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**American  
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Council** Good Chemistry  
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October 13, 2005

US Environmental Protection Agency  
PO Box 1473  
Merrifield, VA 22116  
ATTN: Chemical Right-to-Know Program

Re: Petroleum Additives Panel Health Environmental and Regulatory Task Group HPV Challenge  
Final Submission for the Alkaryl Sulfonate Category

To Whom It May Concern:

On October 8, 2001, the American Chemistry Council, Petroleum Additives Panel, Health, Environmental and Regulatory Task Group (HERTG) submitted a test plan and supporting robust summaries for the following twelve chemicals in the alkaryl sulfonate category:

- Sulfonic acids, petroleum, calcium salts - (CAS # 61789-86-4, referred to in this report as petroleum derived calcium salt)
- Sulfonic acids, petroleum, barium salts - (CAS # 61790-48-5, referred to in this report as petroleum derived barium salt)
- Sulfonic acids, petroleum, sodium salts - (CAS # 68608-26-4, referred to in this report as petroleum derived sodium salt)
- Sulfonic acids, petroleum, calcium salts, overbased - (CAS # 68783-96-0, referred to in this report as petroleum derived calcium salt, overbased)
- Benzenesulfonic acid, mono-C16-C24 alkyl derivatives, calcium salts - (CAS # 70024-69-0, referred to in this report as C16-C24 alkaryl calcium salt derivative)
- Benzenesulfonic acid, mono-C15-C30 branched alkyl and di-C11-C13 branched and linear alkyl derivatives, calcium salts, overbased - (CAS # 71486-79-8, referred to in this report as mixed mono-C15-C30 and di-C11-C13 alkaryl calcium salt, overbased derivative)
- Benzenesulfonic acid, mono-C15-C30 branched alkyl and di-C11-C13 branched and linear alkyl derivatives - (CAS # 71549-79-6, referred to in this report as mixed mono-C15-C30 and di-C11-C13 alkaryl derivative)
- Benzenesulfonic acid, mono and dialkyl derivatives, magnesium salts - (CAS # 71786-47-5, referred to in this report as alkaryl magnesium salt derivative)
- Benzenesulfonic acid, C15-C30 alkyl derivatives, sodium salts - (CAS # 78330-12-8, referred to in this report as C15-C30 alkaryl sodium salt derivative)
- Benzenesulfonic acid, C14-C24 branched and linear alkyl derivatives, calcium salts - (CAS # 115733-09-0, referred to in this report as C14-C24 alkaryl calcium salt derivative)
- Benzenesulfonic acid, C14-C24 branched and linear alkyl derivatives, calcium salts, overbased - (CAS # 115733-10-3, referred to in this report as C14-C24 alkaryl calcium salt, overbased derivative)
- Benzenesulfonic acid, C14-C24 branched and linear alkyl derivatives - (CAS # 115829-36-2, referred to in this report as C14-C24 alkaryl derivative)



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U.S. EPA  
October 13, 2005  
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The HERTG has completed the proposed testing described in the test plan for the alkaryl sulfonate category. Attached to this letter are the HERTG's final HPV submission of the category analysis document and corresponding robust summaries which are submitted to fulfill the HERTG's HPV commitment for the alkaryl sulfonate category.

If you have any questions or comments, please contact me at (703) 741-5607 or via email at Sarah\_McLallen@americanchemistry.com.

Sincerely yours,

Sarah Loftus McLallen  
Manager, HERTG

**Attachments: Final Submission for Alkaryl Sulfonate Category & Robust Summary Document**

201-16048B

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

**Summary prepared by:** Petroleum Additives Panel  
Health & Environmental Research Task Group  
October 2005

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**1.1 Melting and Boiling Points**

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

**2.0 Environmental Fate and Pathways****Category: Alkaryl Sulfonates****2.1 Water Solubility****Robust Summary 3 – WS - 1**

CAS No.	CAS# 115733-09-0
Test Substance Name	C14-24 alkaryl calcium salt derivative
Remarks	For more information on this chemical, see Section 2.0 “General Substance Information” in HERTG’s Final Submission for Alkaryl Sulfonate Category.
Method/Guideline	Flask Method, Method A6 of Commission Directive 92/69/EEC and OECD Method 105
GLP (Y/N)	Yes
Year	2004
Remarks for Test Conditions	<p>The solubility in water of a substance is specified by the saturation mass concentration of the substance in water at a given temperature. A preliminary test was conducted by diluting 0.2645 g of the test material to 600 mL with glass double-distilled water. After shaking at 30°C for 17.5 hours and standing at 20°C for 3 hours, the solution was centrifuged at 13,500 rpm for 20 minutes and analyzed.</p> <p>Based on the preliminary results, mixtures of double distilled water and test substance were added to each of three conical flasks. The flasks were shaken for approximately 24, 48 and 72 hours (one flask/time period) at 100 rpm and 30°C. The flasks were then allowed to stand for approximately 24 hours at 20°C. The contents of each flask was then centrifuged at 13,500 rpm for 20 minutes and transferred to measuring cylinders. The pH of each solution was measured. The concentration of the test material in the sample solutions was determined in duplicate with duplicate injections of each sample by an LCMS method with direct flow injection.</p>
Results	<p>Prior to centrifuging the samples were clear and colorless with excess test material floating on the surface. Following centrifugation the supernatants were clear, colorless and free from excess test material.</p> <p>The water solubility of the test material was determined to be <math>4.79 \times 10^{-4}</math> g/l at <math>20.0 \pm 0.5^\circ\text{C}</math>.</p> <p>The linearity of the detector response in respect to concentration was acceptable over a range of concentrations up to 11.900 mg/L with a correlation coefficient of 0.962. Recovery analysis of the sample procedure was assessed and proved adequate for the test.</p>
Value (g/L) at temperature °C	$4.79 \times 10^{-4}$ g/l at $20.0 \pm 0.5^\circ\text{C}$

Conclusions	The solubility of the test material in double distilled water was found to be $4.79 \times 10^{-4}$ g/l at $20.0 \pm 0.5^\circ\text{C}$ .
Data Quality	Reliable without restriction (Klimisch Code)
References	Determination of Water Solubility SafePharm Laboratories Project No.: 1666/013 (27 Jul 2004)

**Robust Summary 3 - WS - 2**

CAS No.	CAS# 68608-26-4
Test Substance Name	Sulfonic acids, petroleum, sodium salts
Remarks	For more information on this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Alkaryl Sulfonate Category.
Method/Guideline	Flask Method, Method A6 of Commission Directive 92/69/EEC and OECD Method 105
GLP (Y/N)	Yes
Year	2004
Remarks for Test Conditions	<p>The solubility in water of a substance is specified by the saturation mass concentration of the substance in water at a given temperature. A preliminary test was conducted by diluting 0.1305 g of the test material to 600 mL with glass double-distilled water. After shaking at 30°C for 3 hours and standing at 20°C for 3 hours, the solution was centrifuged at 13,500 rpm for 20 minutes and analyzed.</p> <p>Based on the preliminary results and subsequent definitive test results, mixtures of double distilled water and test substance were added to each of nine conical flasks. The flasks were shaken for approximately 24, 48, 72, 96, 120 and 144 hours at 30°C (Flasks 1-6) and for approximately 24, 28 and 72 hrs at 30°C (Flasks 7-9). After standing at 20°C for not less than 24 hours, the contents of each flask were centrifuged and/or filtered as follows: Flasks 1-6 were sampled, centrifuged at 13500 rpm for 20 minutes and the supernatant removed by pipettes. Flasks 7 to 9 were sampled and filtered through 0.2 um filters. The concentration of the test material in the sample solutions was determined in duplicate with duplicate injections of each sample by an HPLC method with Mass Selective detection.</p>
Results	<p>Prior to sampling the samples contained excess test material and were clear and colorless. After sampling and treatment the samples were clear, colorless and visually free from excess test material.</p> <p>The water solubility of the test material was shown to increase with time shaken at 30°C. It appeared that complete saturation of the test samples had not occurred. However the test material contained mineral oil and this could have acted as a co-solvent. There was also the possibility that with time shaken at 30°C, gradual emulsification of the test samples occurred causing an apparent increase in solubility. While the test samples were clear and colorless it is possible that micro-dispersions could have been present and undetected. Filtration of the samples had no effect on solubility. Based</p>

	<p>on these considerations it was determined that the water solubility should be expressed as an upper limit value (based on the sample taken at 144 hrs at 30°C) and that the results should be considered as an overestimate of the water solubility. This was concluded since the experimental value was found to be higher than would be expected based on chemical structure and computer estimation. The computer-based estimate (WSKOWWIN, version 1.41 USEPA, 2000) was calculated to be 0.0144 mg/L at 25°C. This was based on a molecular weight of 432.64 for the total test material with approximately 330 of the molecular weight contributed from alkyl/aromatic groups which did not take into account any mineral oil or impurities in the test material.</p> <p>The water solubility of the test material was determined to be less than or equal to <math>6.38 \times 10^{-3}</math> g/l at <math>20.0 \pm 0.5^\circ\text{C}</math>.</p> <p>The linearity of the detector response in respect to concentration was acceptable over a range of concentrations of 0 to 50 mg/L with a correlation coefficient of 0.998. Recovery analysis of the sample procedure was assessed and proved adequate for the test.</p>
Value (g/L) at temperature °C	Less than or equal to $6.38 \times 10^{-3}$ g/l at $20.0 \pm 0.5^\circ\text{C}$
Conclusions	The solubility of the test material in double distilled water was found to be less than or equal to $6.38 \times 10^{-3}$ g/l at $20.0 \pm 0.5^\circ\text{C}$ .
Data Quality	Reliable with restriction (Klimisch Code). Restriction due to the fact that the water solubility was expressed as an upper limit value and that it was judged that the results should be considered as an overestimate of the water solubility.
References	Determination of Water Solubility SafePharm Laboratories Project No.: 1666/014 (04 Oct 2004)

**Robust Summary 3 – WS - 3**

CAS No.	CAS# 61790-48-5
Test Substance Name	Sulfonic acids, petroleum, barium salts
Remarks	For more information on this chemical, see Section 2.0 “General Substance Information” in HERTG’s Final Submission for Alkaryl Sulfonate Category.
Method/Guideline	Flask Method, Method A6 of Commission Directive 92/69/EEC and OECD Method 105
GLP (Y/N)	Yes
Year	2004
Remarks for Test Conditions	<p>The solubility in water of a substance is specified by the saturation mass concentration of the substance in water at a given temperature. A preliminary test was conducted by diluting 0.1325 g of the test material to 250 mL with glass double-distilled water. After shaking at 30°C for 3 hours and standing at 20°C for 20 hours, the solution was centrifuged at 13,500 rpm for 20 minutes and analyzed.</p> <p>Based on the preliminary results, mixtures of double distilled water and test substance were added to each of three conical flasks. The flasks were shaken for approximately 24, 48 and 72 hours (one flask/time period) at 100 rpm and 30°C. The flasks were then allowed to stand for approximately 24 hours at 20°C. The pH of each solution was measured. The concentration of the test material in the sample solutions was determined in duplicate with duplicate injections of each sample by an LCMS method with direct flow injection.</p>
Results	<p>The samples were clear and colorless and free of excess test material. Prior to treatment the samples were clear and colorless with excess test material present at the bottom of the flasks. The linearity of the detector response in respect to concentration was acceptable over a range of concentrations up to 20 mg/L with a correlation coefficient of 0.998. Recovery analysis of the sample procedure was assessed and proved adequate for the test.</p> <p>The water solubility of the test material was determined to be less than <math>1.03 \times 10^{-3}</math> g/l at <math>20.0 \pm 0.5^\circ\text{C}</math>. A limit value was provided since it was evident from sample spectra that the chromatography peak did not relate to the test material itself but rather to low molecular weight, water soluble fractions or impurities from the test material.</p> <p>Recovery analysis of the sample procedure were assessed and proved adequate for the test.</p>
Value (g/L) at temperature °C	$<1.03 \times 10^{-3}$ g/l at $20.0 \pm 0.5^\circ\text{C}$ .
Conclusions	The solubility of the test material in double distilled water

	was found to be $< 1.03 \times 10^{-3}$ g/l at $20.0 \pm 0.5^\circ\text{C}$ .
Data Quality	Reliable without restriction (Klimisch Code)
References	Determination of Water Solubility SafePharm Laboratories Project No.: 1666/012 (10 Sep 2004)

**Robust Summary 3 – WS – 4**

CAS No.	CAS# 78330-12-8
Test Substance Name	Benzenesulfonic acid,C15-C30 alkyl. derivs., sodium salts
Remarks	For more information on this chemical, see Section 2.0 “General Substance Information” in HERTG’s Final Submission for Alkaryl Sulfonate Category.
Method/Guideline	Flask Method, Method A6 of Commission Directive 92/69/EEC and OECD Method 105
GLP (Y/N)	Yes
Year	2004
Remarks for Test Conditions	<p>The solubility in water of a substance is specified by the saturation mass concentration of the substance in water at a given temperature. A preliminary test was conducted by diluting 0.0098 g of the test material to 250 mL with glass double-distilled water. After shaking at 30°C for 3 hours and standing at 20°C for 19 hours, the solution was centrifuged at 40,000 g for 20 minutes and analyzed.</p> <p>Based on the preliminary results, mixtures of double distilled water and test substance were added to each of three conical flasks. The flasks were shaken for approximately 24, 48 and 72 hours (one flask/time period) at 100 rpm and 30°C. The flasks were then allowed to stand for approximately 24 hours at 20°C. The contents of each flask was filtered through 0.2 µm filters and transferred to measuring cylinders. The pH of each solution was measured. The concentration of the test material in the sample solutions was determined in duplicate with duplicate injections of each sample by an LCMS method with direct flow injection.</p>
Results	<p>The filtered samples were clear and colorless and free of excess test material. Prior to filtration the samples were slightly hazy with excess test material dispersed and suspended in solution. Filtration recoveries showed that approximately 30% of the test material was either lost during analysis or absorbed by the filters. Therefore a recovery correction factor was used at the 10 mg/L nominal concentration level.</p> <p>The water solubility of the test material was determined to be <math>3.82 \times 10^{-2}</math> g/l at 20.0 ±0.5°C.</p> <p>Recovery analysis of the sample procedure were assessed and proved adequate for the test.</p>
Value (g/L) at temperature °C	$3.82 \times 10^{-2}$ g/l at 20.0 ±0.5°C
Conclusions	The solubility of the test material in double distilled water was found to be $3.82 \times 10^{-2}$ g/l at 20.0 ±0.5°C.
Data Quality	Reliable without restriction (Klimisch Code)

References	Determination of Water Solubility SafePharm Laboratories Project No.: 1666/030 (10 Sep 2004)
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**Robust Summary 3 – WS - 5**

CAS No.	CAS# 61789-86-4
Test Substance Name	Sulfonic acids, petroleum, calcium salts
Remarks	For more information on this chemical, see Section 2.0 “General Substance Information” in HERTG’s Final Submission for Alkaryl Sulfonate Category.
Method/Guideline	Flask Method, Method A6 of Commission Directive 92/69/EEC and OECD Method 105
GLP (Y/N)	Yes
Year	2004
Remarks for Test Conditions	<p>The solubility in water of a substance is specified by the saturation mass concentration of the substance in water at a given temperature. A preliminary test was conducted by diluting 0.0511 g of the test material to 250 mL with glass double-distilled water. After shaking at 30°C for 3 hours and standing at 20°C for 18 hours, the solution was centrifuged at 13,500 rpm for 20 minutes and analyzed.</p> <p>Based on the preliminary results, mixtures of double distilled water and test substance were added to each of three sealed conical flasks. The flasks were shaken for approximately 24, 48 and 72 hours (one flask/time period) at 100 rpm and 30°C. The flasks were then allowed to stand for approximately 24 hours at 20°C. The contents of each flask was then centrifuged at 13,500 rpm for 20 minutes and transferred to measuring cylinders. The pH of each solution was measured. The concentration of the test material in the sample solutions was determined in duplicate with duplicate injections of each sample by an LC-MS method with direct flow injection.</p>
Results	<p>Prior to centrifuging the samples were clear and colorless with excess test material floating on the surface. Following centrifugation the supernatants were clear, colorless and free from excess test material.</p> <p>There was a spread of results for all samples ranging from approximately <math>2 \times 10^{-4}</math> to <math>6 \times 10^{-4}</math> g/L. Although this was a relatively wide range in percentage terms it is not in terms of concentration. Excluding one or two data points does not alter the overall result significantly. Given the method of analysis and the difficult nature of the test substance (low water soluble reaction product containing mineral oil) the mean concentration was considered an appropriate expression of the test materials water solubility.</p> <p>The water solubility of the test material was determined to be <math>4.02 \times 10^{-4}</math> g/L at <math>20.0 \pm 0.5^\circ\text{C}</math>.</p>

