneity and for stability. The test material was administered at a dose volume of approximately 7 ml/kg body weight. A vehicle control group consisting of 5-fasted animals/sex was dosed with 7 ml/kg of peanut oil. The animals were observed for signs of toxicity or behavioral changes frequently on the day of treatment. Thereafter, all animals were examined for signs of toxicity twice per day (once/day on weekends), for the 13-day observation period and once on day 14 prior to sacrifice. Individual weights were recorded immediately prior to dosing and at 2, 7 and 14 days after dosing. The surviving animals were euthanized at the conclusion of the observation period. Gross autopsies were performed on all animals after 14 days. Histopathological evaluations were performed on grossly abnormal tissues only.
Results	LD50 > 5.0 g/kg (males and females)
Remarks	Analysis confirmed that the dosing solution was homogeneous and stable for the period of use and that it was prepared at the appropriate concentration. No deaths were observed during the 14-day observation period. Diarrhea was observed in one treated male and in one control male 5 hours post dosing, only. Alopecia with or without

	thinned fur was seen in one vehicle control male (Days 12-14) and in one vehicle control female (Days 10-13) Other than the previous observations, all animals appeared normal throughout the 14-day observation period. No body weight effects occurred. No test material related macroscopic or microscopic findings were evident.
Conclusions	The test article, when administered in peanut oil to 5 male and 5 female Sprague-Dawley rats, had an acute oral LD50 of greater than 5.0 g/kg.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 2/9/00 (RTA-001)

Robust Summary 3-Acute Oral-3

Test Substance	
CAS #	CAS# 61790-48-5
Chemical Name	Petroleum derived barium salt
Remarks	Test material dosed as received, purity is 46 % active.
Method	
Method/Guideline followed	FHSA 16CFR1500.3
Test Type	Acute oral toxicity (Limit Test)
GLP (Y/N)	Y
Year (Study Performed)	1982
Species/Strain	Rats/Sprague-Dawley strain
Sex	Male and female
No. of animals/sex/dose	5
Vehicle	None
Route of administration	Oral (intragastric)
Dose level	2 g/kg
Dose volume	2.4 ml/kg
Control group included	No
Remarks field for test conditions	A single dose of 2.0 g/kg of the undiluted test material was administered intragastrically to five fasted (over night) male and female rats. A control group was not included. The animals were observed for signs of toxicity or behavioral changes frequently on the day of treatment. Thereafter, all animals were examined for signs of toxicity twice per day. Individual weights were recorded on the day of dosing and weekly thereafter. The surviving animals were euthanized at the conclusion of the observation period. Gross autopsies were performed on all animals after 14 days.
Results	LD50 > 2.0 g/kg (males and females)
Remarks	One treated female died on Test Day 5 without exhibiting any clinical symptoms. All remaining animals survived to study termination. The animals exhibited ruffled fur 3 hours post dosing. Urine staining was observed within 24-48 hours of dosing. After 72 hours all animals essentially recovered. No body weight effects were observed. Gross necropsy findings were unremarkable for all animals.
Conclusions	The test article, when administered as received to 5 male and 5 female Sprague-Dawley rats, had an acute oral LD50 of greater than 2.0 g/kg.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 2/10/00 (RTA-003)

Robust Summary 3-Acute Oral-4

Test Substance	
CAS #	CAS# 68608-26-4
Chemical Name	Petroleum derived sodium salt
Remarks	Test material dosed as received, purity not provided.
Method	
Method/Guideline followed	FHSA 16CFR1500.3
Test Type	Acute oral toxicity
GLP (Y/N)	Y
Year (Study Performed)	1983
Species/Strain	Rats/Sprague-Dawley strain
Sex	Male and female
No. of animals/sex/dose	5
Vehicle	None
Route of administration	Oral (intragastric)
Dose level	5 g/kg
Dose volume	5 ml/kg
Control group included	No
Remarks field for test conditions	A single dose of 5.0 g/kg of the undiluted test material was administered intragastrically to five fasted (over night) male and female rats. A control group was not included. The animals were observed for signs of toxicity or behavioral changes frequently on the day of treatment. Thereafter, all animals were examined for signs of toxicity twice per day. Body weights were recorded on the day of dosing and weekly thereafter. The surviving animals were euthanized at the conclusion of the observation period. Gross autopsies were performed on all animals after 14 days.
Results	LD50 > 5.0 g/kg (males and females)
Remarks	No deaths or clinical signs of toxicity were observed during the 14-day observation period. The treated males exhibited a slight body weight decrease during the first week post dosing. These body weights recovered during the second week. Body weight gain in the females was normal at week 1 but was less than expected during the second week of the study. Gross necropsy findings were unremarkable.
Conclusions	The test article, when administered as received to 5 male and 5 female Sprague-Dawley rats, had an acute oral LD50 of greater than 5.0 g/kg.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 6/16/00 (RTA-036)

Robust Summary 3-Acute Oral-5

Test Substance	
CAS #	CAS# 68783-96-0
Chemical Name	Petroleum derived calcium salt, overbased
Remarks	Test material dosed as received, purity not provided.
Method	
Method/Guideline followed	OECD Guideline 401
Test Type	Acute oral toxicity (Limit Test)
GLP (Y/N)	Y
Year (Study Performed)	1984
Species/Strain	Rats/Sprague-Dawley
Sex	Male and female
No. of animals/sex/group	5
Vehicle	None
Route of administration	Oral (intragastric)
Dose level	5 g/kg
Dose volume	4.3 ml/kg
Control group included	Yes
Remarks field for test conditions	A single dose of 5.0 g/kg of the undiluted test material was administered intragastrically to five fasted (over night) male and female rats. Five fasted undosed animals of each sex served as the controls. The animals were observed frequently for any physiological or behavioral abnormalities on the day of dosing and at least twice each weekday for 13 days after treatment. On weekends, observations were made once daily. On day 14 the animals were observed once prior to sacrifice. Individual body weights were recorded on the day of dosing and on days 2, 7 and 14 after dosing. The surviving animals were euthanized at the conclusion of the observation period. Gross autopsies were performed on all animals on Day 14.
Results	LD50 > 5.0 g/kg (males and females)
Remarks	No mortality was observed. Body weights were unremarkable. Slightly reduced food consumption was observed in one treated male (Day 2) and female (Day 1). There were no macroscopic findings associated with treatment.
Conclusions	The test article, when administered as received to 5 male and 5 female Sprague-Dawley rats, had an acute oral LD50 of greater than 5.0 g/kg.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 2/17/00 (RTA-018)

Robust Summary 3-Acute Oral-6

Test Substance	
CAS #	CAS# 71549-79-6
Chemical Name	Mixed C15-C30 and C11-13 alkaryl derivative
Remarks	Test material dosed as received, purity not provided.
Method	
Method/Guideline followed	FHSA 16CFR1500.3
Test Type	Acute oral toxicity
GLP (Y/N)	N
Year (Study Performed)	1978
Species/Strain	Rats/Sherman-Wistar strain
Sex	Male
No. of animals/dose	5
Vehicle	None
Route of administration	Oral (intragastric)
Dose level	1, 2, 4, 8, and 16 ml/kg
Dose volume	1, 2, 4, 8, and 16 ml/kg
Control group included	No
Remarks field for test conditions	A single dose of the undiluted test material was administered intragastrically to five fasted (over night) male rats at each treatment level. A control group was not included. The animals were observed for signs of toxicity or behavioral changes daily. Individual weights were recorded on the day of dosing and at termination. All animals were euthanized at the conclusion of the observation period. Gross autopsies were performed on all animals after 14 days.
Results	LD50 14.9 g/kg (males)
Remarks	Three 16.0 g/kg animals died on test day 3. Animals at this dose level were depressed at 1-hour post dosing and remained in poor health for approximately 7 days before recovering. A reduced mean body weight compared to the other treated groups was observed in this group at termination. No clinical findings or body weight effects were evident in the other dose groups. Gross necropsy findings were unremarkable for all animals.
Conclusions	The test article, when administered as received to male Sherman-Wistar rats, had an acute oral LD50 of 14.9 g/kg.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 6/15/00 (RTA-035)

Robust Summary 3-Acute Oral-7

Test Substance	
CAS #	CAS# 71786-47-5
Chemical Name	Alkaryl magnesium salt derivative
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	FHSA 16CFR1500.3
Test Type	Acute oral toxicity
GLP (Y/N)	N
Year (Study Performed)	1980
Species/Strain	Rats/Sherman-Wistar strain
Sex	Male
No. of animals /dose	5
Vehicle	None
Route of administration	Oral (intragastric)
Dose level	1.0, 2.0, 4.0, 8.0 and 16 g/kg
Dose volume	1.0, 2.0, 4.0, 8.0 and 16 ml/kg
Control group included	No
Remarks field for test conditions	A single dose of the undiluted test material was administered intragastrically to five fasted (over night) male rats at each dose level. A control group was not included. All animals were examined for signs of toxicity daily for 14 days. Individual weights were recorded on the day of dosing and at termination. The surviving animals were euthanized at the conclusion of the observation period. Gross autopsies were performed on all animals after 14 days.
Results	LD50 > 16.0 g/kg (males)
Remarks	No deaths were observed during the 14-day observation period. The animals at 8 and 16 g/kg exhibited ruffled fur for 18-24 hours post dosing. Within 48 hours all animals appeared normal. No body weight effects were observed. Gross necropsy findings were unremarkable.
Conclusions	The test article, when administered as received to male Sherman-Wistar rats, had an acute oral LD50 of greater than 16.0 g/kg.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 6/16/00 (RTA-034)

Robust Summary 3-Acute Oral-8

Test Substance	
CAS #	CAS# Analog of 78330-12-8
Chemical Name	C15-C21 alkaryl sodium salt derivative
Remarks	Test material dosed as received, purity not provided.
Method	
Method/Guideline followed	OECD Guideline 401
Test Type	Acute oral toxicity
GLP (Y/N)	Y
Year (Study Performed)	1982
Species/Strain	Rats/Sprague-Dawley strain
Sex	Male and female
No. of animals/sex/dose	5
Vehicle	None
Route of administration	Oral (intragastric)
Dose level	5 g/kg
Dose volume	4.5 ml/kg
Control group included	No
Remarks field for test conditions	A single dose of 5.0 g/kg of the undiluted test material was administered intragastrically to five fasted (over night) male and female rats. A control group was not included. The animals were observed for signs of toxicity or behavioral changes frequently on the day of treatment. Thereafter, all animals were examined for signs of toxicity twice per day. Individual weights were recorded on the day of dosing and weekly thereafter. The surviving animals were euthanized at the conclusion of the observation period. Gross autopsies were performed on all animals after 14 days.
Results	LD50 > 5.0 g/kg (males and females)
Remarks	No deaths were observed during the 14-day observation period. The animals exhibited ruffled fur 3 hours post dosing. Within 24 hours all animals appeared normal. No body weight effects were observed. Gross necropsy findings were unremarkable.
Conclusions	The test article, when administered as received to 5 male and 5 female Sprague-Dawley rats, had an acute oral LD50 of greater than 5.0 g/kg.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 2/10/00 (RTA-002)

