

201-14923B

**Substance:** Benzoic acid, 2-hydroxy-, Mono-C14-18 Alkyl Derivatives,  
Calcium Salts

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

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## 1.0 Physico-chemical Data

### 1.1 Water Solubility

#### Robust Summary 14-WaterSol-1

<i>Test Substance</i>	
CAS #	114959-46-5
Chemical Name	Benzoic acid, 2-hydroxy-, mono-C14-18 alkyl derivatives, calcium salts
Remarks	Test material purity: 43% active ingredient, 48% highly refined mineral oil, 9% inorganic calcium salts.
Method	
Method/Guideline followed	Water solubility and n-octanol/water partition coefficient were estimated in accordance with guidelines from the UK Health and Safety Executive, the UK Competent Authority for the notification of new substances.
Test Type	Water solubility and partition coefficient (n-octanol/water) estimated based on ultraviolet absorption of aqueous solutions used as an approximate indicator of the concentrations of aromatic rings in water.
GLP (Y/N)	Not specified
Year (Study Performed)	1991
Test Material Solvent	Octanol, hexane and water
Methods	<p>The OECD Shake flask method was determined to not be appropriate for this test material because it is a complex mixture. The test material contains aromatic rings, which absorb strongly in the ultraviolet region. The UV absorptions of aqueous solutions of the test material were, therefore, used as an approximate indicator of the concentrations of those components in water.</p> <p>The solubility of the test material in octanol was examined by addition of the test material to a quantity of octanol.</p> <p>The solubility of the aromatic components in water was determined, in duplicate, by shaking 1.2 g of test material with 10 mL of water for approximately 10 seconds by hand, immersing at 20 °C for 28 hours then centrifuging at 20°C. The layers were separated using a separatory funnel and the aqueous layer was analyzed directly by UV-visible spectrophotometry with standard solutions of the test material in hexane.</p> <p>The solution was not equilibrated for a longer period of time due to concerns regarding test material stability.</p>
Results	The concentration of the aromatic components in water was estimated to be approximately 9 mg/L. This value is an approximation because it assumes that the extinction coefficient for the test material standard is the same as that for the material dissolving in the water. That will not be the case, as some components of the test material will dissolve preferentially to others. Also the mineral oil component of the test material, which was not quantified in the analysis, might

	<p>make some contribution to UV absorption resulting in a possible over estimate of the aromatic component of the aqueous solution. It was estimated that the true water solubility of the aromatic components of the test material lies between 0.9 and 90 mg/L.</p> <p>4.49 g of test material was added to 2.33 g of octanol. A homogeneous solution was formed with a total volume of 7 mL. The result indicates that the solubility in octanol is greater than 0.64 g/mL.</p> <p>Although the aqueous solution will not contain the components of the test material in the same ratio as the hexane standard, the results allow an estimate to be made of the log P<sub>ow</sub> as follows:</p> <p><math>P_{ow} &gt; 0.64/9 \times 10^{-6}</math> or <math>&gt;7 \times 10^4</math>  Log P<sub>ow</sub> &gt; 4.9</p> <p>It was estimated that the true water solubility of the aromatic components of the test material lies between 0.9 and 90 mg/L.</p> <p>Based on the highest water solubility value of 90 mg/L, the log P<sub>ow</sub> is &gt; 3.9. A water solubility value of 0.9 mg/L equates to a log P<sub>ow</sub> of &gt; 5.9.</p>
<u>Conclusions</u>	Log P <sub>ow</sub> > 3.9, allowing for a possible ten fold error in the determination of the solubility of the test material in water. The solubility of the test material in water was approximately 9 mg/L (0.9-90 mg/L) for those species containing a chromophore.
<u>Data Quality</u>	Reliable without restriction.
<u>References</u>	Confidential business information.
<u>Other</u>	Updated: 11/21/2003

## Robust Summary 14-WaterSol-2

<i>Test Substance</i>	
CAS #	114959-46-5 (Test Campaign 2 AI-28)
Chemical Name	Benzoic acid, 2-hydroxy-, mono-C>13 alkyl derivatives, calcium salts
Remarks	<p>Test material purity: 28% active ingredient, 51% highly refined mineral oil, 21% inorganic calcium salts</p> <p>Test material is a complex mixture. Because the various components have different limits of solubility the solubility of the mixture is dependent on loading rate.</p>
Method	
Method/Guideline followed	The water solubility of this test material was determined according to the principles of the EC Test A6 guideline (Official Journal of the European Communities, 1992).
Test Type	Water Solubility
GLP (Y/N)	Yes
Year (Study Performed)	1996
Methods	<p>Tests of the solubility of the test material were carried out in several sets. Loading rates of approximately 1, 10 and 100 g/L were utilized. Stirring times ranged from 6 to 96 hours. The typical procedure for a loading rate of 1 g/L was as follows:</p> <p>Approximately 0.10 g of test material was added to each test flask, 100 mL of water was added to each flask, the flasks were stoppered and placed in a water bath (20°C). Each flask was stirred with a magnetic stirrer for the test period. The vortex extended two-thirds down the depth of the vessel. At the end of the incubation period the flasks were removed and allowed to settle. 25-30 mL of the solution was placed in a centrifuge tube and centrifuged at 3000 rpm for 30 minutes at 30°C.</p> <p>Two analytical approaches were used to determine solubility, total; carbon analysis and UV spectrophotometry. Total carbon analysis provided information about the sum of all carbon containing materials dissolved in water. UV spectrophotometry essentially determined only the calcium alkyl salicylate dissolved in water. Any inorganic calcium salts or mineral oil present in the aqueous phase were expected to have a low or essentially no UV absorbance. Since the test material contains calcium carbonate, inorganic carbon content was measured to determine whether the test material was physically stable in the water phase. Calcium content was also measured to establish whether any dissociated calcium salts were present.</p>
Results	<p>Total carbon analysis indicated that 24 hour vigorous stirring was sufficient to achieve equilibration. The mean values for the 1 g/L loading rate, after 24, 48 and 96 hours of vigorous mixing, were 20, 21 and 21 mg carbon/L, although the 96-hour values were variable (21± 15 mg carbon/L). Mean values for the 100 g/L loading rate after 24, 48 and 96 hours of vigorous mixing were 65, 69 and 71 mg carbon/L.</p> <p>Solubility was dependent on loading rate. The mean carbon content at 1 g/L and</p>

	<p>100 g/L loading rates between 24 and 96 hours of stirring were <math>20 \pm 11</math> mg and <math>69 \pm 4</math> mg carbon/L. Previous studies have shown that the mineral oil component of the test material contributes <math>2.0 \pm 1.0</math> mg carbon/L.</p> <p>The test material has a calculated percent carbon content of 67%; the concentration of components of the test material in the aqueous phase were <math>30 \pm 16</math> mg/L and <math>103 \pm 6</math> mg/L at 1 and 100 g/L loading rates respectively.</p> <p>The solubility of the test material determined by UV analysis was in broad agreement (with a few low values).</p> <p>Inorganic carbon and calcium analysis indicated that the test substance was physically stable in water.</p>
<u>Conclusions</u>	The concentration of components of the test material in the aqueous phase were $30 \pm 16$ mg/L and $103 \pm 6$ mg/L at 1 and 100 g/L loading rates respectively.
<u>Data Quality</u>	Reliable without restriction.
<u>References</u>	Confidential business information.
<u>Other</u>	Updated: 11/27/2003