	The linearity of the detector response in respect to concentration was acceptable over a range of concentrations up to 10 mg/L with a correlation coefficient of 0.999. Recovery analysis of the sample procedure was assessed and proved adequate for the test.
Value (g/L) at temperature °C	4.02 x 10 <sup>-4</sup> g/L at 20.0 ±0.5°C
Conclusions	The solubility of the test material in double distilled water was found to be 4.02 x 10 <sup>-4</sup> g/L at 20.0 ±0.5°C.
Data Quality	Reliable without restriction (Klimisch Code)
References	Determination of Water Solubility SafePharm Laboratories Project No.: 1666/015 (10 Sep 2004)

## 2.2 Biodegradation

### **Robust Summary 3-Biodeg-1**

<u>Test Substance</u>	
CAS #	61789-86-4
Chemical Name	Sulfonic acids, petroleum, calcium salts
Remarks	For more information on this chemical, see Section 2.0 “General Substance Information” in HERTG’s Final Submission for Alkaryl Sulfonate Category.
<u>Method</u>	
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	<p><u>Inoculum:</u> 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.</p> <p><u>Concentration of test chemical:</u> 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.</p> <p><u>Temp of incubation:</u> 23 ± 1°C</p> <p><u>Dosing procedure:</u> 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.</p> <p><u>Sampling frequency:</u> The oxygen uptake was monitored continuously and recorded every 4 hours throughout the test.</p> <p><u>Controls:</u> Yes (blank and positive controls per guideline); abiotic and toxicity checks were not included. Sodium benzoate was used as the positive control.</p> <p><u>Analytical method:</u> Oxygen uptake was measured using a BI-1000 electrolytic respirometer system.</p>

	<u>Method of calculating measured concentrations:</u> N/A <u>Other:</u> The inoculum was pre-adapted to the test substance for 14 days.
<u>Results</u>	
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	
<u>Conclusions</u>	8.6% in 28 days. The reference substance, sodium benzoate, reached a level of 88.8% in the same test period.
<u>Data Quality</u>	(1) Reliable without restriction
<u>References</u>	This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information).
<u>Other</u>	Updated: 9-6-00

### Robust Summary 3-Biodeg-2

<u>Test Substance</u>	
CAS #	71486-79-8
Chemical Name	Benzensulfonic acid, mono-C15-30-branched alkyl and di-C11-13-branched and linear alkyl derivatives, calcium salts, overbased
Remarks	For more information on this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
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	<p><u>Inoculum:</u> 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.</p> <p><u>Concentration of test chemical:</u> 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.</p> <p><u>Temp of incubation:</u> 23 ± 1°C</p> <p><u>Dosing procedure:</u> 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.</p> <p><u>Sampling frequency:</u> The oxygen uptake was monitored continuously and recorded every 4 hours throughout the test.</p> <p><u>Controls:</u> Yes (blank and positive controls per guideline); abiotic and toxicity checks were not included. Sodium benzoate was used as the positive control.</p> <p><u>Analytical method:</u> Oxygen uptake was measured using a BI-1000 electrolytic respirometer system.</p> <p><u>Method of calculating measured concentrations:</u> N/A</p>

	<u>Other</u> : The inoculum was pre-adapted to the test substance for 14 days.
<u>Results</u>	
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	
<b>Conclusions</b>	8.6% in 28 days.
<u>Data Quality</u>	(1) Reliable without restriction
<u>References</u>	This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information).
<u>Other</u>	Updated: 9-6-00

**Robust Summary 3- Biodeg-3**

<b><i>Test Substance</i></b>	
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 <u>alkaryl magnesium salt derivative</u> , in HERTG's Final Submission 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 the chemicals, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Alkaryl Sulfonate Category.
<b><u>Method</u></b>	
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	<p>Inoculum: Activated sewage sludge from domestic WWTP prepared per test guideline. Inoculum was not acclimated.</p> <p>Replicates: Triplicates for test substance, positive control material, and control blank.</p> <p>Temperature of incubation: 20 – 23 °C</p> <p>Dosing procedure: Neat test chemical was gravimetrically determined on glass cover slips, which were then added to culture medium in test vessels.</p> <p>Sampling: Days 2, 4, 7, 10, 14, 17, 21, 24, 29 (after acidification on day 28)</p> <p>Concentration of test substance: Loading into each of 3 test vessels were 19.9, 20.1, and 20.0 mg C/L.</p> <p>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.</p> <p>Analytical method: Titration of residual Ba(OH)<sub>2</sub> (0.05 N initially) in trapping solution, using 0.1N HCl.</p> <p>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.</p>

<b><u>Results</u></b>	
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
<b><u>Conclusions</u></b>	1.5% in 28 days
<b><u>Data Quality</u></b>	(1) Reliable without restriction
<b><u>References</u></b>	This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information).
<b><u>Other</u></b>	Updated: 9-6-00

### Robust Summary 3- Biodeg-4

<i>Test Substance</i>	
CAS #	Analog of 70024-69-0
Chemical Name	Benzenesulfonic acid, mono-C16-C24-alkyl derivatives, calcium salts
Remarks	This substance is an analog for the group of substances in HERTG's Final Submission 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 this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Alkaryl Sulfonate Category.
<u>Method</u>	
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	<p>Inoculum: Activated sludge bacteria from domestic sewage treatment plant used at about 1 drop sludge filtrate inoculum/L basal medium.</p> <p>Concentration of test chemical: 2 mg/L. Inoculum was not pre-acclimated to test substance. Two replicates run per treatment.</p> <p>Temperature of incubation: 20±1 °C</p> <p>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.</p> <p>Sampling: Days 0, 5, 15, and 28 after inoculation.</p> <p>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.</p> <p>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.</p>

Remarks for test conditions, cont'd	<p>Inoculum: Sludge from domestic WWTP used at 10 mL/L basal medium</p> <p>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.</p> <p>Temp of incubation: 23 – 24 °C</p> <p>Dosing procedure: Neat test chemical added by micropipettor to culture medium in vessels immediately prior to addition of sewage and soil inocula</p> <p>Sampling: Days 1, 3, 6, 10, 14, 21, 29 (after acidification on d 28)</p> <p>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</p> <p>Analytical method: Titration of residual Ba(OH)<sub>2</sub> in trapping solution, using HCl</p> <p>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.</p> <p>Other:</p> <ul style="list-style-type: none"> <li>• 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.</li> <li>• Two replicates were run per treatment; values are average of replicates.</li> </ul> <p>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.</p>
<b><u>Results</u></b>	
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

<b><u>Conclusions</u></b>	8.0% Not Readily biodegradable; biodegradation was essentially zero
<b><u>Data Quality</u></b>	(1) Reliable without restriction
<b><u>References</u></b>	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.
<b><u>Other</u></b>	Updated: 9-6-00

### Robust Summary 3-Biodeg-5

<b>Test Substance</b>	
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 <u>petroleum derived calcium salt, overbased</u> , 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 this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
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	<p>Inoculum: Activated sewage sludge from domestic WWTP prepared per test guideline. Inoculum was not acclimated.</p> <p>Replicates: Triplicates for test substance, positive control material, and control blank.</p> <p>Temperature of incubation: 20 – 23 °C</p> <p>Dosing procedure: Neat test chemical was gravimetrically determined on glass cover slips, which were then added to culture medium in test vessels.</p> <p>Sampling: Days 1, 3, 5, 7, 10, 13, 17, 20, 24, 29 (after acidification on day 28)</p> <p>Concentration of test substance: Loadings into 3 test vessels were 19.8, 20.1, and 19.4 mg C/L.</p> <p>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.</p> <p>Analytical method: Titration of residual Ba(OH)<sub>2</sub> (0.05 N initially) in trapping solution, using 0.1N HCl.</p> <p>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.</p>

<b><u>Results</u></b>	
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
<b><u>Conclusions</u></b>	9.1% in 28 days
<b><u>Data Quality</u></b>	(1) Reliable without restriction
<b><u>References</u></b>	This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information).
<b><u>Other</u></b>	Updated: 9-6-00

### 3. Ecotoxicity

### Category: Alkaryl Sulfonates

#### AQUATIC ORGANISMS

#### 3.1 Acute Toxicity to Fish

##### Robust Summary 3-Fish -1

<b><i>Test Substance</i></b>	
CAS #	61789-86-4
Chemical Name	Sulfonic acids, petroleum, Calcium salts
Remarks	For more information on this chemical, see Section 2.0 “General Substance Information” in HERTG’s Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
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 ( <i>Cyprinodon variegatus</i> )
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)	<p>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.</p> <p>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</p>

	<p>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.</p> <p>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</p> <p>Test Levels: Control &amp; 10,000 mg/L WAF loading rate.</p> <p>Test Findings: No mortality or signs of toxicity were noted in the 10,000 WAF test level and the control throughout the entire test.</p> <p>Calculation of LL<sub>50</sub>s: Statistical analysis of survival data not warranted.</p> <p>Test Substance: No undissolved test material was report on the surface of the test vessels during the entire aquatic toxicity test.</p> <p>Reference Substance: Sodium lauryl sulfate (SLS). The 96-h LC<sub>50</sub> was 1.2 mg/L. No information provided on method of calculation.</p>
<b><u>Results</u></b>	Nominal concentrations: 96-h LL <sub>50</sub> >10,000 mg/L. This is equivalent to 96-h LL <sub>0</sub> = 10,000 mg/L (no mortality or toxic signs noted).
Remarks	<p>Measured concentration: n/a</p> <p>Unit: mg/L</p> <p>Statistical results: Statistical analysis of survival data not warranted.</p> <p>Other:</p> <ul style="list-style-type: none"> <li>• Test results reported in original study as “lethal concentrations”</li> </ul>

	<p>are reported in this summary as “lethal loading”, because test results are based on WAF loading rates.</p> <ul style="list-style-type: none"> <li>• Control response was satisfactory.</li> </ul>
<b><u>Conclusions</u></b>	No mortality or signs of toxicity were noted in the 10,000 WAF test level and the control throughout the entire test.
<b><u>Data Quality</u></b>	(1) Reliable without restriction
<b><u>References</u></b>	Nicholson, R.B. (1986) Acute toxicity of CMA Test Material Code 504 to Sheepshead Minnow, <i>Cyprinodon variegatus</i> . Springborn Bionomics Study #10823-0186-6100-500-504, Report #BW-86-04-1983.
<b><u>Other</u></b>	Updated: 9-6-00

**Robust Summary 3-Fish - 2**

<b><u>Test Substance</u></b>	
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 Final Submission 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 this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
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 ( <i>Cyprinodon variegatus</i> )
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)	<p>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.</p> <p>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 accommodated fraction (WAF), was used for the aquatic toxicity test.</p>

	<p>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.</p> <p>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.</p> <p>Test Levels: Control &amp; 10,000 mg/L WAF loading rate.</p> <p>Test Findings: No mortality or signs of toxicity were noted in the 10,000 WAF test level and the control throughout the entire test.</p> <p>Calculation of LL<sub>50</sub>s: Statistical analysis of survival data not warranted because there was no mortality in the study.</p> <p>Test Substance: No undissolved test material was report on the surface of the test vessels during the entire aquatic toxicity test.</p> <p>Reference Substance: Sodium lauryl sulfate (SLS). The 96-h LC<sub>50</sub> was 1.2 mg/L. No information provided on method of calculation.</p>
<b><u>Results</u></b>	Nominal concentrations: 96-h LL <sub>50</sub> >10,000 mg/L. This is equivalent to 96-h LL <sub>0</sub> = 10,000 mg/L (no mortality or toxic signs noted).
Remarks	<p>Measured concentration: n/a</p> <p>Unit: mg/L</p> <p>Statistical results: Statistical analysis of survival data not warranted.</p> <p>Other:</p> <ul style="list-style-type: none"> <li>• 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.</li> <li>• Control response was satisfactory.</li> </ul>

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

**Robust Summary 3-Fish - 3**

<b><u>Test Substance</u></b>	
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	For more information on this chemical, see Section 2.0 “General Substance Information” in HERTG’s Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
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 ( <i>Pimephales promelas</i> )
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)	<p>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: &lt;0.5 g biomass/L, Pretreatment: none, fish held for a minimum of 14 days before testing. No feeding during the test.</p> <p>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).</p>

	<p>Test vessels loosely covered to reduce entry of dust.</p> <p>Dilution Water: Filtered well water collected at Hampton, New Hampshire and adjusted to the appropriate hardness of 176 mg/L as CaCO<sub>3</sub>. 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.</p> <p>Light: 16-h light per day using cool-white fluorescent lights with an intensity of 20 uEin/m<sup>2</sup>.</p> <p>Test Temperature: 21.4 to 22.8 C.</p> <p>Water Chemistry: Dissolved oxygen: 7.3 – 8.6 mg/L, pH: 7.4 - 8.1, conductivity: 860 – 910 umhos/cm. Alkalinity not reported.</p> <p>Element: Mortality</p> <p>Test Levels: Control, 100, 300, &amp; 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.</p> <p>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.</p> <p>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.</p> <p>Reference Substance: No</p>
<b><u>Results</u></b>	Nominal concentrations: 96-h LL <sub>50</sub> >1,000 mg/L. This is equivalent to 96-h LL <sub>0</sub> = 1,000 mg/L (no mortality or toxic signs noted).
Remarks	<p>Measured concentration: n/a</p> <p>Unit: mg/L</p> <p>LC50, LC0, LL50 or LL0 at 48, 72, 96-hours: LL<sub>50</sub> and LL<sub>0</sub> reported</p>

	<p>as LC<sub>50</sub> and NOEC, respectively, although test results were based on WAF loading rate.</p> <p>Statistical results: Statistical analysis of survival data not warranted.</p> <p>Other:</p> <ul style="list-style-type: none"> <li>• 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.</li> <li>• Control response was satisfactory.</li> </ul>
<b><u>Conclusions</u></b>	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.
<b><u>Data Quality</u></b>	(1) Reliable without restriction
<b><u>References</u></b>	Ward, T.J. (1993) Acute Toxicity of The Water Accommodated Fractions (WAFs) of CMA 605 to The Fathead Minnow, <i>Pimephales promelas</i> . T.R. Wilbury Study #9176-CMA/ESI-605.
<b><u>Other</u></b>	Updated: 9-6-00

### Robust Summary 3-Fish - 4

<b><u>Test Substance</u></b>	
CAS #	71786-47-5
Chemical Name	Benzenesulfonic acid, mono and dialkyl derivatives, magnesium salts
Remarks	For more information on this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
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 ( <i>Pimephales promelas</i> )
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)	<p>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: &lt;0.5 g biomass/L, Pretreatment: none, fish held for a minimum of 14 days before testing. No feeding during the test.</p> <p>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.</p>

	<p>Dilution Water: Filtered well water collected at Hampton, New Hampshire and adjusted to the appropriate hardness of 176 mg/L as CaCO<sub>3</sub>. 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.</p> <p>Light: 16-h light per day using cool-white fluorescent lights with an intensity of 20 uEin/m<sup>2</sup>.</p> <p>Test Temperature: 21.6 to 22.8 C.</p> <p>Water Chemistry: Dissolved oxygen: 6.9 – 8.3 mg/L, pH: 7.0 - 7.9, conductivity: 870 – 890 umhos/cm. Alkalinity not reported.</p> <p>Element: Mortality</p> <p>Test Levels: Control, 100, 300, &amp; 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.</p> <p>Test Findings: No mortality or signs of toxicity was observed in all treatments and the control throughout the entire test.</p> <p>Calculation of LL<sub>50</sub>s: Statistical analysis of survival data not warranted.</p> <p>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.</p> <p>Reference Substance: No</p>
<b><u>Results</u></b>	Nominal concentrations: 96-h LL <sub>50</sub> >1,000 mg/L. This is equivalent to 96-h LL <sub>0</sub> = 1,000 mg/L (no mortality or toxic signs noted).
Remarks	<p>Measured concentration: n/a</p> <p>Unit: mg/L</p> <p>Statistical results: Statistical analysis of survival data not warranted.</p>

	<p>Other:</p> <ul style="list-style-type: none"> <li>• 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.</li> <li>• Control response was satisfactory.</li> </ul>
<b><u>Conclusions</u></b>	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.
<b><u>Data Quality</u></b>	(1) Reliable without restriction
<b><u>References</u></b>	Ward, T.J. (1993) Acute Toxicity of The Water Accommodated Fractions (WAFs) of CMA 609 to The Fathead Minnow, <i>Pimephales promelas</i> . T.R. Wilbury Study #9176-CMA/ESI-609.
<b><u>Other</u></b>	Updated: 9-6-00