ROBUST SUMMARY 3-Acute Oral-9

Test Substance	
CAS #	CAS# 115733-09-0
Chemical Name	C14-24 alkaryl calcium salt derivative
Remarks	Test material dosed as received, purity not provided.
Method	
Method/Guideline followed	OECD Guideline 401
Test Type	Acute oral toxicity
GLP (Y/N)	Y
Year (Study Performed)	1981
Species/Strain	Rats/Sprague-Dawley strain
Sex	Male and female
No. of animals/sex/dose	5
Vehicle	None
Route of administration	Oral (intragastric)
Dose level	5 g/kg
Dose volume	Adjusted for specific gravity of 0.94 g/ml
Control group included	No
Remarks field for test conditions	A single dose of 5.0 g/kg of the undiluted test material was administered intragastrically to five fasted (approximately 16 hours) male and female rats. The animals were observed for signs of physiological or behavioral changes at 2 and 4 hours post dose. Thereafter all animals were examined for signs of toxicity twice per day. Individual body weights were recorded on the day of dosing and at termination. The surviving animals were euthanized at the conclusion of the observation period. Gross autopsies were performed on all animals after 14 days.
Results	LD50 > 5.0 g/kg (males and females)
Remarks	No mortality was observed. Two rats exhibited slight diarrhea two hours post dosing. On Day 1., most rats had slight diarrhea and anal stains. Two rats exhibited slight bloody nasal discharge on Days 2 and 3. All rats were normal on days 4 through 14. No other signs of toxicity were observed. Body weights and necropsy findings were unremarkable.
Conclusions	The test article, when administered as received to 5 male and 5 female Sprague-Dawley rats, had an acute oral LD50 of greater than 5.0 g/kg.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 6/18/01

5.1.2 Acute Inhalation Toxicity

Robust Summary 3-Acute Inhalation-1

Test Substance	
CAS #	CAS# 68783-96-0
Chemical Name	Petroleum derived calcium salt, overbased
Purity	35.1% active in oil
Method	
Method/Guideline followed	OECD Guideline 403
Test Type	Acute Inhalation toxicity (Limit Test)
GLP (Y/N)	Y
Year (Study Performed)	1986
Species/Strain	Rats/Sprague-Dawley
Sex	Male and female
No. of animals/sex	5
Vehicle	Oil based material dosed undiluted
Route of administration	Aerosol inhalation (single 4 hour whole body exposure)
Dose level	1.9 mg/L (actual maximum attainable concentration)
Vehicle control group	No
Chamber analysis	Yes
Remarks field for test conditions	One group of five rats/sex was exposed for 4 hours to the test material as a liquid droplet aerosol generated by a pressure spray apparatus delivered into a 100-liter plexi-glass exposure chamber. The actual exposure concentration as measured by gravimetric analysis was 1.9 mg/L. Particle size analyses were performed once/hour using a multi-stage cascade impactor. Animal observations for toxicological signs and mortality were recorded periodically during exposure and twice daily during the 14 day observation period. Individual body weights were recorded on Day1 (immediately prior to exposure) and on Days 2, 3, 5, 8 and 15. Animals were euthanized by exsanguination under ether anesthesia. All animals were subjected to a complete gross necropsy.
Results	LC50 > 1.9 mg/L (males and females)(maximum attainable concentration)
Remarks	The mass median aerodynamic diameter was 4.2 microns with a geometric standard deviation of 1.9 (estimated percent of particles <10 microns=93%). All animals survived the exposure and observation periods. Observations recorded during exposure included reduced activity, matted coat and closed eyes. Observations noted post exposure on Day 1 included lacrimation, nasal discharge, salivation, , rales, matted coat, hunched appearance, soft stool and closed eyes. These findings decreased in incidence over the next week. Animals

	were free of symptoms of exposure during the second week of observation. Several animals exhibited very slight body weight losses on Day 2. Body weights recovered and were unremarkable by Day 5. There were no abnormal postmortem findings evident in any of the animals at study termination.
Conclusions	Following 4-hour whole body exposure to a liquid droplet aerosol of the test material the LC50 in male and female Sprague Dawley rats was >1.9 mg/L. This was the maximum concentration attainable.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 2/18/00 (RTA-023)

5.1.3 Acute Dermal Toxicity

Robust Summary # 3-Acute Dermal-1

Test Substance	
CAS #	CAS# 115733-09-0
Chemical Name	C14-24 alkaryl calcium salt derivative
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD Guideline 402
Test Type	Acute dermal toxicity (Limit Test)
GLP (Y/N)	Y
Year (Study Performed)	1981
Species/Strain	Rabbits/New Zealand White
Sex	Male and female
No. of animals/sex/group	5
Vehicle	None
Route of administration	Dermal
Dose level	5 g/kg
Dose volume	5 ml/kg
Control group included	No
Remarks field for test conditions	This study was conducted prior to the development of Test Guideline 402. This study deviated from Guideline 402 in that animals were abraded prior to dosing. This deviation was not considered sufficient to change the outcome of the study. Approximately 24 hours prior to topical application of the test material, the hair of each animal was closely clipped. On the day of dosing the skin was abraded prior to test material administration. A single dose of 5 g/kg of the undiluted test material was administered dermally to five male and female animals. The test material was kept in contact with the skin for a period of 24 consecutive hours under a gauze patch and elastic bandage. The application site was wiped clean of residual test material at the end of the 24-hour exposure period. The animals were observed for abnormal clinical signs frequently on the day of dosing and once daily for 14 days after treatment. Individual body weights were recorded on the day of dosing and on days 7 and 14. The surviving animals were euthanized at the conclusion of the observation period. Gross necropsies were performed on all animals on Day 14.
Results	LD50 > 5.0 g/kg (males and females
Remarks	Rabbits were normal at 0, 2 and 4 hours post dosing. Upon bandage removal at 24 hours rabbits were distressed. Skin at the dose site was red, swollen and stained with test material. Irritation subsided by day 9, however the skin remained dry, flaky and stained throughout the observation period. All animals

	gained weight during the study. No systemic toxicity was observed. At necropsy 9 rabbits exhibited alopecia, matted fur and flaky skin at or around the test site. One animal had a friable, white, mottled left front liver lobe. One rabbit had a small right testis.
Conclusions	The test article, when administered dermally as received to 5 male and 5 female New Zealand white rabbits had an acute dermal LD50 of greater than 5.0 g/kg. No evidence of systemic toxicity was observed.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 6/16/00 (RTA-033)

Robust Summary 3-Acute Dermal-2

Test Substance	
CAS #	CAS# 61789-86-4
Chemical Name	Petroleum derived calcium salt
Remarks	Test material purity 100%
Method	
Method/Guideline followed	OECD Guideline 402
Test Type	Acute dermal toxicity (Limit Test)
GLP (Y/N)	Y
Year (Study Performed)	1985
Species/Strain	Rabbits/New Zealand White
Sex	Male and female
No. of animals/sex/group	5
Vehicle	None
Route of administration	Dermal
Dose level	5 g/kg
Dose volume	5 ml/kg
Control group included	Yes
Remarks field for test conditions	Approximately 24 hours prior to topical application of the test material, the hair of each animal was closely clipped. Elizabethan type collars were placed on the neck of each rabbit. The skin was left intact. Collars remained on for 24 hours post dosing. Animals were reclipped as needed. A single dose of 5 g/kg of the undiluted test material was administered dermally to five male and female animals. The test material was kept in contact with the skin for a period of 24 consecutive hours under a plastic wrap, which was over wrapped with paper toweling. The application site was wiped cleaned of residual test material at the end of the 24-hour exposure period. Five clipped, untreated animals/sex were wrapped as described above and served as sham controls. The animals were observed for abnormal clinical signs frequently on the day of dosing and twice daily for 13 days after treatment. On Day 14 the animals were observed once prior to sacrifice. Dermal examinations (Draize) were performed on day 1, 7 and 14. Individual body weights were recorded on the day of dosing and on days 2, 7 and 14. The surviving animals were euthanized at the conclusion of the observation period. Gross necropsies were performed on all animals on Day 14. Selected tissues, including the skin were examined microscopically.
Results	LD50 > 5.0 g/kg (males and females)
Remarks	No mortality was observed. Reduced food intake and nasal discharge were observed in treated and control animals. Slight to moderate erythema and edema were observed in both sexes 24 hours after treatment. Slight erythema was observed in three animals on Day 7. Dry and flaky skin was observed in all treated animals on Days 6 and 14. Body weights were unremarkable. Gross pathological findings included dry, flaky skin at the dose site of all treated

	animals. Microscopic examination revealed the presence of trace to mild hyperkeratosis. There were no other treatment related gross or microscopic findings.
Conclusions	The test article, when administered dermally as received to 5 male and 5 female New Zealand white rabbits had an acute dermal LD50 of greater than 5.0 g/kg. No evidence of systemic toxicity was observed.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 2/17/00 (RTA-020)

Robust Summary 3-Acute Dermal-3

Test Substance	
CAS #	CAS# 68783-96-0
Chemical Name	Petroleum derived calcium salt, overbased
Remarks	Test material dosed as received, purity not provided.
Method	
Method/Guideline followed	OECD Guideline 402
Test Type	Acute dermal toxicity (Limit Test)
GLP (Y/N)	Y
Year (Study Performed)	1993
Species/Strain	Rabbits/New Zealand White
Sex	Male and female
No. of animals/sex/group	5
Vehicle	None
Route of administration	Dermal
Dose level	2 g/kg
Dose volume	1.8 ml/kg
Control group included	No
Remarks field for test conditions	Approximately 24 hours prior to topical application of the test material, the hair of each animal on the dorsal surface from the shoulder region to the lumbar region was closely clipped. Elizabethan type collars were placed on the neck of each rabbit. The skin was left intact. Collars remained on for the duration of the study. Animals were reclipped as needed. A single dose of 2 g/kg of the undiluted test material was administered dermally to five male and female animals. The test material was kept in contact with the skin for a period of 24 consecutive hours, on approximately 10% of the total body surface under a semi-occlusive bandage that was covered with an elastic bandage. The application site was wiped clean of residual test material with water at the end of the 24-hour exposure period. The animals were observed for abnormal clinical signs 2 and 4 hours after dosing and once daily for the 14-day study period. Cutaneous examinations (Draize) were performed on day 1 (45 minutes after patch removal) and on Days 3, 7, 10 and 14. Individual body weights were recorded on the day of dosing and on day 7 and 14. The surviving animals were euthanized at the conclusion of the observation period. Gross necropsies were performed on all animals on Day 14.
Results	LD50 > 2.0 g/kg (males and females)
Remarks	No mortality was observed. Clinical observations were unremarkable in 7 of 10 treated animals. Three treated animals exhibited findings consistent with stress and collaring. Findings included sores/scabs in the mouth and in the dorsal cervical area and stool abnormalities. Erythemia was observed in all animals on day 1. Very slight to well defined erythemia was observed in 9 of 10 treated animals on Day 3. On Day 7 one animal exhibited slight erythemia and one

	animal exhibited well-defined erythemia. Slight erythemia was observed in two animals on Days 10 and 14. Edema was not observed in any of the animals. Desquamation was observed in all animals on Day 7 By Day 14 desquamation was observed in six treated animals. All animals exhibited body weight gains during the treatment period. At necropsy 6 of 10 treated animals exhibited desquamation. One animal was noted with tan striations on the liver.
Conclusions	The test article, when administered dermally as received to 5 male and 5 female New Zealand white rabbits had an acute dermal LD50 of greater than 2.0 g/kg. No evidence of systemic toxicity was observed.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 2/17/00 (RTA-019)

Robust Summary 3-Acute Dermal-4

Test Substance	
CAS #	Analog of CAS# 70024-69-0
Chemical Name	C20-C24 alkaryl calcium salt derivative
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD Guideline 402
Test Type	Acute dermal toxicity (Limit Test)
GLP (Y/N)	Y
Year (Study Performed)	1989
Species/Strain	Rats/Sprague Dawley
Sex	Male and female
No. of animals/sex/group	5
Vehicle	None
Route of administration	Dermal
Dose level	2 g/kg
Dose volume	Not specified
Control group included	Yes
Remarks field for test conditions	Approximately 24 hours prior to topical application of the test material, the hair of each control and treated animal was closely clipped. A single dose of 2 g/kg of the undiluted test material was administered dermally to five male and female animals. The test material was kept in contact with the skin for a period of 24 consecutive hours under a plastic film and elastic bandage. Plastic collars were used during the dosing phase. The application site was wiped clean of residual test material at the end of the 24-hour exposure period. The animals were observed for abnormal clinical signs frequently on the day of dosing and once daily for 14 days after treatment. Individual body weights were recorded on the day of dosing and on days 2, 7 and 14. The surviving animals were euthanized at the conclusion of the observation period. Gross necropsies were performed on all animals on Day 14.
Results	LD50 > 2.0 g/kg (males and females)
Remarks	No signs of systemic toxicity were observed. All treated animals exhibited skin irritation. Significant differences in mean body weight were observed between treated and control males on Days 2, 7 and 14. At necropsy, multiple pinpoint scabs were observed in three treated males and one treated female.
Conclusions	The test article, when administered dermally as received to 5 male and 5 female Sprague Dawley rats had an acute dermal LD50 of greater than 2.0 g/kg. No evidence of systemic toxicity was observed.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 10/4/00 (RTA-068)