## 1.2 Partition Coefficient

### Robust Summary 14-Kow-1

<u>Test Substance</u>	
CAS #	114959-46-5
Chemical Name	Benzoic acid, 2-hydroxy-, mono-C14-18 alkyl derivatives, calcium salts
Remarks	Test material purity: 43% active ingredient, 48% highly refined mineral oil, 9% inorganic calcium salts.
Method	
Method/Guideline followed	Water solubility and n-octanol/water partition coefficient were estimated in accordance with guidelines from the UK Health and Safety Executive, the UK Competent Authority for the notification of new substances.
Test Type	Water solubility and partition coefficient (n-octanol/water) estimated based on ultraviolet absorption of aqueous solutions used as an approximate indicator of the concentrations of aromatic rings in water.
GLP (Y/N)	Not specified
Year (Study Performed)	1991
Test Material Solvent	Octanol, hexane and water
Methods	<p>The OECD Shake flask method was determined to not be appropriate for this test material because it is a complex mixture. The test material contains aromatic rings, which absorb strongly in the ultraviolet region. The UV absorptions of aqueous solutions of the test material were, therefore, used as an approximate indicator of the concentrations of those components in water.</p> <p>The solubility of the test material in octanol was examined by addition of the test material to a quantity of octanol.</p> <p>The solubility of the aromatic components in water was determined, in duplicate, by shaking 1.2 g of test material with 10 mL of water for approximately 10 seconds by hand, immersing at 20 °C for 28 hours then centrifuging at 20°C. The layers were separated using a separatory funnel and the aqueous layer was analyzed directly by UV-visible spectrophotometry with standard solutions of the test material in hexane.</p> <p>The solution was not equilibrated for a longer period of time due to concerns regarding test material stability.</p>
Results	<p>The concentration of the aromatic components in water was estimated to be approximately 9 mg/L. This value is an approximation because it assumes that the extinction coefficient for the test material standard is the same as that for the material dissolving in the water. That will not be the case, as some components of the test material will dissolve preferentially to others. Also the mineral oil component of the test material, which was not quantified in the analysis, might make some contribution to UV absorption resulting in a possible over estimate of the aromatic component of the aqueous solution. It was estimated that the true water solubility of the aromatic components of the test material lies between 0.9 and 90 mg/L.</p>

	<p>4.49 g of test material was added to 2.33 g of octanol. A homogeneous solution was formed with a total volume of 7 mL. The result indicates that the solubility in octanol is greater than 0.64 g/mL.</p> <p>Although the aqueous solution will not contain the components of the test material in the same ratio as the hexane standard, the results allow an estimate to be made of the log P<sub>ow</sub> as follows:</p> <p><math>P_{ow} &gt; 0.64/9 \times 10^{-6}</math> or <math>&gt;7 \times 10^4</math>  Log P<sub>ow</sub> &gt; 4.9</p> <p>It was estimated that the true water solubility of the aromatic components of the test material lies between 0.9 and 90 mg/L.</p> <p>Based on the highest water solubility value of 90 mg/L, the log P<sub>ow</sub> is &gt; 3.9. A water solubility value of 0.9 mg/L equates to a log P<sub>ow</sub> of &gt; 5.9.</p>
<u>Conclusions</u>	Log P <sub>ow</sub> > 3.9, allowing for a possible ten fold error in the determination of the solubility of the test material in water. The solubility of the test material in water was approximately 9 mg/L (0.9-90 mg/L) for those species containing a chromophore.
<u>Data Quality</u>	Reliable without restriction.
<u>References</u>	Confidential business information.
<u>Other</u>	Updated: 11/21/2003

### **1.3 Hydrolysis**

#### **Robust Summary 14-Hydro-1**

<u>Test Substance</u>	
CAS #	114959-46-5
Chemical Name	Benzoic acid, 2-hydroxy-, mono-C14-18 alkyl derivatives, calcium salts
Remarks	
Method	
Method/Guideline followed	Other: Technical discussion
Test Type	Not applicable
GLP (Y/N)	Not applicable
Year (Study Performed)	Not applicable
Test Material Solvent	Not applicable
Methods	Not applicable
Results	Not applicable
<u>Conclusions</u>	<p>In the environment, hydrolysis will not contribute to the degradation of Benzoic acid, 2-hydroxy-, mono-C14-18 alkyl derivatives, calcium salts (CAS No. 114959-46-5). Benzoic acid, 2-hydroxy-, mono-C14-18 alkyl derivatives, calcium salts is not amenable to hydrolysis because it does not contain any readily hydrolysable functional groups.</p> <p>Hydrolysis of an organic molecule occurs when a molecule (R-X) reacts with water to form a new carbon-oxygen bond after the carbon-X bond is cleaved. Chemicals that have a potential to hydrolyze include alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters (1). Benzoic acid, 2-hydroxy-, mono-C14-18 alkyl derivatives, calcium salts does not contain any of these functional groups. Therefore, Benzoic acid, 2-hydroxy-, mono-C14-18 alkyl derivatives, calcium salts is not anticipated to undergo hydrolysis, and this degradative process will not contribute to the removal of this material in the environment.</p>
<u>Data Quality</u>	Not applicable
<u>References</u>	<ol style="list-style-type: none"><li>1. Harris, J.C. 1982. "Rate of Hydrolysis," Chapter 7 in: W.J. Lyman, W.F. Reehl, and D.H. Rosenblatt, eds., Handbook of Chemical Property Estimation Methods, McGraw-Hill Book Company, New York, NY, USA.</li></ol>
<u>Other</u>	Updated: 12/17/2003

## 2.0 Environmental Fate and Pathways

### 2.1 Biodegradation

#### **Robust Summary 14-Biodeg-1**

<i>Test Substance</i>	
CAS #	114959-46-5 (test campaign 1 AI-43)
Chemical Name	Benzoic acid, 2-hydroxy-, mono-C14-18 alkyl derivatives, calcium salts
Remarks	Test material purity: 43% active ingredient, 48% highly refined mineral oil, 9% inorganic calcium salts.
<b><u>Method</u></b>	
Method/Guideline Followed	Similar to ISO (1997) Headspace CO <sub>2</sub> Biodegradation Test with modifications recommended by CONCAWE
Test Type (aerobic/anaerobic)	Aerobic
GLP (Y/N)	Y
Year (study performed)	1997
Contact time (units)	56 days
Test apparatus	160 mL capacity serum bottles
Inoculum	<p>Activated sewage sludge from a domestic wastewater treatment plant and soil filtrate were collected. Sieved soil, activated sludge and yeast extract were added to 2 liters of mineral salts medium (OECD) and mixed. Eight adaptation cultures were prepared. Each flask was dosed with the test material (4 mg/L as carbon). The test substance was added on a glass fiber filter. The flasks were then closed with foam stoppers and incubated at 20°C while shaking at 120 rpm. On days 7 and 11, any evaporation loss was made up with distilled water, the pH adjusted, if necessary, to between 7.2 and 7.6, and 8 mg/L as carbon of the test material was added to each flask.</p> <p>On day 14 pre-exposure (Day 0 of the test) the pre-exposed inoculum was coarse filtered and shaken until used. Mineral salts medium (OECD) was inoculated with 10% of the inoculum and 107 mL aliquots were dispensed into sets of 10 replicate 160 mL capacity serum bottles</p>
Replicates:	<p><i>All groups tested in duplicate. Test groups included the following:</i></p> <ol style="list-style-type: none"> <li>1) Blanks</li> <li>2) Test material at 20 mg/L carbon</li> <li>3) Hexadecane at 20 mg/L carbon (reference compound)</li> <li>4) Test material plus hexadecane both at 20 mg/L (inhibition control)</li> <li>5) Mineral oil at 20 mg/L carbon</li> <li>6) Test material at 20 mg/L carbon poisoned by addition of 50 mg/L HgCl<sub>2</sub> on day 0 and 28 (used to correct for any abiotic production of inorganic carbon).</li> </ol>
Temperature of incubation:	20± 1°C