### Robust Summary 3-Fish - 5

<b><u>Test Substance</u></b>	
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 this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
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 ( <i>Cyprinodon variegatus</i> )
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)	<p>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.</p> <p>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 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.</p>

	<p>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.</p> <p>Test Levels: Control &amp; 10,000 mg/L WAF loading rate.</p> <p>Test Findings: No mortality or signs of toxicity were noted in the 10,000 WAF test level and the control throughout the entire test.</p> <p>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.</p> <p>Test Substance: No undissolved test material was seen on the surface of the test vessels during the entire aquatic toxicity test.</p> <p>Reference Substance: Sodium lauryl sulfate (SLS). The 96-h LC<sub>50</sub> was 1.2 mg/L. No information provided on method of calculation.</p>
<b><u>Results</u></b>	Nominal concentrations: 96-h LL <sub>50</sub> >10,000 mg/L. This is equivalent to 96-h LL <sub>0</sub> = 10,000 mg/L (no mortality or toxic signs noted).
Remarks	<p>Measured concentration: n/a</p> <p>Unit: mg/L</p> <p>Statistical results: Statistical analysis of survival data not warranted because there was no mortality in this study.</p> <p>Other:</p> <ul style="list-style-type: none"> <li>• 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.</li> <li>• Control response was satisfactory.</li> </ul>

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

### Robust Summary 3-Fish - 6

<b><u>Test Substance</u></b>	
CAS #	115733-09-0
Chemical Name	Benzenesulfonic acid, C14-C24 branched & linear alkyl derivatives. Calcium salts
Remarks	For more information on this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
Method/Guideline followed	OECD Guideline for Testing of Chemicals #203 Fish Acute Toxicity Test
Test Type	Semi-Static acute toxicity test (renewal)
GLP (Y/N)	Y
Year (Study Performed)	2004
Species/Strain	<i>Oncorhynchus mykiss</i>
Analytical Monitoring	Not performed.
Exposure Period (unit)	96 hours
Statistical methods	An estimate of the lethal loading rates (LL <sub>50</sub> ) was determined by inspection.
Remarks field for test conditions (fill as applicable)	<p>Fingerlings were obtained from a commercial breeder and were acclimated for 13 days. The fish had a mean length of 4.7 cm and a mean weight of 1.54 g at the end of the definitive test.</p> <p>Individual test concentrations were prepared for each test level. A measured volume of test material was added to a measured volume of dilution water and continuously stirred for 24 hours. Following settling for 1 hour the aqueous phase or WAF (water accommodated fraction) was removed by mid-depth siphoning.</p> <p>A sealed 96 hours semi-static test was carried out with daily renewal of the test WAF's. Duplicate 20-liter glass exposure vessels were filled with the WAF. A separate chamber served as the control. Ten fish were placed in each chamber and the chambers were covered to reduce evaporation. The chambers were aerated. The fish were not fed during the study.</p> <p>The fish were observed for toxicity at 3, 24, 48, 72 and 96 hours. At 24, 48 and 72 hours the fish were transferred to fresh WAFs or control water.</p> <p>Dissolved oxygen, water temperature and pH were determined throughout the study. Vortex depth was recorded at the start and end of each mixing period.</p>

<b>Test Concentrations (Nominal)</b>	100mg/L (Water Accommodated Fraction-WAF) test concentration was selected based on a range-finding study.																					
<b>Results</b>	The 96-hour LL <sub>50</sub> (loading level likely to cause 50% mortality) was >100 mg/L WAF. The No Observed Effect Loading rate was 100 mg/L.																					
Remarks	<p>In the range find study no mortality or sublethal effects were observed at 10 or 100 mg/L. During the main study, no toxicity or mortality was observed at 100 mg/L (WAF). The LL<sub>50</sub>s (loading levels likely to cause 50% mortality) were as follows:</p> <table border="1"> <thead> <tr> <th>Time (hours)</th> <th>LL<sub>50</sub> (mg/L)</th> <th>95% Confidence Limits (mg/L)</th> </tr> </thead> <tbody> <tr> <td>3</td> <td>&gt;100</td> <td>-</td> </tr> <tr> <td>6</td> <td>&gt;100</td> <td>-</td> </tr> <tr> <td>24</td> <td>&gt;100</td> <td>-</td> </tr> <tr> <td>48</td> <td>&gt;100</td> <td>-</td> </tr> <tr> <td>72</td> <td>&gt;100</td> <td>-</td> </tr> <tr> <td>96</td> <td>&gt;100</td> <td>-</td> </tr> </tbody> </table> <p>Microscopic inspection of the WAFs showed no micro-dispersions or undissolved test material to be present.  Water chemistry: Temperature: 12.7-14.3 °C; Dissolved Oxygen: 10.0-10.3 mg/L; pH: 7.7-8.2.</p>	Time (hours)	LL <sub>50</sub> (mg/L)	95% Confidence Limits (mg/L)	3	>100	-	6	>100	-	24	>100	-	48	>100	-	72	>100	-	96	>100	-
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<b>Conclusions</b>	The 96 hour LL <sub>50</sub> (loading level likely to cause 50% mortality) was >100 mg/L WAF. The No Observed Effect Loading rate was 100 mg/L.																					
<b>Data Quality</b>	Reliable with restriction (Klimisch Code). Restriction due to the lack of analytical confirmation of test concentrations.																					
<b>References</b>	Acute Toxicity to Rainbow Trout SafePharm Study Number: 1666/066 (03 Mar 2005)																					
<b>Other</b>	Updated: 3/22/2005																					

### Robust Summary 3-Fish - 7

<b><u>Test Substance</u></b>	
CAS #	68608-26-4
Chemical Name	Sulfonic acids, petroleum, sodium salts
Remarks	For more information on this chemical, see Section 2.0 “General Substance Information” in HERTG’s Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
Method/Guideline followed	OECD 203
Test Type	Acute Toxicity to Fish (Water Accommodated Fraction-WAF)
GLP (Y/N)	Y
Year (Study Performed)	2005
Species/Strain	Rainbow Trout ( <i>Oncorhynchus mykiss</i> ) Fingerlings were obtained from a commercial breeder.
Fish Size	Average length 5.5 cm and a mean weight of 1.76 g, loading rate of 0.88 g body weight/L
Number of Fish	Range Find: 3/concentration Definitive Study: 20/concentration (10/replicate)
Analytical Monitoring	No
Nominal Test Substance Concentration Levels	Range Find Study A: 0, 10, 100 mg/L WAF Range Find Study B: 0, 32, 320 mg/L WAF Range Find Study C: 0, 10, 100 mg/L WAF Definitive Study: 0, 100 mg/L WAF
Test Concentration Preparation	Individual test concentrations were prepared for each test level. A measured volume of test material was added to a measured volume of dilution water and continuously stirred for 24 hours. Following settling for 1 hour the aqueous phase or WAF (water accommodated fraction) was removed by mid-depth siphoning.
Exposure Period	96 hours
Exposure Conditions	Static-renewal test conditions. The test preparations were renewed daily.
Vehicle	None
Statistical Analysis	LL <sub>50</sub> values determined by inspection.
Dose Rangefinding Study	Yes
Test Chambers	Covered, 20-liter glass aquaria containing the test solution
Diluent Water	Dechlorinated, softened water
Diluent Water Chemistry	Hardness 100 mg/l as CaCO <sub>3</sub> pH 7.6-8.5.
Diluent Water Chemistry During 96 Hour Exposure Period.	Dissolved Oxygen: 8.3-9.6 mg/L pH: 7.5-8.3
Photoperiod	16 hours of light, 8 hours of dark
Temperature Range	Approximately 14°C during holding period 14.5-14.9°C during exposure period

Remarks field for test conditions	All organisms were observed for mortality and the number of individuals exhibiting clinical signs of toxicity or abnormal behavior at 3, 6, 24, 48, 72, and 96 hours after initiation of test material exposure.
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<b><u>Results</u></b>	<p>At the start of the mixing period, the 100 mg/L loading rate was clear, with an oily slick at the surface. Microscopic examination of the WAF indicated a significant amount of dispersed test material present in the water column. The WAF was therefore filtered through glass wool prior to use. Microscopic examination post filtration showed no particles or micro dispersions present.</p> <p>Cumulative mortality data was as follows:</p> <table border="1" data-bbox="649 441 1469 1092"> <thead> <tr> <th rowspan="2">Concentration mg/L (WAF)</th> <th rowspan="2">mortality (%) Number of Trout</th> <th colspan="2">Cumulative</th> </tr> <tr> <th>24 Hours</th> <th>96 Hours</th> </tr> </thead> <tbody> <tr> <td colspan="4">Range Find A and B</td> </tr> <tr> <td>0</td> <td>3</td> <td>0</td> <td>0</td> </tr> <tr> <td>10</td> <td>3</td> <td>0</td> <td>0</td> </tr> <tr> <td>32</td> <td>3</td> <td>0</td> <td>0</td> </tr> <tr> <td>100</td> <td>3</td> <td>0</td> <td>33.3</td> </tr> <tr> <td>320</td> <td>3</td> <td>0</td> <td>33.3</td> </tr> <tr> <td colspan="4">Range Find C</td> </tr> <tr> <td>0</td> <td>3</td> <td>0</td> <td>0</td> </tr> <tr> <td>10</td> <td>3</td> <td>0</td> <td>0</td> </tr> <tr> <td>100</td> <td>3</td> <td>0</td> <td>0</td> </tr> <tr> <td colspan="4">Definitive Study</td> </tr> <tr> <td>100</td> <td>20 (10/replicate)</td> <td>0</td> <td>0</td> </tr> </tbody> </table> <table border="1" data-bbox="649 1176 1469 1470"> <thead> <tr> <th>Time (hours)</th> <th>Lethal Loading Rate<sub>50</sub> (mg/L)</th> <th>Method of Determination</th> </tr> </thead> <tbody> <tr> <td>3</td> <td>&gt;100</td> <td>Inspection</td> </tr> <tr> <td>6</td> <td>&gt;100</td> <td>Inspection</td> </tr> <tr> <td>24</td> <td>&gt;100</td> <td>Inspection</td> </tr> <tr> <td>48</td> <td>&gt;100</td> <td>Inspection</td> </tr> <tr> <td>72</td> <td>&gt;100</td> <td>Inspection</td> </tr> <tr> <td>96</td> <td>&gt;100</td> <td>Inspection</td> </tr> </tbody> </table> <p>The 96-hour Lethal Loading rate (LL<sub>50</sub>) was &gt;100 mg/L (WAF). The No Observed Effect Level, based on mortality and the absence of any sub lethal effects of exposure was 100 mg/L.</p>	Concentration mg/L (WAF)	mortality (%) Number of Trout	Cumulative		24 Hours	96 Hours	Range Find A and B				0	3	0	0	10	3	0	0	32	3	0	0	100	3	0	33.3	320	3	0	33.3	Range Find C				0	3	0	0	10	3	0	0	100	3	0	0	Definitive Study				100	20 (10/replicate)	0	0	Time (hours)	Lethal Loading Rate <sub>50</sub> (mg/L)	Method of Determination	3	>100	Inspection	6	>100	Inspection	24	>100	Inspection	48	>100	Inspection	72	>100	Inspection	96	>100	Inspection
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<b><u>Data Quality</u></b>	Reliable without restriction (Klimisch Code)																																																																											
<b><u>References</u></b>	Acute Toxicity to Rainbow Trout SafePharm Laboratories Project No.: 1666/062 (21 Jul 2005)																																																																											

<b><i>Other</i></b>	Updated: 8/15/2005

### Robust Summary 3-Fish - 8

<b><u>Test Substance</u></b>	
CAS #	61789-86-4
Chemical Name	Sulfonic acids, petroleum, calcium salts
Remarks	For more information on this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
Method/Guideline followed	OECD 203
Test Type	Acute Toxicity to Fish (Water Accommodated Fraction-WAF)
GLP (Y/N)	Y
Year (Study Performed)	2005
Species/Strain	Rainbow Trout ( <i>Oncorhynchus mykiss</i> ) Fingerlings were obtained from a commercial breeder.
Fish Size	Average length 4.6 cm and a mean weight of 1.26 g, loading rate of 0.63 g body weight/L
Number of Fish	Range Find: 3/concentration Definitive Study 20/concentration (10/replicate)
Analytical Monitoring	No
Nominal Test Substance Concentration Levels	Range Find Study: 0, 10, 100 mg/L WAF Definitive Study: 0, 100 mg/L WAF
Test Concentration Preparation	Individual test concentrations were prepared for each test level. A measured volume of test material was added to a measured volume of dilution water and continuously stirred for 24 hours. Following settling for 1 hour the aqueous phase or WAF (water accommodated fraction) was removed by mid-depth siphoning.
Exposure Period	96 hours
Exposure Conditions	Static-renewal test conditions. The test preparations were renewed daily.
Vehicle	None
Statistical Analysis	LL <sub>50</sub> values determined by inspection.
Dose Range finding Study	Yes
Test Chambers	Covered, 20-liter glass aquaria containing the test solution
Diluent Water	Dechlorinated, softened water
Diluent Water Chemistry	Hardness 100 mg/l as CaCO <sub>3</sub> pH 7.6-8.5.
Diluent Water Chemistry During 96 Hour Exposure Period.	Dissolved Oxygen: 9.2-9.9 mg/L pH: 7.5-8.0
Photoperiod	16 hours of light, 8 hours of dark
Temperature Range	Approximately 14°C during holding period 13.6-14.4°C during exposure period
Remarks field for test conditions	All organisms were observed for mortality and the number of individuals exhibiting clinical signs of toxicity or abnormal behavior at 3, 6, 24, 48,

	72, and 96 hours after initiation of test material exposure.																																											
<b><u>Results</u></b>	<p>At the start of the mixing period, the 100 mg/L loading rate was clear, with an oily slick at the surface. Microscopic examination of the WAF indicated a slight amount of dispersed test material present in the water column. The WAF was therefore filtered through glass wool prior to use. Microscopic examination post filtration showed no particles or micro dispersions present.</p> <p>Cumulative mortality data was as follows:</p> <table border="1"> <thead> <tr> <th rowspan="2">Concentration mg/L (WAF) Range Find</th> <th rowspan="2">mortality (%) Number of Trout</th> <th colspan="2">Cumulative</th> </tr> <tr> <th>24 Hours</th> <th>96 Hours</th> </tr> </thead> <tbody> <tr> <td>10</td> <td>3</td> <td>0</td> <td>0</td> </tr> <tr> <td>100</td> <td>3</td> <td>0</td> <td>0</td> </tr> <tr> <td colspan="4">Definitive Study</td> </tr> <tr> <td>100</td> <td>20 (10/replicate)</td> <td>0</td> <td>0</td> </tr> </tbody> </table> <table border="1"> <thead> <tr> <th>Time (hours)</th> <th>Lethal Loading Rate<sub>50</sub> (mg/L)</th> <th>Method of Determination</th> </tr> </thead> <tbody> <tr> <td>3</td> <td>&gt;100</td> <td>Inspection</td> </tr> <tr> <td>6</td> <td>&gt;100</td> <td>Inspection</td> </tr> <tr> <td>24</td> <td>&gt;100</td> <td>Inspection</td> </tr> <tr> <td>48</td> <td>&gt;100</td> <td>Inspection</td> </tr> <tr> <td>72</td> <td>&gt;100</td> <td>Inspection</td> </tr> <tr> <td>96</td> <td>&gt;100</td> <td>Inspection</td> </tr> </tbody> </table> <p>The 96-hour Lethal Loading rate (LL<sub>50</sub>) was &gt;100 mg/L (WAF). The No Observed Effect Level, based on mortality and the absence of any sub lethal effects of exposure was 100 mg/L.</p>	Concentration mg/L (WAF) Range Find	mortality (%) Number of Trout	Cumulative		24 Hours	96 Hours	10	3	0	0	100	3	0	0	Definitive Study				100	20 (10/replicate)	0	0	Time (hours)	Lethal Loading Rate <sub>50</sub> (mg/L)	Method of Determination	3	>100	Inspection	6	>100	Inspection	24	>100	Inspection	48	>100	Inspection	72	>100	Inspection	96	>100	Inspection
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<b><u>Data Quality</u></b>	Reliable without restriction (Klimisch Code)																																											
<b><u>References</u></b>	Acute Toxicity to Rainbow Trout SafePharm Laboratories Project No.: 1666/065 (5 Aug 2005)																																											
<b><u>Other</u></b>	Updated: 8/15/2005																																											

