FINAL

Index of Robust Summaries
ADBAC Joint Venture HPV Chemicals Challenge

March 1, 2011

Appendix A

Index of Robust Summaries for
ADBAC Joint Venture HPV Chemicals Challenge

Page

Index of Robust Summaries for
ADBAC Joint Venture HPV Chemicals Challenge

Index of Robust Summaries for
ADBAC Joint Venture HPV Chemicals Challenge

Page

Index of Robust Summaries for
ADBAC Joint Venture HPV Chemicals Challenge

Page

4.2 TOXICITY TO AQUATIC INVERTEBRATES

Index of Robust Summaries for
ADBAC Joint Venture HPV Chemicals Challenge

Page

Index of Robust Summaries for
ADBAC Joint Venture HPV Chemicals Challenge

Page

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

5.1.1 ACUTE ORAL TOXICITY

Index of Robust Summaries for
ADBAC Joint Venture HPV Chemicals Challenge

Page

5.1.3 ACUTE DERMAL TOXICITY

5.4. REPEATED DOSE TOXICITY

Index of Robust Summaries for
ADBAC Joint Venture HPV Chemicals Challenge

Index of Robust Summaries for
ADBAC Joint Venture HPV Chemicals Challenge

Page

5.5 GENETIC TOXICITY *IN VITRO*

Index of Robust Summaries for
ADBAC Joint Venture HPV Chemicals Challenge

Page

Page

3.1.1 PHOTODEGRADATION

Test Substance

Identity:	Alkyl dimethyl benzyl ammonium chloride (CAS RN 68424-85-1; Alkyl (C12-16) dimethylbenzylammonium chloride)
Purity:	Non-radiolabeled = 30% aqueous; Radiochemical purity = 98.4%

Method

Method/Guideline followed:	U. S. EPA FIFRA N-161-2
Type:	Thin layer chromatography
GLP:	Yes
Year:	1987
Light source:	Xenon arc lamp
Light spectrum:	285 nm to 750 nm
Relative intensity:	Approximately one-half the intensity of the sun.
Spectrum of substance:	Not provided
Remarks:	A study using ^{14}C-alkyl dimethyl benzyl ammonium chloride (ADBAC) at a nominal concentration of 10 µg/ml was conducted at 25 ˚C in aqueous solution buffered at pH 7. The test substance was exposed to a xenon arc light source for 30 days.

Results

Concentration of substance:	10 µg/ml
Temperature:	25 ˚C ± 1 ˚C
Direct photolysis:	Half-life = Not determined since no significant degradation of the test substance was detected during the 30-day evaluation period. Degradation % = No detectable degradation occurred during the 30-day test period. Quantum yield = Not applicable
Indirect photolysis:	Sensitizer = Acetone Concentration of sensitizer = 1.0% Rate Constant = 0.0636 days (exposed) Half-life = 10.9 days (exposed) Degradation % = 82.6% over a period of 30 days when exposed to light. Little or no degradation occurred in the presence of acetone without exposure to the light source.
Breakdown products:	Yes, only in the presence of a photosensitizer when exposed to light. Essentially all of the ^{14}C-moiety not present as parent compound was found in one degradate.

Remarks:	Based on the data generated during this study, ADBAC was found to be photolytically stable in the absence of a photosensitizer. An accurate estimate of the photolysis rate constants and the half-life for solutions containing no photosensitizer and all dark controls (both sensitized and nonsensitized) could not be determined since no significant degradation of the test substance was detected during the 30-day evaluation period. The overall mean ^{14}C-activity accountability for this study was 99.3% for the nonsensitized samples and 97.8% for the sensitized samples.
Conclusions	ADBAC is photolytically stable in the absence of a photosensitizing agent. In the presence of the energy from a xenon arc lamp and the photosensitizing agent, acetone, it appears that ADBAC breaks down to form a single degradate. (Author of report)
Remarks:	The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability (Klimisch):	1A
Remarks:	Reliable without restriction; guideline study.

References

Carpenter, M. and M. Fennessey. 1988. Determination of the Photolysis rate of ADBAC in pH 7 Buffered Solution at 25 ºC. Unpublished report number 35713. Analytical Bio-Chemistry Laboratories, Inc., Columbia, MO, U.S.

3.1.2 STABILITY IN WATER

Test Substance

Identity:	Alkyl dimethyl benzyl ammonium chloride (CAS RN 68424-85-1; Alkyl (C12-16) dimethylbenzylammonium chloride)
Purity:	Non-radiolabeled = 30% aqueous; Radiochemical purity = 98.4%

Method

Method/Guideline followed:	U. S. EPA FIFRA N-161-1, 40 CFR 158.130
Type:	Thin layer chromatography
GLP:	Yes
Year:	1987
Remarks:	A study using ^{14}C-alkyl dimethyl benzyl ammonium chloride (ADBAC) was conducted at 25 °C in aqueous solutions buffered at pHs 5, 7 and 9. Two pH 7 buffers were employed during the study to evaluate buffer catalysis of the degradation process. All experiments were conducted at a nominal test concentration of 10 µg/ml.

Results

Nominal:	10 µg/ml
Measured value:	pH 5 = 9.35 to 8.17 µg/ml; pH 7 (HEPES) = 9.49 to 7.68 µg/ml; pH 7 (Tris) = 8.99 to 7.75 µg/ml; and pH 9 = 7.65 to 6.79 µg/ml.
Degradation %:	No degradation determined (measured concentration of parent chemical was 96.3% ± 4.47% over a pH range of 5 to 9 at 25 °C after 30 days)
Half-life:	An accurate estimate of the half-life for the hydrolysis could not be determined since no significant degradation of the test compound was detected during the 30-day evaluation period.
Remarks:	

Conclusions ADBAC was found to be hydrolytically stable in the pH range 5 to 9 at 25 °C. The overall mean ^{14}C-activity accountability for this study was 96.3%.

Remarks: The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability (Klimisch):	1A
Remarks:	Reliable without restriction; guideline study.

References

Carpenter, M. and M. Fennessey. 1988. Hydrolysis of ADBAC as a Function of pH at 25 ºC. Unpublished report number 35712. Analytical Bio-Chemistry Laboratories, Inc., Columbia, MO, U.S.

3.3 TRANSPORT AND DISTRIBUTION

Test Substance

Identity: Alkyl(C_{12}-C_{16})dimethylbenzylammonium chloride (ADBAC; CAS RN 68424-85-1), in aqueous solution

Purity**:** Non-radiolabelled, 30% w/w a.s. in aqueous solution
Radiolabelled, 98.4%

Method

Method/Guideline followed: U.S. EPA Guideline subdivision N 163-1

GLP: Yes

Year: 1988

Soil Types: sand, sandy loam, clay loam, silt loam

Remarks:

Results

Soil Types	Adsorption coefficient (K_d)	Mobility coefficient (K_{oc})	Desorption coefficient (K_d)	Mobility coefficient (K_{oc})
Sand	6,172	6,171,657	7173	7137310
Sandy loam	5,123	640,389	96540	12067457
Clay loam	32,429	1,663,039	165556	8490062
Silt loam	10,797	2,159,346	14083	2816590

Conclusions The substance can be classified as immobile in all four soil types.

Remarks: The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability: 1

Remarks: Reliable without restriction; guideline study. Key study.

References

Daly, D. and W. Cranor. (1988) Soil/Sediment Adsorption-Desorption of Alkyl Dimethyl Ammonium Chloride. Report No. 35716. Analytical Bio-Chemistry Laboratories, Inc., Columbia, MO, USA. (Unpublished)

3.3 TRANSPORT AND DISTRIBUTION

Test Substance

Identity:	Active substance (a.s.), alkyl(C_{12}-C_{16})dimethyl-benzylammonium chloride (ADBAC; CAS RN 68424-85-1), in aqueous solution.
Purity:	Non-radiolabelled = 49-51% w/w a.s. in aqueous/alcohol solution Radiolabelled = 99.7% (radiochemical purity)

Method

Method/Guideline followed:	Dutch CTB guideline Section G.2.1, German BBA guideline for the registration of pesticides, part IV, 5-1, the EU Commission Directive 95/36/EC and SETAC-Europe Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticides. The study design is in general agreement with the current OECD 308 guideline.
GLP:	Yes
Year:	2001
Contact Time:	120 days
Test System:	The test system consisted of twenty-two 1000-ml biometer flasks with a soda lime column for trapping CO_2 for each sediment type. The column also contained an oil covered glass wool layer for trapping volatile metabolites. Sediment was added to comprise a layer of 3 cm (60 g and 90 g dry solids for the TNO and Kromme Rijn systems, respectively). The sediment was then covered with a 6 cm layer of associated water. The incubation vessels were pre-incubated, on a rotary shaker (ca 50 rpm), for 16 days. The microbial biomass of the test system was monitored in separate systems in triplicate at the start and at the end of the study period. These separate systems were prepared in 300-ml flasks, with 20 g of dry solids and 152.3 ml of ditch water for the TNO system and 30 g of dry solids and 142.6 ml of ditch water for the Kromme Rijn system, and were treated with an equivalent amount of non-radiolabelled material. Two spare flasks were prepared for measurements of redox potential.
Sampling:	Duplicate whole samples were taken for analysis on days 0, 1, 2, 7, 14, 28, 42, 56, 84 and 120 days. At each sampling interval, the pH, redox potential and oxygen concentrations in the water layer were measured in the incubation vessels taken for analysis. The redox potential in the sediment layer was also determined.

Remarks: The route and rate of aquatic degradation of [^{14}C]-ADBAC was investigated in two representative natural aerobic water/anaerobic sediment systems under laboratory conditions at a temperature of 20°C. Quantification of all liquid extracts and samples was conducted using a liquid scintillation counter (Packard model Tri-Carb CA 2000). Scintillation counting was carried out with automatic quench correction using an external standard for a total accumulation of counts (so that 2 sigma = 2%) or for a maximum of 10 minutes, whichever happened first. One ml of the concentrated sediment extracts was extracted with 2 ml of dichloromethane. This sample was analyzed using a reverse phase isocratic HPLC with mobile phase chloroform:methanol:trifluoro-acetic acid (85:15:0.5 v/v/v).

Results

Remarks: The recovery and distribution of the applied radioactivity from the water/sediment systems was generally acceptable in the study ranging overall from 73.1 to 112.6%. The applied radioactivity quickly dissipated to the sediment layer; radioactivity in the water layer comprised < 5% by 28 days. The total amount of carbon dioxide evolved after 120 days was 17.9% (TNO) and 30.5% (Kromme Rijn).

Conclusions ADBAC rapidly dissipated from the water layer to the sediment layer in water/sediment systems. First order half-lives determined for the dissipation of the active substance from the aqueous phase of the TNO and Kromme Rijn systems were 4.8 and 2.9 days, respectively, and from the entire TNO and Kromme Rijn water/sediment systems were >120 and 34.7 days, respectively (DT_{50} = >120 days, TNO; DT_{50} = 32 days, Kromme Rijn). Levels of applied radioactivity in the water layer were < 3.7% after 28 days. The levels in the sediment layer increased to a maximum of 77.1% in the TNO system and a maximum of 71.1% in the Kromme Rijn system after 3 days. The levels of total carbon dioxide evolved steadily increased to 17.9% (TNO system) and 30.5% (Kromme Rijn system) after 120 days. No metabolites were detected.

Remarks: The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability: 2

Remarks:

References

de Vette, H.Q.M. and van Asten, J.G. (2001) A water/sediment study of alkyldimethylbenzylammonium chloride (ADBAC) using [^{14}C]-ADBAC. TNO Nutrition and Food Research, Delft, The Netherlands. Study No. IMW-99-9072-01 (unpublished).

3.5 BIODEGRADATION

Test Substance

Identity:	Hyamine 3500-80 (CAS RN 68424-85-1; Alkyl (C12-16) dimethylbenzylammonium chloride)
Purity:	80.8% ethanol/water solution

Method

Method/Guideline followed:	Based on "A Procedure and Standards for the Determination of the Biodegradability of Alkyl Benzene Sulfonate and Linear Alkylate Sulfonate." J. Amer. Oil Chem. Soc. 1965. 42:1 - 16.
Test type:	Aerobic semi-continuous activated sludge (SCAS)
GLP:	Yes
Year:	1991
Contact time:	7 Days (following an acclimation period of seven days)
Inoculum:	Activated sludge from Avondale Sewage Treatment Plant, Avondale, PA
Remarks:	The test apparatus consisted of four SCAS aeration chambers containing 1.5 l of activated sludge. The suspended solids level in each unit was adjusted to approximately 2,500 mg/l. The units were aerated at a rate adequate to maintain solids suspension. The stock solution of Hyamine 3500-80 at 1000 mg active/l was added to two test units for a seven-day acclimation period. This consisted of incremental additions until the final test concentration of 10 mg active/l was reached. Two additional units did not receive test substance and served as controls. The testing period was an additional seven days following the acclimation period. Throughout the study all units were fed synthetic sewage. During the test period the effluents withdrawn from each unit were analyzed for soluble organic carbon (SOC).

Results

Degradation:	Approximately 100% (±) after seven days
Results:	Inherently biodegradable

Kinetic:

Percent Carbon Removed

Day	Hyamine 3500-80 Test 1 (%)	Hyamine 3500-80 Test 2 (%)
1	100.59	102.96
2	98.22	106.51
3	100.00	104.73
4	98.22	100.59
5	100.59	102.96
6	101.18	104.73
7	102.96	102.96

Breakdown products: Not stated
Remarks:

Conclusions The average percent SOC removal for ADBAC was > 100%.

Remarks: The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability (Klimisch): 1A
Remarks: Reliable without restriction; guideline study.

References

Corby, J. E. 1992. Semi-Continuous Activated Sludge (SCAS) Removability Test - Hyamine 3500-80. Unpublished report number 91-065. Roy F. Weston, Inc., Lionville, PA, U. S.