5.2 Repeated Dose Toxicity

Robust Summary 3-Four Week Oral 1

Test Substance	
CAS #	Analog of CAS# 70024-69-0
Chemical Name	C20-24 alkaryl calcium salt derivative
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD 407
Test Type	28-day oral toxicity study in rats
GLP (Y/N)	Y
Year (Study Performed)	1989
Species	Rat
Strain	Sprague-Dawley CD, 41 days old at initiation of treatment
Route of administration	Oral gavage (syringe and dosing tube)
Duration of test	29 days of treatment followed by 14 day recovery period in the control and high dose satellite recovery groups
Doses/concentration levels	0, 100, 500 and 1000 mg/kg/day
Sex	Males and females
Exposure period	29-day treatment duration with a 14 day recovery
Frequency of treatment	7 days/week
Control group and treatment	6 rats/sex/group for each dose, and satellite recovery groups of 6 animals/sex for the control and 1000 mg/kg/day dose. Control group received daily doses of peanut oil at 2.0 ml/kg, and treatment groups received the indicated dose of test material diluted in peanut oil at a dose volume of 2.0 ml/kg
Post exposure observation period	14-days
Statistical methods	Body weight, food consumption, feed efficiency, hematology and clinical chemistry parameters, organ weights and organ/body weight ratios were analyzed. Mean values of all dose groups were compared to control at each time interval. Tests included parametric ANOVA with a Dunnett's *post-hoc* test, non-parametric Kruskal-Wallis and a Mann-Whitney U-test, Bartlett's test for equal variances, a Student's *t*-test and Dixon's test for rejection of outlying values.
Dose rangefinding study	Yes (Pilot two-week repeated dose oral toxicity study)
Remarks field for test conditions	Single oral doses were administered for 29 consecutive days using a gavage needle. Clinical observations were made daily. Viability checks were performed twice daily. Body weight were recorded twice weekly during treatment and weekly during recovery. Terminal body weights were recorded. Food consumption were recorded during treatment and recovery. Hematology, clinical chemistry and urinalysis parameters were evaluated at termination of

	treatment and recovery. Macroscopic examinations were performed on all animals. Select organs were weighed. A range of tissues was examined microscopically. Significant deviations from the OECD 407 test guidelines include: • A function observational battery for neurotoxicity was not performed since this test was not part of the OECD 407 guideline at the time the study was performed. • Microscopic pathology was performed as required by OECD 407 guideline in place at the time the study was conducted.
Results	
Remarks	An NOEL of 500 mg/kg/day was established for this study. No test material related mortality was observed. One low dose male was found dead on Day 9. This was attributed to a probable misdosing. A second low dose male was replaced, due to a possible misdosing, on the first day of treatment. Mean serum cholesterol levels were significantly reduced in the 1000 mg/kg males and females at termination of dosing and in the 1000 mg/kg females at the end of the 14-day recovery period. No treatment-related effects were observed on mortality, clinical observations, body weight and body weight gain, food consumption, feed efficiency, hematology, urinalysis, absolute and relative organ weights and macroscopic or microscopic pathology. Statistically significant differences from control were observed for some hematology and clinical chemistry parameters. These values were within clinically normal limits and were not associated with corresponding histopathological changes. They were not considered biologically significant. Chemical analysis of dosing solutions confirmed that they were homogeneously prepared at the desired concentrations.
Conclusions	Little subchronic toxicity was observed over the range of doses administered in this study. Based on a reduction in mean cholesterol values in the males and females treated at the 1000 mg/kg dose level, the NOEL was 500 mg/kg.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 2/11/00 (RTA-006)

Robust Summary 3-Four Week Dermal -2

Test Substance	
CAS #	CAS# 68783-96-0
Chemical Name	Petroleum derived calcium salt, overbased
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD 410
Test Type	28-day dermal toxicity study in rats
GLP (Y/N)	Y
Year (Study Performed)	1995
Species	Rat
Strain	Sprague-Dawley CD, 8-9 weeks of age at initiation of treatment
Route of administration	Dermal, 6 hour/day, to the clipped, unabraided, dorsal surface.
Duration of test	28 days of treatment followed by 14 day recovery period in the high dose satellite recovery group only.
Doses/concentration levels	0, 100, 300 and 1000 mg/kg/day
Sex	Males and females
Frequency of treatment	Once/day, 7 days/week
Control and treatment groups	5 rats/sex in the control group, in each dose level and in the satellite recovery group at the1000 mg/kg/day dose. The control group received no treatment (sham control). The test material was administered undiluted to the treated animal based on individual animal body weight.
Post exposure observation period	14-days (High dose group only)
Dose rangefinding study	Dose levels were selected based on results of a rangefinding study conducted at dose levels up to 1000mg/kg/day. No signs of toxicity were observed.
Statistical methods	Body weight, food consumption, hematology and clinical chemistry parameters, organ weights and organ/body weight ratios were analyzed. Mean values of all dose groups were compared to control at each time interval. Tests included parametric ANOVA with a Dunnett's test and regression analysis for linear response, non-parametric Kruskal-Wallis and Dunn's Summed Rank Test, Jonckheere's test for monotonic trend. A Student's *t*-test was used to compare the satellite group's main study termination and recovery blood values and organ weights.
Remarks field for test conditions	The test material was applied to the clipped, unabraided dorsal surface of the rats for 6 hours/day, 7 days/week for 28 days. The gauze patch was secured to the trunk with non-irritating tape and wrapped with an elastic sleeve. After at least 6 hours the test material residue was removed from the skin with peanut oil and a paper towel. Clinical observations were made daily. Dermal responses were evaluated (Draize) prior to dosing on days 0, 1, 4, 7, 11, 14, 18, 21, and 25; prior to blood collection on day 28 and after sleeve removal on day 0. Satellite animals were also evaluated on Days 32, 35, 40 and 42. Body weight and food consumption were recorded during treatment and recovery.

	Hematology and clinical chemistry parameters were evaluated at termination of treatment and recovery. Macroscopic examinations were performed on all animals. Select organs were weighed. A range of tissues was examined microscopically.
Results	
Remarks	A NOAEL of 1000 mg/kg was established for this study. No mortality occurred during this study. Low incidences of very slight erythemia, desquamation and/or pinpoint scabbing were observed sporadically in the treated animals. All animals were free of edema during the study. Body weights and food consumption data were unremarkable during the treatment and recovery periods. There were no treatment-related differences from control observed in the hematology data of the treated animals following the dosing or recovery periods. Differences from control were noted for several hematology parameters including a statistically significant increase in the mean percentage of neutrophils of the 300 and 1000 mg/kg females and a decrease in mean percentage of lymphocytes in the 1000 mg/kg females compared to control on Day 28. There was a statistically significant decrease in mean percentage of basophils in the satellite females from Day 28 to 42. However these values were within the normal range. In the absence of differences from control in absolute white blood cell counts, these findings were considered unrelated to treatment. There was a statistically significant decrease in the mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration of the male satellite animals from Day 28 to 42. In the absence of other significant findings in mean hemoglobin or red blood cell parameters, these small differences were not considered clinically significant. Serum chemistry values were unremarkable in the treated animals at termination of the treatment and recovery periods. There was a slight increase in the mean aspartate aminotransferase and alanine aminotransferase of the high dose females at Day 28. These increases were attributed to two females with high values. Similar changes were not observed in the satellite females or in the males at Day 28. These increases were not considered related to treatment. There were several differences from control noted at the end of recovery. These values were within the range of normal and similar differences were not evident at the end of the treatment period indicating that these findings were not clinically significant or treatment related. Gross postmortem findings were limited to one 300 mg/kg male with small testes, one control female with discolored lungs and liver and black material in the stomach; and single occurrences of scabs in the 100 and 1000 mg/kg and recovery males. These findings were considered incidental and unrelated to treatment. Tape irritation was observed in a number of animals. There were no alterations in organ weights that were attributed to treatment with the test material. Slight alterations were noted in several organ weights at termination of dosing or recovery. There was a statistically significant decrease in mean absolute brain weight of the 300 mg/kg females compared to control. This finding lacked a dose response and was not considered biologically significant. There was a statistically significant decrease in mean relative adrenal and testes weights of the male satellite animals at termination of recovery compared to control at end of treatment. Compared to the high dose at study termination there was a statistically significant decrease in mean relative

	adrenal, brain and testes weight of the male satellite animals and mean relative adrenal and brain weight of the female satellite animals at recovery termination. These alterations in organ weights were attributed to the cessation of the stress associated with wrapping (adrenal) and the animals continued increase in body weight while organ weights remained constant in adult animals. In the absences of significant organ weight findings following treatment or correlating effects with histopathology these findings were not considered clinically significant. There were no test material related microscopic findings noted in any group. Livers from female rats of all groups (including control) sacrificed after 28 days of treatment exhibited focal necrosis. This finding did not exhibit a dose response. This finding has been seen in other dermal studies and has been attributed to trauma and/or ischemia to the liver resulting from the wrapping and manipulation of the animals. Liver necrosis was not evident in any of the satellite recovery animals. This finding was not considered treatment related. The treated skin of most animals revealed variable amounts of thickening of the epidermis due to acanthosis and hyperkeratosis, sebaceous gland hyperplasia and focal dermal inflammation. These changes occurred in all groups including control. However the severity of these changes tended to be increased in the male treated group rats and in the females of the 300 and 1000 mg/kg groups, suggesting a mild irritating effect of the test material. Following recovery these findings were less severe.
Conclusions	A NOAEL of 1000 mg/kg was established for this study. Under the conditions of this study dermal application of this test material resulted in no signs of overt systemic toxicity.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 2/18/00 (RTA-024)

Robust Summary 3-Four Week Inhalation-3

Test Substance	
CAS #	CAS# 68783-96-0
Chemical Name	Petroleum derived calcium salt, overbased
Remarks	35.1% active in oil
Method	
Method/Guideline followed	OECD 412
Test Type	28-day inhalation toxicity study in rats
GLP (Y/N)	Y
Year (Study Performed)	1986
Species	Rat
Strain	Sprague-Dawley CD, 6-7 weeks of age at initiation of treatment
Route of administration	Aerosol inhalation, whole body exposure
Duration of exposure	6 hours/day
Doses/concentration levels	49.5, 156, 260 mg/m^3 (measured concentration)
Sex	Males and females
Frequency of treatment	5 days/week for 4 weeks
Control and treatment groups	5 rats/sex
Post exposure recovery period	None
Statistical methods	Body weight, food consumption, hematology and clinical chemistry parameters, organ weights and organ/body weight ratios were analyzed. Mean values of all dose groups were compared to control at each time interval. Tests included parametric ANOVA with a Dunnett's test and regression analysis for linear response, non-parametric Kruskal-Wallis and Dunn's Summed Rank Test, Jonckheere's test for monotonic trend
Dose rangefinding study	No
Remarks field for test conditions	Treated animals were exposed to the test material as a liquid droplet aerosol generated by a pressure spray apparatus delivered into a 1000-liter glass and stainless steel exposure chamber. Chamber airflow rates was approximately 200 liters/minute with a chamber 99% equilibration time of 22 minutes. Control animals were exposed to room air only. Chamber exposure concentrations were measured by gravimetric analysis at one and one half-hour intervals. Particle size analyses were performed once/week using a multi-stage cascade impactor. Animal observations for toxicological signs and mortality were recorded periodically during exposure and twice daily during the 14 day observation period. Individual body weights were recorded weekly. Hematology and clinical chemistry evaluations were performed on all animals prior to terminal sacrifice. Animals were euthanized by exsanguination under ether anesthesia. Macroscopic examinations were performed on all animals. Select organs were weighed. A range of tissues was examined microscopically.