### Robust Summary 3-Fish - 9

<b><u>Test Substance</u></b>	
CAS #	61790-48-5
Chemical Name	Sulfonic acids, petroleum, barium salts
Remarks	For more information on this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
Method/Guideline followed	OECD 203
Test Type	Acute Toxicity to Fish (Water Accommodated Fraction-WAF)
GLP (Y/N)	Y
Year (Study Performed)	2005
Species/Strain	Rainbow Trout ( <i>Oncorhynchus mykiss</i> ) Fingerlings were obtained from a commercial breeder.
Fish Size	Average length 4.2 cm and a mean weight of 0.88 g, loading rate of 0.44 g body weight/L
Number of Fish	Range Find: 3/concentration Definitive Study: 20/concentration (10/replicate)
Analytical Monitoring	No
Nominal Test Substance Concentration Levels	Range Find Study: 0, 10, 100 mg/L WAF Definitive Study: 0, 100 mg/L WAF
Test Concentration Preparation	Individual test concentrations were prepared for each test level. A measured volume of test material was added to a measured volume of dilution water and continuously stirred for 24 hours. Following settling for 1 hour the aqueous phase or WAF (water accommodated fraction) was removed by mid-depth siphoning.
Exposure Period	96 hours
Exposure Conditions	Static-renewal test conditions. The test preparations were renewed daily.
Vehicle	None
Statistical Analysis	LL <sub>50</sub> values determined by inspection.
Dose Range finding Study	Yes
Test Chambers	Covered, 20-liter glass aquaria containing the test solution
Diluent Water	Dechlorinated, softened water
Diluent Water Chemistry	Hardness 100 mg/l as CaCO <sub>3</sub> pH 7.6-8.5.
Diluent Water Chemistry During 96 Hour Exposure Period.	Dissolved Oxygen: 9.1-9.9 mg/L pH: 7.6-8.2
Photoperiod	16 hours of light, 8 hours of dark
Temperature Range	Approximately 14 °C during holding period 13.9-14.5 °C during exposure period
Remarks field for test conditions	All organisms were observed for mortality and the number of individuals exhibiting clinical signs of toxicity or abnormal behavior at 3, 6, 24, 48,

	72, and 96 hours after initiation of test material exposure.																																											
<b><u>Results</u></b>	<p>At the start of the mixing period, the 100 mg/L loading rate was clear, with brown oily globules at the surface. Microscopic examination of the WAF indicated small globules of test material were present in the water column. The WAF was therefore filtered through glass wool prior to use. Microscopic examination post filtration showed no globules present.</p> <p>Cumulative mortality data was as follows:</p> <table border="1"> <thead> <tr> <th rowspan="2">Concentration mg/L (WAF) Range Find</th> <th rowspan="2">mortality (%) Number of Trout</th> <th colspan="2">Cumulative</th> </tr> <tr> <th>24 Hours</th> <th>96 Hours</th> </tr> </thead> <tbody> <tr> <td>10</td> <td>3</td> <td>0</td> <td>0</td> </tr> <tr> <td>100</td> <td>3</td> <td>0</td> <td>0</td> </tr> <tr> <td colspan="4">Definitive Study</td> </tr> <tr> <td>100</td> <td>20 (10/replicate)</td> <td>0</td> <td>0</td> </tr> </tbody> </table> <table border="1"> <thead> <tr> <th>Time (hours)</th> <th>Lethal Loading Rate<sub>50</sub> (mg/L)</th> <th>Method of Determination</th> </tr> </thead> <tbody> <tr> <td>3</td> <td>&gt;100</td> <td>Inspection</td> </tr> <tr> <td>6</td> <td>&gt;100</td> <td>Inspection</td> </tr> <tr> <td>24</td> <td>&gt;100</td> <td>Inspection</td> </tr> <tr> <td>48</td> <td>&gt;100</td> <td>Inspection</td> </tr> <tr> <td>72</td> <td>&gt;100</td> <td>Inspection</td> </tr> <tr> <td>96</td> <td>&gt;100</td> <td>Inspection</td> </tr> </tbody> </table> <p>The 96-hour Lethal Loading rate (LL<sub>50</sub>) was &gt;100 mg/L (WAF). The No Observed Effect Level, based on mortality and the absence of any sub lethal effects of exposure was 100 mg/L.</p>	Concentration mg/L (WAF) Range Find	mortality (%) Number of Trout	Cumulative		24 Hours	96 Hours	10	3	0	0	100	3	0	0	Definitive Study				100	20 (10/replicate)	0	0	Time (hours)	Lethal Loading Rate <sub>50</sub> (mg/L)	Method of Determination	3	>100	Inspection	6	>100	Inspection	24	>100	Inspection	48	>100	Inspection	72	>100	Inspection	96	>100	Inspection
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		24 Hours	96 Hours																																									
10	3	0	0																																									
100	3	0	0																																									
Definitive Study																																												
100	20 (10/replicate)	0	0																																									
Time (hours)	Lethal Loading Rate <sub>50</sub> (mg/L)	Method of Determination																																										
3	>100	Inspection																																										
6	>100	Inspection																																										
24	>100	Inspection																																										
48	>100	Inspection																																										
72	>100	Inspection																																										
96	>100	Inspection																																										
<b><u>Conclusions</u></b>	Under the conditions of this study the 96-hour Lethal Loading rate (LL <sub>50</sub> ) was >100 mg/L (WAF). The 96-hour no observed effect level was 100 mg/L (WAF).																																											
<b><u>Data Quality</u></b>	Reliable without restriction (Klimisch Code)																																											
<b><u>References</u></b>	Acute Toxicity to Rainbow Trout SafePharm Laboratories Project No.: 1666/067 (19 Jul 2005)																																											
<b><u>Other</u></b>	Updated: 8/15/2005																																											

### 3.2 Acute Toxicity to Aquatic Invertebrates (e.g. Daphnia)

#### **Robust Summary 3-DAPH - 1**

<b><i>Test Substance</i></b>	
CAS #	115733-09-0
Chemical Name	Benzenesulfonic acid, C14-C24 branched and linear alkyl derivatives, calcium salts
Remarks	For more information on this chemical, see Section 2.0 “General Substance Information” in HERTG’s Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
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 <i>Daphnia</i> 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, <i>Daphnia magna</i>
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)	<p>Test species: Juvenile daphnids less than 24-hours old were produced from laboratory in-house culture.</p> <p>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.</p> <p>Test conditions: Two 250-mL glass beakers that contained 200 mL of test solution were used per treatment. The 250-mL test vessels were</p>

	<p>loosely covered to reduce entry of dust.</p> <p>Light: 16-hour light per day using cool-white fluorescent lights with an intensity of 20 uEin/m<sup>2</sup>.</p> <p>Test temperature: 20.4 – 20.9 C</p> <p>Dilution water: Filtered well water collected at Hampton, New Hampshire and adjusted to the appropriate hardness 176 mg/L as CaCO<sub>3</sub>. 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 &lt;10 mg/L at the end of the test.</p> <p>Water chemistry: Dissolved oxygen: 7.9 - 8.7 mg/L; pH: 7.2 - 8.1; conductivity: 860 – 880 umhos/cm.</p> <p>Element: Immobilization/mortality</p> <p>Test Levels: Control, 100, 300, &amp; 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.</p> <p>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.</p> <p>Calculation of EL<sub>50</sub>s: Statistical analysis of survival data not warranted.</p> <p>Exposure period: 48 hours</p> <p>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.</p>
<b><u>Results</u></b>	Nominal concentrations: 48-h EL <sub>50</sub> >1,000 mg/L. This is equivalent to 48-h EL <sub>0</sub> = 1000 mg/L based on WAF loading rates.
Remarks	<p>Measured concentration: n/a</p> <p>Unit: mg/L</p>

	<p>Statistical results: Not applicable.</p> <p>Other:</p> <ul style="list-style-type: none"> <li>• 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.</li> <li>• 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.</li> <li>• Control response was satisfactory.</li> </ul>
<b><u>Conclusions</u></b>	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.
<b><u>Data Quality</u></b>	(1) Reliable without restriction
<b><u>References</u></b>	Ward, T.J. (1993) Acute Toxicity of the Water Accommodated Fractions (WAFs) of CMA #604 to the Daphnid, <i>Daphnia magna</i> . T.R. Wilbury Study #9178-CMA/ESI-604.
<b><u>Other</u></b>	Updated: 9-6-00

### Robust Summary 3-DAPH-2

<b><u>Test Substance</u></b>	
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	For more information on this chemical, see Section 2.0 “General Substance Information” in HERTG’s Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
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 <i>Daphnia</i> 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, <i>Daphnia magna</i>
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)	<p>Test species: Juvenile daphnids less than 24-hours old were produced from laboratory in-house culture.</p> <p>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.</p> <p>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.</p> <p>Light: 16-hour light per day using cool-white fluorescent lights with</p>

	<p>an intensity of 20 uEin/m.</p> <p>Test temperature: 20.3 – 20.7 C</p> <p>Dilution water: Filtered well water collected at Hampton, New Hampshire and adjusted to the appropriate hardness 176 mg/L as CaCO<sub>3</sub>. 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 &lt;10 mg/L at the end of the test.</p> <p>Water chemistry: Dissolved oxygen: 7.8 - 8.8 mg/L; pH: 7.4 - 8.5; conductivity: 850 – 900 umhos/cm.</p> <p>Element: Immobilization/mortality</p> <p>Test Levels: Control, 100, 300, &amp; 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.</p> <p>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.</p> <p>Calculation of EL<sub>50</sub>s: Statistical analysis of survival data not warranted.</p> <p>Exposure period: 48 hours</p> <p>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.</p>
<b><u>Results</u></b>	Nominal concentrations: 48-h EL <sub>50</sub> >1,000 mg/L. This is equivalent to 48-h EL <sub>0</sub> = 1000 mg/L based on WAF loading rates.
Remarks	<p>Measured concentration: n/a</p> <p>Unit: mg/L</p> <p>Statistical results: Not applicable because immobilization seen at high</p>

	<p>dose was not significant.</p> <p>Other:</p> <ul style="list-style-type: none"> <li>• 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.</li> <li>• Control response was satisfactory.</li> </ul>
<b><u>Conclusions</u></b>	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.
<b><u>Data Quality</u></b>	(1) Reliable without restriction
<b><u>References</u></b>	Ward, T.J. (1993) Acute Toxicity of the Water Accommodated Fractions (WAFs) of CMA #605 to the Daphnid, <i>Daphnia magna</i> . T.R. Wilbury Study #9178-CMA/ESI-605.
<b><u>Other</u></b>	Updated: 9-6-00

### Robust Summary 3-DAPH-3

<b><u>Test Substance</u></b>	
CAS #	71786-47-5
Chemical Name	Benzenesulfonic acid, mono and dialkyl derivatives, magnesium salts
Remarks	For more information on this chemical, see Section 2.0 “General Substance Information” in HERTG’s Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
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 <i>Daphnia</i> 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, <i>Daphnia magna</i>
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)	<p>Test species: Juvenile daphnids less than 24-hours old were produced from laboratory in-house culture.</p> <p>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.</p> <p>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.</p> <p>Light: 16-hour light per day using cool-white fluorescent lights with</p>

	<p>an intensity of 20 uEin/m<sup>2</sup>.</p> <p>Test temperature: 19.7 – 20.9 C</p> <p>Dilution water: Filtered well water collected at Hampton, New Hampshire and adjusted to the appropriate hardness 176 mg/L as CaCO<sub>3</sub>. 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 &lt;10 mg/L at the end of the test.</p> <p>Water chemistry: Dissolved oxygen: 7.9 - 9.3 mg/L; pH: 7.0 - 8.4; conductivity: 870 – 910 umhos/cm.</p> <p>Element: Immobilization/mortality</p> <p>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.</p> <p>Calculation of EL<sub>50</sub>s: Statistical analysis of survival data not warranted.</p> <p>Exposure period: 48 hours</p> <p>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.</p>
<b><u>Results</u></b>	<p>Nominal concentrations: 48-h EL<sub>50</sub> &gt;1,000 mg/L. This is equivalent to 48-h EL<sub>0</sub> = 1000 mg/L based on WAF loading rates (no immobilization noted).</p>
Remarks	<p>Measured concentration: n/a</p> <p>Unit: mg/L</p> <p>Statistical results: Not applicable because there was no immobilization.</p> <p>Other:</p> <ul style="list-style-type: none"> <li>• 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.</li> </ul>

	<ul style="list-style-type: none"> <li>• Control response was satisfactory.</li> </ul>
<b><u>Conclusions</u></b>	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.
<b><u>Data Quality</u></b>	(1) Reliable without restriction
<b><u>References</u></b>	Ward, T.J. (1993) Acute Toxicity of the Water Accommodated Fractions (WAFs) of CMA #609 to the Daphnid, <i>Daphnia magna</i> . T.R. Wilbury Study #9178-CMA/ESI-609.
<b><u>Other</u></b>	Updated: 9-6-00

**Robust Summary 3-DAPH- 4**

Test Substance	
CAS #	68608-26-4
Chemical Name	Sulfonic acids, petroleum, sodium salts
Remarks	For more information on this chemical, see Section 2.0 “General Substance Information” in HERTG’s Final Submission for Alkaryl Sulfonate Category.
Method	
Method/Guideline followed	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)	2005
Species/Strain	Daphnia magna
Analytical Monitoring	Test material concentrations of exposure solutions were not determined.
Exposure Period (unit)	48 hours
Positive Control	Potassium dichromate at 0, 0.32, 0.56, 1.0, 1.8, 3.2 mg/L (Conducted during December 2004).
Statistical methods	EL50 values calculated using the trimmed Spearman-Kärber method (ToxCalc software 1999).
Remarks field for test conditions (fill as applicable)	<p>Twenty-four hours old Daphnia magna derived from in house cultures were used for the study. Individual water accommodated fractions (WAFs) were prepared for each test level. Appropriate amounts of test material was added to a measured volume of reconstituted water in a glass vessel and stirred for 24 hours. Stirring was accomplished using a magnetic stirrer at a stirring rate that produced a slight dimple at the surface of the water. Following the mixing period, the test solutions were allowed to stand for 1 hour before the water phase was gently siphoned (first 75-100 mL discarded) from the mixing vessel by mid-depth siphoning into the test vessels.</p> <p>The test chambers were covered, 250 ml vessels that contained 200 ml of test solution. Ten daphnids/time point were distributed into each concentration for the range finding study. Five daphnids/replicate/time point (4 replicates) were used in the definitive study. Test vessels were covered to reduce evaporation and were maintained at 20.6 to 21.1°C with a photoperiod of 16 hours light and 8 hours dark. Daphnia were not fed nor were cultures aerated during exposure. Control groups were handled in the same manner as the test groups. Test preparations were not renewed during the exposure period.</p> <p>Water temperature was recorded daily throughout the test. Dissolved oxygen concentration and pH were recorded at the start and end of the study.</p> <p>Any immobilization or adverse reactions to exposure were recorded at 24 and 48 hours after the start of exposure. Daphnia were considered immobilized if they were unable to swim for approximately 15 seconds after gentle agitation.</p>
Test Concentrations	Range Find Study A: 0, 10, 100 mg/L WAF Range Find Study B: 0, 1.0, 10 mg/L WAF Range Find Study C: 0, 1.0, 10, 100 mg/L WAF Definitive Study: 0, 100 mg/L WAF
Results	The 48-hour EL <sub>50</sub> (Effective Loading rate) was determined to be >100 mg/L (WAF).