3.5 BIODEGRADATION

Test Substance

Identity:	Hyamine 3500-80 (CAS RN 68424-85-1; Alkyl (C12-16) dimethylbenzylammonium chloride)
Purity:	80.8% ethanol/water solution

Method

Method/Guideline followed:	Not reported
Test type:	Aerobic CO_2 production test
GLP:	Yes
Year:	1991
Contact time:	28 days
Inoculum:	Acclimated activated sludge, originally from Avondale Sewage Treatment Plant, Avondale, PA, was obtained from a previous SCAS assay conducted with Hyamine 3500-80 (10 mg active ingredient per liter) by collection of the sludge from the test units on the final day of the testing.
Remarks:	The test apparatus consisted of four glass four-liter Erlenmeyer flasks containing two liters of modified biochemical oxygen demand (BOD) water. A stock solution of the test substance was prepared in deionized water (ASTM Type II) at a concentration of 1000 mg active/l. This solution was volumetrically added to the respective flasks at concentrations of 5 and 10 mg active/l. One flask receiving no test substance (control blank) and one flask receiving d-glucose at a concentration of 20 mg active/l (reference chemical) were included in the test design. The flasks were placed on a rotary platform shaker and mixed at 110 ± 10 rpm for the duration of the study. The CO_2 produced in each flask reacted with 0.024 N $Ba(OH)_2$ and precipitated as $BaCO_3$. The amount of CO_2 produced was determined by titrating the remaining $Ba(OH)_2$ with 0.05 N standardized hydrochloric acid (HCl). After 28 days, the contents of the flasks were acidified with concentrated sulfuric acid (H_2SO_4) and aerated overnight. One final titration was performed. Temperature ranged from 22.1 ^{o}C to 23.0 ^{o}C throughout study.

Results

Degradation: Degradation was between 82 and 85% on Day 28.
Results: Ultimately biodegradable.

Test Substance	Concentration (mg active/L)	Final %TCO_2	Final SOC (ml/L)
Control (blank)	-	-	0.4
d-glucose	20	80.6	2.2
Hyamine 3500-80	5	84.0	0.7
Hyamine 3500-80	10	82.6	0.6

Kinetic:

% TCO_2 Over Time			
Day	d-glucose	Hyamine 3500-80 5 mg/kg	Hyamine 3500-80 10 mg/kg
2	20.6	9.3	1.8
5	39.2	43.8	39.1
8	50.6	58.5	54.2
11	61.0	75.4	67.9
14	64.9	85.0	76.0
17	69.2	88.5	80.2
20	73.4	87.2	81.6
23	77.0	85.6	81.9
28	80.1	85.0	82.3
28	80.6	84.0	82.6

Breakdown products: Not required by this guideline
Remarks:

Conclusions The test material was considered biodegradable in this assay.

Remarks: The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability (Klimisch): 1A
Remarks: Reliable without restriction; guideline study.

References

Corby, J. E. 1992. CO_2 Production Test - Hyamine 3500-80. Unpublished report number 91-066. Roy F. Weston, Inc., Lionville, PA, U.S.

3.5 BIODEGRADATION

Test Substance

Identity: Alkyl dimethyl benzyl ammonium chloride (CAS RN 68391-01-5; Alkyl (C12-18) dimethylbenzylammonium chloride)

Purity: Not stated

Method

Method/Guideline followed: EEC Guideline 84/449 - Method C.5

Test Type: Aerobic inherent biodegradability

GLP: No

Year: 2002

Contact Time: 28 days

Inoculum: Activated sludge, non-adapted

Remarks: The study was carried out in accordance with EEC Guideline 84/449 – Method C5 – Official Journal L252 (19-09-84) and is similar to OECD Guideline 301B. Aniline was used as a positive control at 10 mg/l. The test substance was evaluated at 5 or 10 mg/l. The contact time was 28 days.

Results

Degradation: 5 mg/l: 72% degradation after 28 days
10 mg/l: 5% degradation after 28 days

Results: 5 mg/l: 72% degradation after 28 days
10 mg/l: 5% degradation after 28 days

Kinetic:

Day	% degradation ADBAC (5 mg/l)	% degradation ADBAC (10 mg/l)	% degradation Control
0	0	0	0
5	9.5	0.65	33.55
15	55.3	4.65	76.45
28	71.6	5.0	85.8

Breakdown Products: Not stated

Remarks: 72% of the test substance had degraded after 28 days at 5 mg/l meeting the 10-day window and 5% degraded at 10 mg/l clearly showing toxicity to the organisms at the higher concentration. 86% degradation of the control substance occurred during the study. Read-across from Catigene T50 (C12-C18 ADBAC) is appropriate. Many studies have shown that longer alkyl chains tend to biodegrade more slowly than shorter chains. Therefore, the use of C12-C18 ADBAC represents a "worst-case" scenario for

	biodegradation. The alkyl chains of C12-C16 ADBAC are shorter than the surrogate and would be expected to degrade at least as rapidly as the surrogate chemical.
Conclusions	The test substance is readily biodegradable.
Remarks:	The endpoint has been adequately characterized (ADBAC Joint Venture).
Data Quality	
Reliability:	1
Remarks:	Reliable without restriction; guideline study. Key study
References	Bazzon, M and Deschamps, F. (2002) Biotic degradation: biodegradability evaluation in aqueous medium: ultimate aerobia of the referenced compounds, CATIGENE T 50 for Stepan Europe, by INERIS; INERIS Study 506223 (Unpublished).

3.5 BIODEGRADATION

Test Substance

Identity:	BTC 8358 F (CAS RN 68391-01-5; Alkyl (C12-18) dimethylbenzylammonium chloride)
Purity:	Not stated

Method

Method/Guideline followed:	OECD Guideline 301B
Test Type:	Aerobic ready biodegradability
GLP:	No
Year:	2004
Contact Time:	28 days
Inoculum:	$>10^6$ CFU/ml; consisting of micro-organisms in mud collected from Station Epuration Wavre 2nd Stage, a household water treating plant
Remarks:	A clearly chemically defined medium, without any other organic carbon sources, was prepared. Three-litre flasks containing the test medium, inoculum from a household water treating plant and test or reference substance (10-20 mg TOC/l) were maintained for 28 days in the dark. Test concentration was prepared based on 62.8% total organic carbon (TOC) of this test substance. Air free from carbon dioxide was passed through the solutions using a flow of 50 to 100 ml/min. Carbon dioxide (CO_2) released from biodegradation was trapped in a series of 100-ml absorber flasks containing barium hydroxide ($Ba(OH)_2$). The CO_2, trapped as barium carbonate precipitate, was determined by titration of the solution (with phenolphthalein) in flask closest to the test flask, at which point, another flask was added to end of the absorber flask series to replace the one removed for titration. The titrations were carried out every two to three days for the first 10 days and every three to five days thereafter until Day 28. On Day 28, the pH of the test solutions was measured. The test solutions were acidified (with 1 ml of HCl) to drive off any remaining CO_2. The last titration was performed on day 29. Biodegradability was calculated from the released CO_2 over time in the test and reference flasks relative to that which was released in the blank (an inoculum control flask prepared as above with inoculum, but without test or reference substance).

Results

Degradation: 95.5% degradation after 28 days

Results: 95.5% degradation after 28 days

Kinetic:

Day	Reference Substance		Test Substance	
	% CO_2 Produced	% CO_2 Total	% CO_2 Produced	% CO_2 Total
0	0.00	0.00	0.0	0.0
1	14.16	14.16	1.2	1.2
3	28.54	42.69	1.0	2.2
6	21.20	63.89	9.5	11.6
8	8.77	72.67	16.0	27.7
10	3.72	76.39	20.2	47.9
13	3.26	79.65	17.4	65.3
15	2.53	82.18	7.5	72.8
17	1.47	83.65	5.1	77.9
20	2.31	85.95	3.9	81.8
22	1.15	87.11	1.6	83.5
24	0.25	87.36	4.4	87.8
28	0.16	87.52	1.8	89.6
29	1.35	88.87	5.8	95.5

Breakdown Products: Not stated

Remarks: The results indicated that 95.5% total CO_2 was produced in test vessels, compared to 88.9% in the reference standard. CO_2 production in the blank (inoculum control) was 39.2 mg. The pH measured at the start and end of the test period was 7.3 and 7.8, respectively. The temperature throughout the test ranged from 20.8 to 21.4°C.

Conclusions The test material was determined to be readily biodegradable.

Remarks: The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability: 1

Remarks: Reliable without restriction; guideline study. Key study

References

Van Dievoet, F. and V. Bouillon. (2005) Biodegradability Test Report According to OECD 301 B – Modified. Report No. ST49132.01.01, dated January 19, 2004 for Stephan Europe, Voreppe, France; from BfB Oil Research S.A., Gembloux, Belguim. (Unpublished)

3.5 BIODEGRADATION

Test Substance

Identity:	Benzyloctadecyldimethylammonium chloride (CAS RN 122-19-0; Benzyldimethyloctadecylammonium chloride)
Purity:	Not stated

Method

Method/Guideline followed:	OECD guideline 301C, MITI test.
Test Type:	Aerobic ready biodegradability
GLP:	No
Year:	1995
Contact Time:	10 days
Inoculum:	Activated sludge
Remarks:	The review article provided few specific details of the test methodology other than to say the results were from standard biodegradability tests.

Results

Degradation:	0% degradation in the MITI test
Results:	0% degradation, with a conclusion that the material is not readily biodegradable under the MITI test conditions
Kinetic:	Not stated
Breakdown Products:	Not stated
Remarks:	The article further states that low biodegradation was due at least in part to inhibitory effects of the test substance on microbial flora capable of utilizing the test substance. Lower initial concentrations in tests would show a greater propensity for biodegradation.

Conclusions No biodegradation was observed, which was attributed to inhibitory effects of the test substance on the microbial flora.

Remarks: The endpoint was adequately characterized in the article (ADBAC Joint Venture).

Data Quality

Reliability:	2D
Remarks:	Reliable with restrictions; endpoint was described in a review article on the biodegradation of cationic surfactants including data on CAS RN 112-19-0.

References

Van Ginkel, C. G. 1995. Biodegradability of Cationic Surfactants. pp. 183 – 203. In Biodegradability of Surfactants. Karsa, D. R., and M. R. Porter, eds. Chapman & Hall, London, UK.

3.5 BIODEGRADATION

Test Substance

Identity:	Benzyloctadecyldimethylammonium chloride (CAS RN 122-19-0; Benzyldimethyloctadecylammonium chloride)
Purity:	Not stated

Method

Method/Guideline followed:	Semi-Continuous Activated Sludge test systems and guidelines of US Soap & Detergent Association and OECD.
Test Type:	Aerobic inherent biodegradability
GLP:	Not stated
Year:	1997
Contact Time:	Not stated
Inoculum:	Activated sludge
Remarks:	The review article provided few specific details of the test methodology other than to say the results were from standard test methodologies cited above.

Results

Degradation:	Degradation achieved 94% in the SCAS test.
Results:	Degradation achieved 94% in the SCAS test.
Kinetic:	Not stated
Breakdown Products:	Not stated
Remarks:	

Conclusions	The test material was biodegradable in the test system.
Remarks:	The endpoint was adequately characterized in a review article (ADBAC Joint Venture).

Data Quality

Reliability:	2A
Remarks:	Acceptable, well-documented publication/study report which meets basic scientific principles.

References — Boethling, R.S., P.H. Howard, W. Stiteler, and A. Hueber. 1997. Does the Semi-Continuous Activated Sludge (SCAS) Test Predict Removal in Secondary Treatment? Chemosphere 35(10):2119 - 2130.

4.1 ACUTE TOXICITY TO FISH

Test Substance

Identity:	Alkyl dimethyl benzyl ammonium chloride (CAS RN 68424-85-1; Alkyl (C12-16) dimethylbenzylammonium chloride)
Purity:	Non-radiolabeled = 80.8% ethanol/water solution; Radiochemical purity = 97.99%

Method

Method/Guideline followed:	U. S. EPA FIFRA 72-3(a)
Type:	Static: daily renewal
GLP:	Yes
Year:	1992
Species/Strain/Supplier:	*Cyprinodon variegatus* (Sheepshead minnow)/Wildlife International Cultures, Easton, MD
Analytical monitoring:	Yes
Exposure period:	96 hours
Statistical methods:	Binomial probability with the nonlinear interpolation method of C. E. Stephan (1977)
Remarks:	Juvenile Sheepshead minnows (10 per group) were exposed to mean measured concentrations of 0, 0.42, 0.68, 1.1, 1.8 and 2.8 ppm alkyl dimethyl benzyl ammonium chloride in a 96-hour static-renewal acute toxicity test. Test chambers were Teflon®-lined, 25-L, polyethylene aquaria filled with approximately 15 liters of water. The dilution water was saltwater collected at Indian River Inlet, Delaware and diluted to the appropriate salinity with Wildlife International Ltd. well water. Test concentrations were replicated so that 20 organisms (10 per test chamber) were exposed to each concentration. Approximately 90% of the solution in each test chamber was replaced with fresh solution every 24 hours. Test conditions were as follows: dissolved oxygen ranged from 6.2 to 8.3 mg/l, temperature ranged from 21.0 to 22.2 °C, pH ranged from 7.9 to 8.1 and the salinity was 25 parts per thousand. Fish were observed approximately 2.5, 24, 48, 72 and 96 hours after the start of the test to evaluate the number of mortalities and the number of individuals exhibiting clinical signs of toxicity or abnormal behavior. At the end of the test, the average standard length and wet weight of ten control fish were 20 mm (ranging from 16 to 26 mm) and 0.21 g (ranging from 0.11 to 0.37 g), respectively. Instantaneous loading, representing the biomass of fish per liter of test water at a given time, was 0.14 g of fish/l. Radioactivity (considered equivalent to measured concentrations of the test

substance) was determined in duplicate 10-ml samples at the beginning and end of the pretest period and at approximately 24, 48, 72 and 96 hours after the start of the test.

Results

Nominal concentrations (mg/l): 0, 0.39, 0.65, 1.1, 1.8 and 3.0 ppm (mg/l)
Measured concentrations (mg/l): 0, 0.42, 0.68, 1.1, 1.8 and 2.8 ppm (mg/l)
Unit: ppm
Element value: LC_{50} (24-hour) = 1.3 ppm (mg/l)
LC_{50} (48-hour) = 0.89 ppm (mg/l)
LC_{50} (72-hour) = 0.86 ppm (mg/l)
LC_{50} (96-hour) = 0.86 ppm (mg/l) (95% confidence limit = 0.68 and 1.1 ppm)
Statistical results: Described below
Remarks: All LC_{50} values were based on mean measured concentrations. The 96-hour no-observable-effect concentration and no mortality concentration was 0.68 ppm. None of the fish in the control group died during the test and all appeared healthy and normal throughout the test period. No fish died in the 0.42 and 0.68 ppm treated groups and all appeared normal throughout the test. Cumulative mortalities were as follows:

Cumulative mortalities					
Concentration (ppm)	**2.5 h**	**24 h**	**48 h**	**72 h**	**96 h**
1.1	0/20	4/20	19/20	20/20	20/20
1.8	0/20	20/20	20/20	20/20	20/20
2.8	20/20	20/20	20/20	20/20	20/20

Signs of abnormal behavior among fish exposed to the test substance at 1.1 ppm were observed by 24 hours, when 31% of the surviving fish were surfacing for unusually long periods of time. At 1.8 ppm all fish were dead within 24 hours of test initiation and those exposed to 2.8 ppm were dead within approximately 2.5 hours of test initiation.