Study initiation:	<p>The test material, hexadecane and mineral oil were added as measured weights on filters. The liquid to headspace ratio was 2:1. The bottles were sealed and incubated while shaking at 120 rpm.</p> <p>CO<sub>2</sub> evolution during incubation was determined by measuring inorganic carbon production. On days 7, 14, 28, 42 and 56 duplicate bottles from each group were acidified with 1 mL concentrated orthophosphoric acid, injected through the stopper, and shaken for 1 hour. The inorganic carbon concentration of the headspace was determined by duplicate injections of 1 mL headspace gas into a carbon analyzer that had been calibrated against a 1.0% w/w CO<sub>2</sub> in N<sub>2</sub> standard.</p>
Sampling:	Days 7, 14, 28, 42 and 56
Concentration of test substance:	20 mg carbon /L
Controls:	Blank and positive controls used per guideline. Positive control was hexadecane at a loading of 20 mg carbon/L.
Method of calculating biodegradation values:	Biodegradation was calculated as net inorganic carbon (IC) production and expressed as a percentage of the theoretical maximum inorganic production (ThIC), based on the quantity of test substance (as carbon) added initially. ThIC is analogous to the term ThCO <sub>2</sub> used in the CO <sub>2</sub> evolution (modified Sturm) ready biodegradability test (OECD, 1992)

<p><u>Results</u></p>	<p>Steady biodegradation of the test material occurred over the incubation period and mineralization of CO<sub>2</sub> was still occurring at the end of the test.</p> <table border="0" data-bbox="630 302 1321 737"> <thead> <tr> <th data-bbox="732 302 906 338">Study Group</th> <th data-bbox="1032 302 1321 373">Mean Biodegradation at 56 Days (%ThIC)</th> </tr> </thead> <tbody> <tr> <td data-bbox="630 373 808 409">Test Material</td> <td data-bbox="1159 373 1192 409">63</td> </tr> <tr> <td data-bbox="630 409 959 483">Test Material plus HgCl<sub>2</sub> (poisoned controls)</td> <td data-bbox="1159 428 1192 464">0</td> </tr> <tr> <td data-bbox="630 483 943 556">Mineral Oil (Carrier Oil Control)</td> <td data-bbox="1159 501 1192 537">65</td> </tr> <tr> <td data-bbox="630 556 919 630">Hexadecane (Positive Control)</td> <td data-bbox="1159 575 1192 611">94</td> </tr> <tr> <td data-bbox="630 630 935 737">Test Material plus hexadecane (inhibition control)</td> <td data-bbox="1159 669 1192 705">69</td> </tr> </tbody> </table> <p>A value of <math>\geq 60\%</math> was used in this study as the pass level as this is the pass level for ready biodegradability in the CO<sub>2</sub> evolution test (Modified Strum Test). As the final extent of biodegradation of the test material exceeded 60% the test material was considered to have “inherent, ultimate, biodegradability”.</p> <p>All assay validity criteria were met.</p>	Study Group	Mean Biodegradation at 56 Days (%ThIC)	Test Material	63	Test Material plus HgCl <sub>2</sub> (poisoned controls)	0	Mineral Oil (Carrier Oil Control)	65	Hexadecane (Positive Control)	94	Test Material plus hexadecane (inhibition control)	69
Study Group	Mean Biodegradation at 56 Days (%ThIC)												
Test Material	63												
Test Material plus HgCl <sub>2</sub> (poisoned controls)	0												
Mineral Oil (Carrier Oil Control)	65												
Hexadecane (Positive Control)	94												
Test Material plus hexadecane (inhibition control)	69												
<p><u>Conclusions</u></p>	<p>The test substance was considered to have “inherent, ultimate, biodegradability”.</p>												
<p><u>Data Quality</u></p>	<p>(1) Reliable without restriction</p>												
<p><u>References</u></p>	<p>Confidential Business Information</p>												
<p><u>Other</u></p>	<p>Updated: 11/21/2003</p>												

### 3.0 AQUATIC ORGANISMS

#### 3.1 Acute and Prolonged Toxicity to Fish **Robust Summary 14-Fish-1**

<b><i>Test Substance</i></b>	
CAS #	114959-46-5
Chemical Name	Benzoic acid, 2-hydroxy-, mono-C14-18 alkyl derivatives, calcium salts
Remarks	Test material purity: 28% active ingredient, 51% highly refined mineral oil, 21% inorganic calcium salts
<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)	1995
Species/Strain	<i>Oncorhynchus mykiss</i>
Analytical Monitoring	Concentrations of dissolved components of the test substance were below the limit of determination (0.4 mg/L) of the analytical method.
Exposure Period (unit)	96 hours
Statistical methods	Based on the results, statistical analysis of the survival data was not performed.
Remarks field for test conditions (fill as applicable)	<p>Fingerlings were obtained from a commercial breeder and were acclimated for nine days. The control fish had a mean length of 4.4 cm and a mean weight of 0.77 g.</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 (1 cm vortex) for 70-74 hours in a sealed 22-liter vessel. Following settling for 1-2 hour and separation of any surface film from the water phase, the water phase was used as the test solution in the study.</p> <p>A sealed 96 hours semi-static test was carried out with daily renewal of the test WAF's. Three 11-liter glass aspirators were filled with each WAF. A fourth chamber served as the control. Seven fish were placed in each chamber and the chambers were sealed ensuring that there was no headspace. 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 and pH were determined throughout the study. Total hardness and chlorine concentration were determined for each fresh batch of control water. Water temperature was monitored throughout the study.</p>

Test Concentrations (Nominal)	0, 220, 460 and 1000 mg/L (Water Accommodated Fraction-WAF) Test concentrations were selected based on a range-finding study.
<b><u>Results</u></b>	The 24, 48, 72 and 96 hour LL <sub>50</sub> 's (loading levels likely to cause 50% mortality) were all >1000 mg/L WAF.
Remarks	Range finding results indicated that the 48-hour LL <sub>50</sub> was >1000 mg/L. During the main study, no toxicity was observed at dose levels up to and including 1000 mg/L (WAF). The 24, 48, 72 and 96 hour LL <sub>50</sub> 's (loading levels likely to cause 50% mortality) were therefore all >1000 mg/L WAF. Water chemistry: Temperature: 16.0-16.2 °C; Dissolved Oxygen: 8.2-9.4 mg/L; pH: 7.1-7.6; Total Hardness: 266-278 mg/L as CaCO <sub>3</sub> Residual Chlorine <0.02 mg/L.
<b><u>Conclusions</u></b>	The 24, 48, 72 and 96 hour LL <sub>50</sub> 's (loading levels likely to cause 50% mortality) were all >1000 mg/L WAF.
<b><u>Data Quality</u></b>	Reliable with restriction (Klimisch Code). Restriction due to the lack of analytical confirmation of test concentrations.
<b><u>References</u></b>	Unpublished confidential business information
<b><u>Other</u></b>	Updated: 11/21/2003