Remarks	<p>Temperature was maintained at 20.3 to 21.0°C throughout the test. No treatment related differences were observed in oxygen concentration or pH during the study.</p> <p>After 24 hours stirring and a 1-hour standing period the 100 mg/L loading rate was a clear colorless water column with test material floating at the water surface. After siphoning and for the duration of the test, the loading rate was a clear colorless solution. No micro-dispersions or undissolved test material were present upon microscopic examination.</p> <p>Cumulative immobilization data was as follows:</p> <table border="1" data-bbox="649 588 1461 1176"> <thead> <tr> <th colspan="5">Cumulative Immobilization (%)</th> </tr> <tr> <th>Concentration mg/L (WAF)</th> <th>Number of Daphnia</th> <th>24 Hours</th> <th>48 Hours</th> <th></th> </tr> </thead> <tbody> <tr> <td colspan="5">Range Find Study A</td> </tr> <tr> <td>0</td> <td>10/interval</td> <td>0</td> <td>0</td> <td></td> </tr> <tr> <td>10</td> <td>10/interval</td> <td>4</td> <td>10</td> <td></td> </tr> <tr> <td>100</td> <td>10/interval</td> <td>5</td> <td>10</td> <td></td> </tr> <tr> <td colspan="5">Range Find Studies B and C</td> </tr> <tr> <td>0</td> <td>10/interval</td> <td>0</td> <td>0</td> <td></td> </tr> <tr> <td>1.0</td> <td>10/interval</td> <td>0</td> <td>0</td> <td></td> </tr> <tr> <td>10</td> <td>10/interval</td> <td>0</td> <td>0</td> <td></td> </tr> <tr> <td>100</td> <td>10/interval</td> <td>0</td> <td>0</td> <td></td> </tr> <tr> <td colspan="5">Definitive Study</td> </tr> <tr> <td>100</td> <td>20/interval</td> <td>0</td> <td>0</td> <td></td> </tr> </tbody> </table> <p>In the initial range find study immobilization was observed at 10 and 100 mg/L WAF. However, in follow up range find studies B and C no immobilization was observed at concentrations ranging from 1.0 to 100 mg/L WAF. Based on these results the definitive study was conducted at 100 mg/L WAF. In the definitive study no immobilization was observed.</p> <p>The results in the initial range find study could not be explained from an examination of the data and were attributed to biological variation by the Study Director.</p> <p>The 24 and 48-hour EL<sub>50</sub> (Effective Loading rate) were determined to be &gt;100 mg/L (WAF).</p> <p>The no observed effect-loading rate at 24 and 48-hours was 100 mg/L (WAF).</p> <p>The results of the positive control were within the normal range for potassium dichromate. The mean positive control 48-hour EC<sub>50</sub> was 0.78 mg/L.</p>	Cumulative Immobilization (%)					Concentration mg/L (WAF)	Number of Daphnia	24 Hours	48 Hours		Range Find Study A					0	10/interval	0	0		10	10/interval	4	10		100	10/interval	5	10		Range Find Studies B and C					0	10/interval	0	0		1.0	10/interval	0	0		10	10/interval	0	0		100	10/interval	0	0		Definitive Study					100	20/interval	0	0	
Cumulative Immobilization (%)																																																																		
Concentration mg/L (WAF)	Number of Daphnia	24 Hours	48 Hours																																																															
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Conclusions	The 24 and 48-hour EL <sub>50</sub> (Effective Loading rate) was determined to be >100																																																																	

	mg/L (WAF). The no observed effect-loading rate at 24 and 48-hours was 100 mg/L (WAF).
Data Quality	Reliable with restriction (Klimisch Code). Restriction due to findings noted during the initial range find study.
References	Acute Toxicity to <i>Daphnia Magna</i> SafePharm Laboratories Project No.: 1666/061 (21 Jul 2005)
Other	Updated: 8/15/2005

### Robust Summary 3-DAPH- 5

Test Substance	
CAS #	61789-86-4
Chemical Name	Sulfonic acids, petroleum, calcium salts
Remarks	For more information on this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Alkaryl Sulfonate Category.
Method	
Method/Guideline followed	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)	2005
Species/Strain	Daphnia magna
Analytical Monitoring	Test material concentrations of exposure solutions were not determined.
Exposure Period (unit)	48 hours
Positive Control	Potassium dichromate at 0, 0.32, 0.56, 1.0, 1.8, 3.2 mg/L (Conducted during December 2004).
Statistical methods	EL50 values calculated using the trimmed Spearman-Kärber method (ToxCalc software 1999).
Remarks field for test conditions (fill as applicable)	<p>Twenty-four hours old Daphnia magna derived from in house cultures were used for the study. Individual water accommodated fractions (WAFs) were prepared for each test level. Appropriate amounts of test material was added to a measured volume of reconstituted water in a glass vessel and stirred for 24 hours. Stirring was accomplished using a magnetic stirrer at a stirring rate that produced a slight dimple at the surface of the water. Following the mixing period, the test solutions were allowed to stand for 1 hour before the water phase was gently siphoned (first 75-100 mL discarded) from the mixing vessel by mid-depth siphoning into the test vessels.</p> <p>The test chambers were covered, 250 ml vessels that contained 200 ml of test solution. Ten daphnids/time point were distributed into each concentration for the range finding study. Five daphnids/replicate/time point (4 replicates) were used in the definitive study. Test vessels were covered to reduce evaporation and were maintained at 20.3 to 21.0°C with a photoperiod of 16 hours light and 8 hours dark. Daphnia were not fed nor were cultures aerated during exposure. Control groups were handled in the same manner as the test groups. Test preparations were not renewed during the exposure period.</p> <p>Water temperature was recorded daily throughout the test. Dissolved oxygen concentration and pH were recorded at the start and end of the study.</p> <p>Any immobilization or adverse reactions to exposure were recorded at 24 and 48 hours after the start of exposure. Daphnia were considered immobilized if they were unable to swim for approximately 15 seconds after gentle agitation.</p>
Test Concentrations	Range Find Study: 0, 10, 100 mg/L WAF Definitive Study: 0, 100 mg/L WAF
Results	The 48-hour EL <sub>50</sub> (Effective Loading rate) was determined to be >100 mg/L (WAF).
Remarks	Temperature was maintained at 20.3 to 21.0°C throughout the test. No

	<p>treatment related differences were observed in oxygen concentration or pH during the study.</p> <p>After 24 hours stirring and a 1-hour standing period the 100 mg/L loading rate was a clear colorless water column with brown oily test material floating at the water surface. After siphoning and for the duration of the test, the loading rate was a clear colorless solution. No micro-dispersions or undissolved test material were present upon microscopic examination.</p> <p>Cumulative immobilization data was as follows:</p> <table border="1"> <thead> <tr> <th rowspan="2">Concentration mg/L (WAF) Range Find</th> <th colspan="3">Cumulative Immobilization (%)</th> </tr> <tr> <th>Number of Daphnia</th> <th>24 Hours</th> <th>48 Hours</th> </tr> </thead> <tbody> <tr> <td>0</td> <td>10/interval</td> <td>0</td> <td>0</td> </tr> <tr> <td>10</td> <td>10/interval</td> <td>0</td> <td>0</td> </tr> <tr> <td>100</td> <td>10/interval</td> <td>0</td> <td>0</td> </tr> <tr> <td colspan="4">Definitive Study</td> </tr> <tr> <td>100</td> <td>20/interval</td> <td>0</td> <td>0</td> </tr> </tbody> </table> <p>No immobilization was observed.</p> <p>The 24 and 48-hour EL<sub>50</sub> (Effective Loading rate) were determined to be &gt;100 mg/L (WAF).</p> <p>The no observed effect-loading rate at 24 and 48-hours was 100 mg/L (WAF).</p> <p>The results of the positive control were within the normal range for potassium dichromate. The mean positive control 48-hour EC<sub>50</sub> was 0.78 mg/L.</p>	Concentration mg/L (WAF) Range Find	Cumulative Immobilization (%)			Number of Daphnia	24 Hours	48 Hours	0	10/interval	0	0	10	10/interval	0	0	100	10/interval	0	0	Definitive Study				100	20/interval	0	0
Concentration mg/L (WAF) Range Find	Cumulative Immobilization (%)																											
	Number of Daphnia	24 Hours	48 Hours																									
0	10/interval	0	0																									
10	10/interval	0	0																									
100	10/interval	0	0																									
Definitive Study																												
100	20/interval	0	0																									
Conclusions	The 24 and 48-hour EL <sub>50</sub> (Effective Loading rate) was determined to be >100 mg/L (WAF). The no observed effect-loading rate at 24 and 48-hours was 100 mg/L (WAF).																											
Data Quality	Reliable without restriction (Klimisch Code).																											
References	Acute Toxicity to <i>Daphnia Magna</i> SafePharm Laboratories Project No.: 1666/064 (21 Jul 2005)																											
Other	Updated: 8/15/2005																											

### 3.3 Toxicity to Aquatic Plants (e.g. Algae)

#### **Robust Summary 3-ALG - 1**

<b><u>Test Substance</u></b>	
CAS #	115733-09-0
Chemical Name	Benzenesulfonic acid, C14-C24 branched and linear alkyl derivatives, calcium salts
Remarks	For more information on this chemical, see Section 2.0 “General Substance Information” in HERTG’s Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
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, <i>Pseudokirchneriella subcapitata</i> formerly called <i>Selenastrum capricornutum</i>
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)	<p>Test Species: Cells taken from a log-growth phase in-house culture of <i>Pseudokirchneriella subcapitata</i> that was originally purchased from University of Texas at Austin alga collection.</p> <p>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</p>

	<p>toxicity test.</p> <p>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.</p> <p>Light: Cool-white fluorescent lights provided a light intensity of 47-50 <math>\mu\text{Ein}/\text{m}^2\text{sec}</math> 24-h per day.</p> <p>Test temperature: 23.3 to 24.0 C.</p> <p>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 &lt;1.0 and &lt;10 mg/L, respectively. Test media pH was 7.5 - 9.8 at 0-hour and 8.3 - 10.1 after 96 hours.</p> <p>Test Levels: Control, 100, 300 &amp; 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.</p> <p>Calculation of <math>\text{EL}_{50}</math>s and NOELs: Binomial nonlinear interpolation methods (Stephan, 1983) were used to calculate <math>\text{EC}_{50}</math>s (i.e., <math>\text{EL}_{50}</math>s). A parametric one-way analysis of variance (ANOVA) and Dunnett's test were used to calculate the no-observed effect level.</p> <p>Method of calculating mean measured concentrations: not applicable</p> <p>Exposure period: 96 hours</p> <p>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.</p>
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<b><u>Results</u></b>	Nominal concentrations: 72- & 96-h EL <sub>50</sub> >1,000 mg/L and 72- & 96-h NOEL = 1,000 mg/L based on both growth rate and biomass measurements.
<b>Remarks</b>	<p>Measured concentration: n/a</p> <p>Unit: mg/L</p> <p>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.</p> <p>Other:</p> <ul style="list-style-type: none"> <li>• 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.</li> <li>• Control response was satisfactory.</li> </ul>
<b><u>Conclusions</u></b>	The test material was not toxic to freshwater alga at loading rates up to and including 1,000 mg/L.
<b><u>Data Quality</u></b>	(1) Reliable without restriction
<b><u>References</u></b>	<p>Ward, T.J. (1994) Acute Toxicity of the Water Accommodated Fractions (WAFs) of CMA #604 to the Freshwater Alga, <i>Selenastrum capricornutum</i>. T.R. Wilbury Study #73-CM-604.</p> <p>Stephan, C.E. (1983). Computer Program for the Calculation of LC50 Values. U.S. EPA. Duluth, MN. Personal Communication.</p>
<b><u>Other</u></b>	Updated: 9-6-00

### Robust Summary 3-ALG-2

<b><u>Test Substance</u></b>	
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	For more information on this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
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, <i>Pseudokirchneriella subcapitata</i> formerly called <i>Selenastrum capricornutum</i>
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 <sub>50</sub> 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)	<p>Test Species: Cells taken from a log-growth phase in-house culture of <i>Pseudokirchneriella subcapitata</i> that was originally purchased from University of Texas at Austin alga collection.</p> <p>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.</p> <p>Test Conditions: A static test was conducted; i.e., there was no daily</p>

	<p>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.</p> <p>Light: Cool-white fluorescent lights provided a light intensity of 47-50 uEin/m<sup>2</sup>sec 24-h per day.</p> <p>Test temperature: 24.0 to 24.2 C.</p> <p>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 &lt;1.0 and &lt;10 mg/L, respectively. Test media pH was 7.3 - 10.8 at 0-hour and 9.7 - 10.8 after 96 hours.</p> <p>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.</p> <p>Calculation of EL<sub>50</sub>s and NOELs: Binomial nonlinear interpolation methods (Stephan, 1983) were used to calculate EC<sub>50</sub>s (i.e., EL<sub>50</sub>s). A parametric one-way analysis of variance (ANOVA) and Dunnett's test were used to calculate the no-observed effect level.</p> <p>Method of calculating mean measured concentrations: not applicable</p> <p>Exposure period: 96 hours</p> <p>Analytical monitoring: At the beginning and end of the test, TOC measurements were between non-detect (&lt;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.</p>
<b><u>Results</u></b>	Nominal concentrations: 72- & 96-h EL <sub>50</sub> >1,000 mg/L, based on both growth rate and biomass measurements. 72- & 96-h NOEL = 1000 mg/L.
Remarks	Measured concentration: n/a

	<p>Unit: mg/L</p> <p>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.</p> <p>Statistical results: A parametric one-way analysis of variance (ANOVA) and Dunnett's test were used to calculate the no-observed effect level.</p> <p>Other:</p> <ul style="list-style-type: none"> <li>• 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.</li> <li>• Control response was satisfactory.</li> </ul>
<b><u>Conclusions</u></b>	The test material was not toxic to freshwater alga at loading rates up to and including 1,000 mg/L.
<b><u>Data Quality</u></b>	(1) Reliable without restriction
<b><u>References</u></b>	<p>Ward, T.J. (1994) Acute Toxicity of the Water Accommodated Fractions (WAFs) of CMA #605 to the Freshwater Alga, <i>Selenastrum capricornutum</i>. T.R. Wilbury Study #73-CM-605.</p> <p>Stephan, C.E. (1983). Computer Program for the Calculation of LC50 Values. U.S. EPA. Duluth, MN. Personal Communication.</p>
<b><u>Other</u></b>	Updated: 9-6-00

**Robust Summary 3-ALG-3**

<b><u>Test Substance</u></b>	
CAS #	71786-47-5
Chemical Name	Benzenesulfonic acid, mono and dialkyl derivatives, magnesium salts
Remarks	For more information on this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
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, <i>Pseudokirchneriella subcapitata</i> formerly called <i>Selenastrum capricornutum</i>
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 <sub>50</sub> 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)	<p>Test Species: Cells taken from a log-growth phase in-house culture of <i>Pseudokirchneriella subcapitata</i> that was originally purchased from University of Texas at Austin alga collection.</p> <p>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.</p> <p>Test Conditions: A static test was conducted; i.e., there was no daily</p>

	<p>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.</p> <p>Light: Cool-white fluorescent lights provided a light intensity of 47-50 uEin/m<sup>2</sup>sec 24-h per day.</p> <p>Test temperature: 24.0 C.</p> <p>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 &lt;1.0 and &lt;10 mg/L, respectively. Test media pH was 7.5 – 9.9 at 0-hour and 8.3 - 10.8 after 96 hours.</p> <p>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.</p> <p>Calculation of EL<sub>50</sub>s and NOELs: Binomial nonlinear interpolation methods (Stephan, 1983) were used to calculate EC<sub>50</sub>s (i.e., EL<sub>50</sub>s). A parametric one-way analysis of variance (ANOVA) and Dunnett's test were used to calculate the no-observed effect level.</p> <p>Method of calculating mean measured concentrations: not applicable</p> <p>Exposure period: 96 hours</p> <p>Analytical monitoring: At the beginning and end of the test, TOC measurements were non-detect (&lt;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.</p>
<b><u>Results</u></b>	<p>Nominal concentrations: 72-h EL<sub>50</sub>s = 1,400 mg/L and &gt;1,500 mg/L based on biomass and growth rate, respectively. 96-h EL<sub>50</sub>s = 1,100 mg/L and &gt;1,500 mg/L, based on biomass and growth rate, respectively. The 72-h and 96-hr NOEL = 1,000 mg/L.</p>
Remarks	<p>Measured concentration: n/a</p>

	<p>Unit: mg/L</p> <p>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<sup>3</sup>. 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<sup>3</sup>) between 72 – 96 hours upon which test concentration growth was compared. This produced an erroneous measurement of NOEL.</p> <p>Other:</p> <ul style="list-style-type: none"> <li>• 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.</li> <li>• 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.</li> <li>• Control response was satisfactory.</li> </ul>
<b><u>Conclusions</u></b>	The test material was not toxic to freshwater alga at loading rates up to and including 1,000 mg/L.
<b><u>Data Quality</u></b>	(1) Reliable without restriction
<b><u>References</u></b>	<p>Ward, T.J. (1994) Acute Toxicity of the Water Accommodated Fractions (WAFs) of CMA #609 to the Freshwater Alga, <i>Selenastrum capricornutum</i>. T.R. Wilbury Study #73-CM-609.</p> <p>Stephan, C.E. (1983). Computer Program for the Calculation of LC50 Values. U.S. EPA. Duluth, MN. Personal Communication.</p>
<b><u>Other</u></b>	Updated: 9-6-00