Conclusions The 96-hour LC_{50} for ADBAC was determined to be 0.86 mg/l.

Remarks: The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability (Klimisch): 1A

Remarks: Reliable without restriction; guideline study.

References

Sved, D. W., J. P. Swigert and G. J. Smith. 1992. A 96-Hour Static-Renewal Acute Toxicity Test with Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) in the Sheepshead Minnow (*Cyprinodon variegatus*). Unpublished report number 350A-102. Wildlife International LTD., Easton, MD, U.S.

4.1 ACUTE TOXICITY TO FISH

Test Substance

Identity:	Alkyl dimethyl benzyl ammonium chloride (CAS RN 68424-85-1; Alkyl (C12-16) dimethylbenzylammonium chloride. An incorrect CAS RN, 68391-01-5, was included in the study report.)
Purity:	Non-radiolabeled = 81.9% ethanol/water solution; Radiochemical purity = 97.4%

Method

Method/Guideline followed:	U. S. EPA TSCA 797.1400
Type:	Static: daily renewal
GLP:	Yes
Year:	1993
Species/Strain/Supplier:	*Pimephales promelas* (Fathead minnow)/lot #4093 FHM from ABC Laboratories' in-house culture in Columbia, MO
Analytical monitoring:	Yes
Exposure period:	96 hours
Statistical methods:	Computerized LC_{50} program developed by Stephan *et al.* *(*1978). This program calculated the 24-hour LC_{50} value using the binomial method, the 48-hour LC_{50} value using the probit method and the 72- and 96-hour LC_{50} values using the moving average method.
Remarks:	Twenty fathead minnows per concentration were exposed to mean measured test concentrations of 0 (dilution water control), 0.096, 0.18, 0.31, 0.57, and 1.0 mg/l alkyl dimethyl benzyl ammonium chloride. The dilution water was prepared by blending naturally hard well water with well water that had been demineralized by reverse osmosis. The tests were conducted in five-gallon glass vessels containing 15 liters of hard blended water. Two replicates of each test concentration and control were included in the definitive study. The test and control solutions were renewed at 24, 48 and 72 hours. The wet weight and standard length of the control fish at study termination were (mean ± standard deviation) 0.08 ± 0.05 g and 18 ± 3 mm, respectively. The dilution water total hardness ranged from 148 to 152 mg/l as $CaCO_3$, the total alkalinity ranged from 160 to 162 mg/l as $CaCO_3$, pH ranged from 7.9 to 8.2, temperature was 22 to 23°C and the conductivity ranged from 320 to 360 μMhos/cm. Observations for mortality and sublethal responses were made once every 24 hours during the 96-hour test.

Samples for radioactivity counting as a measure of analytical concentration were collected at 0, 24, 48, 72 and 96 hours.

Results

Nominal concentrations (mg/l): 0, 0.10, 0.18, 0.32, 0.56 and 1.0 mg/l
Measured concentrations (mg/l): 0, 0.096, 0.18, 0.31, 0.57 and 1.0 mg/l
Unit: mg/l
Element value: LC_{50} (24-hour) = 0.39 mg/l (95% confidence limits = 0.31 – 0.57 mg/l)
LC_{50} (48-hour) = 0.34 mg/l (95% confidence limits = 0.30 – 0.40 mg/l)
LC_{50} (72-hour) = 0.28 mg/l (95% confidence limits = 0.23 – 0.34 mg/l)
LC_{50} (96-hour) = 0.28 mg/l (95% confidence limits = 0.23 – 0.34 mg/l)
Statistical results: Described below
Remarks: The LC_{50} values were based on the mean measured concentrations. The 96-hour no-observed-effect concentration (NOEC) was 0.096 mg/l. The 96-hour dose-response slope was 4.8. All of the control fish appeared normal and survived for the duration of the test. No sublethal/abnormal effects were noted in the fish treated with the test substance. Cumulative mortalities were as follows:

Cumulative mortalities				
Concentration (mg/l)	**24 h**	**48 h**	**72 h**	**96 h**
0.096	0/20	0/20	1/20	1/20
0.18	0/20	1/20	4/20	4/20
0.31	2/20	5/20	6/20	6/20
0.57	20/20	20/20	20/20	20/20
1.00	20/20	20/20	20/20	20/20

There was one mortality in the 0.096 mg/l concentration at 72 hours. However, there were no other mortalities or sublethal/abnormal effects in the 0.096 mg/l concentration during the test. Therefore, the single mortality in that concentration was not considered in estimating the NOEC.

Conclusions The 96-hour LC_{50} for ADBAC was determined to be 0.28 mg/l.
Remarks: The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability (Klimisch):	1A
Remarks:	Reliable without restriction; guideline study.

References

Sword, M. C. and L. Stuerman. 1994. Static-Renewal Acute Toxicity of Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) to Fathead Minnow (*Pimephales promelas*). Unpublished report number 41237. ABC Laboratories, Columbia, MO, U.S.

4.1 ACUTE TOXICITY TO FISH

Test Substance

Identity:	Alkyl dimethyl benzyl ammonium chloride (CAS RN 68424-85-1; Alkyl (C12-16) dimethylbenzylammonium chloride. An incorrect CAS RN, 68391-01-5, was included in the study report.)
Purity:	Non-radiolabeled = 81.9% ethanol/water solution; Radiochemical purity = 97.4%

Method

Method/Guideline followed:	U. S. EPA TSCA 797.1400
Type:	Static: daily renewal
GLP:	Yes
Year:	1993
Species/Strain/Supplier:	*Pimephales promelas* (Fathead Minnow)/lot # 4093 FHM from ABC Laboratories' in-house culture in Columbia, MO
Analytical monitoring:	Yes
Exposure period:	96 hours
Statistical methods:	Computerized LC_{50} program developed by Stephan *et al.* (1978). This program calculated the LC_{50} values using the binomial method.
Remarks:	Twenty fathead minnows per concentration were exposed to mean measured test concentrations of 0 (dilution water control), 0 + 10 mg/l humic acid (humic acid control), 0.30, 0.53, 0.98, 1.8 and 3.2 mg/l alkyl dimethyl benzyl ammonium chloride amended with 10 mg/l humic acid. The dilution water was prepared by blending naturally hard well water with well water that had been demineralized by reverse osmosis. The tests were conducted in five-gallon glass vessels containing 15 liters of hard blended water. Two replicates of each test concentration and control were included in the definitive study. The test and control solutions were renewed at 24, 48 and 72 hours. The wet weight and length of the control fish at study termination were (mean ± standard deviation) 0.10 ± 0.05 g and 19 ± 3 mm, respectively. The dilution water total hardness ranged from 148 to 152 mg/l as $CaCO_3$, total alkalinity ranged from 160 to 162 mg/l as $CaCO_3$, pH ranged from 7.9 to 8.2, and conductivity ranged from 320 to 360 μMhos/cm. Water temperature was 22 to 23 ºC in the test solutions. Observations for mortality and sublethal responses were made once every 24 hours during the 96-hour test. Samples for radioactivity counting as a measure of analytical concentration were collected at 0, 24, 48, 72 and 96 hours.

Results

Nominal concentrations (mg/l): 0, humic acid control, 0.32, 0.56, 1.0, 1.8 and 3.2 mg/l
Measured concentrations (mg/l): 0, < MQL (humic acid control), 0.30, 0.53, 0.98, 1.8 and 3.2 mg/l
Unit: mg/l
Element value: LC_{50}: 24-, 48-, 72- and 96-hour = 0.81, 0.81, 0.79 and 0.77 mg/l, respectively (95% confidence limits = 0.53 – 0.98 mg/l) based on mean measured concentrations
Statistical results: Described below
Remarks: The 96-hour no-observed-effect concentration and no mortality concentration was 0.53 mg/l. The 96-hour dose-response slope was 14. All of the control and humic acid control fish appeared normal and survived for the duration of the test. All fish exposed to 0.30 and 0.53 mg/l of the test substance survived and appeared normal throughout the test. Cumulative mortalities were as follows:

Cumulative mortalities				
Concentration (mg/l)	**24 h**	**48 h**	**72 h**	**96 h**
0.98	16/20	16/20	17/20	18/20
1.80	20/20	20/20	20/20	20/20
3.20	20/20	20/20	20/20	20/20

Conclusions

The 96-hour LC_{50} for ADBAC was determined to be 0.77 mg/l.
Remarks: The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability (Klimisch): 1A
Remarks: Reliable without restriction; guideline study.

References

Sword, M. C. and L. Stuerman. 1994. Static-Renewal Acute Toxicity of Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) to Fathead Minnow (*Pimephales promelas*) in Dilution Water Amended with 10 mg/l Humic Acid. Unpublished report number 41236. ABC Laboratories, Columbia, MO, U.S.

4.1 ACUTE TOXICITY TO FISH

Test Substance

Identity:	Alkyl dimethyl benzyl ammonium chloride (CAS RN 68391-01-5; Alkyl (C12-18) dimethylbenzylammonium chloride)
Purity:	Non-radiolabeled = 81.9% ethanol/water solution; Radiochemical purity = 97.4%

Method

Method/Guideline followed:	U. S. EPA TSCA 797.1400
Type:	Static: daily renewal
GLP:	Yes
Year:	1993
Species/Strain/Supplier:	*Pimephales promelas* (Fathead minnow)/lot #4093 FHM from ABC Laboratories' in-house culture in Columbia, MO
Analytical monitoring:	Yes
Exposure period:	96 hours
Statistical methods:	Computerized LC_{50} program developed by Stephan *et al.* *(*1978). This program calculated the LC_{50} values using the binomial method.
Remarks:	Twenty fathead minnows per concentration were exposed to mean measured test concentrations of 0 (dilution water control), 0 + 20 mg/l humic acid (humic acid control), and 0.30, 0.54, 0.99, 1.8, and 3.2 mg/l alkyl dimethyl benzyl ammonium chloride amended with 20 mg/l humic acid. The dilution water was prepared by blending naturally hard well water with well water that had been demineralized by reverse osmosis. The tests were conducted in five-gallon glass vessels containing 15 liters of hard blended water. Two replicates of each test concentration and control were included in the definitive study. The test and control solutions were renewed at 24, 48 and 72 hours. The wet weight and length of the control fish at study termination were (mean ± standard deviation) 0.13 ± 0.05 g and 21 ± 3 mm, respectively. The dilution water total hardness ranged from 146 to 148 mg/l as $CaCO_3$, total alkalinity ranged from 158 to 162 mg/l as $CaCO_3$, pH ranged from 7.8 to 7.9, and conductivity ranged from 320 to 340 µMhos/cm. Water temperature was 22 to 23ºC in the test solutions. Observations for mortality and sublethal responses were made once every 24 hours during the 96-hour test. Samples for radioactivity counting as a measure of analytical concentration were collected at 0, 24, 48, 72 and 96 hours.

Results

Nominal concentrations (mg/l): 0, humic acid control, 0.32, 0.56, 1.0, 1.8 and 3.2 mg/l

Measured concentrations (mg/l): 0, < MQL (humic acid control), 0.30, 0.54, 0.99, 1.8 and 3.2 mg/l

Unit: mg/l

Element value: LC_{50} 24-, 48-, 72- and 96-hour = 1.4 mg/l (95% confidence limits = 0.99 to 1.8 mg/l) based on mean measured concentrations

Statistical results: Described below

Remarks: The 96-hour no-observable-effect concentration and no mortality concentration was 0.99 mg/l. The 96-hour dose-response slope was 15. All of the control fish appeared normal and survived for the duration of the test. No sublethal/abnormal effects were noted in any control fish or in surviving fish exposed to the test substance. No mortality occurred in the groups of fish exposed to the test substance at 0.30, 0.54 and 0.99 mg/l. Cumulative mortalities were as follows:

Cumulative mortalities				
Concentration (mg/l)	**24 h**	**48 h**	**72 h**	**96 h**
1.8	19/20	19/20	19/20	19/20
3.2	20/20	20/20	20/20	20/20

Conclusions

The 96-hour LC_{50} for ADBAC was determined to be 1.4 mg/l.

Remarks: The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability (Klimisch): 1A

Remarks: Reliable without restriction; guideline study.

References

Sword, M. C. and L. Stuerman. 1994. Static-Renewal Acute Toxicity of Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) to Fathead Minnow (*Pimephales promelas*) in Dilution Water Amended with 20 mg/l Humic Acid. Unpublished report number 41235. ABC Laboratories, Columbia, MO, U.S.

4.1 ACUTE TOXICITY TO FISH

Test Substance

Identity:	Alkyl dimethyl benzyl ammonium chloride (CAS RN 68424-85-1; Alkyl (C12-16) dimethylbenzylammonium chloride)
Purity:	Non-radiolabeled = 30% aqueous; Radiochemical purity = 96.5% (by TLC) and 95.5% (by HPLC)

Method

Method/guideline followed:	U. S. EPA, FIFRA Subdivision E, Guideline 72-1, Hazard Evaluation: Wildlife and Aquatic Organisms
Type:	Static: daily renewal
GLP:	Yes
Year:	1990
Species/Strain/Supplier:	Bluegill sunfish (*Lepomis macrochirus*)/Kurtz's Fish Hatchery, Elverson, PA
Analytical monitoring:	Yes
Exposure period:	96 hours
Statistical methods:	Binominal
Remarks:	Groups of juvenile freshwater bluegill sunfish were treated with the test substance at nominal concentrations of 0 (dilution water control), 180, 320, 490, 560 and 750 µg/l for 96 hours under static-renewal conditions. Two chamber replicates per concentration, each containing ten fish, were run. The day before test initiation, the average weight of ten fish randomly sampled from the culture holding tank was 0.76 g (range = 0.57 to 0.98 g). Total length averaged 36 mm (range = 33 to 41 mm). At test termination, the average weight of the fish from the dilution water control was 0.76 g (range = 0.53 to 1.45 g). The average length was 39 mm (range = 35 to 47 mm). Fish loading in the dilution water control exposure chambers was 0.35 g/l. The test chambers consisted of 25 l glass aquaria containing 22 l of test solution with an approximate depth of 21.7 cm. The test was conducted in a temperature controlled water bath at a temperature of 22 ± 1ºC. A 16-hour light:8-hour dark photoperiod was maintained. Light intensity ranged from 74.2 to 81.3 fc the day prior to test initiation. All test solutions were renewed 24, 48 and 72 hours after test initiation. Water quality characteristics of the test solutions were measured at test initiation, at the 24-, 48- and 72-hour renewal events, and at test termination. Mean water quality characteristics including pH, dissolved oxygen concentration, temperature, specific

conductance, total hardness and total alkalinity were 7.0, 7.7 mg/l, 21.7 ºC, 192 µmhos/cm, 54 mg/l as $CaCO_3$ and 89 mg/l as $CaCO_3$, respectively. Test solutions used were prepared from two stock solutions of the radiolabeled and non-radiolabeled test substance diluted with Millipore water. All test solution replicates were sampled daily for measurement of radioactivity by liquid-scintillation counting. Results of measurements of the mean measured radioactivity concentrations (considered equivalent to test substance concentration) ranged from 85 – 110% of nominal concentrations. Fish were observed for symptoms of toxicity and mortality at 24, 48, 72 and 96 hours.