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

#### Robust Summary 14-Aquatic Invertebrates Tox – 1

<b><i>Test Substance</i></b>	
CAS #	114959-46-5 (Test Campaign 2 AI-28)
Chemical Name	Benzoic acid, 2-hydroxy-, mono-C14-18 alkyl derivatives, calcium salts
Remarks	Test material purity: 28% active ingredient, 51% highly refined mineral oil, 21% inorganic calcium salts
<b>Method</b>	
Method/Guideline followed	OECD Guideline for Testing of Chemicals #202 <i>Daphnia</i> sp. Acute Immobilization Test and Reproduction Test (1984).
Test Type	Static acute toxicity test (non-renewal)
GLP (Y/N)	Y
Year (Study Performed)	1995
Species/Strain	<i>Daphnia magna</i>
Analytical Monitoring	Concentrations of dissolved components of the test substance were below the limit of determination (0.4 mg/L) of the analytical method.
Exposure Period (unit)	48 hours
Statistical methods	Based on the results, statistical analysis of the survival data was not performed.
Remarks field for test conditions (fill as applicable)	<p>Juvenile daphnids less than 24-hours old were produced from laboratory in-house culture.</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 (1 cm vortex) for 47 hours in a sealed 2.25-liter vessel. Following settling for 1-1.5 hour and separation of any surface film from the water phase, the water phase was used as the test solution in the study.</p> <p>Twenty daphnids, less than 24 hours old were distributed into each concentration (10 daphnids/replicate). Test chambers consisted of 150 mL Erlenmeyer flasks filled with each WAF test solution. Test chambers were sealed during the study. Control test chambers were handled in an identical fashion. Daphnids were observed at 24 and 48 hours for immobility. Daphnia were considered to be immobilized if, after a brief stirring, they did not swim during a 15 second period of observation. Dissolved oxygen and pH were determined at time 0 and at 48 hours. Total hardness of control water was determined at the start of the test. Water temperature was monitored throughout the study.</p>

Test Concentrations (Nominal)	0, 100, 220, 460 and 1000 mg/L (Water Accommodated Fraction-WAF) Test concentrations were selected based on a range-finding study.
<b><u>Results</u></b>	The 48 hour EL <sub>50</sub> was >1000 mg/L
Remarks	Range finding results indicated that the 48 hour EL <sub>50</sub> was >1000 mg/L. During the main study, no immobilization of <i>Daphnia magna</i> was observed at dose levels up to and including 1000 mg/L (WAF) at both 24 and 48 hours. The 24 and 48 hour EL <sub>50</sub> 's (loading levels likely to cause 50% immobilization) were therefore >1000 mg/L WAF. Water chemistry: Temperature: 19.6-20.3°C; Dissolved oxygen: 8.9-9.1 mg/L; pH: 7.9-8.2; Total Hardness: 186 mg/L as CaCO <sub>3</sub> .
<b><u>Conclusions</u></b>	The 48 hour EL <sub>50</sub> was >1000 mg/L
<b><u>Data Quality</u></b>	Reliable with restriction (Klimisch Code). Restriction due to the lack of analytical confirmation of test concentrations.
<b><u>References</u></b>	Unpublished confidential business information
<b><u>Other</u></b>	Updated: 11/21/2003

## Robust Summary 14-Aquatic Invertebrates Tox – 2

<b><u>Test Substance</u></b>	
CAS #	114959-46-5 (test campaign AI 43)
Chemical Name	Benzoic acid, 2-hydroxy-, mono-C14-18 alkyl derivatives, calcium salts
Remarks	Test material purity: 43% active ingredient, 48% highly refined mineral oil, 9% inorganic calcium salts.
<b>Method</b>	
Method/Guideline followed	OECD Guideline for Testing of Chemicals #202 <i>Daphnia</i> sp. Acute Immobilization Test and Reproduction Test (1984).
Test Type	Static acute toxicity test (non-renewal)
GLP (Y/N)	Y
Year (Study Performed)	1987
Species/Strain	<i>Daphnia magna</i>
Analytical Monitoring	Not performed
Exposure Period (unit)	48 hours
Statistical methods	Statistical analysis of the survival data was not performed.
Remarks field for test conditions (fill as applicable)	<p>Juvenile daphnids less than 24-hours old were produced from laboratory in-house culture.</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 23 hours in a sealed vessel. Care was taken to ensure that emulsions did not form. Following settling for 1 hour and separation of any surface film from the water phase, the water phase was used as the test solution in the study.</p> <p>Thirty daphnids, less than 24 hours old were distributed into each concentration (10 daphnids/replicate). Test chambers consisted of 150 mL Pyrex crystallizing dishes containing 100 mL of test solution. Test chambers were not covered during the study. Control test chambers were handled in an identical fashion. Daphnids were observed at 24 and 48 hours for immobility and abnormal effects. Daphnia were considered to be immobilized if, after a brief stirring, they did not swim during a 10 second period of observation. Temperature, dissolved oxygen, water hardness and pH were determined at time 0 and at 48 hours.</p> <p>Light cycles were maintained at 16-hour light per day.</p>
Test Concentrations (Nominal)	0, 10, 100 and 1000 mg/L (Water Accommodated Fraction-WAF)
<b><u>Results</u></b>	
Remarks	<p>Immobilization of all <i>Daphnia magna</i> was observed at 1000 mg/L (WAF) at both 24 and 48 hours. Immobilization of all <i>Daphnia magna</i> was observed at 100 mg/L (WAF) at 48 hours. The no observed effect level was 10 mg/L (WAF).</p> <p>Water chemistry: Temperature: 18-22°C; Dissolved oxygen: 8.8-9.2</p>

	mg/L; pH: 8.0-8.1; Total Hardness: 170 mg/L as CaCO <sub>3</sub> .
<b><u>Conclusions</u></b>	The 100 and 1000 mg/L (WAF) concentrations resulted in 100% immobilization at 48 hours. The 10 mg/L (WAF) concentration was the no observed effect level.
<b><u>Data Quality</u></b>	Reliable with restriction (Klimisch Code). Restriction due to the lack of analytical confirmation of test concentrations.
<b><u>References</u></b>	Unpublished confidential business information
<b><u>Other</u></b>	Updated: 11/21/2003