Results	
Remarks	The actual exposure concentrations measured by gravimetric analysis were 49.5, 156, 260 mg/m^3. The mass median aerodynamic diameter of the aerosol ranged from 3.3 to 3.7 microns, with an average geometric standard deviation range of 2.0 to 2.1. These data confirmed that the aerosol was respirable in the rat (estimated percent of particles <10 microns=93%). There was no test material exposure related mortality during the study. One low dose animal escaped from its cage and was euthanized. One control male died during blood collection immediately prior to its scheduled sacrifice. Red nasal discharge, matted coat and decreased activity were noted at the two higher concentrations. The mean body weight gain of the high dose males was slightly reduced over the four weeks of study. Body weights and gains in the other groups were unremarkable. Clinical chemistry and hematology data exhibited no patterns indicative of a treatment-related effect. Several incidental statistically significant differences from control were observed these included: increased hematocrit (low dose females), creatinine phosphokinase (low and high dose females) and sodium (high dose females). These differences were not attributed to treatment. Dose related increases in absolute and relative (to body weight) lung weights were observed in the mid and high dose males and females. Increases were statistically significant, with the exception of mid dose female absolute lung weight. Microscopically the accumulation of intraalveolar macrophages (males: 3,5,5,5; females: 5,5,5,5) and hyperplasia/hypertrophy of bronchiole epithelium (males: 3,4,5,5; females: 4,5,5,5) were seen in the control and treated groups. While these findings were observed in control and treated animals the severity of the lesions exhibited a dose response in the mid and high dose groups and was considered treatment related. Differences in severity between the control and low dose group were equivocal. Based on these findings the lowest dose level (49.5 mg/m^3) is considered the NOAEL by this reviewer.
Conclusions	Under the conditions of this study inhalation exposure of this test material resulted in minimal toxicity over the range of doses administered. A NOAEL of 49.5 mg/m^3 was established for this study based on the slight, dose related increase observed in the severity of microscopic pulmonary findings and increased lung weights.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 2/24/00 (RTA-025)

Robust Summary 3-Four Week Dermal 4

Test Substance	
CAS #	CAS# 71786-47-5
Chemical Name	Alkaryl magnesium salt derivative
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD 410
Test Type	28-day dermal toxicity study in rabbits
GLP (Y/N)	Y
Year (Study Performed)	1981
Species	Rabbit
Strain	New Zealand White (SPF) (approximately 2 kg in body weight at initiation)
Route of administration	Dermal, 6 hour/day, 5 days/week, to the clipped, unabraided, dorsal surface.
Duration of test	20 days of treatment followed by 4 week recovery period
Doses/concentration levels	0, 25 and 100% (w/v) (OECD Guideline 410 suggests three treated groups and a control be included in this study design. The lowest dose level should be free of toxic effects. These suggestions were not met in this study.)
Vehicle control	Primol 205
Dose volume	2 mL/kg/day
Sex	Males and females
Frequency of treatment	Once/day, 5 days/week for a total of 20 doses.
Vehicle control and treatment groups	15 rabbits/sex in the vehicle control group and in both treated groups. Five of the initial 15 animals/sex/group served as recovery animals. The control group received the vehicle. An untreated control group was not included in the study. The test material was administered undiluted to the treated animal in the high dose group. The animals in the low dose group received the test material diluted in the vehicle. Doses were administered based on individual animal body weights.
Post exposure observation period	4 weeks
Statistical methods	Body weight, food consumption, hematology and clinical chemistry parameters, organ weights and organ/body weight ratios were analyzed. Mean values of all dose groups were compared to control at each time interval. Tests included parametric ANOVA with a Dunnett's test and regression analysis for linear response, non-parametric Kruskal-Wallis and Dunn's Summed Rank Test, Jonckheere's test for monotonic trend.
Remarks field for test conditions	The test material was applied to the clipped, unabraided dorsal surface of the rabbits for 6 hours/day, 5 days/week for 20 days. Elizabethan collars were used to prevent ingestion. (OECD Guideline 410 suggests the use of a gauze patch over the treatment site secured to the trunk with non-irritating tape and wrapped with an elastic sleeve. This procedure was not used during this study. This is considered a minor deviation from the Guideline.) After approximately 6 hours the test material residue was removed from the skin with a paper towel, if

	necessary. Clinical observations were made weekly. Dermal responses were evaluated daily during treatment (7 days/week; prior to dosing on dosing days) and recovery. Body weight was recorded weekly during treatment and recovery. (OECD Guideline 410 suggests the recording of food consumption. This parameter was not recorded during this study. This is considered a minor deviation from the guideline.) Hematology and clinical chemistry parameters were evaluated pretest and at termination of treatment and recovery. Macroscopic examinations were performed on all animals. Select organs were weighed. A range of tissues was examined microscopically in the control and high dose animals sacrificed at the end of the treatment period and for all found dead and moribund sacrifice animals. In addition the liver, testes and epididymides were evaluated in all low dose animals.
Results	
Remarks	One control and four high dose animals died or were sacrificed early during this study. One control female was sacrificed moribund during recovery (test day 35). Two high dose males were sacrificed moribund during the treatment period (test days 23 and 32). One high dose male was found dead (test day 49) during recovery. One high dose female was sacrificed moribund during recovery (test day 39). The cause of death of these animals was not established. Alopecia was observed in many of the low and high dose males and females during the last two to three weeks of treatment and during the first two to three weeks of recovery. Several high dose males and females exhibited this finding throughout recovery. Erythema, edema, atonia, desquamation, fissuring and exfoliation were observed in all of the low and high dose animals throughout the treatment period. Most of these findings were evident during recovery with a decreasing severity and incidence. These data did not exhibit a strong dose response. Erythema and desquamation were observed in the control males and females during the treatment and recovery periods. These findings were less severe then those observed in the treated animals. As in the treated groups severity and incidences decreased with time during recovery. The mean body weights of the low dose males and females were slightly lower (~5%) than control during the last two weeks of treatment and during the first week of recovery. The mean body weights of the high dose males and females were lower than control (5-15%) during the last two weeks of treatment and throughout recovery. Some of the difference from control observed during recovery may be due to the small number of animals (2-5) available in each group and the normal variability expected in rabbit weight. The mean total leukocyte count of the low and high dose males and females were statistically significantly lower than control at termination of the treatment period. Low and high dose males and high dose females were also slightly reduced at the end of recovery. In addition the mean hemoglobin and hematocrit values and the mean erythrocyte count of the high dose females were significantly reduced following treatment but not following recovery. The low and high dose males and females exhibited slight or statistically significant, dose-related decreases in total protein and globulin and increased albumin/globulin ratios at termination of treatment. In addition albumin was

	slightly reduced in the high dose females. At termination of the recovery period the mean globulin level of the low dose females was significantly reduced and the albumin/globulin ratios of the low and high dose females were slightly (statistically significantly) increased compared to control. At termination of treatment the low and high dose males exhibited increases in mean SGOT and alkaline phosphatase. The low and high dose females exhibited increases in SGOT and SGPT. These enzymes were unremarkable following recovery. (The changes observed in SGOT and SGPT were not discussed in the original final report of this study.) Treatment related decreases were observed in the absolute and relative (to body weight) testes and epididymides weights of the low and high dose males at the end of the treatment and recovery periods. Absolute testes weights were decreased -21 and -35%, compared to control, in the low and high dose groups following treatment and -22 (low dose) and -58% (high dose) following recovery. Treatment related increases were observed in the absolute and relative (to body weight) liver weights of the low and high dose males (+5/+30%-absolute weight) and females (+12/+23%-absolute weight) following treatment and in the high dose males (+14%-absolute weight) following recovery. Macroscopic examinations revealed dermal findings consistent with those observed during the in life examinations. The testes of many low and high dose animals were noted to be small in size at the end of the treatment period. This observation was recorded in one high dose recovery animal. These data are consistent with the reduced testes weights observed in the low and high dose groups. Microscopic evaluations revealed treatment related morphologic changes in the skin, testes, epididymides and possibly the liver. Compound related microscopic lesions were seen in the treated skin of the high dose animals at termination of the treatment period (high dose recovery and low dose treatment and recovery animals were not examined). Treated skin findings included slight to moderately severe hyperkeratosis and epithelial hyperplasia. Findings in males and females were comparable. Possible treatment related liver findings were observed in the high dose group only. Findings present at termination of dosing but not following recovery included the presence of multifocal areas of minimal to moderate hepatocellular degeneration usually accompanied by multifocal areas of necrosis and/or multifocal areas of coarse cytoplasmic vacuolation of hepatocytes. Testicular changes were observed in the high dose males only following treatment and recovery. No changes were evident in the low dose males. Alterations observed in the high dose included aspermatogenesis, reduced numbers of spermatids, and multifocal to diffuse tubular hypoplasia. Epithelial hypoplasia of the epididymis accompanied the testicular changes in many animals at termination of treatment but not following recovery. There were no other findings observed in this study that were considered treatment related.

Conclusions	Based on the findings observed during this study this reviewer has concluded that an NOAEL was not established for this study.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 4/13/00 (RTA-029)

Robust Summary 3-Four Week Dermal 5

Test Substance	
CAS #	CAS# 71786-47-5
Chemical Name	Alkaryl magnesium salt derivative
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD 410
Test Type	28-day dermal toxicity study in rats
GLP (Y/N)	Y
Year (Study Performed)	1995
Species	Rat
Strain	Sprague-Dawley CD, 8-9 weeks of age at initiation of treatment
Route of administration	Dermal, 6 hour/day, to the clipped, unabraided, dorsal surface.
Duration of test	28 days of treatment followed by 14 day recovery period in the high dose satellite recovery group only.
Doses/concentration levels	0, 100, 300 and 1000 mg/kg/day
Sex	Males and females
Frequency of treatment	Once/day, 7 days/week
Control and treatment groups	5 rats/sex in the control group, in each dose level and in the satellite recovery group at the1000 mg/kg/day dose. The control group received no treatment (sham control). The test material was administered undiluted to the treated animal based on individual animal body weight.
Post exposure observation period	14-days (High dose group only)
Statistical methods	Body weight, food consumption, hematology and clinical chemistry parameters, organ weights and organ/body weight ratios were analyzed. Mean values of all dose groups were compared to control at each time interval. Tests included parametric ANOVA with a Dunnett's test and regression analysis for linear response, non-parametric Kruskal-Wallis and Dunn's Summed Rank Test, Jonckheere's test for monotonic trend. A Student's *t*-test was used to compare the satellite groups main study termination and recovery blood values and organ weights.
Dose rangefinding study	Dose levels were selected based on results of a rangefinding study conducted at dose levels up to 1000mg/kg/day. No signs of toxicity were observed.
Remarks field for test conditions	The test material was applied to the clipped, unabraided dorsal surface of the rats for 6 hours/day, 7 days/week for 28 days. The gauze patch was secured to the trunk with non-irritating tape and wrapped with an elastic sleeve. After at least 6 hours the test material residue was removed from the skin with peanut oil and a paper towel. Clinical observations were made daily. Dermal responses were evaluated (Draize) prior to dosing on days 0, 1, 4, 7, 11, 14, 18, 21, and 25; prior to blood collection on day 28 and after sleeve removal on day 0. Satellite animals were also evaluated on Days 32, 35, 40 and 42. Body weight and food consumption were recorded during treatment and recovery. Hematology and clinical chemistry parameters were evaluated at termination of