Results

Nominal concentrations (mg/l): 0, 0.180, 0.320, 0.490, 0.560 and 0.750 mg/l
Measured concentrations (mg/l): 0, 0.1973, 0.3171, 0.4555, 0.515 and 0.638 mg/l
Unit: µg/l converted to mg/l
Element Value: LC_{50} (24-hour) = 0.540 mg/l (95% confidence intervals = 0.456 – 0.638 mg/l)
LC_{50} (48-, 72- and 96-hour) = 0.515 mg/l
(95% confidence intervals = 0.456 – 0.638 mg/l)
Statistical results: Described above
Remarks: Percent mortality and calculated 24-, 48-, 72- and 96-hour LC_{50} values were based on the corrected mean measured ADBAC concentrations. No mortality occurred in the dilution water control or test substance concentrations of 0.456 mg/l or less during the 96-hour exposure period. Immobilization and erratic swimming were noted for several fish in the 0.515 mg/l dose level on day 1. The following cumulative percent mortality was observed:

Mean measured concentration (mg/l)	**Percent mortality at:**			
	24 hours	**48 hours**	**72 hours**	**96 hours**
0.6384	100	100	100	100
0.515	30	50	50	50
All other dose levels	0	0	0	0

Based on mortality and non-lethal toxic symptoms, the no-observed-effect concentration (NOEC) for the test substance was 0.456 mg/l.

Conclusions

The 96-hour LC_{50} for ADBAC was determined to be 0.515 mg/l.

Remarks: The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability: 1A

Remarks: Reliable without restriction, guideline study.

References

Pate, H. O. and D. O. McIntyre. 1991. Daily Static-Renewal Acute 96-Hour Toxicity Test of Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) to Bluegill Sunfish. Study number SC890050. Battelle Columbus Division, Columbus, OH, U.S.

4.1 ACUTE TOXICITY TO FISH

Test Substance

Identity:	Alkyl dimethyl benzyl ammonium chloride (CAS RN 68424-85-1; Alkyl (C12-16) dimethylbenzylammonium chloride)
Purity:	Non-radiolabeled = 30% aqueous; Radiochemical purity = 96.5% (by TLC) and 95.5% (by HPLC)

Method

Method/guideline followed:	U. S. EPA, FIFRA Subdivision E, Guideline 72-1, Hazard Evaluation: Wildlife and Aquatic Organisms
Type:	Static: daily renewal
GLP:	Yes
Year:	1990
Species/Strain/Supplier:	Rainbow trout (*Oncorhynchus mykiss*)/Trout Lodge, McMillin, WA
Analytical monitoring:	Yes
Exposure period:	96 hours
Statistical methods:	Binominal (24-, 48- and 72-hour intervals) and probit (96-hour interval)
Remarks:	Groups of juvenile freshwater rainbow trout were treated with the test substance at nominal concentrations of 0 (dilution water control), 750, 1000, 1250, 1500 and 1750 µg/l for 96 hours under static-renewal conditions. Two chamber replicates per concentration, each containing ten fish, were run. The day before test initiation, the average weight of ten fish randomly sampled from the culture holding tank was 1.65 g (range = 1.29 to 2.11 g). Total length averaged 57 mm (range = 50 to 62 mm). At test termination, the average weight of the fish from the dilution water control was 1.54 g (range = 1.01 to 2.17 g). Average length was 57 mm (range = 50 to 64 mm). Fish loading in the dilution water control exposure chambers was 0.7 g/l. The test chambers consisted of 25-liter glass aquaria containing 22 l of test solution with an approximate depth of 21.7 cm. The test was conducted in a temperature controlled water bath at a temperature of 12 ± 1ºC. A 16-hour light:8-hour dark photoperiod was maintained. Light intensity ranged from 74.6 to 92.9 fc the day prior to test initiation. All test solutions were renewed 24, 48 and 72 hours after test initiation. Water quality characteristics of the test solutions were measured at test initiation, at the 24-, 48- and 72-hour renewal events, and at test termination. Mean water quality characteristics including

pH, dissolved oxygen concentration, temperature, specific conductance, total hardness and total alkalinity were 7.1, 8.4 mg/l, 12.4ºC, 158 µmhos/cm, 59 mg/l as $CaCO_3$ and 82 mg/l as $CaCO_3$, respectively. Test solutions used were prepared from two stock solutions of the radiolabeled and non-radiolabeled test substance diluted with Millipore water. All test solution replicates were sampled daily for measurement of radioactivity by liquid-scintillation counting. Results of measurements of the mean measured radioactivity concentrations (considered equivalent to test substance concentration) ranged from 85 – 110% of nominal concentrations. Fish were observed for symptoms of toxicity and mortality at 24, 48, 72 and 96 hours.

Results

Nominal concentrations (mg/l):	0, 0.75 1.00, 1.25, 1.50 and 1.75 mg/l
Measured concentrations (mg/l):	0, 0.619, 0.864, 1.029, 1.204 and 1.354 mg/l
Unit:	µg/l converted to mg/l
Element value:	24-hour LC_{50} > 1.354 mg/l (95% confidence intervals not determined) 48-hour LC_{50} = 1.175 mg/l (95% confidence intervals = 1.029 – 1.354 mg/l) 72-hour LC_{50} = 1.066 mg/l (95% confidence intervals = 0.864 – 1.204 mg/l) 96-hour LC_{50} = 0.930 mg/l (95% confidence intervals = 0.866 –0.984 mg/l)
Statistical results:	Described above
Remarks:	Percent mortality and calculated 24-, 48-, 72- and 96-hour LC_{50} values were based on the corrected mean measured ADBAC concentrations. Three hours after test initiation, three fish in the 1.354 mg/l dose group were swimming erratically on the water surface. All other fish were normal in behavior and appearance. No mortality or non-lethal toxic symptoms occurred in the dilution water control or test substance concentration of 0.619 mg/l during the 96-hour exposure. The following cumulative percent mortality was observed:

Mean measured concentration (mg/l)	Percent mortality at:			
	24 hours	48 hours	72 hours	96 hours
1.354	10	95	100	100
1.204	0	60	100	100
1.029	0	5	30	60
0.864	0	0	25	40
0.619	0	0	0	0

Based on mortality and non-lethal toxic symptoms, the no-observed-effect concentration (NOEC) for the test substance was 0.619 mg/l.

Conclusions

The 96-hour LC_{50} for ADBAC was determined to be 0.930 mg/l.

Remarks: The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability: 1A

Remarks: Reliable without restriction, guideline study.

References

Pate, H. O. and D. O. McIntyre. 1991. Daily Static-Renewal Acute 96-Hour Toxicity Test of Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) to Rainbow Trout. Study number SC890051. Battelle Columbus Division, Columbus, OH, U.S.

4.2 TOXICITY TO AQUATIC INVERTEBRATES

Test Substance

Identity:	Alkyl dimethyl benzyl ammonium chloride (CAS RN 68424-85-1; Alkyl (C12-16) dimethylbenzylammonium chloride)
Purity:	Non-radiolabeled = 80.8% ethanol/water solution; Radiochemical purity = 98.74%

Method

Method/Guideline followed:	U. S. EPA FIFRA 72-3(b)
Test type:	Static
GLP:	Yes
Year:	1992
Species/Strain:	*Crassostrea virginica* (Eastern oyster)/from Horn Point Environmental Laboratory, Cambridge, MD
Analytical monitoring:	Yes
Exposure period:	48 hours
Test details:	Described below
Statistical methods:	Moving average method
Remarks:	Embryo larvae (< 3 hours post-fertilization) of the Eastern oyster were exposed to mean measured concentrations of 0, 25.0, 40.8, 58.6, 89.7, and 145 ppb alkyl dimethyl benzyl ammonium chloride (based on total radioactivity) in a 48-hour static acute toxicity test. Additional concentrations of 233, 389, 648, 1080 and 1800 ppb were tested, but a full range of response, from no observed effects to almost 100% effect was achieved following evaluation of the data from the five lowest concentrations (25.0 to 145 ppb); therefore, only data from these dose levels are reported and discussed. The mean initial density of the oyster embryos was 228 embryos/10 ml of solution. The control had four replicate test chambers and each treatment group had three replicate test chambers. The dilution water used in the test was saltwater collected at the Horn Point Environmental Laboratory from the Choptank River and filtered to 2 microns. Dissolved oxygen ranged from 6.2 to 7.2 mg/l, temperature ranged from 24.5 to 25.5 °C, pH ranged from 7.6 to 7.8, and salinity was 15 parts per thousand at study initiation. Test containers were 250 ml glass beakers containing 200 ml of dilution water that had been aerated overnight. The appropriate volume of dispensing stock solution (containing radiolabeled and non-labeled ADBAC) was added to each chamber for the treated groups and stirred for approximately 2 minutes using a glass rod. No additions were added to the control or surrogate test

chambers. Fluorescent tubes, that emitted wavelength similar to natural sunlight (e.g. Chroma 50) provided 16 hours of light and 8 hours of darkness. Light intensity at the water surface was approximately 250 lux. Radioactivity (considered equivalent to measured concentrations of the test substance) was determined in duplicate 10-ml samples at the beginning and 24 hours into the pretest period and at approximately 24 and 48 hours after the start of the test.

Results

Nominal concentrations (mg/l): 0, 18.1, 30.2, 50.4, 84.0 and 140 ppb (0, 0.0181, 0.0302, 0.0504, 0.084, 0.14 mg/l)
Measured concentrations (mg/l): 0, 25.0, 40.8, 58.6, 89.7 and 145 ppb (0, 0.025, 0.0408, 0.0586, 0.0897, 0.145 mg/l)

Unit: ppb

EC_{50} (48 h): = 47.6 ppb (0.0476 mg/l)
(95% confidence interval = 40.8 to 58.6 ppb)

LC_{50} (48 h): = 55.2 ppb (0.0552 mg/l)
(95% confidence interval = 52.1 to 58.5 ppb)

NOEC = 25.0 ppb (0.025 mg/l)

Remarks: The mean number of larvae surviving the 48-hour test period in the controls was 119 embryos/10 ml, representing a 52.2% survival. This survival rate was considered within the range of normal survival for embryos fertilized at the Horn Point Environmental Laboratory with the fertilization process used. Survival rates for all treatment groups were normalized using the control survival rate of 52.2%. Survival of the larvae at the 25.0 ppb test concentration was not different from the controls. Embryo survival in the 40.8 through 145 ppb test concentrations was concentration dependent and mortality appeared to be treatment-related. At the 145 ppb test concentration, mortality was > 95%. Normal development of embryos appeared to be unaffected at the 25.0 ppb test level. The percentage of abnormally developed larvae observed increased in the 40.8 ppb and higher test concentrations, was concentration dependent, and appeared to be treatment-related.

Conclusions

The 48-hour EC_{50} value for ADBAC was determined to be 47.6 ppb (0.0476 mg/l) and the 48-hour LC_{50} value was 55.2 ppb (0.0552 mg/l).

Remarks: The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability (Klimisch):	1A
Remarks:	Reliable without restriction; guideline study.

References

Sved, D. W., J. P. Swigert and G. J. Smith. 1992. A 48-Hour Static Acute Toxicity Test with Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) in Embryo Larvae of the Eastern Oyster (*Crassostrea virginica*). Unpublished report number 350A-103. Wildlife International LTD., Easton, MD, U.S.

4.2 TOXICITY TO AQUATIC INVERTEBRATES

Test Substance

Identity:	Alkyl dimethyl benzyl ammonium chloride (CAS RN 68424-85-1; Alkyl (C12-16) dimethylbenzylammonium chloride)
Purity:	Non-radiolabeled = 80.8% ethanol/water solution; Radiochemical purity = 98.74%

Method

Method/Guideline followed:	U. S. EPA FIFRA 72-3(c)
Test type:	Static: daily renewal
GLP:	Yes
Year:	1992
Species/Strain:	*Mysidopsis bahia* (Saltwater mysid)/Wildlife International Ltd. cultures, Easton, MD
Analytical monitoring:	Yes
Exposure period:	96 hours
Test details:	Described below
Statistical methods:	Binomial probability with the nonlinear interpolation or the probit method.
Remarks:	Juvenile saltwater mysids were exposed to mean measured concentrations of 0, 0.030, 0.047, 0.081, 0.13, 0.22 and 0.35 ppm alkyl dimethyl benzyl ammonium chloride (based on total radioactivity) in a 96-hour static renewal acute toxicity test. The control and each treatment group had two replicate test chambers. Ten mysids were exposed in each chamber for a total of 20 mysids per concentration. Test chambers were 2-liter glass beakers and the test compartments that held the mysids were 500-liter glass beakers with nylon screen-covered holes. The dilution water was saltwater collected at Indian River Inlet, Delaware and diluted to the appropriate salinity with Wildlife International Ltd. well water. Dissolved oxygen ranged from 6.1 to 6.8 mg/l, temperature ranged from 24.5 to 25.5 °C, pH ranged from 7.9 to 8.0, and salinity was 25 parts per thousand at study initiation. Chambers were conditioned overnight prior to introduction of the test organisms. Fluorescent tubes that emitted wavelength similar to natural sunlight provided 16 hours of light and 8 hours of darkness. Light intensity at the water surface was approximately 240 to 380 lux. All mysids were observed approximately 4, 24, 48, 72 and 96 hours after the start of the test to evaluate the number of mortalities and the number of individuals exhibiting clinical signs of toxicity or abnormal behavior. Radioactivity (considered

	equivalent to measured concentrations of the test substance) was determined in duplicate 10-ml samples at the beginning and end of the pretest period and at approximately 24, 48, 72 and 96 hours after the start of the test.