### Robust Summary 14-Aquatic Invertebrates Tox – 3

<b><u>Test Substance</u></b>	
CAS #	114959-46-5
Chemical Name	Benzoic acid, 2-hydroxy-, mono-C14-18 alkyl derivatives, calcium salts
Remarks	Test material purity: 43% active ingredient, 48% highly refined mineral oil, 9% inorganic calcium salts.
<b>Method</b>	
Method/Guideline followed	OECD Guideline for Testing of Chemicals #202 <i>Daphnia</i> sp. Acute Immobilization Test and Reproduction Test (1984).
Test Type	Chronic toxicity and reproduction test (Water Accommodated Fraction (WAF) renewal)
GLP (Y/N)	Y
Year (Study Performed)	1994
Species/Strain	<i>Daphnia magna</i>
Analytical Monitoring	Previous experience showed that at a loading rate of 1 mg/L the amount of test material going into solution would be below the limit of determination of available analytical techniques.
Exposure Period (unit)	21 days
Statistical methods	The mortality of the parent generation was not analyzed statistically. The mean number of offspring per day produced by daphnids exposed to the test material was compared to control using Wilcoxon's rank-Sum test.
Remarks field for test conditions (fill as applicable)	<p>Juvenile daphnids less than 24-hours old were produced from laboratory in-house culture.</p> <p>A measured volume of test material was added to a measured volume of dilution water and continuously stirred for 22-25 hours in a sealed vessel. Care was taken to ensure that an emulsion did not form. Following settling for 1 hour the aqueous phase was separated for use as the test medium. Water for use as the control medium was treated in the same way with out the addition of the test material. Soil extract was added at 20ml/L to the control and test mediums. Fresh WAFs were prepared at 1-3 day intervals throughout the study.</p> <p>The study was conducted in Erlenmeyer flasks, each completely filled with 150 mL of test medium and sealed. Twelve replicates were used containing 1 mg/L WAF. Twelve control replicates were also utilized. One daphnid, less than 24 hours old, was added to each flask. The daphnia were fed daily.</p> <p>At 24-hour intervals the number of immobilized daphnia were recorded. At 1 to 3 day intervals throughout the test the live daphnia were transferred to flasks containing freshly prepared media. At each change over of test media, live young from each flask were removed and counted.</p>

	<p>The pH and concentration of dissolved oxygen were determined in each batch of fresh media and in the used media immediately after transfer of the daphnia to fresh solutions. Total hardness and alkalinity of each batch of fresh control medium was determined. Water temperature was recorded in a blank flask, adjacent to the test flasks, hourly during the study.</p> <p>Light cycles were maintained at 16-hour light per day.</p>																								
Test Concentrations (Nominal)	0 and 1 mg/L (Water Accommodated Fraction-WAF)																								
<b><u>Results</u></b>																									
Remarks	<p>Mortality rates were 50% in the WAF treated group and less than 10% in the control group.</p> <p>Reproduction of <i>Daphnia magna</i> exposed to 1 mg/L WAF was significantly greater than control after 8 days of exposure, significantly less after 15 days of exposure and not significantly different from control after 21 days of exposure. It was concluded by the Study Director that exposure to the WAF did not significantly affect the number of young produced per individual daphnid.</p> <p>Cumulative Number (Range) of live Young Produced/Adult</p> <table border="1"> <thead> <tr> <th></th> <th>Day 8</th> <th>Day 13</th> <th>Day 21</th> </tr> </thead> <tbody> <tr> <td>Control</td> <td>14-25</td> <td>37-68</td> <td>59-95</td> </tr> <tr> <td>1 mg/L WAF</td> <td>17-35</td> <td>17-34</td> <td>45-74</td> </tr> </tbody> </table> <p>Number (Range) of live Young Produced/Adult/Day</p> <table border="1"> <thead> <tr> <th></th> <th>Day 8</th> <th>Day 13</th> <th>Day 21</th> </tr> </thead> <tbody> <tr> <td>Control</td> <td>1.8-3.1</td> <td>3.3-4.5</td> <td>2.8-4.5</td> </tr> <tr> <td>1 mg/L WAF</td> <td>2.1-4.4</td> <td>1.6-3.5</td> <td>1.7-3.5</td> </tr> </tbody> </table> <p>Water chemistry: Temperature: 19-22°C; Dissolved oxygen: 8.4-9.5 mg/L; pH: 7.7-8.9; Total hardness: 174-198 mg/L as CaCO<sub>3</sub>; Alkalinity: 105-122 mg/L.</p>		Day 8	Day 13	Day 21	Control	14-25	37-68	59-95	1 mg/L WAF	17-35	17-34	45-74		Day 8	Day 13	Day 21	Control	1.8-3.1	3.3-4.5	2.8-4.5	1 mg/L WAF	2.1-4.4	1.6-3.5	1.7-3.5
	Day 8	Day 13	Day 21																						
Control	14-25	37-68	59-95																						
1 mg/L WAF	17-35	17-34	45-74																						
	Day 8	Day 13	Day 21																						
Control	1.8-3.1	3.3-4.5	2.8-4.5																						
1 mg/L WAF	2.1-4.4	1.6-3.5	1.7-3.5																						
<b><u>Conclusions</u></b>	Daphnia magna exposed to 1 mg/L WAF for 21 days exhibited increased mortality compared to control. However, the reproductive performance of surviving daphnids was not significantly affected compared to control animals.																								
<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: 11/21/2003																								

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

#### Robust Summary 14-Aquatic Plant Tox - 1

<u>Test Substance</u>	
CAS #	114959-46-5 (test campaign 1 AI 43)
Chemical Name	Benzoic acid, 2-hydroxy-, mono-C14-18 alkyl derivatives, calcium salts
Remarks	Test material purity: 43% active ingredient, 48% highly refined mineral oil, 9% inorganic calcium salts.
Method	
Method/Guideline followed	Miller, W., Green, J., The <i>Selenastrum capricornutum</i> algal assay bottle test (1978).
Test Type	Static acute toxicity test (Water Accommodated Fraction (WAF))
GLP (Y/N)	Not specified
Year (Study Performed)	1982
Species/Strain	Freshwater algae, <i>Pseudokirchneriella subcapitata</i> formerly called <i>Selenastrum capricornutum</i> derived from a strain (ATCC 22662) obtained from the American Type Culture Collection, Maryland, USA.
Element basis (# of cells/mL)	Approximately 5,000 cells/mL
Exposure period/duration	96 hours
Analytical monitoring	None
Statistical methods	Mean relative growth rate for each culture was calculated.
Remarks field for test conditions (fill as applicable)	<p>Test Species: Cells taken from an in-house culture of <i>Pseudokirchneriella subcapitata</i> that was originally obtained from the American Type Culture Collection, Maryland, USA.</p> <p>Test System: A measured weight (100 g) of test material was added to a measured volume of distilled water (1L) and shaken for 24 hours. The supernatant was used as the stock solution.</p> <p>Test Conditions: A static test was conducted; i.e., there was no daily renewal of test solution. Two 50-mL replicates per treatment, inoculum ~5,000 cells/mL. Cell counts performed on days 2 and 4.</p> <p>Test Levels: Control, 0.1, 0.5, 2, 10, 50, 200 and 1000 mg/L WAF loading rates.</p> <p>Light: Not specified.</p> <p>Test temperature: Not specified.</p> <p>Dilution Water: Not specified.</p> <p>Method of calculating mean measured concentrations: Not applicable.</p> <p>Exposure period: 96 hours</p>

	Analytical monitoring: Not performed																		
<u>Results</u>	48-96 hour EL <sub>50</sub> >1000 mg/L (WAF)																		
<u>Remarks</u>	<p>Test Findings: At 96-hours the mean relative growth rates in the control and treated groups were as follows:</p> <table border="1"> <thead> <tr> <th>WAF Concentration (mg/L)</th> <th>Mean Relative Growth Rate (% of Control)</th> </tr> </thead> <tbody> <tr> <td>0</td> <td>-</td> </tr> <tr> <td>0.1</td> <td>103</td> </tr> <tr> <td>0.5</td> <td>100</td> </tr> <tr> <td>2</td> <td>99</td> </tr> <tr> <td>10</td> <td>97</td> </tr> <tr> <td>50</td> <td>101</td> </tr> <tr> <td>200</td> <td>101</td> </tr> <tr> <td>1000</td> <td>83</td> </tr> </tbody> </table> <p>The 48-96 hour EL<sub>50</sub> was greater than 1000 mg/L (WAF)</p>	WAF Concentration (mg/L)	Mean Relative Growth Rate (% of Control)	0	-	0.1	103	0.5	100	2	99	10	97	50	101	200	101	1000	83
WAF Concentration (mg/L)	Mean Relative Growth Rate (% of Control)																		
0	-																		
0.1	103																		
0.5	100																		
2	99																		
10	97																		
50	101																		
200	101																		
1000	83																		
<u>Conclusions</u>	48-96 hour EL <sub>50</sub> >1000 mg/L (WAF)																		
<u>Data Quality</u>	Reliable with restriction. Restriction due to the lack of analytical characterization of the WAF and due to the limited methodology contained in the report.																		
<u>References</u>	Confidential business information.																		
<u>Other</u>	Updated: 11/21/2003																		