**Robust Summary 3-ALG-4**

<u>Test Substance</u>	
CAS #	68608-26-4
Chemical Name	Sulfonic acids, petroleum, sodium salts
Remarks	For more information on this chemical, see Section 2.0 “General Substance Information” in HERTG’s Final Submission for Alkaryl Sulfonate Category.
Method	
Method/Guideline followed	OECD Guideline for Testing of Chemicals #201 Alga, Growth Inhibition Test (1984).
Test Type	Static acute toxicity test (Water Accommodated Fraction-WAF)
GLP (Y/N)	Y
Year (Study Performed)	2005
Species/Strain	Freshwater algae, <i>Scenedesmus subspicatus</i> /CCAP 276/20
Element basis (# of cells/mL)	Approximately $2.13 \times 10^6$ cells/mL, 5 mL used to inoculate 1 liter of medium for an initial cell density of $10^4$ cells/mL.
Exposure period/duration	72 hours
Range find test	Yes
Analytical monitoring	Not performed
Statistical methods	A Students t-test incorporating Bartlett’s test for homogeneity of variance was used to compare the area under the growth curve data of the treated and control groups at 72 hours.
Remarks field for test conditions (fill as applicable)	<p>Test Species: Cultures obtained from the Culture Collection of Algae and Protozoa (CCAP), Institute of Freshwater Ecology, The Ferry House, Far Sawrey, Ambleside, Cumbria, U.K.</p> <p>Loading Concentration:  Range Find Study: 0, 10 and 100 mg/L (WAF)  Definitive Study: 0 and 100 mg/L (WAF)</p> <p>Test System: A measured weight of test material was added to a measured volume of culture media in a glass vessel and stirred for 24 hours. Stirring was accomplished using a magnetic stirrer. Mixing speed was adjusted such that a slight vortex formed causing a slight dimple at the media surface. Following the mixing period, the test solution was allowed to stand for one hour. A small amount of the WAF was removed and examined microscopically for the presence of micro-dispersions or globules of test material. Microscopic examination of the 100 mg/L WAF confirmed that there was a significant amount of dispersed test material present in the media. The 100 mg/L WAF was therefore filtered through glass wool prior to use. Microscopic examination post filtration showed there to be micro dispersions or undissolved test material present. Given that the test material exhibited no toxicity, this was considered to have had no effect on the outcome of the study. The siphoned phase (i.e., WAF) was used for the aquatic toxicity test.</p> <p>Test Conditions: A static test was conducted; i.e., there was no daily renewal of test solution. Two (range find study) or six (definitive study) 100-mL replicates</p>

	<p>per treatment, inoculum ~10,000 cells/mL. The 250-mL conical flasks were plugged with polyurethane foam bungs. During the test all treatment and control flasks were randomly placed on an orbital shaker adjusted to approximately 150 cycles per minute under constant light (24 hours/day) for 72 hours. Cell densities were determined using a hemocytometer and light microscopic at 0, 24, 48 and 72 hours. pH was determined at 0 and 72 hours.</p> <p><b>Light: Continuous illumination approximately 7000 lux.</b></p> <p>Test temperature: 24.0° C.</p> <p><b>Culture Media: As specified in the guideline.</b></p> <p>Method of calculating mean measured concentrations: Not applicable.</p> <p>Exposure period: 72 hours</p>
<u>Results</u>	<p>Range Find Study: No effect on growth at 10 or 100 mg/L WAF.</p> <p>Definitive Study: Neither growth nor biomass were affected by the presence of the test material over a 72 hour period at 100 mg/L WAF.</p> <p>The E<sub>b</sub>L<sub>50</sub>, the loading rate that reduced biomass by 50%, was &gt;100 mg/L WAF. The E<sub>r</sub>L<sub>50</sub>, the loading rate that reduced specific growth by 50%, was &gt;100 mg/L WAF.</p> <p>The No Observed Effect Loading Rate (NOEL) was 100 mg/L WAF.</p> <p>The cell concentrations of the control cultures increased by a factor of 43 during the study meeting the guideline requirement of at least a factor of 16 after 72 hours.</p> <p>All test and control cultures were inspected microscopically at 72 hours. No abnormalities were observed in any of the control or treated cultures. Control culture pH increased from 7.3 at 0 hour to 7.8 at 72 hours.</p>
<u>Conclusions</u>	<p>Both the growth and the biomass of <i>Scenedesmus subspicatus</i> (CCAP 276/20) were unaffected by the presence of the test material over the 72-hour exposure period. The E<sub>b</sub>L<sub>50</sub>, the loading rate that reduced biomass by 50% was &gt;100 mg/L WAF. The E<sub>r</sub>L<sub>50</sub>, the loading rate that reduced specific growth by 50% was &gt;100 mg/L WAF. The No Observed Effect Loading Rate (NOEL) was 100 mg/L WAF.</p>
<u>Data Quality</u>	(1) Reliable without restriction
<u>References</u>	Algal Inhibition Test SafePharm Laboratories Project No.: 1666/060 (20 Jul 2005)
<u>Other</u>	Updated: 8/16/2005

### Robust Summary 3-ALG-5

<u>Test Substance</u>	
CAS #	61789-86-4
Chemical Name	Sulfonic acids, petroleum, calcium salts
Remarks	For more information on this chemical, see Section 2.0 “General Substance Information” in HERTG’s Final Submission for Alkaryl Sulfonate Category.
Method	
Method/Guideline followed	OECD Guideline for Testing of Chemicals #201 Alga, Growth Inhibition Test (1984).
Test Type	Static acute toxicity test (Water Accommodated Fraction-WAF)
GLP (Y/N)	Y
Year (Study Performed)	2005
Species/Strain	Freshwater algae, <i>Scenedesmus subspicatus</i> /CCAP 276/20
Element basis (# of cells/mL)	Approximately $2.13 \times 10^6$ cells/mL, 5 mL used to inoculate 1 liter of medium for an initial cell density of $10^4$ cells/mL.
Exposure period/duration	72 hours
Range find test	Yes
Analytical monitoring	Not performed
Statistical methods	A Students t-test incorporating Bartlett’s test for homogeneity of variance was used to compare the area under the growth curve data of the treated and control groups at 72 hours.
Remarks field for test conditions (fill as applicable)	<p>Test Species: Cultures obtained from the Culture Collection of Algae and Protozoa (CCAP), Institute of Freshwater Ecology, The Ferry House, Far Sawrey, Ambleside, Cumbria, U.K.</p> <p>Loading Concentration:  Range Find Study: 0, 10 and 100 mg/L (WAF)  Definitive Study: 0 and 100 mg/L (WAF)</p> <p>Test System: A measured weight of test material was added to a measured volume of culture media in a glass vessel and stirred for 24 hours. Stirring was accomplished using a magnetic stirrer. Mixing speed was adjusted such that a slight vortex formed causing a slight dimple at the media surface. Following the mixing period, the test solution was allowed to stand for one hour. The test media was clear and colorless with test material floating on the surface. A small amount of the WAF was removed and examined microscopically for the presence of micro-dispersions or globules of test material. Microscopic examination of the WAF confirmed that there were no micro-dispersions of test material present. The siphoned phase (i.e., WAF) was used for the aquatic toxicity test.</p> <p>Test Conditions: A static test was conducted; i.e., there was no daily renewal of test solution. Two (range find study) or six (definitive study) 100-mL replicates per treatment, inoculum ~10,000 cells/mL. The 250-mL conical flasks were</p>

	<p>plugged with polyurethane foam bungs. During the test all treatment and control flasks were randomly placed on an orbital shaker adjusted to approximately 150 cycles per minute under constant light (24 hours/day) for 72 hours. Cell densities were determined using a Coulter Multisizer Particle Counter at 0, 24, 48 and 72 hours. pH was determined at 0 and 72 hours.</p> <p><b>Light: Continuous illumination approximately 7000 lux.</b></p> <p>Test temperature: 24.0° C.</p> <p><b>Culture Media: As specified in the guideline.</b></p> <p>Method of calculating mean measured concentrations: Not applicable.</p> <p>Exposure period: 72 hours</p>
<u>Results</u>	<p>Range Find Study: No effect on growth at 10 or 100 mg/L WAF.</p> <p>Definitive Study: Neither growth nor biomass were affected by the presence of the test material over a 72 hour period at 100 mg/L WAF.</p> <p>The E<sub>b</sub>L<sub>50</sub>, the loading rate that reduced biomass by 50%, was &gt;100 mg/L WAF. The E<sub>r</sub>L<sub>50</sub>, the loading rate that reduced specific growth by 50%, was &gt;100 mg/L WAF.</p> <p>The No Observed Effect Loading Rate (NOEL) was 100 mg/L WAF.</p> <p>The cell concentrations of the control cultures increased by a factor of 40 during the study meeting the guideline requirement of at least a factor of 16 after 72 hours.</p> <p>All test and control cultures were inspected microscopically at 72 hours. No abnormalities were observed in any of the control or treated cultures. Control culture pH increased from 7.2 at 0 hour to 7.6 at 72 hours.</p>
<u>Conclusions</u>	<p>Both the growth and the biomass of <i>Scenedesmus subspicatus</i> (CCAP 276/20) were unaffected by the presence of the test material over the 72-hour exposure period. The E<sub>b</sub>L<sub>50</sub>, the loading rate that reduced biomass by 50% was &gt;100 mg/L WAF. The E<sub>r</sub>L<sub>50</sub>, the loading rate that reduced specific growth by 50% was &gt;100 mg/L WAF. The No Observed Effect Loading Rate (NOEL) was 100 mg/L WAF.</p>
<u>Data Quality</u>	(1) Reliable without restriction
<u>References</u>	Algal Inhibition SafePharm Laboratories Project No.: 1666/063 (20 Jul 2005)
<u>Other</u>	Updated: 8/16/2005

#### 4. Mammalian Toxicity

#### Category: Alkaryl Sulfonates

##### 4.1 Acute Toxicity

##### 4.1.1 Acute Oral Toxicity

##### **Robust Summary 3-Acute Oral -1**

<b><i>Test Substance</i></b>	
CAS #	Analog of 70024-69-0
Chemical Name	C20-24 alkaryl calcium salt derivative
Remarks	This substance is an analog for the group of substances in HERTG's Final Submission 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 this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
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 (intra-gastric)
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 intra-gastrically 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.

<b><u>Results</u></b>	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.
<b><u>Conclusions</u></b>	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.
<b><u>Data Quality</u></b>	Reliable without restriction (Klimisch Code)
<b><u>References</u></b>	Unpublished confidential business information
<b><u>Other</u></b>	Updated: 2/10/00 (RTA-004)

**Robust Summary 3-Acute Oral-2**

<b><u>Test Substance</u></b>	
CAS #	CAS# 61789-86-4
Chemical Name	Petroleum derived calcium salt
Remarks	For more information on this chemical, see Section 2.0 “General Substance Information” in HERTG’s Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
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 CrI:CD® (SD)BR
Sex	Male and female
No. of animals/sex/dose	5
Vehicle	Peanut oil
Route of administration	Oral (intra-gastric)
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 intra-gastrically 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.
<b><u>Results</u></b>	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

	<p>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.</p>
<b><u>Conclusions</u></b>	<p>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.</p>
<b><u>Data Quality</u></b>	<p>Reliable without restriction (Klimisch Code)</p>
<b><u>References</u></b>	<p>Unpublished confidential business information</p>
<b><u>Other</u></b>	<p>Updated: 2/9/00 (RTA-001)</p>

**Robust Summary 3-Acute Oral-3**

<b><u>Test Substance</u></b>	
CAS #	CAS# 61790-48-5
Chemical Name	Petroleum derived barium salt
Remarks	For more information on this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
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 (intra-gastric)
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 intra-gastrically 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.
<b><u>Results</u></b>	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.
<b><u>Conclusions</u></b>	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.
<b><u>Data Quality</u></b>	Reliable without restriction (Klimisch Code)
<b><u>References</u></b>	Unpublished confidential business information
<b><u>Other</u></b>	Updated: 2/10/00 (RTA-003)

### Robust Summary 3-Acute Oral-4

<b><u>Test Substance</u></b>	
CAS #	CAS# 68608-26-4
Chemical Name	Petroleum derived sodium salt
Remarks	For more information on this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
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 (intra-gastric)
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 intra-gastrically 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.
<b><u>Results</u></b>	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.

<b><u>Conclusions</u></b>	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.
<b><u>Data Quality</u></b>	Reliable without restriction (Klimisch Code)
<b><u>References</u></b>	Unpublished confidential business information
<b><u>Other</u></b>	Updated: 6/16/00 (RTA-036)

**Robust Summary 3-Acute Oral-5**

<b><u>Test Substance</u></b>	
CAS #	CAS# 68783-96-0
Chemical Name	Petroleum derived calcium salt, overbased
Remarks	For more information on this chemical, see Section 2.0 “General Substance Information” in HERTG’s Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
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 (intra-gastric)
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 intra-gastrically 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.
<b><u>Results</u></b>	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.

<b><u>Conclusions</u></b>	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.
<b><u>Data Quality</u></b>	Reliable without restriction (Klimisch Code)
<b><u>References</u></b>	Unpublished confidential business information
<b><u>Other</u></b>	Updated: 2/17/00 (RTA-018)

**Robust Summary 3-Acute Oral-6**

<b><u>Test Substance</u></b>	
CAS #	CAS# 71549-79-6
Chemical Name	Mixed C15-C30 and C11-13 alkaryl derivative
Remarks	For more information on this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
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 (intra-gastric)
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 intra-gastrically 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.
<b><u>Results</u></b>	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.
<b><u>Conclusions</u></b>	The test article, when administered as received to male Sherman-Wistar rats, had an acute oral LD50 of 14.9 g/kg.
<b><u>Data Quality</u></b>	Reliable without restriction (Klimisch Code)
<b><u>References</u></b>	Unpublished confidential business information
<b><u>Other</u></b>	Updated: 6/15/00 (RTA-035)

### Robust Summary 3-Acute Oral-7

<b><u>Test Substance</u></b>	
CAS #	CAS# 71786-47-5
Chemical Name	Alkaryl magnesium salt derivative
Remarks	For more information on this chemical, see Section 2.0 “General Substance Information” in HERTG’s Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
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 (intra-gastric)
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 intra-gastrically 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.
<b><u>Results</u></b>	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.
<b><u>Conclusions</u></b>	The test article, when administered as received to male Sherman-Wistar rats, had an acute oral LD50 of greater than 16.0 g/kg.
<b><u>Data Quality</u></b>	Reliable without restriction (Klimisch Code)
<b><u>References</u></b>	Unpublished confidential business information
<b><u>Other</u></b>	Updated: 6/16/00 (RTA-034)

**Robust Summary 3-Acute Oral-8**

<b><u>Test Substance</u></b>	
CAS #	Analog of 78330-12-8
Chemical Name	C15-C21 alkaryl sodium salt derivative
Remarks	For more information on this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
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 (intra-gastric)
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 intra-gastrically 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.
<b><u>Results</u></b>	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.
<b><u>Conclusions</u></b>	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.
<b><u>Data Quality</u></b>	Reliable without restriction (Klimisch Code)
<b><u>References</u></b>	Unpublished confidential business information
<b><u>Other</u></b>	Updated: 2/10/00 (RTA-002)

### Robust Summary 3-Acute Oral-9

<b><u>Test Substance</u></b>	
CAS #	CAS# 115733-09-0
Chemical Name	C14-24 alkaryl calcium salt derivative
Remarks	For more information on this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
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 (intra-gastric)
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 intra-gastrically 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.
<b><u>Results</u></b>	
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.

<b><u>Conclusions</u></b>	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.
<b><u>Data Quality</u></b>	Reliable without restriction (Klimisch Code)
<b><u>References</u></b>	Unpublished confidential business information
<b><u>Other</u></b>	Updated: 6/18/01

#### 4.1.2 Acute Dermal Toxicity

##### Robust Summary 3-Acute Dermal-1

<b><u>Test Substance</u></b>	
CAS #	CAS# 115733-09-0
Chemical Name	C14-24 alkaryl calcium salt derivative
Remarks	For more information on this chemical, see Section 2.0 “General Substance Information” in HERTG’s Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
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	<p>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.</p> <p>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.</p> <p>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.</p>
<b><u>Results</u></b>	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.
<b><u>Conclusions</u></b>	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.
<b><u>Data Quality</u></b>	Reliable without restriction (Klimisch Code)
<b><u>References</u></b>	Unpublished confidential business information
<b><u>Other</u></b>	Updated: 6/16/00 (RTA-033)

**Robust Summary 3-Acute Dermal-2**

<b><u>Test Substance</u></b>	
CAS #	CAS# 61789-86-4
Chemical Name	Petroleum derived calcium salt
Remarks	For more information on this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
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.
<b><u>Results</u></b>	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.
<b><u>Conclusions</u></b>	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.
<b><u>Data Quality</u></b>	Reliable without restriction (Klimisch Code)
<b><u>References</u></b>	Unpublished confidential business information
<b><u>Other</u></b>	Updated: 2/17/00 (RTA-020)

**Robust Summary 3-Acute Dermal-3**

<b><u>Test Substance</u></b>	
CAS #	CAS# 68783-96-0
Chemical Name	Petroleum derived calcium salt, overbased
Remarks	For more information on this chemical, see Section 2.0 “General Substance Information” in HERTG’s Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
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.
<b><u>Results</u></b>	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. Erythema was observed in all animals on day 1. Very slight to well defined erythema was observed in 9 of 10 treated

	<p>animals on Day 3. On Day 7 one animal exhibited slight erythema and one animal exhibited well-defined erythema. Slight erythema 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.</p>
<b><u>Conclusions</u></b>	<p>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.</p>
<b><u>Data Quality</u></b>	<p>Reliable without restriction (Klimisch Code)</p>
<b><u>References</u></b>	<p>Unpublished confidential business information</p>
<b><u>Other</u></b>	<p>Updated: 2/17/00 (RTA-019)</p>

**Robust Summary 3-Acute Dermal-4**

<b><u>Test Substance</u></b>	
CAS #	Analog of 70024-69-0
Chemical Name	C20-C24 alkaryl calcium salt derivative
Remarks	This substance is an analog for the group of substances in HERTG's Final Submission 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 this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Alkaryl Sulfonate Category..
<b>Method</b>	
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.
<b><u>Results</u></b>	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.