Results

Nominal concentrations (mg/l):	0, 0.029, 0.048, 0.080, 0.13, 0.22 and 0.37 ppm (mg/l)
Measured concentrations (mg/l):	0, 0.030, 0.047, 0.081, 0.13, 0.22 and 0.35 ppm (mg/l)
Unit:	ppm (mg/l)
LC_{50} (24 hour):	> 0.35 ppm (mg/l)
LC_{50} (48 hour):	= 0.14 ppm (mg/l)
LC_{50} (72 hour):	= 0.092 ppm (mg/l)
LC_{50} (96 hour):	= 0.092 ppm (mg/l) (95% confidence limits = 0.081 and 0.13 ppm)
NOEC	0.047 ppm (mg/l)
Remarks:	No mysids died in the control, 0.030 and 0.047 ppm groups. The percent mortalities at 24, 48, 72 and 96 hours were as follows: at 0.081 ppm 0, 5, 25 and 25%, respectively; at 0.13 ppm 0, 20, 100 and 100%, respectively; at 0.22 ppm 5, 90, 100 and 100%, respectively; and at 0.35 ppm 15% mortality occurred by 24 hours and 100% mortality occurred by approximately 48 hours of exposure. The mysids in the 0, 0.30 and 0.47 ppm groups appeared normal throughout the test period. No aberrant behavior was observed in the surviving mysids exposed to 0.081 ppm for 96 hours. At 0.13 ppm, 12% (2 of 16) of the surviving mysids were moribund at 48 hours. At 0.22 ppm, all surviving mysids (2 of 2) were moribund at the 48-hour period. At the 0.35 ppm, 94% (16 of 17) of the surviving mysids were lethargic at 24 hours and the other surviving mysid was moribund.

Conclusions

	The 96-hour LC_{50} value was 92 ppb (0.092 mg/l).
Remarks:	The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability (Klimisch):	1A
Remarks:	Reliable without restriction; guideline study.

References

Sved, D. W., J. P. Swigert and G. J. Smith. 1992. A 96-Hour Static-Renewal Acute Toxicity Test with Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) in the Saltwater Mysid (*Mysidopsis bahia*). Unpublished report number 350A-101A. Wildlife International LTD., Easton MD, U.S.

4.2 TOXICITY TO AQUATIC INVERTEBRATES

Test Substance

Identity:	Alkyl dimethyl benzyl ammonium chloride (CAS RN 68424-85-1; Alkyl (C12-16) dimethylbenzylammonium chloride)
Purity:	Radiochemical purity = 96.5% (by TLC) and 95.5% (by HPLC)

Method

Method/guideline followed:	U. S. EPA, FIFRA Subdivision E, Guideline 72-2 Hazard Evaluation: Wildlife and Aquatic Organisms
Test Type:	Static: daily-renewal
GLP:	Yes
Year:	1990
Species/Strain:	*Daphnia magna*/in-house cultures (the original *Daphnia* broodstock was obtained from the U. S. EPA Environmental Research Laboratory, Duluth, MN)
Analytical monitoring:	Yes
Exposure period:	48 hours
Test details:	Described below
Statistical methods:	Binominal (24-hour interval) and probit (48-hour interval)
Remarks:	*Daphnia magna* neonates (juvenile daphnids ≤ 24 hours old) were treated with the test substance at nominal concentrations of 0 (dilution water control), 10, 18, 27, 32 and 57 µg/l for 48 hours under static-renewal conditions. Each chamber contained five *Daphnia* with four replicate chambers per concentration. *Daphnia* were obtained from in-house cultures that had been maintained by the laboratory for several years. *Daphnia* used for this particular study were from 16-day-old cultures. The water used in rearing and as dilution and control water for the test was moderately hard, reconstituted water. The base water used in the preparation of the reconstituted water was Millipore Milli-Q™ deionized reverse osmosis treated well water. The hardness, alkalinity, pH and specific conductance of the test water used to initiate the test were 96 mg/l as $CaCO_3$, 60 mg/l as $CaCO_3$, 7.5 pH units and 264 µmhos/cm, respectively. Test chambers consisted of 250 ml glass Griffin beakers containing 200 ml of test solution with an approximate depth of 63 mm. Chambers were conditioned with the appropriate nominal concentrations of the test substance prior to test initiation. *Daphnia* loading in 200 ml of test solution was approximately 0.0075 g/l. The test was conducted in a temperature controlled water bath at a temperature of

20 ± 1 ºC. A 16-hour light:8-hour dark photoperiod was maintained. Light intensity measured at the level of the test chambers was 52 fc. All test solutions were renewed 24 hours after test initiation. Water quality characteristics of the test solutions were measured on days 0, 1 and 2. Mean water quality characteristics including pH, dissolved oxygen concentration, temperature, specific conductance, total hardness and total alkalinity were 8.1, 8.6 mg/l, 20.8 ºC, 268 µmhos/cm, 86 mg/l as $CaCO_3$ and 64 mg/l as $CaCO_3$, respectively. Stock solutions for preparation of test substance concentrations were prepared by diluting an aliquot of test substance in Millipore water. All test solution replicates were sampled daily for measurement of the radioactivity by liquid-scintillation counting. Results of measurements of the mean measured radioactivity concentrations (considered equivalent to concentrations of the test substance) ranged from 60 – 90% of nominal concentrations. *Daphnia* were observed for symptoms of toxicity and mortality (or immobilization) at 24 and 48 hours.

Results

Nominal concentrations (mg/l): 0, 0.01, 0.018, 0.027, 0.032 and 0.057 mg/l
Measured concentrations (mg/l): 0, 0.0060, 0.0149, 0.0227, 0.0272 and 0.0516 mg/l
Unit: µg/l were converted to mg/l
EC_{50} (48-hour): 0.0058 mg/l

(95% confidence intervals = 0.0036 – 0.0075 mg/l)
NOEC (48 hour): < 0.006 mg/l
Statistical results: Described above
Remarks: The EC_{50} values, 95% confidence limits and NOEC values were based on corrected mean measured concentrations as µg/l of ^{14}C-ADBAC. The 24-hour EC_{50} was calculated to be 0.0194 mg/l (95% confidence intervals = 0.0149 – 0.0272 mg/l). The dilution water control exhibited 10% mortality at 48 hours, which is an acceptable level of control mortality for acute tests by convention. The following cumulative percent mortality was observed:

Mean measured concentration (mg/l)	Percent mortality at:	
	24 hours	48 hours
0.0516	100	100
0.0272	100	100
0.0227	65	100
0.0149	25	95
0.0060	5	53
0.0	0	10

Conclusions The 48-hour EC_{50} value was 5.8 ppb (0.0058 mg/l).

Remarks: The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability (Klimisch): 1A

Remarks: Reliable without restriction, guideline study.

References

Pate, H. O. and D. O. McIntyre. 1991. Daily Static-Renewal Acute 48-Hour Toxicity Test of Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) to *Daphnia magna*. Study number SC890052. Battelle Columbus Division, Columbus, OH, U. S.

4.2 TOXICITY TO AQUATIC INVERTEBRATES

Test Substance

Identity:	Barquat MS-100 (CAS RN 68424-85-1; Alkyl (C12-16) dimethylbenzylammonium chloride)
Purity:	51.0%

Method

Method/guideline followed:	EC Methods for Determination of Ecotoxicity, Annex to Directive 92/69/EEC (O.J. No. L383A, 1992) Part C, Method 2 "Acute Toxicity for *Daphnia*"; The OECD Guideline for Testing of Chemicals No. 202, "*Daphnia* sp., Acute Immobilisation Test" (2004); USA EPA OPPTS Method 850.1010 "Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids" Public Draft April 1996.
Test Type:	Static: no renewal
GLP:	Yes
Year:	2007
Species/Strain:	*Daphnia magna*/in-house cultures (the original *Daphnia* broodstock was obtained from the Institute National de Recherche Chimique Appliqué (IRChA), France.)
Analytical monitoring:	Yes
Exposure period:	48 hours
Test details:	Described below
Statistical methods:	Statistical analysis was performed using the SAFEStat LD_{50} application, SAS 8.2 (SAS Institute, 1999). The "no observed effect concentration" (NOEC) was derived by direct inspection of the data on treatment-related effects.
Remarks:	Twenty juvenile daphnids (<24 hours old) were exposed in each control and test group at the following nominal concentrations: 0.007, 0.012, 0.019, 0.031, and 0.052 mg a.i./l. The *Daphnia* were placed at random into glass vessels, four replicates of five animals per vessel, each containing approximately 150 mL of medium (resulting in a loading rate of 30 mL medium per organism). Each vessel was loosely covered with a watch glass. The temperature of the test area was 20 ± 1ºC. A 16-hour light: 8-hour dark photoperiod was maintained, with 60-minute periods of subdued lighting at the beginning and end of each phase. No supplementary aeration was employed and no feed was given during the exposure period. The test organisms were maintained and the tests conducted in softened Elendt M4 medium. The medium was prepared in deionised water produced by reverse osmosis. A stock

solution for preparation of the test substance concentrations was prepared by diluting an aliquot of the test substance in the dilution medium. Mean water quality parameters, including temperature, pH, dissolved oxygen, total hardness and alkalinity remained within acceptable limits throughout the study. Stock cultures of *Daphnia magna* were maintained in glass vessels containing approximately 0.8 litres of Elendt M4 culture medium in a temperature-controlled laboratory at nominally 20 ± 2ºC. A photoperiod of 16 hours light: 8 hours dark was maintained, with 60-minute transition periods of subdued lighting at the beginning and end of each light phase. The light intensity of the culture area was 395 lux.

Results

Nominal concentrations (mg/l): 0, 0.007, 0.012, 0.019, 0.031 and 0.052 mg a.i./l

EC_0: 0.012 mg a.i./l

EC_{50} (24-hour): 0.0226 mg a.i./l (95% confidence limits of 0.019 and 0.024 mg a.i./l

EC_{50} (48-hour): 0.0160 mg a.i./l (95% confidence intervals = 0.0144 – 0.0177 mg a.i./l)

NOEC (48 hour): 0.012 mg a.i./l

Statistical results: Described above

Remarks: Test results were expressed in terms of the nominal concentrations. No adverse effects were observed in the control replicates during the study. The following cumulative percent mortality was observed:

Concentration (mg a.i./l)	**Percent Immobile at:**	
	24 hours	**48 hours**
0.052	100	100
0.031	100	100
0.019	15	85
0.012	0	5
0.007	0	0
0.0	0	0

Conclusions The NOEC was 0.012 mg a.i./l

Remarks: The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability: 1

Remarks: Reliable without restriction, guideline study. Key study.

References

Jenkins, C. A. (2007). N-Alkyl(C_{12}-C_{16})-N,N-Dimethyl-N-Benzylammonium Chloride (ADBAC) (Supplied as Barquat DM 50): Acute Toxicity to *Daphnia magna*. Huntingdon Life Sciences, Ltd., Huntingdon, Cambridgeshire, England. Report No. ADB 0037/072526 (unpublished).

4.3 TOXICITY TO AQUATIC PLANTS

Test Substance

Identity:	Barquat MB 50 (CAS RN 68424-85-1; Alkyl (C12-16) dimethylbenzylammonium chloride)
Purity:	Non-radiolabelled, 49-51% w/w a.s. in aqueous solution Radiolabelled, 99.7%

Method

Method/guideline followed:	OECD Guideline No. 201 "Algal Growth Inhibition Test"
GLP:	Yes
Year:	2000
Species/Strain:	*Selenastrum capricornutum* (obtained from the CCAP, Cumbria, England)
Analytical monitoring:	Yes
Exposure period:	72 hours
Test details:	Described below
Statistical methods:	The effect concentrations based on growth rate and exponential growth (E_rC_x) were calculated using a parametric model assuming a constant error per measurement and was based on a method validated in an international ring test. Effect concentrations based on area under the growth curve (E_bC_x) were calculated by linear interpolation. The NOEC was determined based on visual inspection of the data relative to specific acceptability criteria for the control group. DEBtox software was used to calculate no-effect concentration NEC) from the Profile Ln Likelihood function.
Remarks:	An inoculum algae was added to each test chamber to achieve nominal density of 10,000 cells/ml. Flasks were continuously shaken at 100 rpm. The test was carried out at 22.3°C average, and a pH of 8.2. Flasks were continuously shaken at 100 rpm. Illumination was 60-120 umol $s^{-1}.m^{-2}$ using fluorescent lights. Algal cell densities were determined in the treatments and control at 0, 23, 47 and 71 hours. The pH was measured in all cultures after 71 hours. All other parameters were also within the guideline recommendations.

Results

Nominal concentrations (µg/l):	0, 3.7, 13, 24, 43, 136 and 421 µg a.s./l
Measured concentrations (µg/l):	< LOQ, 1.2, 5.1, 11, 22, 98 and 382 µg/l
Unit:	µg/l

EC_{10}:	E_rC_{10} = 9 µg/l
	E_bC_{10} < 1.2 µg/l
EC_{50}:	E_rC_{50} = 49 µg/l
	E_bC_{50} = 14 µg/l
EC_{90}:	E_rC_{90} = 270 µg/l
	E_bC_{90} = 57 µg/l
NOEC:	≤ 0.0012 mg/l
Statistical results:	Described above
Remarks:	

Conclusions The NOEC was ≤ 0.0012 mg/l.

Remarks: The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability: 1

Remarks: Reliable without restriction, guideline study. Key study.

References

Mayer, P, H. Oldersma and J. A. Schoonmade (2001). Determination of the effect of Alkyldimethylbenzylammonium Chloride (ADBAC) on the growth of fresh water green alga *Selenastrum capricornutum* (OECD Guideline No. 201 and EU C.3). TNO Chemistry, Delft, The Netherlands. Report no. 99-9072-03 (unpublished).