## Robust Summary 14-Aquatic Plant Tox – 2

<u>Test Substance</u>	
CAS #	114959-46-5 (test campaign 2 AI-28)
Chemical Name	Benzoic acid, 2-hydroxy-, mono-C14-18 alkyl derivatives, calcium salts
Remarks	Test material purity: 28% active ingredient, 51% highly refined mineral oil, 21% inorganic calcium salts
Method	
Method/Guideline followed	OECD Guideline for Testing of Chemicals #201 Alga Growth Inhibition Test
Test Type	Static acute toxicity test (Water Accommodated Fraction (WAF))
GLP (Y/N)	Y
Year (Study Performed)	1995
Species/Strain	Freshwater algae, <i>Raphidocelis subcapitata</i> (Nygaard) formerly called <i>Selenastrum capricornutum</i> derived from a strain (CCAP 278/4) obtained from the Institute of Freshwater Ecology, Windermere, England.
Element basis (# of cells/mL)	Approximately 5,000 cells/mL
Exposure period/duration	72 hours
Analytical monitoring	Concentrations of dissolved components of the test substance were below the limit of determination (0.4 mg/L) of the analytical method.
Statistical methods	The area under the growth curve and the average specific growth rate were determined.
Remarks field for test conditions (fill as applicable)	<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 (1 cm vortex) for 45 hours in a sealed 2.3-liter vessel. Following settling for 1.5-2 hour and separation of any surface film from the water phase, the water phase was used as the test solution in the study.</p> <p>A 72-hour sealed static test was carried out in 287 mL full volume Erlenmeyer flasks filled with each WAF test solution. Four flasks were prepared for each of the seven WAFs along with seven control flasks containing algal growth medium only. Three out of every set of four flasks containing WAF and six of the control flasks were inoculated with 5,000 cells/mL. The remaining flasks were used to determine background particle counts in the absence of <i>Raphidocelis subcapitata</i>. Two marbles were placed in each flask to ensure good mixing during incubation. Test chambers were sealed and incubated in a cool orbital incubator (100 cycles/min) under constant illumination (~4950 lux). The pH was determined at time 0 and at 72 hours. Air temperature in the test incubator was monitored throughout the study. Cell counts were made at the start of the study and then at approximately 24-hour intervals. Cell counts were made using a Coulter counter.</p> <p>Test Levels: Control, 10, 22, 46, 100, 220, 460 and 1000 mg/L WAF loading rates.</p>

<u>Results</u>	0-72 hour EL <sub>50</sub> >1000 mg/L (WAF)												
Remarks	<p>Range finding results indicated that the 72-hour EL<sub>50</sub> ranged from 100 to &gt;1000 mg/L. In the main study, culture growth was inhibited by a maximum of 22% when expressed in terms of the area under the growth curve and by 6.5% when expressed in terms of the average specific growth rate. The 72 hour EL<sub>50</sub> value for both end points was therefore &gt;1000 mg/L, the highest loading rate tested.</p> <p>The 24, 48 and 72 hour no observed effect loading rates (NOEL) determined for the area under the growth curve and the average specific growth rate were as follows:</p> <table border="1"> <thead> <tr> <th>Exposure Period (Hours)</th> <th>Area Under Growth Curve NOEL (mg/L)</th> <th>Average Specific Growth Rate NOEL (mg/L)</th> </tr> </thead> <tbody> <tr> <td>0-24</td> <td>&gt;1000</td> <td>&gt;1000</td> </tr> <tr> <td>0-48</td> <td>&lt;10</td> <td>100</td> </tr> <tr> <td>0-72</td> <td>&lt;10</td> <td>100</td> </tr> </tbody> </table> <p>The 0-48 hour and 0-72 hour NOELs of &lt;10 mg/L were based on only 9.4% and 14% growth inhibition being identified as statistically significantly different from control.</p> <p>Incubation temperature: 23.3-23.8°C pH range: 7.3 (0 hour) to 9.6 (72 hours)</p>	Exposure Period (Hours)	Area Under Growth Curve NOEL (mg/L)	Average Specific Growth Rate NOEL (mg/L)	0-24	>1000	>1000	0-48	<10	100	0-72	<10	100
Exposure Period (Hours)	Area Under Growth Curve NOEL (mg/L)	Average Specific Growth Rate NOEL (mg/L)											
0-24	>1000	>1000											
0-48	<10	100											
0-72	<10	100											
<u>Conclusions</u>	0-72 hour EL <sub>50</sub> >1000 mg/L (WAF)												
<u>Data Quality</u>	Reliable with restriction. Restriction due to the lack of analytical characterization of the WAF.												
<u>References</u>	Confidential business information.												
<u>Other</u>	Updated: 11/21/2003												

## 4. Toxicity

### 4.1 Acute Toxicity

#### 4.1.1 Acute Oral Toxicity

##### **Robust Summary 14-Acute Oral –1**

<b><u>Test Substance</u></b>	
CAS #	114959-46-5 (test campaign 2 AI 28)
Chemical Name	Benzoic acid, 2-hydroxy-, mono-C14-18 alkyl derivatives, calcium salts
Remarks	Test material purity: 28% active ingredient, 51% highly refined mineral oil, 21% inorganic calcium salts
<b>Method</b>	
Method/Guideline followed	OECD Guideline 401
Test Type	Acute oral toxicity
GLP (Y/N)	Y
Year (Study Performed)	1991
Species/Strain	Rats/Fisher 344
Sex	Male/Female
No. of animals/dose	5 /sex
Vehicle	None
Route of administration	Oral (intra-gastric)
Dose level	5000 mg/kg
Dose volume	4.76 mL/kg
Control group	No
Chemical analysis of dosing solution	No
Remarks field for test conditions	A single dose of the undiluted test material was administered intra-gastrically to five fasted male and female rats at a dose level of 5000 mg/kg. Clinical observations were conducted seven times on the day of test material administration and twice daily thereafter for 14 days. Individual body weights were recorded on the day of dosing, on day 8 and at termination. All animals were euthanized, and gross necropsies were performed, at the conclusion of the observation period.
<b><u>Results</u></b>	LD <sub>50</sub> >5000 mg/kg
Remarks	All treated animal survived the 14-day duration of the study. All animals developed a hunched posture, diarrhea and an unkempt appearance within 2.5 hours of dosing. All rats exhibited yellow anogenital staining by day 2. All animals recovered by day 4. All animals exhibited weight gain over the 14-day observation period. No treatment related macroscopic findings were evident.