	treatment and recovery. Macroscopic examinations were performed on all animals. Select organs were weighed. A range of tissues was examined microscopically.
Results	
Remarks	A NOAEL of 1000 mg/kg was established for this study. No treatment related mortality was observed. One 300 mg/kg female was found dead on Day 19. This death was attributed to the wrapping procedure. One 1000 mg/kg male died following blood collection at study termination. Desquamation was observed in one 300 mg/kg female on days 4 and 7. No other significant clinical in life or dermal observations were observed. Body weights and food consumption data were unremarkable during the treatment and recovery periods. There were no treatment-related differences from control observed in the hematology data of the treated animals following the dosing or recovery periods. Differences from control were noted for several hematology parameters including a decrease in mean percentage of eosinophils in low and mid dose males at termination of treatment. These values were within the normal range and did not exhibit a dose response. Following the recovery period there were several statistically significant differences from control noted in the hematology parameters of the satellite animals. These included decreases in mean white blood cell count, absolute lymphocytes and basophils in the females; an increase in mean percentage of large unclassified cells in males and females; and an increase in mean corpuscular hemoglobin in the females. All of these differences were within the expected range of normal and were not considered clinically significant. Increases observed in mean prothrombin time and activated partial thromboplastin time (APTT) in the male recovery animals and in mean APTT of the female recovery animals were attributed to variations in bleeding technique. Serum chemistry values were unremarkable in the treated animals at termination of the treatment period. Following recovery there were a number of small, but statistically significant differences observed in serum chemistry parameters of the satellite animals. These included a decrease in mean blood urea nitrogen (males), sodium (males) and chloride (females) and increases in phosphorus and billirubin (males) and calcium, total billirubin and triglycerides (females). All of these findings were within the range of normal values and were comparable to control following the termination of dosing. These findings were not considered clinically significant or related to treatment. One female (300 mg/kg) which died on Day 19 exhibited an enlarged liver, ascites in the abdominal cavity and a reddened jejunum. This death was attributed to the wrapping procedure. There were no gross postmortem observations or alterations in organ weights that were attributed to treatment with the test material. Slight alterations were noted in several organ weights at termination of dosing or recovery. These included a statistically significant increase in absolute and relative liver weight in the 100 mg/kg females and statistically significant decreases in relative brain and ovary weights in the 1000mg/kg females at termination of recovery. These findings did not correlate with any histopathological findings and were not attributed to treatment. There were no test material related microscopic findings noted in any group. One male and one female (1000 mg/kg) has epidermal acanthosis/hyperkeratosis. The

	male also exhibited slight focal epithelial spongiosis. Similar lesions were observed in the skin of one untreated control female. These findings were attributed to the repeated clipping and tape irritation in both treated and sham control animals. No skin changes were noted following recovery. Livers from rats of all groups (including control) sacrificed after 28 days of treatment exhibited focal or multifocal necrosis. This was an acute change which was characterized by coagulative necrosis of hepatocytes and occurred in a random fashion. This finding has been seen in other dermal studies and has been attributed to trauma and/or ischemia to the liver resulting from the wrapping and manipulation of the animals. Liver necrosis was not evident in any of the satellite recovery animals.
Conclusions	A NOAEL of 1000 mg/kg was established for this study. Dermal application of this test material resulted in no signs of overt toxicity.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 2/15/00 (RTA-009)

5.3 Genetic Toxicity:

Robust Summary 3-Gentox-1

Test Substance	
CAS #	Analog of CAS# 70024-69-0
Chemical Name	C20-24 alkaryl calcium salt derivative
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD Guideline 474
Test Type	Mammalian Erythrocyte Micronucleus Test
GLP (Y/N)	Y
Year (Study Performed)	1989
Species	Mouse
Strain	Swiss Albino Crl: CD-1 (ICR) BR 50 days of age at initiation of treatment
Route of administration	Intraperitoneal
Duration of test	Single dose followed by 72-hour evaluation period.
Doses/concentration levels	0, 100, 200, 400 and 500 mg/kg
Dose volume	5 ml/kg
Sex	Males and females
Frequency of treatment	Once
Control and treatment groups	Peanut oil vehicle control: 18/sex; triethylenemelamine positive control: 0.25 mg/kg, 5/sex; 100 and 500 mg/kg: 15/sex; 200 and 400 mg/kg: 18/sex
Statistical methods	Animal to animal variability in spontaneous frequency of micronucleated polychromatic erythrocytes were evaluated in vehicle controls. Statistically significant differences were evaluated in the frequency of micronucleated polychromatic erythrocytes between treated groups and vehicle controls. NCE/PCE (normochromatic erythrocytes/polychromatic erythrocytes) ratios in treated and control groups were compared. Tests included dispersion test of Amphlett and Delow, and Margolin, Fishers exact test, binomial approximation, Cochran-Armitage test for trend, a one-way analysis of variance and Dunnett's procedure.
Dose rangefinding study	A rangefinding study was conducted at 200, 400 and 600 mg/kg. Mortality and physical observations were evaluated.
Remarks field for test conditions	All animals were observed frequently for physiological or behavioral abnormalities on the day of dosing and at least twice daily thereafter. Body weights taken on first day of the study prior to treatment and at sacrifice. Macroscopic pathology performed on all animals at sacrifice. Five/sex from each treatment group and vehicle control group were sacrificed for bone marrow sampling 24, 48 and 72 hours post treatment. Positive controls sampled at 24 hours only. NCE/PCE ratio and %PCE of total erythrocytes were calculated by counting a total of $\geq$1000 erythrocytes/animal. A total of 1000 PCE /animal were evaluated for the presence of micronuclei. (Guideline calls for 2000/animal to be evaluated.) The number of micronuclei in NCEs was also determined.

Results	
Remarks	During the dose rangefinding study mortality (9 of 10 animals) was observed at 600 mg/kg but not at lower dose levels. Signs of toxicity observed at all dose levels included reduced feces, reduced food consumption, hyperactivity and phonation. Decreased motor activity was observed at 400 and 600 mg/kg. Based on these results dose levels of 100, 200, 400 and 500 mg/kg were selected for the main study. During the main study toxicity was observed at 400 and 500 mg/kg. At 500 mg/kg 5 males and 4 females of 15/sex died prior to the scheduled sampling time. At 400 mg/kg 1 of 18 treated females died on Day 3. Other clinical signs of toxicity included palpebral closure, decreased motor activity and weakness. Cytotoxicity was observed in both sexes. A statistically significant increase in NCE/PCE ratio was observed in males at 500 mg/kg at 24 hours. Elevated ratios were also observed in individual animals of both sexes in other groups. Altered proportions of erythrocytes to nucleated cells were noted for both sexes in the treated groups. No biological or statistical significant increase in the number of micronucleated-PCE was observed in any treated group compared to the vehicle control. All values for individual animals were within the expected range of micronucleated-PCE/1000 PCE expected for control animals. The variability in response observed in the treated animals was similar to that observed in the vehicle controls. The positive control exhibited a statistically significant increase in micronuclei as expected. Chemical analysis confirmed that the dosing solution preparation procedure utilized for this study resulted in homogeneous solutions of appropriate concentration.
Conclusions	Under the conditions of this study the test material did not induce micronuclei in bone marrow erythrocytes of mice. The genotoxicity NOEL was 500 mg/kg.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 2/10/00 (RTA-005)

Robust Summary 3-Gentox:-2

Test Substance	
CAS #	CAS# 68783-96-0
Chemical Name	Petroleum derived calcium salt, overbased
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD Guideline 474
Test Type	Mammalian Erythrocyte Micronucleus Test
GLP (Y/N)	Y
Year (Study Performed)	1995
Species	Mouse
Strain	Swiss Albino CD-1; 10-12 weeks of age at initiation of dosing
Route of administration	Oral gavage
Duration of test	Three treatment days followed by a 24-hour holding period.
Doses/concentration levels	0, 500, 1000, 2000 mg/kg
Dose volume	10 ml/kg
Sex	Males and females
Frequency of treatment	Three treatments administered approximately 24 hours apart.
Control and treatment groups	Peanut oil vehicle control: 5/sex; Cyclophosphamide positive control: 20 mg/kg (in water), 5/sex; 500, 1000 and 2000 mg/kg: 5/sex
Statistical methods	Data summarized by sex and dose group/time point. Analysis performed using an analysis of variance, Dunnett's test, Cochran-Armitage test for linear trend Wilk's Criterion or Kolomogorov-Smirnov statistic, Kruskal-Wallis, Dunn's Summed Rank Test and Jonkheere's test of ordered response.
Dose Rangefinding Studies	Doses: 0.5, 1.0 and 2.0 g/kg; 2/sex/dose sacrificed 24 hours after dosing. Percent polychromatic erythrocytes (PCE) was determined by counting 1000 cells. Number of micronuclei/1000 PCE determined.
Remarks field for test conditions	All animals were observed after dosing for signs of toxicity. Animals were examined twice daily for viability. Body weights were recorded prior to initiation of dosing. The animals from each group were sacrificed for bone marrow sampling 24 hours after the third dose. Necropsies were not performed. 2000 PCEs from each animal were examined for the presence of micronuclei. The percent of PCE in the total population of erythrocytes was determined for each animal by counting a total of 1000 polychromatic and normochromatic erythrocytes. If the test article induced neither a statistically significant dose response nor a statistically significant increase at any dose level above concurrent vehicle, at any sampling point, it was considered negative.
Results	
Remarks	All dose rangefinding animals survived and were free of clinical signs. Bone marrow toxicity was not observed at any dose levels tested. Therefore 2000

	mg/kg was selected as the high dose for the micronucleus assay. The mid and low doses were selected to be 1/2 and 1/4 of the high dose. In the main study, all vehicle, positive control and treated animals were normal after dosing and remained healthy until sacrifice. There were no dose related increases or statistical differences in micronuclei formation observed at any dose level. Cytotoxicity was not observed since there were no atatistically significant decreases in the percentage of polychromatioc erythrocytes compared to the vehicle control. The positive control induced a statistically significant increase in mean micronucleated PCEs in both sexes compared to the vehicle controls which indicated the positive control was clastogenic and responded appropriately. The positive control also induced cytotoxicity. Chemical analysis confirmed the uniformity and stability of the test material in peanut oil for at least 9 days at all three concentrations. Concentration verification analysis confirmed that each dose level was within 3% of nominal concentration.
Conclusions	The test material was not genotoxic under the conditions of this study. The genotoxicity NOEL was 2000 mg/kg.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 2/18/00 (RTA-022)