4.3 TOXICITY TO AQUATIC PLANTS

Test Substance

Identity:	Barquat MB-80 (CAS RN 68424-85-1; Alkyl (C12-16) dimethylbenzylammonium chloride)
Purity:	80.9% a.i. w/w, Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC; 40% C12, 50% C14, 10% C16; CAS RN 68424-85-1), in aqueous/ethanol solution

Method

Method/guideline followed:	U.S. Environmental Protection Agency Series 850 – Ecological Effects Test Guideline OPPTS Number 850.5400
Test Type:	Static
GLP:	Yes
Year:	2005
Species/Strain:	*Skeletonema costatum* (obtained from Provasoli-Guillard National Center for Culture of Marine Phytoplankton, West booth Harbor, Maine, USA; subsequently maintained in culture medium at Wildlife International, Ltd., Easton, Maryland, USA)
Analytical monitoring:	Yes
Exposure period:	96 hours
Test details:	Described below
Statistical methods:	The calculation of cell densities, areas under the growth curve, growth rates and percent inhibition values, as well as all statistical analyses, were conducted using "The SAS System for Windows", Version 8.02. Non-linear regression was used to calculate EC_{50} values and their corresponding 95% confidence intervals for cell density (EC_{50}), biomass (E_bC_{50}) and growth rate (E_rC_{50}) for each 24-hour exposure period, when possible. The data were evaluated for normality and homogeneity of variance (p=0.05) using the Shapiro-Wilk's and Levene's tests, respectively. The treatment groups then were compared to the negative control using analysis of variance (ANOVA) and Dunnett's test (p=0.05).
Remarks:	The test organism, a marine diatom (*Skeletonema costatum*), was exposed to a geometric series of five test concentrations and a negative (culture medium) control under static conditions for 96 hours. Three replicate test chambers were maintained in each treatment and control group. Algal cell densities were determined in the treatments and control at approximately 0, 24, 48, 72 and

96 hours. At test termination, the algal cells were examined for gross morphology. At 96 hours, the test substance concentrations resulting in maximum inhibitory effects were further test in a recovery phase to determine if growth inhibition observed during test was reversible. If reversible, the effects would be algistatic versus algicidal. Due to the decline in test substance concentrations during the exposure period, effect values were reported in terms of Day 0 measured concentrations (0.0075, 0.016, 0.035, 0.066, 0.16 and 0.51 mg a.i./l). An inoculum of algae was added to each test chamber to achieve a nominal algal density of 77,000 cells/ml at test initiation. Test vessels were sterile 250-ml glass beakers pretreated for approximately 24 hours, and contained 100 ml of test solution or control medium. Test vessels were shaken continuously at 100 rpm. Each test concentration and the negative control were tested in triplicate. Temperatures measured twice daily ranged from 18.9 to 20.4°C. The pH measurements of the test solutions at test initiation were 8.1 and at exposure termination ranged from 8.0 to 8.7. The light intensity on Day 0 ranged from 4090 to 4890 lux.

Results

Nominal concentrations (mg/l):	0, 0.0085, 0.017, 0.034, 0.068, 0.14 and 0.30 mg a.i./l
Measured concentrations (mg/l):	Day 0: <LOQ, 0.0075, 0.016, 0.035, 0.066, 0.16 and 0.51 mg a.i./l Day 4: <LOQ, 0.0056, 0.0046, 0.021, 0.095, 0.11 and 0.73 mg a.i./l
Unit:	mg/l (as active ingredient, a.i.)
EC_{50} (72-hour):	0.056 mg a.i./l (95% confidence intervals = 0.033 – 0.096 mg a.i./l)
EC_{50} (96-hour):	0.063 mg a.i./l (95% confidence intervals = 0.040 – 0.098 mg a.i./l)
E_bC_{50} (72-hour):	0.058 mg a.i./l (95% confidence intervals = 0.042 – 0.081 mg a.i./l)
E_bC_{50} (96-hour):	0.058 mg a.i./l (95% confidence intervals = 0.041 – 0.082 mg a.i./l)
E_rC_{50} (72-hour):	0.078 mg a.i./l (95% confidence intervals = 0.061 – 0.10 mg a.i./l)
E_rC_{50} (96-hour):	0.089 mg a.i./l (95% confidence intervals = 0.066 – 0.12 mg a.i./l)
NOAEC (72- and 96-hour):	0.035 mg a.i./l
Statistical results:	Described above

Remarks:	The 0.16 and 0.51 mg a.i./l treatment groups were maximally inhibited at the end of the 96-hour exposure period. Aliquots of the 0.16 and 0.51 mg a.i./l test solutions were diluted with algal medium and cultured for 9 days. At the termination of the recovery phase (nine days), there was a no visible growth present at either concentration. Growth in the negative control group was 1.7×10^6 cells/ml by 96 hours, demonstrating logarithmic growth.
Conclusions	The 72-hr EC_{50}, based on cell density, was 0.056 mg a.i./l, with a 95% confidence interval of 0.033 and 0.096 mg a.i./l. The 72-hr E_bC_{50} and E_rC_{50} were 0.058 and 0.078 mg a.i./l, respectively. The 96-hr EC_{50}, based on cell density, was 0.063 mg a.i./l, with a 95% confidence interval of 0.040 to 0.098 mg a.i./l. The 96-hr E_bC_{50} and E_rC_{50} were 0.058 and 0.089 mg a.i./l, respectively. The 72- and 96-hr NOAEC, based on cell density, area under the growth curve (biomass) and growth rate, was 0.035 mg a.i./l.
Remarks:	The endpoint has been adequately characterized (ADBAC Joint Venture).
Data Quality	
Reliability (Klimisch):	1
Remarks:	Reliable without restriction, guideline study. Key study.
References	Desjardins, D., J.A. MacGregor and H.O. Krueger. (2005) A 96-Hour Toxicity Test of Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC; 40% C12, 50% C14, 10% C16; CAS RN 68424-85-1) with the Marine Diatom (*Skeletonema costatum*). Report No. 350A-104, Wildlife International, Ltd., Easton, MD, USA. (Unpublished)

4.3 TOXICITY TO AQUATIC PLANTS

Test Substance

Identity:	Barquat MB-80 (CAS RN 68424-85-1; Alkyl (C12-16) dimethylbenzylammonium chloride)
Purity:	The purity of the a.s. is typically >93%; the a.s. is supplied in aqueous/alcohol solution of 50% or 80% a.s. Doses are based on a.s. (i.e. corrected for the dilution in alcohol/water)

Method

Method/guideline followed:	U.S. EPA OPPTS 850.4400 and OECD Revised Proposal for a New Guideline 221
Test Type:	Static
GLP:	Yes
Year:	2005
Species/Strain:	Duckweed, *Lemna gibba* G3, were obtained from the United States Department of Agriculture and have been maintained in culture medium at Wildlife International, Ltd., Easton, Maryland.
Analytical monitoring:	Yes
Exposure period:	7 days
Test details:	Described below
Statistical methods:	The calculations of mean frond numbers, growth rates, biomass and percent inhibition values and the percentages of necrotic, chlorotic and dead fronds were performed using "Microsoft Excel 2000", while statistical analyses were conducted using "TOXSTAT Version 3.5". Day 7 EC_{50} values were determined using linear interpolation with treatment response (frond number, biomass (dry weight) and growth rate based on frond number and biomass) and exposure concentration data. The Day 7 frond numbers, growth rates and biomass were evaluated for normality and homogeneity of variances (p = 0.01) using the Shapiro-Wilk's and Bartlett's tests, respectively. Treatment groups were compared to the control groups (p = 0.05) using analysis of variance (ANOVA) and Dunnett's t-test.
Remarks:	The test organism was exposed to a geometric series of six test concentrations and a negative (culture medium) control under static conditions for seven days. Three replicate test chambers were maintained in each treatment and control group. The number of fronds were recorded on Days 0, 3, 5 and 7 (test termination). The total number of plants was also recorded at test termination. Observations for effects

such as chlorosis, necrosis, death, small fronds, curled fronds, root destruction and breakup of duckweed colonies were performed on Days 0, 3, 5, and 7 (termination). Biomass (dry weight in milligrams) was determined on Day 1 from three representative samples (15 fronds/5 plants) of the inoculum culture and on Day 7 with the plant material from each test and control chamber after completion of the final-day frond count. Growth rate was determined in reference to the number of fronds and biomass. Test vessels were sterile 250-ml glass beakers pretreated for approximately 24 hours and holding 100 ml of test solution or control medium. Five plants totaling approximately 15 fronds were added to each test and control chamber. Each test concentration and the negative control were tested in triplicate. The test was carried out at 25 ± 2°C, 5000 ± 750 lux (continuous). The pH of the medium in each treatment group and control was measured at test initiation and exposure termination.

Results

Nominal concentrations (mg/l):	0, 0.019, 0.043, 0.094, 0.21, 0.45 and 1.0 mg a.s./l
Measured concentrations (mg/l):	Day 0: <LOQ, 0.019, 0.043, 0.090, 0.21, 0.41 and 1.0 mg/l Day 4: <LOQ, <LOQ, <LOQ, <LOQ, 0.0085, 0.074 and 0.44 mg a.s./l
Unit:	mg/l (as active substance, a.s.)
EC_{50}:	0.12 mg a.s./l (95% confidence interval = 0.045 – 0.17 mg a.s./l
NOAEC:	0.019 mg a.s/l
E_bC_{50}:	0.13 mg a.s./l (95% confidence interval = 0.030 – 0.18 mg a.s./l)
NOAEC (biomass):	0.043 mg a.s./l
E_rC_{50} (frond number):	0.25 mg a.s./l (95% confidence interval = 0.16 – 0.33 mg a.s./l)
E_rC_{50}(biomass):	0.37 mg a.s./l (95% confidence interval = 0.31 – 0.45 mg a.s./l)
NOAEC (growth rate):	0.043 mg a.s./l
Statistical results:	Described above
Remarks:	Duckweed plants in each negative control replicate appeared healthy and exhibited normal growth throughout the test. Percent inhibition of frond number in the 0.019, 0.043, 0.090, 0.21, 0.41 and 1.0 mg a.s./l treatment groups at exposure termination was 3.1, 13, 41, 72, 81 and 87%, respectively. A treatment related reduction in frond number was apparent in the five highest concentrations. Percent inhibition of biomass in the 0.019, 0.043, 0.090,

0.21, 0.41 and 1.0 mg a.s./l treatment groups at test termination was 1.8, 13, 40, 68, 85 and 96%, respectively, when compared to the control. Treatment related effects on biomass were apparent in the four highest concentrations. Percent inhibition of growth rate based on frond number in the 0.019, 0.043, 0.090, 0.21, 0.41 and 1.0 mg a.s./l treatment groups at exposure termination was 1.1, 5.1, 20, 47, 61 and 76%, respectively. Percent inhibition of growth rate based on biomass in the 0.019, 0.043, 0.090, 0.21, 0.41 and 1.0 mg a.s./l treatment groups at test termination was 0.63, 4.4, 16, 34, 54 and 81%, respectively, when compared to the control. Treatment related effects on growth rate based on both frond number and biomass were apparent in the four highest concentrations.

Conclusions The 7-day NOAEC for frond number was 0.019 mg a.s./l. The 7-day NOAEC for biomass was 0.043 mg a.s./l. The 7-day NOAEC for growth rate based on frond number and biomass was 0.043 mg a.s./l.

Remarks: The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability (Klimisch): 1

Remarks: Reliable without restriction, guideline study. Key study

References

Desjardins, D., J.A. McGregor and H.O. Krueger. (2005) A 7-Day Toxicity Test of Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC; 40% C_{12}, 50% C_{14}, 10% C_{16}; CAS RN 68424-85-1) with Duckweed (Lemna gibba G3). Study Number 350A-105. Wildlife International, Ltd., Easton, MD, USA. (Unpublished)

4.5.1 CHRONIC TOXICITY TO FISH

Test Substance

Identity:	Alkyl dimethyl benzyl ammonium chloride (CAS RN 68424-85-1; Alkyl (C12-16) dimethylbenzylammonium chloride)
Purity:	Non-radiolabeled = 30% aqueous; Radiochemical purity = 96.5% (by TLC) and 95.5% (by HPLC)

Method

Method/Guideline followed:	U. S. EPA FIFRA 72-4(a)
Test:	Static: daily renewal
GLP:	Yes
Year:	1991
Species/Strain/Supplier:	*Pimephales promelas* (Fathead minnow)/original source of the brood stocks was the U. S. EPA-Environmental Monitoring and Support Laboratory (Newtown, OH)
Analytical monitoring:	Yes
Exposure period:	28-day post-hatch (34-day total: egg plus post-hatch exposure)
Statistical methods:	The LC_{50} values were estimated using TOXSTAT v.2 (Peltier and Weber). IC_{50} calculated using monotonic regression analysis. The 28-day post-hatch survival data were analyzed using Fisher's Exact Test. Analysis of variance (ANOVA) and Dunnett's multiple comparison tests were used (SAS-Version 6.03) for comparing hatchability and weight data.
Remarks:	Dilution waster consisted of water from two deep wells. Water was treated to remove iron and organic impurities, passed through reverse-osmosis purifiers, and treated to obtain a hardness of approximately 75 mg/l as $CaCO_3$. All test solutions were renewed daily and prepared immediately before use. Exposures were conducted without aeration. Fathead minnow eggs [20 eggs per replicate with 4 replicates per group (total of 80 eggs per concentration)] were exposed to mean measured concentrations of 0, 32.3, 75.9, 134.2, 186.8, 273.2 and 488.7 µg/l ^{14}C-alkyl dimethyl benzyl ammonium chloride. Sample concentrations of ^{14}C-ADBAC were verified by liquid scintillation counting. After 7 days, surviving fry from two replicates were thinned to 10 animals per replicate for each exposure group (total of 20 animals per concentration) and exposed to the same concentrations for a 28-day post-hatch static renewal toxicity test. Observations of symptoms of toxicity and mortality were made daily

	throughout the study. The effects on hatchability, mortality, and growth were based on mean measured concentrations. Dissolved oxygen ranged from 5.0 to 9.2 mg/l, temperature ranged from 24.2 to 26.3 °C, and pH ranged from 6.7 to 7.6.

Results

Nominal concentrations (mg/l):	0, 27, 74, 135, 180, 270 and 490 µg/l
Measured concentrations (mg/l):	0, 32.2, 75.9, 134.2, 186.8, 273.2 and 488.7 µg/l
Unit:	µg/l
LOEC (hatchability)	488.7 µg/l
NOEC (hatchability)	273.2 µg/l
LC_{50} (7-day post-hatch)	= 198 µg/l (95% confidence limit = 164 – 231 µg/l)
LC_{50} (14-day post-hatch)	= 104 µg/l (95% confidence limit = 72 – 138 µg/l)
LC_{50} (21-day post-hatch)	= 98 µg/l (95% confidence limit = 68 – 130 µg/l)
LC_{50} (28-day post-hatch)	= 94 µg/l (95% confidence limit = 64 – 126 µg/l)
NOEC (survival)	= 32.2 µg/l
NOEC (growth)	> 32.2 µg/l (lowest concentration without a significant reduction in survival
Remarks:	The average dry weight of surviving fathead minnows generally increased as the concentration of the test substance increased and in each case was higher than the control. This trend of increased growth also correlated with decreased numbers of fish present in each test concentration suggesting food and space may have been the reason for the increased weights in high concentrations that were lethal to the less tolerant or weaker fish. Since no reduction in growth was observed at any of the test concentrations, the IC_{50} and LOEC values were not determined.

Conclusions	The NOEC was determined to be 273 µg/l for hatchability, 32.2 µg/l for survival and > 32.2 µg/l for growth.
Remarks:	The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability (Klimisch):	1A
Remarks:	Reliable without restriction; guideline study.