<b><u>Conclusions</u></b>	The test article, when administered to 5 male and 5 female rats, had an acute oral LD <sub>50</sub> of >5000 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: 11/21/2003

### **4.1.3. Acute Dermal Toxicity**

#### **Robust Summary 14-Acute Dermal –1**

<b><u>Test Substance</u></b>	
CAS #	114959-46-5 (test campaign 2 AI 28)
Chemical Name	Benzoic acid, 2-hydroxy-, mono-C14-18 alkyl derivatives, calcium salts
Remarks	Test material purity: 28% active ingredient, 51% highly refined mineral oil, 21% inorganic calcium salts
<b>Method</b>	
Method/Guideline followed	OECD Guideline 402
Test Type	Acute dermal toxicity
GLP (Y/N)	Y
Year (Study Performed)	1991
Species/Strain	Rats/Fisher 344
Sex	Male/Female
No. of animals/dose	5 /sex
Vehicle	None
Route of administration	Dermal
Dose level	2000 mg/kg
Control group	No
Chemical analysis of dosing solution	No
Remarks field for test conditions	On the day prior to dosing the dorsal fur was removed using electric clippers. On day 1 the animals were weighed and a single dose of the test material was applied to the skin. The test material was covered with a gauze dressing and waterproof adhesive tape. The animals were then individually housed. Following 24 hours the dressings were removed and the dose site washed with warm dilute detergent solution. The animals were dried and returned to group housing. Clinical observations were conducted six times on the day of test material administration and twice daily thereafter for 14 days. Individual body weights were recorded on the day of dosing, on day 8 and at termination. All animals were euthanized and gross necropsies were performed, at the conclusion of the observation period.
<b><u>Results</u></b>	LD <sub>50</sub> >2000 mg/kg
Remarks	All treated animals survived the 14-day duration of the study. Female rats exhibited yellow anogenital staining from day 2. All animals recovered by day 4. Dose sites were stained brown and, in male rats, developed erythema by day 2. The treated skin was normal from day 4. All animals exhibited weight gain over the 14-day observation period. No treatment related macroscopic findings were evident.

<b><u>Conclusions</u></b>	The test article, when administered to 5 male and 5 female rats, had an acute dermal LD <sub>50</sub> of >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: 11/21/2003

## 4.2 Genetic Toxicity:

### Robust Summary 14-Gentox-1

<b><u>Test Substance</u></b>																																																									
CAS #	CAS# 114959-46-5 (test campaign 1 AI-43)																																																								
Chemical Name	Benzoic acid, 2-hydroxy-, mono-C14-18 alkyl derivatives, calcium salts																																																								
Remarks	Test material purity: 43% active ingredient, 48% highly refined mineral oil, 9% inorganic calcium salts.																																																								
<b>Method</b>																																																									
Method/Guideline followed	OECD Guideline 471																																																								
Test Type	Bacterial Reverse Mutation Assay																																																								
GLP (Y/N)	Not Specified																																																								
Year (Study Performed)	1982																																																								
Test System	<i>Salmonella typhimurium</i> and <i>Escherichia Coli</i>																																																								
Strains Tested	<i>Salmonella typhimurium</i> tester strains TA98, TA100, TA1535, TA1537, TA1538; <i>Escherichia Coli</i> tester strains WP2 and WP2 <i>uvrA</i>																																																								
Exposure Method	Plate incorporation																																																								
Test Substance Doses/concentration levels	31.25, 62.5, 125, 250, 500, 1000, and 2000 ug/plate																																																								
Metabolic Activation	With and without S9 fraction mixture																																																								
Vehicle	The test material contained 38% mineral oil and was formulated in water containing 20% w/v Tween 80.																																																								
Tester strain, activation status, Positive Controls and concentration level	<table border="0"> <tr><td>TA98</td><td>+S9</td><td>benzo(a)pyrene</td><td>20.0 ug/plate</td></tr> <tr><td>TA98</td><td>-S9</td><td>benzo(a)pyrene</td><td>20.0 ug/plate</td></tr> <tr><td>TA100</td><td>+S9</td><td>benzo(a)pyrene</td><td>20.0 ug/plate</td></tr> <tr><td>TA100</td><td>-S9</td><td>benzo(a)pyrene</td><td>20.0 ug/plate</td></tr> <tr><td>TA1535</td><td>+S9</td><td>sodium azide</td><td>5.0 ug/plate</td></tr> <tr><td>TA1535</td><td>-S9</td><td>sodium azide</td><td>5.0 ug/plate</td></tr> <tr><td>TA1537</td><td>+S9</td><td>Neutral Red</td><td>20.0 ug/plate</td></tr> <tr><td>TA1537</td><td>-S9</td><td>Neutral Red</td><td>20.0 ug/plate</td></tr> <tr><td>TA1538</td><td>+S9</td><td>benzo(a)pyrene</td><td>20.0 ug/plate</td></tr> <tr><td>TA1538</td><td>-S9</td><td>benzo(a)pyrene</td><td>20.0 ug/plate</td></tr> <tr><td>WP2</td><td>+S9</td><td>4-nitroquinoline-N-oxide</td><td>20.0 ug/plate</td></tr> <tr><td>WP2</td><td>-S9</td><td>4-nitroquinoline-N-oxide</td><td>20.0 ug/plate</td></tr> <tr><td>WP2<i>uvrA</i></td><td>+S9</td><td>4-nitroquinoline-N-oxide</td><td>20.0 ug/plate</td></tr> <tr><td>WP2<i>uvrA</i></td><td>-S9</td><td>4-nitroquinoline-N-oxide</td><td>20.0 ug/plate</td></tr> </table>	TA98	+S9	benzo(a)pyrene	20.0 ug/plate	TA98	-S9	benzo(a)pyrene	20.0 ug/plate	TA100	+S9	benzo(a)pyrene	20.0 ug/plate	TA100	-S9	benzo(a)pyrene	20.0 ug/plate	TA1535	+S9	sodium azide	5.0 ug/plate	TA1535	-S9	sodium azide	5.0 ug/plate	TA1537	+S9	Neutral Red	20.0 ug/plate	TA1537	-S9	Neutral Red	20.0 ug/plate	TA1538	+S9	benzo(a)pyrene	20.0 ug/plate	TA1538	-S9	benzo(a)pyrene	20.0 ug/plate	WP2	+S9	4-nitroquinoline-N-oxide	20.0 ug/plate	WP2	-S9	4-nitroquinoline-N-oxide	20.0 ug/plate	WP2 <i>uvrA</i>	+S9	4-nitroquinoline-N-oxide	20.0 ug/plate	WP2 <i>uvrA</i>	-S9	4-nitroquinoline-N-oxide	20.0 ug/plate
TA98	+S9	benzo(a)pyrene	20.0 ug/plate																																																						
TA98	-S9	benzo(a)pyrene	20.0 ug/plate																																																						
TA100	+S9	benzo(a)pyrene	20.0 ug/plate																																																						
TA100	-S9	benzo(a)pyrene	20.0 ug/plate																																																						
TA1535	+S9	sodium azide	5.0 ug/plate																																																						
TA1535	-S9	sodium azide	5.0 ug/plate																																																						
TA1537	+S9	Neutral Red	20.0 ug/plate																																																						
TA1537	-S9	Neutral Red	20.0 ug/plate																																																						
TA1538	+S9	benzo(a)pyrene	20.0 ug/plate																																																						
TA1538	-S9	benzo(a)pyrene	20.0 ug/plate																																																						
WP2	+S9	4-nitroquinoline-N-oxide	20.0 ug/plate																																																						
WP2	-S9	4-nitroquinoline-N-oxide	20.0 ug/plate																																																						
WP2 <i>uvrA</i>	+S9	4-nitroquinoline-N-oxide	20.0 ug/plate																																																						
WP2 <i>uvrA</i>	-S9	4-nitroquinoline-N-oxide	20.0 ug/plate																																																						
Vehicle Control	Vehicle control samples were prepared so that the concentration of mineral oil was comparable to that present at the highest concentration of the test substance. The vehicle control also contained water and Tween 80.																																																								
Statistical Analysis	Mean revertant colony count and standard deviation were determined for each dose point.																																																								
Dose Rangefinding Study	Conducted using tester strain TA100. Doses of test material ranged to 4,000 ug/plate. Cytotoxicity was evaluated.																																																								
S9 Optimization Study	Not specified																																																								