Robust Summary 3-Gentox: -3

Test Substance	
CAS #	CAS# 68783-96-0
Chemical Name	Petroleum derived calcium salt, overbased
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD Guideline 476
Test Type	Mouse Lymphoma Mutagenicity Screen
GLP (Y/N)	Y
Year (Study Performed)	1984
Test System	L5178Y-3.7.2C mouse lymphoma cells
Culture Preparation and Maintenance	Cells were stored frozen in liquid nitrogen. Cultures were incubated at 37°C with shaking. Cultures were diluted daily to a cell density of approximately 3 x 10^5 cells/mL. Cultures were checked for bacterial and fungal contamination. Prior to use cultures were treated with methotrexate to reduce the frequency of spontaneously occurring $TK^{-/-}$ cells.
Exposure Method	Dilution
Test Substance Doses/concentration levels	Concentrations of 500, 1000, 1500, 2000, 4000 and 5000 ug/mL were evaluated with and without metabolic activation.
Metabolic Activation	Aroclor induced rat liver
Vehicle	Dimethyl sulfoxide (DMSO) 10 ul/mL
Positive Control concentration levels by activation status	With activation: 7,12-dimethylbenzanthracene (DMBA) 5 ug/mL Without activation: ethylmethanesulfonate (EMS) 744 ug/mL
Statistical Analysis	Means and standard deviations were determined.
Test Substance Solubility	Test substance solubility in the vehicle was determined during a solubility test.
Dose range finding study	Test substance (dose levels from 1 to 10,000 ug/mL) and vehicle control tested with and without activation. Cultures were exposed to the test substance and incubated for approximately four hours, then washed and cultured for two days. Cell culture density was determined 24 and 48 hours post exposure. Treated cell suspension growth at each dose level was compared to the negative solvent control.
Remarks field for test conditions	Prior to study initiation the solubility of the test substance and of the positive control materials in the vehicle (DMSO) was confirmed. A pretest dose range finding study was conducted at concentrations up to 10,000 ug/mL with and without metabolic activation. In the main study there were two treatment sets for each concentration of test substance, with (+S9) and without (-S9) metabolic activation. DMBA (positive control) was tested with activation and EMS (positive control) was tested without activation. The test material was prepared so that the highest and lowest concentrations would yield percent total growth of approximately 10% and 90% respectfully. The test material was added to cells with and without

	activation and incubated for four hours. Cells were then washed and placed in suspension cultures for two days with a cell population adjustment at 24 hours. The cells were then plated in a restrictive media containing trifluorothymidine (TFT) which allows $TK^{-/-}$ cells to grow. Cells were also plated in a non-restrictive media that indicated cell viability. Plates were incubated at $37^{O}C$ in a humidified 5% CO_2 atmosphere for 10-12 days. Following incubation all plates were scored for total number of colonies/plate. The frequency of mutation by dose was determined by comparing the average number of colonies in the mutagenicity plates to the average number of colonies in the corresponding viability plates. For the study to be acceptable the following criteria must be met: mutation frequency of positive controls with or without activation should be twice that of the solvent control; the negative control spontaneous mutation frequency should be in the range of 0.2 to $2.0/10^4$ cells; negative control plating efficiency should be at or above 50% and the test material should be tested to the level of approximately 10% total growth or to the limits of solubility or to a high dose of 100 mg/mL.
Results	The test substance was not mutagenic in this assay with or without metabolic activation.
Remarks	The dose rangefinding study indicated significant toxicity (<90% total growth) at 500 ug/mL with and without metabolic activation. Based on these results the test material was evaluated for mutagenicity at concentrations ranging from 500 to 5000 ug/mL. Six cultures with and without activation were selected for cloning at 500, 1000, 1500, 2000, 4000 and 5000 ug/mL. None of the cultures treated with test material with or without activation exhibited mutant frequencies significantly different from the average mutant frequency of the negative (solvent) controls at a percent total growth of 10% or greater. Positive and vehicle control group responses were appropriate and met the criteria outlined above.
Conclusions	The test material was not genotoxic under the conditions of this study.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 4/11/00 (RTA-028)

Robust Summary 3-Gentox-4

Test Substance	
CAS #	CAS# 68783-96-0
Chemical Name	Petroleum derived calcium salt, overbased
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD Guideline 471
Test Type	Bacterial Reverse Mutation Assay
GLP (Y/N)	Y
Year (Study Performed)	1995
Test System	*Salmonella typhimurium*
Strains Tested	TA98, TA100, TA1535, TA1537, TA1538
Exposure Method	Plate incorporation
Test Substance Doses/concentration levels	250, 500, 1000, 2500 and 5000 ug/plate (initial assay) 1000, 2000, 3000, 4000 and 5000 ug/plate (repeat assay)
Metabolic Activation	With and without (0.5 mL S9 fraction mix of livers of Aroclor 1254 pretreated Sprague Dawley rats. –S9 groups received 0.5mL saline).
Vehicles	Tetrahydrofuran (THF, for test material), Dimethylsulfoxide (DMSO, for positive control substances)
Positive Controls and concentration levels by tester strain and activation status	9-Aminoacridine (9AA), 100 ug/plate- TA1537 without S9 2-Aminoanthracine (2AA), 2.5 ug/plate-all strains with S9 N-Methyl-N-Nitro-N-Nitrosoguanidine (MNNG), 10 ug/plate-TA100, TA1535 without S9 2-Nitrofluorene (2NF), 5 ug/plate-TA98, TA1538 without S9
Vehicle Controls	Tetrahydrofuran 25 uL/plate Dimethylsulfoxide 100 uL/plate
Statistical Analysis	Mean revertant colony count and standard deviation were determined for each dose point.
Dose Range finding Study	Conducted using tester strain TA100 at concentrations up to 5000 ug/plate with and without metabolic activation. Cytotoxicity was evaluated.
Remarks field for test conditions	This study was conducted according to OECD Guideline 471 (1983). Revision to this Guideline in 1997 suggests the addition of the *E. coli* WP2 uvrA or *S. typhimurium* TA 102 tester strains. Since this study was conducted prior to this revision, these strains were not included. Prior to study initiation the solubility of the test substance in the vehicle (tetrahydrofuran) was confirmed. A pretest dose range finding study was conducted using tester strain TA100 at concentrations up to 5000 ug/plate with and without metabolic activation. In the main study there were two treatment sets for each tester strain, with and without metabolic activation. Each of the five tester strains was dosed with five concentrations of test substance (250, 500, 1000, 2500 and 5000 ug/plate), two vehicle controls (THF and DMSO), a nontreated control and a positive control. Three plates/dose

	group/strain/treatment set were evaluated. The results of the initial assay were verified by repeating the assay at dose levels of 1000, 2000, 3000, 4000 and 5000 ug/plate. After 2 days of incubation all plates in the initial and repeat assays were evaluated for gross toxic effects and total revertant colony numbers.
Results	The test substance was not mutagenic in this assay with or without metabolic activation.
Remarks	Toxicity (notable reduction in background lawn and/or 50% reduction in the number of revertant colonies compared to vehicle control) was not observed at any concentration tested with or without metabolic activation in the range finding study. However at the 5000 and 2000 ug/plate levels a haze attributed to the test substance was present. These findings resulted in the selection of concentrations of 250, 500, 1000, 2500 and 5000 ug/plate for the initial study. The test substance did not induce significant increases in revertant colonies (equal to or greater than three times the THF control) in any of the tester strains, at any dose level, with or without metabolic activation in the initial or repeat assays. Beading of the test substance was observed at 5000 ug/plate in all tester strains (with/without activation) and at 4000 ug/plate in tester strain TA1537 (with/without activation) in the repeat assay. The positive controls produced at least a three-fold increase in revertant colonies when compared with the DMSO control in each respective strain. The nontreated and vehicle controls responded appropriately. The 5000 ug/plate concentration of test substance in THF was evaluated analytically for concentration in both the initial and repeat assays. Analysis conformed that the test substance concentration was within 7% of the nominal concentration for both assays.
Conclusions	The test substance was not mutagenic in any strain of *Salmonella typhimurium* tested, including at least one dose above the solubility of the test substance. The genotoxicity NOEL was 5000 ug/plate.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 2/18/00 (RTA-021)

Robust Summary 3-Gentox–-5

Test Substance	
CAS #	CAS# 71786-47-5
Chemical Name	Alkaryl magnesium salt derivative
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD Guideline 473
Test Type	*In Vitro* Chromosomal Aberration Assay in CHO Cells
GLP (Y/N)	Y
Year (Study Performed)	1995
Test System	Chinese hamster ovary cells
Clone Tested	WBL
Culture Preparation and Maintenance	Cells were thawed and cultured in McCoy's 5A Medium containing 10% fetal bovine serum and 2 mM L-glutamine at 37°C, in 4-6% CO_2 in air. Cultures were seeded at 1.2 x 10^6 cells (16-hour harvest) and 0.8 x 10^6 (40-hour harvest) approximately 1 day prior to dosing. Fetal bovine serum was excluded from activated cultures.
Exposure Method	Dilution
Test Substance Doses/concentration levels	A 50 uL sample of concentrations of 10, 20, 40, 80, 120, 160 ug/mL was evaluated with and without metabolic activation.
Metabolic Activation	With and without (0.015 mL/ mL serum free medium) S9 fraction mix of livers of Aroclor 1254 pretreated Sprague Dawley rats and 0.06 mL/ mL serum free medium cofactor mix (13.4 mg/mL NADP and 25 mg/mL DL-Isocitric Acid in distilled water).
Vehicles	Tetrahydrofuran (THF, for test material), acetone (for positive control substances)
Vehicle and Positive Control concentration levels by activation status	Acetone, 5 ug/mL with and without activation Tetrahydrofuran, 5 ug/mL with and without activation N-Methyl-N-Nitro-N-Nitrosoguanidine (MNNG), 0.6 ug/mL without activation 7,12-Dimethylbenz[a]anthracene (DMBA), 10 ug/mL with activation
Statistical Analysis	The number of cells with at least one aberrant chromosome and the number of cells examined in each replicate were used for statistical analysis. The number of aberrant individual chromosomes/cell was not analyzed. Positive control groups were compared to vehicle control by Fisher Exact Test. Each pair of replicates was compared by Fisher Exact Test. Differences between control and treated groups were compared using Fisher Exact Test and if necessary a 2x2 Fisher Tests. A permutation test was performed to test for dose related trends. Significance levels of less than 0.05 were reported.
Test Substance Solubility	Test substance solubility in the vehicle was determined.
Culture Medium Solubility Test	The solubility of the test substance in the culture medium was established at concentrations of 10, 20, 39, 78, 156, 313, 625, 1250, and 2500 ug/mL. Visual and microscopic examinations were made for precipitation at 0, 30 and 180 minutes post preparation. Concentrations showing signs of insolubility at any

	of these time points were considered unsuitable for dosing.
Dose range finding study	Test substance and vehicle controls tested in duplicate cultures each with and without activation. Test substance tested at concentrations of 2.5, 5, 10, 20, 40, 60, 80, 120, and 160 ug/ml. Cytotoxicity and mitotic indices were evaluated.
Remarks field for test conditions	Prior to study initiation the solubility of the test substance and of the positive control materials in the vehicles (tetrahydrofuran/acetone) was confirmed. A pretest dose range finding study was conducted at concentrations up to 160 ug/mL with and without metabolic activation. In the main study there were two treatment sets for each concentration of test substance, with (+S9) and without (-S9) metabolic activation. DMBA (positive control) was tested with activation and MNNG (positive control) was tested without activation. Prepared cultures were treated with test substance or control material and were incubated for 16 hours. A repeat assay was performed using 16 and 40 hour harvest time points. Vehicle, MNNG and DMBA cultures were incubated for 16 hours only. Two to three hours prior to the 16 and 40 hour harvest the spindle inhibitor, Colcemid, was added to each culture to obtain a final concentration of 0.2 ug/mL. Harvested cells were evaluated microscopically for percent confluency, morphology and estimated number of mitotic cells prior to harvest. The test substance treated groups, selected for chromosome analysis based on cell count data and the presence of participate, were as follows: Initial assay +S9 (16 hour harvest) 20, 40 80 ug/mL Initial assay -S9 (16 hour harvest) 20, 40 80 ug/mL Repeat assay +S9 (16 hour harvest) 20, 40 80 ug/mL Repeat assay -S9 (16 hour harvest) 20, 40 80 ug/mL Repeat assay +S9 (40 hour harvest) 80, 120, 160 ug/mL Repeat assay -S9 (40 hour harvest) 80, 120, 160 ug/mL Slides were prepared for these groups using Giemsa stain. Two slides/treatment group were evaluated. 200 metaphase cells (100 per culture) each containing 19-23 chromosomes per treatment group were scored. Chromosomes were counted for each cell. Chromosome aberrations, either chromosome or chromatid type were recorded. The following observations were recorded and excluded from the total aberration frequency: gaps, polyploid and endoreduplicated cells, pulverized chromosomes, Robertsonian translocations, translocations and abnormal monocentric chromosomes. The percent of aberrant cells and the frequency of aberration (%) per treatment group were determined. In order for a test substance to be considered to have induced a positive response compared to vehicle control a statistically significant dose related increase in the percentage of aberrant cells along with a mean percentage of aberrant cells in excess of 5% in at least one treatment group were required. Or, a reproducible and statistically significant response in at least one treatment group with a mean % of aberrant cells exceeding 5% was observed. Test substance concentration verification was performed on the highest stock concentration in both the initial and repeated assays. Results were within 8% of nominal.