<b><u>Conclusions</u></b>	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.
<b><u>Data Quality</u></b>	Reliable without restriction (Klimisch Code)
<b><u>References</u></b>	Unpublished confidential business information
<b><u>Other</u></b>	Updated: 10/4/00 (RTA-068)

### 4.1.3 Acute Inhalation Toxicity

#### Robust Summary 3-Acute Inhalation-1

<b><u>Test Substance</u></b>	
CAS #	CAS# 68783-96-0
Chemical Name	Petroleum derived calcium salt, overbased
Remarks	For more information on this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
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.
<b><u>Results</u></b>	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.
<b><u>Conclusions</u></b>	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.
<b><u>Data Quality</u></b>	Reliable without restriction (Klimisch Code)
<b><u>References</u></b>	Unpublished confidential business information
<b><u>Other</u></b>	Updated: 2/18/00 (RTA-023)

## 4.2 Repeated Dose Toxicity

### Robust Summary 3-Repeated Tox - 1

<b><i>Test Substance</i></b>	
CAS #	Analog of 70024-69-0
Chemical Name	C20-24 alkaryl calcium salt derivative
Remarks	This substance is an analog for the group of substances in HERTG's Final Submission 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 this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
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 <i>post-hoc</i> test, non-parametric Kruskal-Wallis and a Mann-Whitney U-test, Bartlett's test for equal variances, a Student's <i>t</i> -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.

	<p>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.</p> <p>Significant deviations from the OECD 407 test guidelines include:</p> <ul style="list-style-type: none"> <li>• 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.</li> <li>• Microscopic pathology was performed as required by OECD 407 guideline in place at the time the study was conducted.</li> </ul>
<b><u>Results</u></b>	
Remarks	<p>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.</p> <p>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.</p>
<b><u>Conclusions</u></b>	<p>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.</p>
<b><u>Data Quality</u></b>	Reliable without restriction (Klimisch Code)
<b><u>References</u></b>	Unpublished confidential business information
<b><u>Other</u></b>	Updated: 2/11/00 (RTA-006)

### Robust Summary 3-Repeated Tox -2

<b><u>Test Substance</u></b>	
CAS #	CAS# 68783-96-0
Chemical Name	Petroleum derived calcium salt, overbased
Remarks	For more information on this chemical, see Section 2.0 “General Substance Information” in HERTG’s Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
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, unabraded, 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 the 1000 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 <i>t</i> -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, unabraded 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.
<b><u>Results</u></b>	
Remarks	<p>A NOAEL of 1000 mg/kg was established for this study. No mortality occurred during this study. Low incidences of very slight erythema, 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</p>

	<p>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.</p>
<b><u>Conclusions</u></b>	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.
<b><u>Data Quality</u></b>	Reliable without restriction (Klimisch Code)
<b><u>References</u></b>	Unpublished confidential business information
<b><u>Other</u></b>	Updated: 2/18/00 (RTA-024)

**Robust Summary 3-Repeated Tox-3**

<b><u>Test Substance</u></b>	
CAS #	CAS# 68783-96-0
Chemical Name	Petroleum derived calcium salt, overbased
Remarks	For more information on this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
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 <sup>3</sup> (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.

<b><u>Results</u></b>	
<b>Remarks</b>	<p>The actual exposure concentrations measured by gravimetric analysis were 49.5, 156, 260 mg/m<sup>3</sup>. 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 &lt;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<sup>3</sup>) is considered the NOAEL by this reviewer.</p>
<b><u>Conclusions</u></b>	<p>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<sup>3</sup> was established for this study based on the slight, dose related increase observed in the severity of microscopic pulmonary findings and increased lung weights.</p>
<b><u>Data Quality</u></b>	Reliable without restriction (Klimisch Code)
<b><u>References</u></b>	Unpublished confidential business information
<b><u>Other</u></b>	Updated: 2/24/00 (RTA-025)

### Robust Summary 3-Repeated Tox - 4

<b><u>Test Substance</u></b>	
CAS #	CAS# 71786-47-5
Chemical Name	Alkaryl magnesium salt derivative
Remarks	For more information on this chemical, see Section 2.0 “General Substance Information” in HERTG’s Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
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

	<p>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.</p>
<p><b><u>Results</u></b></p>	
<p>Remarks</p>	<p>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 than 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.</p> <p>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</p>

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.

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

### Robust Summary 3-Repeated Tox - 5

<b><u>Test Substance</u></b>	
CAS #	CAS# 71786-47-5
Chemical Name	Alkaryl magnesium salt derivative
Remarks	For more information on this chemical, see Section 2.0 “General Substance Information” in HERTG’s Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
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, unabraded, 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 the 1000 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 <i>t</i> -test was used to compare the satellite group’s 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, unabraded 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.
<b><u>Results</u></b>	
Remarks	<p>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</p>

	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.
<b><u>Conclusions</u></b>	A NOAEL of 1000 mg/kg was established for this study. Dermal application of this test material resulted in no signs of overt toxicity.
<b><u>Data Quality</u></b>	Reliable without restriction (Klimisch Code)
<b><u>References</u></b>	Unpublished confidential business information
<b><u>Other</u></b>	Updated: 2/15/00 (RTA-009)

**.Robust Summary 3-Repeated Tox - 6**

<b><u>Test Substance</u></b>	
CAS #	CAS# 115733-09-0
Chemical Name	Benzenesulfonic acid, C14-C24 branched & linear alkyl derivs. Calcium salts
Remarks	For more information on this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
Method/Guideline followed	OECD 407; OPPTS 870.3050
Test Type	28-day oral toxicity study in rats
GLP (Y/N)	Y
Year (Study Performed)	2002-2003
Species	Rat
Strain	Sprague-Dawley CD, 6-7 weeks of age at initiation of treatment
Route of administration	Oral gavage
Duration of test	28 days of treatment followed by 14 day recovery period in the control and high dose groups
Doses/concentration levels	0, 50, 150, 500 and 1000 mg/kg/day
Dose Formulation Analysis	Analysis performed for dosing solution stability, homogeneity and concentration.
Sex	Males and females
Exposure period	28-day treatment duration with a 14 day recovery
Frequency of treatment	Once daily, 7 days/week
Control group and treatment	5 rats/sex/group for each dose, and recovery groups of 5 animals/sex for the control and 1000 mg/kg/day dose. Control group received daily doses of corn oil at 5.0 ml/kg, and treatment groups received the indicated dose of test material diluted in corn oil at a dose volume of 5.0 ml/kg
Post exposure observation period	14-days
Statistical methods	Body weight, body weight change, food consumption, functional observational battery observations, hematology and clinical chemistry parameters, organ weights, organ/body and organ to brain weight ratios were analyzed. Mean values of all dose groups were compared to control at each time interval. Tests included parametric ANOVA with a Tukey-Kramer test, non-parametric Kruskal-Wallis and a Dunn's test, Fisher's Exact test, Chi-Square test, and Levene's test as appropriate.
Remarks field for test conditions	Single oral gavage doses were administered for 28 consecutive days. Clinical observations were made daily, between one-half and two hours following dosing and once daily during recovery. Viability checks were performed twice daily. Ophthalmology examinations were performed during study weeks 3 and 5. An abbreviated functional observation battery (FOB) was performed once prior to

	<p>dosing initiation and once during weeks 0, 1 and 2. A full FOB assessment (home cage, removal from home cage, open field, manipulative tests and motor activity) was performed during weeks 3 and 5. All FOB assessments were performed blind. Body weights were recorded on test days -2, 0, 7, 14, 21, 27, 35 and 41. Fasted body weights were recorded at necropsy. Food consumption was 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. All tissues were examined microscopically in the control and high dose groups. Stomachs were evaluated in all animals.</p>
<p><b><u>Results</u></b></p>	
<p>Remarks</p>	<p>An NOAEL (no observed adverse effect levels) of 1000 mg/kg/day was established for males and of 150 mg/kg/day was established for females for this study.</p> <p>Body weight effects were limited to the males at 500 and 1000 mg/kg/day and included decreased weight gain during the third study week. Overall weight gain was reduced, compared to control, in the 500 and 1000 mg/kg males by approximately 9 and 6% at the end of treatment. The weight gain of the 1000 mg/kg/day males was approximately 6% lower than control at the end of recovery. Statistically significant reductions in mean food consumption were observed in the 500 mg/kg/day males during week 3 of the treatment period and in the 1000 mg/kg/day females during week 2 of the treatment period.</p> <p>Notable microscopic changes were limited to irritation of the nonglandular stomach in the 500 and 1000 mg/kg/day males and in the 150, 500 and 1000 mg/kg/day females. This finding was transient and was not evident in the recovery animals. In the 500 mg/kg/day males, minimal edema in the submucosa was observed in 2 of 5 animals. In the 1000 mg/kg/day males minimal to mild edema in the submucosa and minimal epithelial hyperplasia were observed in 3 of 5 animals at the end of treatment. No stomach abnormalities were evident after recovery.</p> <p>In the 150 mg/kg/day females, minimal edema in the submucosa was observed in 2 of 5 animals. In the 500 mg/kg/day females, mild edema in the submucosa, minimal hemorrhage, minimal epithelial hyperplasia, mild inflammation and a mild ulcer were observed in 1 of 5 animals. In the 1000 mg/kg/day females minimal edema in the submucosa was observed in 1 of 5 animals and minimal to mild epithelial hyperplasia was observed in 2 of 5 animals at the end of treatment. No stomach abnormalities were evident after recovery. No stomach abnormalities were evident after recovery.</p>

	<p>No mortality, significant clinical abnormalities, meaningful neurological changes, ophthalmoscopic changes, hematology, clinical chemistry, urinalysis, absolute and relative organ weights and macroscopic pathology were observed during treatment or recovery.</p> <p>Chemical analysis of dosing solutions confirmed that they were homogeneously prepared and stable at the desired concentrations. Weekly concentration analysis confirmed that the dosing solutions were prepared appropriately.</p>
<b><u>Conclusions</u></b>	Little subchronic toxicity was observed over the range of doses administered in this study. Based on the microscopic data the Study Director concluded that the NOAEL was 1000 mg/kg/day in males and 150 mg/kg/day in females.
<b><u>Data Quality</u></b>	Reliable without restriction (Klimisch Code)
<b><u>References</u></b>	Unpublished confidential business information
<b><u>Other</u></b>	Updated: 6/7/2005; SLI Study No.: 3580.1

### Robust Summary 3-Repeated Tox - 7

<b><u>Test Substance</u></b>	
CAS #	CAS# 68783-96-0
Chemical Name	Petroleum derived calcium salt, overbased
Remarks	For more information on this chemical, see Section 2.0 “General Substance Information” in HERTG’s Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
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 the 1000 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 <i>t</i> -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

	<p>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.</p>
<p><b><u>Results</u></b></p>	
<p>Remarks</p>	<p>A NOAEL of 1000 mg/kg was established for this study. No mortality occurred during this study. Low incidences of very slight erythema, 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</p>

	<p>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 absence 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.</p>
<b><u>Conclusions</u></b>	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.
<b><u>Data Quality</u></b>	Reliable without restriction (Klimisch Code)
<b><u>References</u></b>	Unpublished confidential business information
<b><u>Other</u></b>	Updated: 2/18/00 (RTA-024)

### 4.3 Toxicity to Reproduction

#### Robust Summary 5-ReproTox-1

<b>Test Substance</b>	
CAS #	CAS# 115733-09-0
Chemical Name	Benzenesulfonic acid, C14-C24 branched & linear alkyl derivs. Calcium salts
Remarks	For more information on this chemical, see Section 2.0 “General Substance Information” in HERTG’s Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
Method/Guideline followed	OECD 415
Test Type	Oral (Gavage) One-Generation Reproductive Toxicity Study
GLP (Y/N)	Y
Year (Study Performed)	2003-2004
Species	Rat
Strain	Sprague-Dawley CrI: CD®(SD) IGS BR rats, Males approximately 7 weeks of age at initiation of treatment. Females approximately 8 weeks of age at initiation of treatment.
Route of administration	Orally by gastric intubation
Duration of test	F <sub>0</sub> males- 70 days pre-mating; mating period through completion of parturition. F <sub>0</sub> females- 14 days pre-mating; mating; 25 days of gestation and 20 days of lactation. F <sub>1</sub> pups- gestation through day 20 of lactation.
Doses/concentration levels	0, 50, 167 and 500 mg/kg/day
Vehicle control	<b>Corn Oil</b>
Sex	<b>Males and Females</b>
Frequency of treatment	Once/day, 7 days/week
Analytical confirmation of concentration.	Homogeneity, stability and weekly dose concentration confirmation.
Control and treatment groups	28 F <sub>0</sub> rats/sex/group in the control, low, mid and high dose groups.
Mating	1 male mated to 1 female from the same group until evidence of mating (presence of copulatory plug or sperm) was observed. If evidence of mating was not observed mating was discontinued after three weeks.
Post exposure observation period	<b>None</b>
Statistical methods	Body weights, body weight changes, food consumption, semen parameters, organ weights, number of days to mating, gestation length, pup viability data, total pups delivered, pup body weights and mean live litter size were analyzed by ANOVA followed, as needed, by

	Dunnett's test. Count data were analyzed by Chi-Square test followed by Fisher's Exact Test for copulation and fertility indices, pup sex ratios, number of live and dead pups/group and pup survival. All analysis were two-tailed with a minimum significance level of 5%.
Dose rangefinding study	Yes (28 Day oral gavage study conducted according to OECD Test Guideline 407.)
Remarks field for test conditions	<p><b>F<sub>0</sub> Generation:</b>  All F<sub>0</sub> males were dosed for 70 days prior to mating and through the completion of parturition. All F<sub>0</sub> females were dosed for 14 days prior to mating and through day 20 of lactation. All F<sub>0</sub> animals were examined twice daily for appearance and behavior. Detailed clinical observations were performed weekly and cage side observations were performed daily approximately 30 to 120 minutes post dosing. Body weights were recorded weekly for both sexes prior to mating; maternal body weights were recorded on gestation days 0, 7, 14 and 21 as well as on lactation days 1, 4, 7, 14 and 21. Food consumption was recorded on the same days as body weights except during the mating period and during lactation. Animals were paired 1:1 for mating. Positive evidence of mating was confirmed by the presence of sperm or a vaginal copulatory plug (day 0 of gestation). If evidence of mating was not present after three weeks, mating was discontinued. All of the surviving F<sub>0</sub> females were allowed to deliver and rear their pups to lactation day 21. The offspring were potentially exposed to the test substance in utero and through nursing during lactation days 1-21 until euthanization on post-natal day 21. The surviving F<sub>0</sub> dams were necropsied on lactation day 21, following a minimum of 60 days of dosing. The surviving F<sub>0</sub> males were necropsied at the conclusion of parturition following a minimum of 96 days of dosing. F<sub>0</sub> females that failed to deliver were necropsied on post-mating day 25 (with evidence of mating) or 25 days following the termination of the mating period (with no evidence of mating).</p> <p><b>Organ weights were determined and microscopic examinations were conducted for all surviving control and high dose F<sub>0</sub> animals. Tissues examined microscopically included the liver, kidney, brain, right epididymides, cervix, coagulation gland, ovaries, pituitary, prostate, seminal vesicles, testes, uterus, vagina and gross lesions.</b></p> <p><b>F<sub>0</sub> animals from all groups found dead or sacrificed early were subjected to a gross necropsy and the microscopic evaluation of all tissues.</b></p> <p><b>Sperm was collected from all surviving F<sub>0</sub> males and evaluated for sperm count, concentration, motility and morphology assessment.</b></p> <p><b>F<sub>1</sub> Generation:</b></p>