References

McIntyre, D. O. and H. O. Pate. 1992. Static-Renewal Early Life Stage Toxicity Test of Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) to Fathead Minnows. Unpublished report number SC890057. Battelle Columbus Operations, Columbus, OH, U.S.

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

Test Substance

Identity:	Alkyl dimethyl benzyl ammonium chloride (CAS RN 68424-85-1; Alkyl (C12-16) dimethylbenzylammonium chloride)
Purity:	Radiochemical purity = 96.5% (by TLC) and 95.5% (by HPLC)

Method

Method/Guideline followed:	U. S. EPA FIFRA 72-4(b)
Test type:	Static: daily renewal
GLP:	Yes
Year:	1990
Analytical procedures:	Yes
Species/Strain:	*Daphnia magna*/originally obtained from U. S. EPA's Environmental Research Laboratory, Duluth, MN
Test details:	Described below
Statistical methods:	Fisher's Exact Test, analysis of variance, Bonferroni's multiple comparison test
Remarks:	*Daphnia magna* (< 24 hours old) were exposed to mean analytical concentrations of 0, 0.41, 0.71, 1.33, 2.31 and 4.15 µg/l alkyl dimethyl benzyl ammonium chloride in a 21-day daily static-renewal test (without aeration). The base water used was Millipore Milli-Q™ deionized reverse osmosis treated well water. Water quality data indicated dissolved oxygen ranged from 5.6 to 9.6 mg/l, pH ranged from 7.4 to 8.4 and the temperature ranged from 18.1 to 22.0 °C. The mean (and ranges) for total hardness, total alkalinity and specific conductivity of the control and highest test concentration water was 160.5 mg/l as $CaCO_3$ (124 to 228 mg/l), 133.0 mg/l as $CaCO_3$ (112 to 168 mg/l) and 531.6 µmhos/cm (464 to 695 µmhos/cm), respectively. Ten 250 ml test vessels contained 200 ml of test solution were used for each concentration and control. Test solutions were renewed daily and prepared immediately before use. Test concentrations were analyzed using liquid scintillation counting. Observations on daphnid behavior, the number of live and dead (or immobilized) organisms, and the number of young produced were made at each renewal day and at test termination.

Results

Nominal concentrations (mg/l):	0.5, 0.9, 1.6, 2.8 and 5.0 µg/l	
Measured concentrations (mg/l):	0.41, 0.71, 1.33, 2.31 and 4.15 µg/l	
Unit:	µg/l	
NOEC/LOEC:	Mortality:	NOEC $\geq$ 4.15 µg/l
		LOEC > 4.15 µg/l
	Reproduction:	NOEC = 4.15 µg/l
		LOEC = 5.02 µg/l
	Growth:	NOEC $\geq$ 4.15 µg/l
		LOEC > 4.15 µg/l
Remarks:	No effects on survival, reproduction or growth were observed at measured concentrations up to and including 4.15 µg/l. However, reproduction was clearly affected at measured concentrations $\geq$ 5.02 µg/l in a 9-day range-finding study. Therefore, while an effect level was not demonstrated in this study, the principle objective was still accomplished since the no-effect levels for reproduction and growth were clearly defined at levels just below concentrations that have been shown to produce effects on reproduction and survival. The maximum acceptable toxicant concentration (MATC) was 4.56 µg/l.	

Conclusions The chronic NOEC for ADBAC was determined to be 4.15 ug/l for mortality, reproduction, and growth.

Remarks: The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability (Klimisch): 1A

Remarks: Reliable without restriction; guideline study.

References

McIntyre, D. O. and H. O. Pate. 1992. Daily Static-Renewal Chronic 21-Day Toxicity Test of Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) to *Daphnia magna*. Unpublished report number SC890056. Battelle Columbus Operations, Columbus, OH, U.S.

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

Test Substance

Identity:	Alkyl dimethyl benzyl ammonium chloride (CAS RN 68424-85-1; Alkyl (C12-16) dimethylbenzylammonium chloride)
Purity:	Non-radiolabeled = 81.9% ethanol/water solution; Radiochemical purity = 97.4%

Method

Method/Guideline followed:	ASTM Document # E 1383-93 and U. S. EPA-600/3-75-009
Test type:	Static
GLP:	Yes
Year:	1993
Analytical procedures:	Yes
Species/Strain:	Midge, *Chironomus tentans*/lots # CT-21893 and # CT-21893, obtained from an ABC in-house culture
Test details:	Described below
Statistical methods:	Computerized LC_{50} program developed by Stephan *et al.* (1978)
Remarks:	*Chironomus tentans* (second-instar larvae) were exposed to a natural sediment containing mean measured concentrations of 0 (untreated control), 120, 260, 520, 1200, and 2100 mg of ^{14}C ADBAC equivalents/kg sediment in an aerated 28-day static test. Ten larvae were placed in each of six replicate test chambers/test concentration for a total of 60 larvae per treatment level. Chambers contained 300 ml of treated sediment and 1500 ml synthetic hard water. The water was prepared by blending ABC Laboratory well water with well water treated by reverse osmosis, for a final hardness of 130 to 160 mg/l ($CaCO_3$). Alkalinity, hardness and conductivity all tended to increase with increased concentration of test chemical and ranged from 94 to 196 mg/l $CaCO_3$, 68 to 160 mg/l $CaCO_3$ and 250 to 430 μMhos/cm, respectively, for all groups. The respective water column temperature, dissolved oxygen, and pH ranges were 19 to 21 °C, 2.6 to 8.6 mg/l, and 6.7 to 8.2, respectively. Six analytical (abiotic) replicates of each concentration were used for the analysis of ^{14}C residue analysis of water column, interstitial water (by centrifugation) and sediment. Duplicate replicates (abiotic) were processed on days 0, 14 and 28 for analysis of ^{14}C residues in each of the three matrices. Larvae were observed in triplicate on days 14 and 28. Larvae survival and growth were quantified on days 14 and

28, and emergence success was evaluated on day 28. An LC_{50} was calculated for days 14 and 28, and a 14-day EC_{50} value was calculated based on decreased observed midge size and mortality. NOEC and LOEC values were calculated based on more sensitive parameters, 14-day larval weights and 28-day average time to emergence. All results were reported as measured concentrations of DDAC corrected for purity and equivalent oven-dry weight of sediment solids, EDW, when appropriate.

Results

Nominal concentrations (mg/kg): 0, 125, 250, 500, 1000 and 2000 mg/kg sediment
Measured concentrations (mg/kg): 0, 120, 260, 520, 1200 and 2100 mg/kg sediment
Unit: mg/kg sediment

LC_{50}/NOEC/ LOEC/ MATC:	14-day:	28-day:
	Mortality	
	LC_{50} = 548 mg/kg	LC_{50} = 479 mg/kg
(95% Confidence interval):	(458 to 656 mg/kg)	(377 to 600 mg/kg)
	NOEC = 260 mg/kg	NOEC = 520 mg/kg
	LOEC = 520 mg/kg	LOEC = 1200 mg/kg
	MATC = 368 mg/kg	MATC = 790 mg/kg
	Growth	
	14-day:	28-day:
	NOEC = 260 mg/kg	NOEC = 520 mg/kg*
	LOEC = 520 mg/kg	LOEC = 1200 mg/kg*

* Based on emergence

Remarks: The average survival for *Chironomus tentans* in controls at day 14 was 97 ± 6% and after 28 days survival of controls was 77 ± 21%. Mean survival for the 120, 250, and 520 mg/kg treatment groups was 93%, 93% and 77%, respectively. The mean survival in the 1200 and 2100 mg/kg treatment groups was 7 and 0%, respectively, which was significantly lower than control values. Concentration-response slopes were calculated to be 5.13 for day 14 and 4.22 for day 28. The mean wet and dry weights of control larvae at day 14 were 23.3 and 4.1 mg larva/replicate, respectively. The 520 and 1200 mg/kg treatment levels had significantly lower larval weights (wet and dry) than controls. There was a general decrease in mean larval weight with increasing sediment concentration of ADBAC, but the difference was not statistically significant for the 120 or 260 mg/kg treatments.

Conclusions The chronic NOEC based on emergence for ADBAC was determined to be 520 mg/kg of sediment.

Remarks: The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability (Klimisch): 1A

Remarks: Reliable without restriction; guideline study.

References

England, D. C. and T. Leak. 1995. Chronic Toxicity of Sediment-Incorporated ADBAC to *Chironomus tentans*. Unpublished report number 41004. ABC Laboratories, Inc., Columbia, MO, U.S.A.

Stephan, C.E., K.A. Busch, R. Smith, J. Burke and R.W. Andrew. 1978. A computer program for calculating an LC50. U.S. Environmental Protection Agency, Duluth, Minnesota, pre-publication manuscript, August, 1978.

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

Test Substance

Identity:	Barquat MB-80 (CAS RN 68424-85-1; Alkyl (C12-16) dimethylbenzylammonium chloride)
Purity:	Non-radiolabelled: 80.5% a.i. w/w, Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC; 40% C_{12}, 50% C_{14}, 10% C_{16}; CAS RN 68424-85-1), in aqueous/ethanol solution Radiolabelled: >99%

Method

Method/Guideline followed:	U.S. Environmental Protection Agency Series 850 – Ecological Effects Test Guidelines OPPTS Number 850.1350
Test type:	Flow-through
GLP:	Yes
Year:	2006
Analytical procedures:	Yes
Species/Strain:	Neonate saltwater mysids, Mysidopsis bahia (recently renamed Americamysis bahia), obtained from cultures maintained by Wildlife International, Ltd., Easton, Maryland.
Test details:	Described below
Statistical methods:	Test endpoints analyzed statistically for first-generation mysids were survival, reproduction (the number of live young produced per reproductive day), and growth (total body length and dry weight). Discrete-variable data (e.g. survival) were analyzed using Chi-square and Fisher's Exact tests. Since no differences were detected between the two control groups (t-test, $p > 0.05$), the control data for survival, reproduction and growth were pooled respectively for comparison with the treatment groups. Survival observed in the 80 µg a.i./l treatment group from test initiation to pairing (Day 0 - 15) was significantly decreased ($p < 0.05$); therefore, the 80 µg a.i./l treatment group was excluded from the statistical analyses for post-pairing survival (Day 16 - 28), reproduction, total body length and dry weight. Reproduction and growth (total body length and dry weight) were considered to be continuous variables. All continuous-variable data were evaluated for normality using the Shapiro-Wilk's test and for homogeneity of variance using Bartlett's or Levene's test ($\alpha = 0.01$). The data passed the assumptions of normality and homogeneity; therefore, analysis of variance

	(ANOVA) and the Bonferroni's test were used to evaluate differences between the treatment and pooled control means (p = 0.05). All statistical tests were performed using a personal computer with TOXSTAT or SAS software.
Remarks*:*	The test organism, *Mysidopsis bahia*, was exposed to a series of five nominal test concentrations (5.0, 10, 20, 40 and 80 µg a.i./L), a negative control (dilution water) and a solvent control (0.1 mL DMF/L dilution water) under flow-through conditions for 28 days. The low, middle and high treatment concentrations were radiolabelled for test concentration verification by liquid scintillation counts on Days -1 (pretest), 0, 7, 14, 21 and 28. Actual concentrations for the two unlabelled test solutions (10 and 40 µg a.i./L) were estimated based on a linear regression of the measured radiolabelled test concentrations against nominal concentrations. Four replicate test chambers were maintained in each treatment and control group. At test initiation, 15 neonate mysids (<24 hours old) were impartially distributed to each treatment and control chamber. On Day 15 of the test, when all mysids had reached sexual maturity, female and male adults in each control and treatment group were paired and the reproduction of the paired mysids was monitored through Day 28. Males not paired on Day 15 were reserved for replacement of paired males which died at the respective treatment level during the reproduction period. Environmental conditions were monitored over the course of the study. Observations of mortality, clinical signs of toxicity, and reproduction were made daily. The number of live young produced by each adult mysid pair each day were counted and removed. At test termination, the total body lengths and dry weights of all surviving first-generation mysids were individually measured.

Results

Nominal concentrations:	0 (negative control), 0 (solvent control), 5.0, 10, 20, 40 and 80 µg a.i./l
Measured concentrations:	<LOQ (negative control), <LOQ (solvent control), 4.0, 8.0, 16, 31 and 59 µg a.i./l
Unit:	µg/l (as active ingredient, a.i.)
NOEC:	8.0 µg a.i./l
LOEC:	16 µg a.i./l
MATC:	11 µg a.i./l
Remarks:	No adverse effects were observed in the control replicates during the study. Survival of juvenile mysids (Day 0 - 15)

and adult mysids (Day 16 - 28) and reproduction (mean number of young produced per reproductive day) and mysid growth (mean total body length and dry weight) are summarized by treatment group in the following tables:

Nominal Test Concentration (µg a.i./L)	Mysid Survival			
	Juvenile Mysids (Days 0 - 15)		Adult Mysids (Days 16 - 28)	
	Total No. Alive/ Total No. Exposed	Percent Survival (%)	Total No. Alive/ Total No. Exposed	Percent Survival (%)
Negative Control	58/60	97	44/50	88
Solvent Control	59/60	98	41/44	93
4.0	59/60	98	45/48	94
8.0	58/60	97	47/51	92
16	58/60	97	50/52	96
31	60/60	100	46/53	87
59	40/60	67 [1]	26/32	81 [2]

[1] Statistically significantly ($p < 0.05$) different from the pooled control groups (Fisher's Exact Test).
[2] This treatment group was not included in the statistical analysis due to significantly reduced survival from test initiation to pairing relative to that of the pooled controls.

Mean measured test concentration (µg a.i./l)	Mean number of young/Reproductive day [1] (± sd)	Mean total Body length [1] (mm ± sd)	Mean dry weight [1] (mg ± sd)
Negative Control	0.455 ± 0.024	7.59 ± 0.126	0.644 ± 0.032
Solvent Control	0.520 ± 0.066	7.49 ± 0.164	0.633 ± 0.099
4.0	0.463 ± 0.098	7.64 ± 0.157	0.660 ± 0.079
8.0	0.492 ± 0.109	7.77 ± 0.214	0.683 ± 0.058
16	0.333 ± 0.043 [2]	7.60 ± 0.023	0.651 ± 0.038
31	0.301 ± 0.051 [2]	7.54 ± 0.055	0.625 ± 0.067
59 [3]	0.246 ± 0.093	7.66 ± 0.200	0.682 ± 0.107

[1] Results were generated using Excel 2000. Manual calculations may differ slightly.
[2] Statistically significantly ($p < 0.05$) difference from the pooled control groups (Bonferroni's t-test).
[3] This treatment group was not included in the statistical analysis due to significantly reduced survival from test initiation to pairing relative to that of the pooled controls.