Results	The test substance was not mutagenic in this assay with or without metabolic activation.
Remarks	In the culture medium solubility test precipitate and/or cloudiness were present with and without metabolic activation at concentrations of 39 ug/mL and 78 ug/mL and greater. In the pretest toxicity assay, a greater than 50% reduction in cell counts or mitotic activity was not observed at concentrations up to 160 ug/mL. The doses selected for the initial assay were 10, 20, 40, 80, 120 and 160 ug/mL. Cell survival was not significantly reduced when compared to the vehicle control in the initial assay. Cell survival was reduced by at least 50% compared to vehicle control in the repeat assay (40-hr harvest) without metabolic activation at the 160 ug/mL concentration. A greater than 50% reduction in mitotic index was not observed in either the initial or repeat assays at any concentration tested. Precipitation was observed at concentrations greater than 80 ug/mL in the chromosomal aberration assay. Therefore, the highest concentration evaluated at 16 hours was 80 ug/mL. There were no statistically significant differences in the number of chromosomal aberrations at 16 hours with activation and at 40 hours with and without metabolic activation. In the initial 16-hour harvest without activation a statistically significant increase was observed with one dose level different from the vehicle control. However this finding was not evident in the repeat 16-hour harvest without activation. The observed initial increase was not reproducible and was not considered biologically significant. Positive and vehicle control group responses were as expected. The positive control group had a statistically significant higher percentage of aberrant cells than the vehicle control group with and without activation at each harvest interval.
Conclusions	The test material was not genotoxic under the conditions of this study.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 4/10/00 (RTA-026)

Robust Summary 3-Gentox--6

Test Substance	
CAS #	CAS# 71786-47-5
Chemical Name	Alkaryl magnesium salt derivative
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD Guideline 474
Test Type	Mammalian Erythrocyte Micronucleus Test
GLP (Y/N)	Y
Year (Study Performed)	1995
Species	Mouse
Strain	CD-1, 10-12 weeks of age at initiation of treatment
Route of administration	Oral gavage
Duration of test	Three treatments administered approximately 24 hours apart followed by a 24-hour hold period prior to bone marrow sample collection.
Doses/concentration levels	0, 500, 1000 and 2000 mg/kg
Dose volume	10 ml/kg
Sex	Males and females
Frequency of treatment	Three treatments administered approximately 24 hours apart.
Control and treatment groups	Peanut oil vehicle control: 5/sex; cyclophosphamide (in water) positive control: 20 mg/kg, 5/sex; 500, 1000, 2000 mg/kg, 5/sex/kg.
Statistical methods	The following parameters were recorded and evaluated; the ratio of polychromatic to normochromatic erythrocytes, number of polychromatic erythrocytes with micronuclei and number of polychromatic erythrocytes scored. Statistical analysis included means and standard deviations of the micronuclei data and a test of equality of group means. Tests included a one-way analysis of variance, Duncan's Multiple Range test and regression analysis. Residuals from the ANOVA were analyzed by Wilk's Criterion or the Kolomogorov-Smirnov statistic. Nonparametric analyses included the Kruskal-Wallis one way ANOVA followed by Dunn's Summed Rank Test. Dose response was evaluated by Jonkheere's test of ordered response.
Dose range finding study	A dose range finding study was conducted at 500, 1000 and 2000 mg/kg. Percent polychromatic erythrocytes (PCE) were determined by counting 1000 cells. Number of micronuclei/1000 PCE determined.
Remarks field for test conditions	All animals observed for viability twice daily during the dosing period. Detailed clinical observations recorded after each test substance administration. Body weights recorded prior to initiation of dosing. Twenty-four hours after the third dose the animals were sacrificed for bone marrow sampling. Necropsies were not performed. A total of 2000 polychromatic erythrocytes/animal were evaluated for the presence of micronuclei. The percent of PCE in the total population of erythrocytes was determined for each animal by counting the total polychromatic and normochromatic erythrocytes.

Results	
Remarks	All dose rangefinding animals survived and were free of clinical signs. Bone marrow toxicity was not observed at any dose levels tested. Therefore 2000 mg/kg was selected as the high dose for the micronucleus assay. The mid and low doses were selected to be 1/2 and 1/4 of the high dose. All animals survived to scheduled sacrifice and were free of clinical signs. The responses of the vehicle control and positive control groups were appropriate and support the validity of the assay results. The positive control induced a significant increase in mean number of micronucleated polychromatic erythrocytes. In addition it induced cytotoxicity. There were no dose-related increases or statistical differences in micronuclei formation observed at any dose level of the test material. Cytotoxicity was not observed. There were no statistical decreases in the percentage of polychromatic erythrocytes compared to the vehicle control. Chemical analysis of dosing solutions confirmed that they were homogeneously prepared at the desired concentrations and that they were stable for the intended period of use.
Conclusions	Under the conditions of this study the test material did not induce micronuclei in bone marrow erythrocytes and did not induce cytotoxicity in the bone marrow of CD-1 mice. The genotoxicity NOEL was 2000 mg/kg.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 2/11/00 (RTA-007)

Robust Summary 3-Gentox-7

Test Substance	
CAS #	CAS# 71786-47-5
Chemical Name	Alkaryl magnesium salt derivative
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD Guideline 471
Test Type	Bacterial Reverse Mutation Assay
GLP (Y/N)	Y
Year (Study Performed)	1995
Test System	*Salmonella typhimurium*
Strains Tested	TA98, TA100, TA1535, TA1537, TA1538
Exposure Method	Plate incorporation
Test Substance Doses/concentration levels	62.5, 125, 250, 500 and 1000 ug/plate
Metabolic Activation	With and without (0.5 mL S9 fraction mix of livers of Aroclor 1254 pretreated Sprague Dawley rats. –S9 groups received 0.5mL saline.
Vehicles	Tetrahydrofuran (THF, for test material), Dimethylsulfoxide (DMSO, for positive control substances)
Positive Controls and concentration levels by tester strain and activation status	9-Aminoacridine (9AA), 100 ug/plate- TA1537 without S9 2-Aminoanthracine (2AA), 2.5 ug/plate-all strains with S9 N-Methyl-N-Nitro-N-Nitrosoguanidine (MNNG), 10 ug/plate-TA100, TA1535 without S9 2-Nitrofluorene (2NF), 5 ug/plate-TA98, TA1538 without S9
Vehicle Controls	Tetrahydrofuran 25 uL/plate Dimethylsulfoxide 100 uL/plate
Statistical Analysis	Mean revertant colony count and standard deviation were determined for each dose point.
Dose rangefinding study	Conducted using tester strain TA100 at concentrations up to 5000 ug/plate with and without metabolic activation.
Remarks field for test conditions	This study was conducted according to OECD Guideline 471 (1983). Revision to this Guideline in 1997 suggests the addition of the *E. coli* WP2 uvrA or *S. typhimurium* TA 102 tester strains. Since this study was conducted prior to this revision, these strains were not included. Prior to study initiation the solubility of the test substance in the vehicle (tetrahydrofuran) was confirmed. A pretest dose range finding study was conducted using tester strain TA100 at concentrations up to 5000 ug/plate with and without metabolic activation. In the main study there were two treatment sets for each tester strain, with (+S9) and without (-S9) metabolic activation. Each of the five tester strains was dosed with five concentrations of test substance, two vehicle controls (THF and DMSO), a nontreated control and a positive control. Three plates/dose group/strain/treatment set were evaluated. The results of the initial assay were verified by repeating the assay. After 2

	days of incubation all plates in the initial assay and the TA1537 and TA1538 plates in the repeat assay were refrigerated. These plates were evaluated for gross toxic effects and total revertant colony numbers on the following day. In the repeat assay TA98, TA100 and TA1535 were evaluated after 2 days of incubation.
Results	The test substance was not mutagenic in this assay with or without metabolic activation.
Remarks	In the range finding study toxicity (notable reduction in background lawn and/or 50% reduction in the number of revertant colonies compared to vehicle control) was not observed at any concentration tested with or without metabolic activation. However the 5000 and 2000 ug/plate levels were difficult to evaluate due to test substance interference. At 1000 and 500 ug/plate precipitate was observed on the plates. These findings resulted in the selection of concentrations ranging from 62.5 to 1000 ug/plate for the main study. The test substance did not induce significant increases in revertant colonies (equal to or greater than three times the THF control) in any of the tester strains, at any dose level, with or without metabolic activation in the initial or repeat assays. A greater than 50% reduction in mean number of revertant colonies compared to THF were observed in the initial assay in TA1537 without activation at 250 ug/plate. In TA1535 with activation, no background /no revertants was noted in the initial assay for all three plates at 250 and 500 and in two plates at 1000 ug/plate. The significance of these reductions is difficult to interpret since the findings were inconsistent between assays and dose levels. Precipitate was seen on all plates at 1000 ug/plate (+/- S9) in the initial and repeat assays. The positive controls produced at least a three-fold increase in revertant colonies compared with the DMSO control in their respective strains. Nontreated and vehicle controls were acceptable and were consistent with data from previous assays. The 1000 ug/plate concentration of test substance in THF was evaluated analytically for stability, concentration and homogeneity. Analysis conformed that the test substance was stable and homogeneous in THF for the intended period of use. The 1000 ug/plate solution was prepared and assayed twice during the study. The result for the first preparation was 121% above nominal. The result of the second preparation was 15% above nominal.
Conclusions	The test substance was not mutagenic in any strain of *Salmonella typhimurium* tested, including at least one dose above the solubility of the test substance. The genotoxicity NOEL was 1000 ug/plate.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 2/14/00 (RTA-008)

Robust Summary 3-Gentox-8

Test Substance	
CAS #	CAS# Analog of 78330-12-8
Chemical Name	C15-C21 alkaryl sodium salt derivative
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD Guideline 471
Test Type	Bacterial Reverse Mutation Assay
GLP (Y/N)	Y
Year (Study Performed)	1983
Test System	*Salmonella typhimurium*
Strains Tested	*Salmonella typhimurium* tester strains TA98, TA100, TA1535, TA1537 and TA 1538
Exposure Method	Plate incorporation
Test Substance Doses/concentration levels	0.1, 0.3, 1.0, 3.0 and 10 mg/plate
Metabolic Activation	With and without 25 ul/plate S9 fraction mix of livers of Aroclor 1254 pretreated Sprague Dawley rats)
Vehicle	Sterile distilled water
Tester strain, activation status, Positive Controls and concentration level	TA98 +S9 2-aminoanthracene 2.0 ug/plate TA98 -S9 2-nitroflourene 10.0 ug/plate TA100 +S9 2-aminoanthracene 2.0 ug/plate TA100 -S9 sodium azide 1.0 ug/plate TA1535 +S9 2-aminoanthracene 2.0 ug/plate TA1535 -S9 sodium azide 1.0 ug/plate TA1537 +S9 2-aminoanthracene 2.0 ug/plate TA1537 -S9 9-Aminoacridine 50.0 ug/plate TA1538 +S9 2-aminoanthracene 2.0 ug/plate TA1538 -S9 2-nitroflourene 10.0 ug/plate
Vehicle Control	Sterile distilled water
Statistical Analysis	Mean revertant colony count and standard deviation were determined for each dose point.
Dose Range finding Study	Conducted using tester strain TA100 at dose levels of test material ranging from 0.005 to 10 mg/plate without S9.
S9 Optimization Study	Conducted using tester strains TA98 and TA100, and a dose level of test material of 10 mg/plate and concentrations of S9 mix ranging from 25 to 250 ul S-9/plate.
Remarks field for test conditions	This study was conducted according to OECD Guideline 471 (1983). Revisions to this Guideline in 1997 suggest the addition of the *E. coli* WP2 uvrA or *S. typhimurium* TA 102 tester strains. Since this study was conducted prior to this revision, these strains were not included. In the main study there were two treatment sets for each tester strain, with (+S9)

	and without (-S9) metabolic activation. Each of the tester strains was dosed with five concentrations of test substance, vehicle controls, and a positive control. Three plates/dose group/strain/treatment set were evaluated. 0.1 ml of test material, positive control or vehicle control were added to each plate along with 0.1 ml of tester strain, S9 mix (if needed) and 2.0 ml of top agar. This was overlaid onto the surface of 25 ml minimal bottom agar in a petri dish. Plates were incubated for 48 hours at 37°C. The condition of the bacterial background lawn was evaluated for cytotoxicity and test article precipitate.
Results	The test substance was not mutagenic in this assay with or without metabolic activation.
Remarks	Slight cytotoxicity was observed in the dose range finding study with tester strain TA100 without metabolic activation. The S9 optimization study was performed using TA98 and TA100 at 10 mg/plate and concentrations of S9 mix of 25-250 ul. In the absence of any effect 25 ul S9 mix/plate was used in the mutagenicity study. In the main study the test material was not mutagenic to any strain. It was slightly cytotoxic to TA100 in the absence of metabolic activation. Positive control responses were acceptable.
Conclusions	Under the conditions of this study, the test material was not mutagenic with or without metabolic activation.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 10/4/00 (RTA-070)