	<p><b>On lactation day 4 each litter was randomly culled to a maximum of eight pups, 4/sex/litter, when possible. Detailed pup examinations were performed on lactation days 0, 4, 7, 14 and 21. Pup sex was determined on lactation day 0 and verified on lactation days 4, 7, 14 and 21. Individual pup weights were determined on lactation days 1, 4, 7 14 and 21. Pups that were stillborn, cannibalized or found dead were subjected to a gross necropsy with emphasis on developmental morphology. Pups culled on day 4 were subjected to an abbreviated gross necropsy with emphasis on the reproductive system. All surviving pups were euthanized on lactation day 21 and examined macroscopically. All internal gross lesions were preserved for possible future microscopic examination.</b></p>
<p><b><u>Results</u></b></p>	<p>Results of the homogeneity analysis indicate that the test article was homogeneous in the vehicle and stable for ten days when stored under ambient conditions. Concentration analysis confirmed that the test article was at the appropriate concentration in the dosing solutions.</p> <p>F<sub>0</sub> Generation:  F<sub>0</sub> males exhibited a dose related increase in post dosing salivation and dark material around the nose in the mid and high dose groups The remaining F<sub>0</sub> male parameters were unremarkable including: mean body weight and food consumption, mating and fertility indices, absolute and relative organ weights, sperm evaluation parameters and macro and microscopic pathology.</p> <p>The clinical signs of the F<sub>0</sub> females were generally unremarkable. There were no toxicologically meaningful differences between the control low, mid and high dose groups with respect to F<sub>0</sub> female mean body weights, body weight change, food consumption, mating and fertility indices, precoital intervals or gestation length. A macroscopic finding observed in two high dose and one mid female sacrificed on post mating day 25 was a finding of negative ammonium sulfide staining in animals that failed to deliver and were euthanized on gestation day 25.</p> <p>No other remarkable findings were noted in the F<sub>0</sub> females at necropsy and no meaningful microscopic lesions were observed in any of the treated F<sub>0</sub> females.</p> <p>F<sub>1</sub> Generation:  No treatment related findings were noted in the F<sub>1</sub> pups during lactation. No treatment related gross necropsy findings were evident in any of the F<sub>1</sub> pups examined (stillborn, dead during lactation, culled or examined at scheduled sacrifice on lactation day 21.)</p>

<b><u>Conclusions</u></b>	Based on the results of this study the Study Director concluded that the 500 mg/kg/day dose level was the no observed adverse effect level (NOAEL) for parental F <sub>0</sub> and F <sub>1</sub> pup toxicity.
<b><u>Data Quality</u></b>	Reliable without restriction (Klimisch Code)
<b><u>References</u></b>	An Oral (Gavage) Study One – Generation Reproduction Toxicity Study in Sprague Dawley Rats with CASRN 115733-09-0. SLI Study No.: 3626.1 (March 11, 2004)
<b><u>Other</u></b>	Updated: 4/26/2005

#### **4.4 Genetic Toxicity:**

##### **Robust Summary 3-Gentox-1**

<b><i>Test Substance</i></b>	
CAS #	Analog of 70024-69-0
Chemical Name	C20-24 alkaryl calcium salt derivative
Remarks	This substance is an analog for the group of substances in HERTG's Final Submission 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 this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
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.
<b><u>Results</u></b>	
Remarks	<p>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.</p> <p>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.</p>
<b><u>Conclusions</u></b>	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.
<b><u>Data Quality</u></b>	Reliable without restriction (Klimisch Code)
<b><u>References</u></b>	Unpublished confidential business information
<b><u>Other</u></b>	Updated: 2/10/00 (RTA-005)

### Robust Summary 3-Gentox-2

<b><u>Test Substance</u></b>	
CAS #	CAS# 68783-96-0
Chemical Name	Petroleum derived calcium salt, overbased
Remarks	For more information on this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
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.
<b><u>Results</u></b>	
Remarks	All dose rangefinding animals survived and were free of clinical signs. Bone

	<p>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.</p> <p>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 statistically significant decreases in the percentage of polychromatic 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.</p>
<b><u>Conclusions</u></b>	The test material was not genotoxic under the conditions of this study. The genotoxicity NOEL was 2000 mg/kg.
<b><u>Data Quality</u></b>	Reliable without restriction (Klimisch Code)
<b><u>References</u></b>	Unpublished confidential business information
<b><u>Other</u></b>	Updated: 2/18/00 (RTA-022)

### Robust Summary 3-Gentox -3

<b><u>Test Substance</u></b>	
CAS #	CAS# 68783-96-0
Chemical Name	Petroleum derived calcium salt, overbased
Remarks	For more information on this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
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 $3 \times 10^5$ 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 <sup>-</sup> 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	<p>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.</p> <p>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%</p>

	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 <sup>-</sup> cells to grow. Cells were also plated in a non-restrictive media that indicated cell viability. Plates were incubated at 37°C in a humidified 5% CO <sub>2</sub> 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 <sup>4</sup> 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.
<b><u>Results</u></b>	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.
<b><u>Conclusions</u></b>	The test material was not genotoxic under the conditions of this study.
<b><u>Data Quality</u></b>	Reliable without restriction (Klimisch Code)
<b><u>References</u></b>	Unpublished confidential business information
<b><u>Other</u></b>	Updated: 4/11/00 (RTA-028)

### Robust Summary 3-Gentox-4

<b><u>Test Substance</u></b>	
CAS #	CAS# 68783-96-0
Chemical Name	Petroleum derived calcium salt, overbased
Remarks	For more information on this chemical, see Section 2.0 “General Substance Information” in HERTG’s Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
Method/Guideline followed	OECD Guideline 471
Test Type	Bacterial Reverse Mutation Assay
GLP (Y/N)	Y
Year (Study Performed)	1995
Test System	<i>Salmonella typhimurium</i>
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-Aminoanthracene (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 <i>E. coli</i> WP2 <u>uvrA</u> or <i>S. typhimurium</i> 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.
<b><u>Results</u></b>	The test substance was not mutagenic in this assay with or without metabolic activation.
Remarks	<p>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.</p> <p>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.</p>
<b><u>Conclusions</u></b>	The test substance was not mutagenic in any strain of <i>Salmonella typhimurium</i> tested, including at least one dose above the solubility of the test substance. The genotoxicity NOEL was 5000 ug/plate.
<b><u>Data Quality</u></b>	Reliable without restriction (Klimisch Code)
<b><u>References</u></b>	Unpublished confidential business information
<b><u>Other</u></b>	Updated: 2/18/00 (RTA-021)

### Robust Summary 3-Gentox-5

<b><u>Test Substance</u></b>	
CAS #	CAS# 71786-47-5
Chemical Name	Alkaryl magnesium salt derivative
Remarks	For more information on this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
Method/Guideline followed	OECD Guideline 473
Test Type	<i>In Vitro</i> 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 <sub>2</sub> in air. Cultures were seeded at 1.2 x 10 <sup>6</sup> cells (16-hour harvest) and 0.8 x 10 <sup>6</sup> (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	<p>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.</p> <p>The test substance treated groups, selected for chromosome analysis based on cell count data and the presence of participate, were as follows:</p> <p>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</p> <p>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</p>

	nominal.
<b><u>Results</u></b>	The test substance was not mutagenic in this assay with or without metabolic activation.
Remarks	<p>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.</p> <p>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.</p>
<b><u>Conclusions</u></b>	The test material was not genotoxic under the conditions of this study.
<b><u>Data Quality</u></b>	Reliable without restriction (Klimisch Code)
<b><u>References</u></b>	Unpublished confidential business information
<b><u>Other</u></b>	Updated: 4/10/00 (RTA-026)

### Robust Summary 3-Gentox-6

<b><u>Test Substance</u></b>	
CAS #	CAS# 71786-47-5
Chemical Name	Alkaryl magnesium salt derivative
Remarks	For more information on this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
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.

<b><u>Results</u></b>	
Remarks	<p>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.</p> <p>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.</p>
<b><u>Conclusions</u></b>	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.
<b><u>Data Quality</u></b>	Reliable without restriction (Klimisch Code)
<b><u>References</u></b>	Unpublished confidential business information
<b><u>Other</u></b>	Updated: 2/11/00 (RTA-007)

### Robust Summary 3-Gentox-7

<b><u>Test Substance</u></b>	
CAS #	CAS# 71786-47-5
Chemical Name	Alkaryl magnesium salt derivative
Remarks	For more information on this chemical, see Section 2.0 “General Substance Information” in HERTG’s Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
Method/Guideline followed	OECD Guideline 471
Test Type	Bacterial Reverse Mutation Assay
GLP (Y/N)	Y
Year (Study Performed)	1995
Test System	<i>Salmonella typhimurium</i>
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-Aminoanthracene (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	<p>This study was conducted according to OECD Guideline 471 (1983). Revision to this Guideline in 1997 suggests the addition of the <i>E. coli</i> WP2 <u>uvrA</u> or <i>S. typhimurium</i> TA 102 tester strains. Since this study was conducted prior to this revision, these strains were not included.</p> <p>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.</p>

	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.
<b><u>Results</u></b>	The test substance was not mutagenic in this assay with or without metabolic activation.
Remarks	<p>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.</p> <p>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.</p>
<b><u>Conclusions</u></b>	The test substance was not mutagenic in any strain of <i>Salmonella typhimurium</i> tested, including at least one dose above the solubility of the test substance. The genotoxicity NOEL was 1000 ug/plate.
<b><u>Data Quality</u></b>	Reliable without restriction (Klimisch Code)
<b><u>References</u></b>	Unpublished confidential business information
<b><u>Other</u></b>	Updated: 2/14/00 (RTA-008)

### Robust Summary 3-Gentox-8

<b><u>Test Substance</u></b>																																									
CAS #	CAS# Analog of 78330-12-8																																								
Chemical Name	C15-C21 alkaryl sodium salt derivative																																								
Remarks	For more information on this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Alkaryl Sulfonate Category.																																								
<b>Method</b>																																									
Method/Guideline followed	OECD Guideline 471																																								
Test Type	Bacterial Reverse Mutation Assay																																								
GLP (Y/N)	Y																																								
Year (Study Performed)	1983																																								
Test System	<i>Salmonella typhimurium</i>																																								
Strains Tested	<i>Salmonella typhimurium</i> 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	<table border="0"> <tr> <td>TA98</td> <td>+S9</td> <td>2-aminoanthracene</td> <td>2.0 ug/plate</td> </tr> <tr> <td>TA98</td> <td>-S9</td> <td>2-nitroflourene</td> <td>10.0 ug/plate</td> </tr> <tr> <td>TA100</td> <td>+S9</td> <td>2-aminoanthracene</td> <td>2.0 ug/plate</td> </tr> <tr> <td>TA100</td> <td>-S9</td> <td>sodium azide</td> <td>1.0 ug/plate</td> </tr> <tr> <td>TA1535</td> <td>+S9</td> <td>2-aminoanthracene</td> <td>2.0 ug/plate</td> </tr> <tr> <td>TA1535</td> <td>-S9</td> <td>sodium azide</td> <td>1.0 ug/plate</td> </tr> <tr> <td>TA1537</td> <td>+S9</td> <td>2-aminoanthracene</td> <td>2.0 ug/plate</td> </tr> <tr> <td>TA1537</td> <td>-S9</td> <td>9-Aminoacridine</td> <td>50.0 ug/plate</td> </tr> <tr> <td>TA1538</td> <td>+S9</td> <td>2-aminoanthracene</td> <td>2.0 ug/plate</td> </tr> <tr> <td>TA1538</td> <td>-S9</td> <td>2-nitroflourene</td> <td>10.0 ug/plate</td> </tr> </table>	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
TA98	+S9	2-aminoanthracene	2.0 ug/plate																																						
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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 <i>E. coli</i> WP2 <u>uvrA</u> or <i>S. typhimurium</i> 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.
<b><u>Results</u></b>	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.
<b><u>Conclusions</u></b>	Under the conditions of this study, the test material was not mutagenic with or without metabolic activation.
<b><u>Data Quality</u></b>	Reliable without restriction (Klimisch Code)
<b><u>References</u></b>	Unpublished confidential business information
<b><u>Other</u></b>	Updated: 10/4/00 (RTA-070)

### Robust Summary 3 – Gentox-9

<b><u>Test Substance</u></b>																																									
CAS #	Analog of 70024-69-0																																								
Chemical Name	C20-C24 alkaryl calcium salt derivative																																								
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 Final Submission 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 this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Alkaryl Sulfonate Category.																																								
<b>Method</b>																																									
Method/Guideline followed	OECD Guideline 471																																								
Test Type	Bacterial Reverse Mutation Assay																																								
GLP (Y/N)	Y																																								
Year (Study Performed)	1989																																								
Test System	<i>Salmonella typhimurium</i> and <i>Escherichia Coli</i>																																								
Strains Tested	<i>Salmonella typhimurium</i> tester strains TA98, TA100, TA1535, TA1537; <i>Escherichia Coli</i> tester strain WP2uvrA																																								
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	<table border="0"> <tr> <td>TA98</td> <td>+S9</td> <td>2-aminoanthracene</td> <td>2.0 ug/plate</td> </tr> <tr> <td>TA98</td> <td>-S9</td> <td>2-nitroflourene</td> <td>10.0 ug/plate</td> </tr> <tr> <td>TA100</td> <td>+S9</td> <td>2-aminoanthracene</td> <td>2.0 ug/plate</td> </tr> <tr> <td>TA100</td> <td>-S9</td> <td>sodium azide</td> <td>1.0 ug/plate</td> </tr> <tr> <td>TA1535</td> <td>+S9</td> <td>2-aminoanthracene</td> <td>2.0 ug/plate</td> </tr> <tr> <td>TA1535</td> <td>-S9</td> <td>sodium azide</td> <td>1.0 ug/plate</td> </tr> <tr> <td>TA1537</td> <td>+S9</td> <td>2-aminoanthracene</td> <td>2.0 ug/plate</td> </tr> <tr> <td>TA1537</td> <td>-S9</td> <td>ICR-191</td> <td>2.0 ug/plate</td> </tr> <tr> <td>WP2uvrA</td> <td>+S9</td> <td>2-aminoanthracene</td> <td>80.0 ug/plate</td> </tr> <tr> <td>WP2uvrA</td> <td>-S9</td> <td>ICR-191</td> <td>50.0 ug/plate</td> </tr> </table>	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	WP2uvrA	+S9	2-aminoanthracene	80.0 ug/plate	WP2uvrA	-S9	ICR-191	50.0 ug/plate
TA98	+S9	2-aminoanthracene	2.0 ug/plate																																						
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TA1535	+S9	2-aminoanthracene	2.0 ug/plate																																						
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TA1537	+S9	2-aminoanthracene	2.0 ug/plate																																						
TA1537	-S9	ICR-191	2.0 ug/plate																																						
WP2uvrA	+S9	2-aminoanthracene	80.0 ug/plate																																						
WP2uvrA	-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	In the main study there were two treatment sets for each tester strain,																																								

conditions	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.
<b><u>Results</u></b>	The test substance was not genotoxic in this assay with or without metabolic activation.
Remarks	<p>No cytotoxicity was observed in the dose rangefinding study with tester strains TA100 and WP2uvrA 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.</p> <p>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 WP2uvrA 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.</p>
<b><u>Conclusions</u></b>	Under the conditions of this study, the test material was not mutagenic with or without metabolic activation.
<b><u>Data Quality</u></b>	Reliable without restriction (Klimisch Code)
<b><u>References</u></b>	Unpublished confidential business information
<b><u>Other</u></b>	Updated: 10/4/00 (RTA-069)

### Robust Summary 3-Gentox - 10

<b><u>Test Substance</u></b>	
CAS #	CAS# 68783-96-0
Chemical Name	Petroleum derived calcium salt, overbased
Remarks	For more information on this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Alkaryl Sulfonate Category.
<b>Method</b>	
Method/Guideline followed	OECD Guideline 473
Test Type	<i>In Vitro</i> 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 <sub>2</sub> in air. Cultures were seeded at 1.2 x 10 <sup>6</sup> cells (16-hour harvest) and 0.8 x 10 <sup>6</sup> (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	<p>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.</p> <p>The test substance treated groups, selected for chromosome analysis based on cell count data and the presence of participate, were as follows:</p> <p>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</p> <p>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</p>

	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.
<b><u>Results</u></b>	The test substance was not mutagenic in this assay with or without metabolic activation.
Remarks	<p>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.</p> <p>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.</p>
<b><u>Conclusions</u></b>	The test material was not genotoxic under the conditions of this study.
<b><u>Data Quality</u></b>	Reliable without restriction (Klimisch Code)
<b><u>References</u></b>	Unpublished confidential business information
<b><u>Other</u></b>	Updated: 4/11/00 (RTA-027)