Remarks field for test conditions	<p>This study was conducted prior to the development of OECD Guideline No. 471. This study deviates from the guideline in that Tester Strain TA 1538, not called for in the guideline, was included.</p> <p>The study was conducted in duplicate. 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 eight concentrations of test substance, vehicle controls, and a positive control. Three plates/dose group/strain/treatment set were evaluated. 20 ul of test material, positive control or vehicle control were added to each plate along with each tester strain, S9 mix (if needed) and top agar. Plates were incubated for 48 hours at 37°C. The condition of the bacterial background lawn was evaluated for cytotoxicity and test article precipitate.</p>
<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 range finding study with tester strain TA100. Test material precipitate was not observed.</p> <p>In the mutagenicity assays all data were acceptable and no positive increases in the number of revertants/plate were observed with any of the tester strains with or without metabolic activation. No cytotoxicity was observed up to 4,000 ug/plate with any tester strain with or without activation. The positive control for each respective test strain exhibited at least a 2.5-fold increase (with or without S9) over the mean value of the vehicle control for a given strain, confirming the expected positive control response.</p>
<b><u>Conclusions</u></b>	Under the conditions of this study, the test material was not mutagenic.
<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: 11/21/2003

## Robust Summary 14-Gentox-2

<b><u>Test Substance</u></b>																																					
CAS #	114959-46-5 (test campaign 2 AI-28)																																				
Chemical Name	Benzoic acid, 2-hydroxy-, mono-C14-18 alkyl derivatives, calcium salts																																				
Remarks	Test material purity: 28% active ingredient, 51% highly refined mineral oil, 21% inorganic calcium salts																																				
<b>Method</b>																																					
Method/Guideline followed	OECD Guideline 471																																				
Test Type	Bacterial Reverse Mutation Assay																																				
GLP (Y/N)	Not Specified																																				
Year (Study Performed)	1992																																				
Test System	<i>Salmonella typhimurium</i> and <i>Escherichia Coli</i>																																				
Strains Tested	<i>Salmonella typhimurium</i> tester strains TA98, TA100, TA1535, TA1537, TA1538; <i>Escherichia Coli</i> tester strain WP2uvrA pKM101																																				
Exposure Method	Plate incorporation																																				
Test Substance Doses/concentration levels	0, 31.25, 62.5, 125, 250, 500, 1000, 2000 and 5000 ug/plate																																				
Metabolic Activation	With and without S9 fraction mixture obtained from Fisher 344 rats pretreated with Aroclor 1254																																				
Vehicle	The test material contained mineral oil and was prepared as a formulation in 5% Tween 80 in 1:1 heptane:acetone.																																				
Tester strain, activation status, Positive Control and concentration level	<table border="0"> <tbody> <tr> <td>TA98</td> <td>+S9 benzo(a)pyrene</td> <td>10.0 ug/plate</td> </tr> <tr> <td>TA98</td> <td>- S9 2-nitrofluorene</td> <td>5.0 ug/plate</td> </tr> <tr> <td>TA100</td> <td>+S9 benzo(a)pyrene</td> <td>10.0 ug/plate</td> </tr> <tr> <td>TA100</td> <td>- S9 sodium azide</td> <td>5.0 ug/plate</td> </tr> <tr> <td>TA1535</td> <td>+S9 2-aminoanthracene</td> <td>5.0 ug/plate</td> </tr> <tr> <td>TA1535</td> <td>- S9 sodium azide</td> <td>2.0 ug/plate</td> </tr> <tr> <td>TA1537</td> <td>+S9 neutral red</td> <td>5.0 ug/plate</td> </tr> <tr> <td>TA1537</td> <td>-S9 9-aminoacridine</td> <td>5.0 ug/plate</td> </tr> <tr> <td>TA1538</td> <td>+S9 benzo(a)pyrene</td> <td>10.0 ug/plate</td> </tr> <tr> <td>TA1538</td> <td>- S9 2-nitrofluorene</td> <td>5.0 ug/plate</td> </tr> <tr> <td>WP2uvrA pKM101</td> <td>+S9 benzo(a)pyrene</td> <td>10.0 ug/plate</td> </tr> <tr> <td>WP2uvrA pKM101</td> <td>-S9 potassium dichromate</td> <td>20.0 ug/plate</td> </tr> </tbody> </table>	TA98	+S9 benzo(a)pyrene	10.0 ug/plate	TA98	- S9 2-nitrofluorene	5.0 ug/plate	TA100	+S9 benzo(a)pyrene	10.0 ug/plate	TA100	- S9 sodium azide	5.0 ug/plate	TA1535	+S9 2-aminoanthracene	5.0 ug/plate	TA1535	- S9 sodium azide	2.0 ug/plate	TA1537	+S9 neutral red	5.0 ug/plate	TA1537	-S9 9-aminoacridine	5.0 ug/plate	TA1538	+S9 benzo(a)pyrene	10.0 ug/plate	TA1538	- S9 2-nitrofluorene	5.0 ug/plate	WP2uvrA pKM101	+S9 benzo(a)pyrene	10.0 ug/plate	WP2uvrA pKM101	-S9 potassium dichromate	20.0 ug/plate
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Statistical Analysis	Mean revertant colony count and standard deviation were determined for each dose point.																																				
Dose Rangefinding Study	Not specified																																				
S9 Optimization Study	Not specified																																				
Remarks field for test conditions	<p>This study was conducted prior to the development of OECD Guideline No. 471. This study deviates from the guideline in that Tester Strain TA 1538, not called for in the guideline, was included.</p> <p>There were two treatment sets for each tester strain, with (+S9) and</p>																																				

	without (-S9) metabolic activation. Each of the tester strains was dosed with nine concentrations of test substance, vehicle and positive controls. Three plates/dose group/strain/treatment set were evaluated. Test material (20 ul), positive control or vehicle control were added to each plate along with each tester strain, S9 mix (if needed) and top agar. Plates were incubated for 48-72 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>The test material formed smears on the surface of the top agar at 1000 ug/plate and above indicating that it was not miscible in the aqueous test system at these treatment levels. Microscopic examination of the background lawn showed no evidence of cytotoxicity at concentrations up to 5000 ug/plate with or without metabolic activation.</p> <p>In the mutagenicity assays all data were acceptable and no positive increases in the number of revertants/plate were observed with any of the tester strains with or without metabolic activation. The positive control for each respective test strain exhibited at least a 7-fold increase (with or without S9) over the mean value of the vehicle control for a given strain, confirming the expected positive control response.</p>
<b><u>Conclusions</u></b>	Under the conditions of this study, the test material was not mutagenic.
<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: 11/21/2003

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