Robust Summary 3-Gentox-9

Test Substance	
CAS #	Analog of CAS# 70024-69-0
Chemical Name	C20-C24 alkaryl calcium salt derivative
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD Guideline 471
Test Type	Bacterial Reverse Mutation Assay
GLP (Y/N)	Y
Year (Study Performed)	1989
Test System	*Salmonella typhimurium* and *Escherichia Coli*
Strains Tested	*Salmonella typhimurium* tester strains TA98, TA100, TA1535, TA1537; *Escherichia Coli* tester strain WP2*uvr*A
Exposure Method	Plate incorporation
Test Substance Doses/concentration levels	0.1, 0.33, 1.0, 3.33 and 10 mg/plate
Metabolic Activation	With and without (500 ul of 10% S9 fraction mix of livers of Aroclor 1254 pretreated Sprague Dawley rats)
Vehicle	Pluronic F127 25% w/w in ethanol
Tester strain, activation status, Positive Controls and concentration level	TA98 +S9 2-aminoanthracene 2.0 ug/plate TA98 -S9 2-nitroflourene 10.0 ug/plate TA100 +S9 2-aminoanthracene 2.0 ug/plate TA100 -S9 sodium azide 1.0 ug/plate TA1535 +S9 2-aminoanthracene 2.0 ug/plate TA1535 -S9 sodium azide 1.0 ug/plate TA1537 +S9 2-aminoanthracene 2.0 ug/plate TA1537 -S9 ICR-191 2.0 ug/plate WP2*uvr*A +S9 2-aminoanthracene 80.0 ug/plate WP2*uvr*A –S9 ICR-191 50.0 ug/plate
Vehicle Control	Pluronic F127 25% w/w in ethanol
Statistical Analysis	Mean revertant colony count and standard deviation were determined for each dose point.
Dose Rangefinding Study	Conducted using tester strains TA98 and TA100, and dose levels of test material ranging from 0.003 to 10 mg/plate.
S9 Optimization Study	Conducted using tester strains TA98 and TA100, and a dose level of test material of 10 mg/plate and concentrations of S9 mix ranging from 25 to 400 ul S-9/plate. Cytotoxicity was evaluated.
Remarks field for test conditions	In the main study there were two treatment sets for each tester strain, with (+S9) and without (-S9) metabolic activation. Each of the tester strains was dosed with five concentrations of test substance, vehicle controls, and a positive control. Three plates/dose group/strain/treatment set were evaluated. The results of the initial

	assay were confirmed in a second independent experiment. 100 ul of test material, positive control or vehicle control were added to each plate along with 100 ul of tester strain, S9 mix (if needed) and 2.0 ml of top agar. This was overlaid onto the surface of 25 ml minimal bottom agar in a petri dish. Plates were incubated for 48 hours at 37°C. The condition of the bacterial background lawn was evaluated for cytotoxicity and test article precipitate.
Results	The test substance was not genotoxic in this assay with or without metabolic activation.
Remarks	No cytotoxicity was observed in the dose rangefinding study with tester strains TA100 and WP2*uvr*A with or without metabolic activation as evidenced by normal background lawn and no reduction in the number of revertants/plate. The S9 optimization study was performed using TA98 and TA100 with the highest non-cytotoxic dose of test article, (10,000 ug/plate) and concentrations of S9 mix of 25-400 ul. In the absence of any effect 25 ul S9 mix/plate was used in the mutagenicity study. The test material formed a stable emulsion with the vehicle and the dilutions were well dispersed in the top agar. However after incubation test material was visible at all dose levels in the top layer. The test material was not cytotoxic to any tester strain. In the repeat study statistically significant increases in revertant colonies were observed in TA1535 without metabolic activation and in WP2*uvr*A with metabolic activation. However since these findings were not found during the first experiment they were not considered biologically significant. The positive control for each respective test strain exhibited at least a 3-fold increase (with or without S9) over the mean value of the vehicle control for a given strain, confirming the expected positive control response. Dosing solution analysis confirmed that high dose concentration was acceptable.
Conclusions	Under the conditions of this study, the test material was not mutagenic with or without metabolic activation.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 10/4/00 (RTA-069)

Robust Summary 3-Gentox10

Test Substance	
CAS #	CAS# 68783-96-0
Chemical Name	Petroleum derived calcium salt, overbased
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD Guideline 473
Test Type	*In Vitro* Chromosomal Aberration Assay in CHO Cells
GLP (Y/N)	Y
Year (Study Performed)	1995
Test System	Chinese hamster ovary cells
Clone Tested	WBL
Culture Preparation and Maintenance	Cells were thawed and cultured in McCoy's 5A Medium containing 10% fetal bovine serum and 2 mM L-glutamine at 37°C, in 4-6% CO_2 in air. Cultures were seeded at 1.2 x 10^6 cells (16-hour harvest) and 0.8 x 10^6 (40-hour harvest) approximately 1 day prior to dosing. Fetal bovine serum was excluded from activated cultures.
Exposure Method	Dilution
Test Substance Doses/concentration levels	A 50 uL sample of concentrations of 10, 20, 40, 80, 120, 160 ug/mL was evaluated with and without metabolic activation.
Metabolic Activation	With and without (0.015 mL/ mL serum free medium) S9 fraction mix of livers of Aroclor 1254 pretreated Sprague Dawley rats and 0.06 mL/ mL serum free medium cofactor mix (13.4 mg/mL NADP and 25 mg/mL DL-Isocitric Acid in distilled water).
Vehicles	Tetrahydrofuran (THF, for test material), acetone (for positive control substances)
Vehicle and Positive Control concentration levels by activation status	Acetone, 5 ug/mL with and without activation Tetrahydrofuran, 5 ug/mL with and without activation N-Methyl-N-Nitro-N-Nitrosoguanidine (MNNG), 0.6 ug/mL without activation 7,12-Dimethylbenz[a]anthracene (DMBA), 10 ug/mL with activation
Statistical Analysis	The number of cells with at least one aberrant chromosome and the number of cells examined in each replicate were used for statistical analysis. The number of aberrant individual chromosomes/cell was not analyzed. Positive control groups were compared to vehicle control by Fisher Exact Test. Each pair of replicates was compared by Fisher Exact Test. Differences between control and treated groups were compared using Fisher Exact Test and if necessary a 2x2 Fisher Tests. A permutation test was performed to test for dose related trends. Significance levels of less than 0.05 were reported.
Test Substance Solubility	Test substance solubility in the vehicle was determined.
Culture Medium Solubility Test	The solubility of the test substance in the culture medium was established at concentrations of 10, 20, 39, 78, 156, 313, 625, 1250, and 2500 ug/mL. Visual and microscopic examinations were made for precipitation at 0, 30 and 180 minutes post preparation. Concentrations showing signs of insolubility at any

	of these time points were considered unsuitable for dosing.
Dose rangefinding study	Test substance and vehicle controls tested in duplicate cultures each with and without activation. Test substance tested at concentrations of 0.625, 1.25, 2.5, 5, 10, 20, 40, 80 and 160 ug/ml. Cytotoxicity and mitotic indices were evaluated.
Remarks field for test conditions	Prior to study initiation the solubility of the test substance and of the positive control materials in the vehicles (tetrahydrofuran/acetone) was confirmed. A pretest dose range finding study was conducted at concentrations up to 160 ug/mL with and without metabolic activation. In the main study there were two treatment sets for each concentration of test substance, with (+S9) and without (-S9) metabolic activation. DMBA (positive control) was tested with activation and MNNG (positive control) was tested without activation. Prepared cultures were treated with test substance or control material and were incubated for 16 hours. A repeat assay was performed using 16 and 40 hour harvest time points. Vehicle, MNNG and DMBA cultures were incubated for 16 hours only. Two to three hours prior to the 16 and 40-hour harvest the spindle inhibitor, Colcemid, was added to each culture to obtain a final concentration of 0.2 ug/mL. Harvested cells were evaluated microscopically for percent confluency, morphology and estimated number of mitotic cells prior to harvest. The test substance treated groups, selected for chromosome analysis based on cell count data and the presence of participate, were as follows: Initial assay +S9 (16 hour harvest) 10, 20, 40 ug/mL Initial assay -S9 (16 hour harvest) 10, 20, 40 ug/mL Repeat assay +S9 (16 hour harvest) 10, 20, 40 ug/mL Repeat assay -S9 (16 hour harvest) 10, 20, 40 ug/mL Repeat assay +S9 (40 hour harvest) 10, 20, 40 ug/mL Repeat assay -S9 (40 hour harvest) 10, 20, 40 ug/mL Slides were prepared for these groups using Giemsa stain. Two slides/treatment group were evaluated. 200 metaphase cells (100 per culture) each containing 19-23 chromosomes per treatment group were scored. Chromosomes were counted for each cell. Chromosome aberrations, either chromosome or chromatid type were recorded. The following observations were recorded and excluded from the total aberration frequency: gaps, polyploid and endoreduplicated cells, pulverized chromosomes, Robertsonian translocations, translocations and abnormal monocentric chromosomes. The percent of aberrant cells and the frequency of aberration (%) per treatment group were determined. In order for a test substance to be considered to have induced a positive response compared to vehicle control a statistically significant dose related increase in the percentage of aberrant cells along with a mean percentage of aberrant cells in excess of 5% in at least one treatment group were required. Or, a reproducible and statistically significant response in at least one treatment group with a mean % of aberrant cells exceeding 5% was observed. Test substance concentration verification, uniformity and stability were performed on the highest stock concentration in both the initial and/or repeated

	assays. Results were within 6% of nominal. Samples were homogeneous and stable for the intended period of use.
Results	The test substance was not mutagenic in this assay with or without metabolic activation.
Remarks	In the culture medium solubility test precipitate and/or cloudiness were present with and without metabolic activation at concentrations of 39 ug/mL and greater. In the pretest toxicity assay there was an 81% reduction (compared to vehicle control) in cell survival at 160 ug/mL without metabolic activation. The doses selected for the initial assay were 10, 20, 40, 80, 120 and 160 ug/mL. A greater than 50% reduction in cell survival and/or mitotic index was not observed in either the initial or repeat assays. Precipitation was observed at concentrations greater than 40 ug/mL in the chromosomal aberration assay. Therefore, 40 ug/mL was considered to be the limit of solubility for the test substance and was selected as the highest test concentration to be evaluated. There were no statistically significant differences in the number of chromosomal aberrations between the treated and vehicle control groups in either the initial or repeat assay at any dose level evaluated (10, 20 and 40 ug/mL with and without metabolic activation). In the initial 16-hour harvest, there were statistically significant increases with dose in the percent of aberrant cells for both the activated and nonactivated evaluations. These trends were not reproducible in the repeat 16-hour harvest and therefore were not considered biologically significant. Positive and vehicle control group responses were as expected. The positive control groups have frequencies of aberrations outside the normal range of the vehicle control and at least twice the vehicle control value.
Conclusions	The test material was not genotoxic under the conditions of this study.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 4/11/00 (RTA-027)