Conclusions

Saltwater mysids (Mysidopsis bahia) were exposed for 28 days to ADBAC at mean measured or estimated concentrations from 4.0 to 59 µg a.i./l. There were no statistically significant treatment-related effects on survival, reproduction, total length, or dry weight at concentrations ≤ 8.0 µg a.i./l. Reproduction was the most sensitive biological endpoint measured in this study. At concentrations ≥16 µg a.i./l, reproduction was significantly reduced compared to reproduction in the pooled controls. Consequently, the NOEC (based on reproduction) was 8.0 µg a.i./l and the LOEC was 16 µg a.i./l. The MATC was calculated to be 11 µg a.i./l.

Remarks:	The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability (Klimisch):	1A
Remarks:	Reliable without restriction; guideline study. Key Study

References

Blankinship, A.S., T. Z. Kendall and H.O. Krueger. (2006) A Flow-Through Chronic Toxicity Test of Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC; 40% C_{12}, 50% C_{14}, 10% C_{16}; CAS RN 68424-85-1) with the Saltwater Mysid (*Mysidopsis bahia*), Report No. 350A-106, Wildlife International, Ltd., Easton, MD, USA. (Unpublished)

5.1.1 ACUTE ORAL TOXICITY

Test Substance

Identity:	Barquat MB-80 (CAS RN 68424-85-1; Alkyl (C12-16) dimethylbenzylammonium chloride)
Purity:	80% ethanol/water solution

Method

Method/Guideline followed:	Not reported
Type:	LD_{50}
GLP:	No
Year:	1975
Species/Strain:	Rat/strain not stated
Sex:	Male and female
No. of animals per sex per dose:	5 males and females combined per dose level
Vehicle:	Propylene glycol
Route of administration:	Oral gavage
Remarks:	Forty-five rats were fasted 24 hours prior to dosing. Nine dose levels, 0.25, 0.32, 0.40, 0.50, 1.0, 2.0, 4.0, 8.0 and 16.0 ml/kg. A total of five males and females combined in each dose group were used. Dose groups from 2.0 to 16.0 ml/kg were dosed with the undiluted test substance, all other dose levels used propylene glycol as a vehicle. Rats were observed for 14 days post-dosing. No postmortem or histopathology examinations were performed.

Results

Value:	LD_{50} = approximately 344 mg/kg
Number of deaths:	0.25 ml/kg = 0/5 0.32 ml/kg = 0/5 0.40 ml/kg = 1/5 0.50 ml/kg = 5/5 1.0 ml/kg = 5/5 2.0 ml/kg = 5/5 4.0 ml/kg = 5/5 8.0 ml/kg = 5/5 16.0 ml/kg = 5/5
Remarks:	The LD_{50} value reported above was adjusted for 100% active ingredient. The calculated LD_{50} value of the 80% solution was 0.43 ml/kg with 95% confidence limits of 0.39 ml/kg to 0.47 ml/kg. All animals dosed with 0.25 to 0.50 ml/kg exhibited lethargy and slight to moderate diarrhea. The severity of the symptoms increased proportionately to the dose level received. Surviving animals returned to normal within five days following dosing. At 1.0 and 2.0 ml/kg, the animals were extremely

lethargic and died within 10 hours. Animals in the 4.0 to 16.0 ml/kg dose groups lapsed into comas within minutes after dosing and died within four hours. One animal in the 0.40 ml/kg dose group was found dead the day after dosing. Three rats in the 0.50 ml/kg group died on the day of dosing and the remaining two rats died one day after dosing.

Conclusions The acute oral LD_{50} for ADBAC was determined to be 0.43 ml/kg of an 80% ethanol/water solution.

Remarks: The endpoint has been adequately characterized (ADBAC Joint Venture

Data Quality

Reliability (Klimisch): 2C

Remarks: Reliable with restrictions; comparable to guideline study with acceptable restrictions.

References

Wallace, J. M. 1975. Acute Oral LD_{50} Toxicity Study. Bio-Toxicology Laboratories, Inc., Moorestown, NJ, U. S.

5.1.1 ACUTE ORAL TOXICITY

Test Substance

Identity:	BTC 471 (CAS RN 68391-01-5; Alkyl (C12-18) dimethylbenzylammonium chloride)
Purity:	50% w/w a.s.

Method

Method/Guideline followed:	Not reported
Type:	LD_{50}
GLP:	No
Year:	1976
Species/Strain:	Rat/Wistar derived albino
Sex:	Male and female
No. of animals per sex per dose:	5/sex/group
Vehicle:	water
Route of administration:	Oral gavage
Remarks:	Five male and five female rats per group were dosed at 50, 500 or 5000 mg/kg. Water was used as the vehicle. Rats were observed for 14 days post-dosing. No postmortem or histapathology examinations were performed.

Results

Value:	LD_{50} = 850 mg/kg/bw
Number of deaths:	50 ml/kg = 0/10 500 ml/kg = 2/10 5000 ml/kg = 10/10
Remarks:	Doses of Alkyldimethylethylbenzylammonium Chloride greater than or equal to 500 mg/kg were lethal to rats within 2 days of dosing. No animals died at the lowest dose level of 50 mg/kg/bw.

Conclusions Alkyldimethylbenzylammonium Chloride is classified as harmful if swallowed on the basis of this study and is assigned the symbol Xn and risk phrase R22.

Remarks:	The endpoint has been adequately characterized (ADBAC Joint Venture

Data Quality

Reliability (Klimisch):	2C
Remarks:	Reliable with restrictions; comparable to guideline study

References Bailey, D.E. (1976) Acute Oral Toxicity in Rats. Food and Drug Research Laboratories, Inc. Waverly, NY, USA. Study No. 5130b (unpublished).

5.1.3 ACUTE DERMAL TOXICITY

Test Substance

Identity:	Barquat MB-80 (CAS RN 68424-85-1; Alkyl (C12-16) dimethylbenzylammonium chloride)
Purity:	80% ethanol/water solution

Method

Method/Guideline followed:	U. S. EPA 16 CFR 1500.40
Type:	LD_{50}
GLP:	No
Year:	1977
Species/Strain:	Rabbit/strain not stated
Sex:	Male and female
No. of animals per sex per dose:	4
Vehicle:	None
Route of administration:	Dermal
Remarks:	Twelve male and 12 female rabbits were tested. One half of the animals in each sex had their skin abraded on one side. Three groups each of four rabbits/sex were dosed with neat test substance. Group 1 received 5 ml/kg, Group 2 received 4 ml/kg and Group 3 received 3 ml/kg. After dosing, the sites were covered with gauze and each animal was wrapped in a sleeve for 24 hours. After removal of the dressing, animals were washed with warm water and dried. Animals were observed for 14 days for toxic effects.

Results

Value:	LD_{50} = 3.56 ml/kg with 95% confidence limit of 3.01 ml/kg to 4.20 ml/kg
Number of deaths:	5 ml/kg = 7/8 4 ml/kg = 6/8 3 ml/kg = 1/8
Remarks:	The majority of the deaths in the 5 ml/kg group (5 animals) occurred after 1 or 2 days. Two additional deaths occurred in the 5 ml/kg group after 7 and 12 days. Deaths in the 4 ml/kg group occurred on days 2, 3, 4 and 6. The one animal in the 3 ml/kg group died after 9 days. All test animals in all groups showed severe erythema and edema at the site of application immediately after dosing.

Conclusions The acute dermal LD_{50} for ADBAC was determined to be 3.56 ml/kg of an 80% ethanol/water solution.

Remarks: The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability (Klimisch):	2C
Remarks:	Reliable with restrictions; comparable to guideline study with acceptable restrictions.

References

Levenstein, I. 1977. Dermal LD_{50}. Unpublished report number 73130. Leberco Laboratories, Roselle Park, NJ, U. S.

5.1.3 ACUTE DERMAL TOXICITY

Test Substance

Identity:	BTC 471 (CAS RN 68391-01-5; Alkyl (C12-18) dimethylbenzylammonium chloride)
Purity:	50% w/w a.s.

Method

Method/Guideline followed:	Hagan, E.C. (1959) Acute Toxicity; Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, pp. 17-25.
Type:	LD_{50}
GLP:	No
Year:	1976
Species/Strain:	Rabbit/New Zealand white
Sex:	Male and female
No. of animals per sex per dose:	5/group (mixed sex)
Vehicle:	None
Route of administration:	Topical occluded; abraded skin
Remarks:	A total of twenty-five rabbits were tested. Each rabbit was given a single dose of the test substance at dose levels of 0.5, 1.0, 2.0, 2.52 and 3.96 g/kg. Applications were made to the skin, mildly shaved and abraded under 1"x1" gauze patches over 10% of the body surface. After dosing, the sites were covered with an impermeable plastic wrapping for 24 hours. After removal of the dressing, the test sites were gently cleansed and the animals were observed for 14 days for toxic effects. A complete gross necropsy was performed on all animals.

Results

Value:	LD_{50} = 2.3 g/kg with 95% confidence limit of 1.46 to 3.63 g/kg
Number of deaths:	0.5 g/kg = 0/5 1.0 g/kg = 1/5 2.0 g/kg = 3/5 2.52 g/kg = 2/5 3.96 g/kg = 2/8
Remarks:	Gross necropsy observations were recorded; however, no consistent findings were noted. Depression was noted in 1 and 2 animals in the 2.0 and 3.96 g/kg dose groups, respectively.

Conclusions The acute dermal LD_{50} for ADBAC was determined to be 2.3 g/kg

Remarks: The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability (Klimisch):	2C
Remarks:	Reliable with restrictions; comparable to guideline study

References

Palanker, A.L. (1976) Acute Dermal LD50. Consumer Product Testing Company. Fairfield, NJ, USA. Study No 7677-7 (unpublished).

5.4. REPEATED DOSE TOXICITY

Test Substance

Identity:	Alkyl dimethyl benzyl ammonium chloride (CAS RN 68424-85-1; Alkyl (C12-16) dimethylbenzylammonium chloride. Incorrect CAS RN, 68391-01-5, was included in the study report.)
Purity:	Lot # 6158-59-60 = 80.51% ethanol/water solution (used weeks 1 - 4) Lot # SC132-65 = 79.7% ethanol/water solution (used weeks 5 - 14)
Remarks:	Two separate batches of the test substance were utilized because detailed analysis of the initial shipment revealed a higher than normal level of an impurity. The low levels of the impurity actually in the treated diet (approximately 14 ppm in the high dose group) and the short exposure were not expected to have any impact on the results or interpretation of the findings in this study.

Method

Method/Guideline followed:	U. S. EPA FIFRA 82-1
Test type:	Oral
GLP:	Yes
Year:	1987
Species:	Mouse
Strain:	CD-1®
Route of administration:	Oral feed
Duration of test:	93 to 94 days
Doses/concentration levels:	100, 500, 1000, 4000 and 8000 ppm
Sex:	Male and female
Exposure period:	93 days (males) and 94 days (females)
Frequency of treatment:	7 days/week
Control group and treatment:	Yes, concurrent, no treatment, fed basal diet
Postexposure observation period:	None
Statistical methods:	Parametric variables were compared for the dose and control groups using Levene's test for homogeneity of variance, by analysis of variance and by separate variance or pooled variance t-tests. Non-parametric data were analyzed by the Kruskal-Wallis test or by the Wilcoxon rank sum test as modified by Mann-Whitney. Frequency data were compared using Fisher's exact tests.
Remarks:	Dietary concentrations of ADBAC were based on the percent active ingredient. Observations for mortality were made twice daily. Detailed clinical observations were performed once each week and observations for overt signs were made on all other days. Body weights and food

consumption data were collected weekly for all animals. All mice were examined for gross pathologic changes. Histopathologic examinations were performed on selected tissues from 10 randomly selected animals/group from all dose groups.

Results

NOAEL (NOEL):	NOAEL = 1000 ppm (approximately 192 mg/kg/day)
LOAEL (LOEL):	LOAEL < 4000 ppm
Actual dose received:	18, 85 and 174 mg/kg/day for males in the 100, 500 and 1000 ppm groups, respectively 21, 102 and 210 mg/kg/day for females in the 100, 500 and 1000 ppm groups, respectively Mean intake for both sexes was approximately 20, 94, and 192 mg/kg/day, respectively. Due to mortality in the 4000 and 8000 ppm groups, actual dose received could not be calculated.
Toxic response/effects:	Described below
Statistical results:	See below
Remarks:	The steep dose response from 100% mortality in the 4000 ppm group to minimal or no effects in the 1000 ppm group precluded a definition of an adequate LOAEL in this study. No males in the 4000 ppm group, and no males or females in the 8000 ppm group survived beyond the 11th day of treatment. One female in the 4000 ppm group survived to terminal sacrifice. Clinical signs of toxicity were restricted to the animals that died, and were related to general cachexia and gross necropsy observations of increased amounts of liquid or semisolid material throughout the gastrointestinal tract. Death was attributed to ileus and shock. Treatment with 1000 ppm or less of the test substance in the diet resulted in no overt toxic responses. Minor decreases in body weights, observed in females in the 1000 ppm group (relative to controls), may have been treatment-related. No other changes were observed in any measurements throughout the study in males or females from the 100, 500 or 1000 ppm groups.

Conclusions	The oral NOAEL for ADBAC in this range-finding study was 1000 ppm in the diet (approximately 192 mg/kg/day).
Remarks:	The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability (Klimisch):	1A
Remarks:	Reliable without restriction; guideline study.

References

Van Miller, J. P. and E. V. Weaver. 1988. Ninety-Day Dose Range-Finding Study with Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) in Mice. Unpublished report number 51-504. Bushy Run Research Center, Export, PA, U. S.

5.4 REPEATED DOSE TOXICITY

Test Substance

Identity:	Alkyl dimethyl benzyl ammonium chloride (CAS RN 68424-85-1; Alkyl (C12-16) dimethylbenzylammonium chloride. An incorrect CAS RN, 68391-01-5, was included in the study report.)
Purity:	Lot # 6158-59-60 = 80.51% ethanol/water solution (used weeks 1 - 4) Lot # SC132-65 = 79.7% ethanol/water solution (used weeks 5 - 14)
Remarks:	Two separate batches of the test substance were utilized because detailed analysis of the initial shipment revealed a higher than normal level of an impurity. The low levels of the impurity actually in the treated diet (approximately 14 ppm in the high dose group) and the short exposure were not expected to have any impact on the results or interpretation of the findings in this study.

Method

Method/Guideline followed:	U. S. EPA FIFRA 82-1
Test type:	Oral
GLP:	Yes
Year:	1987
Species:	Rat
Strain:	Sprague-Dawley CD®
Route of administration:	Oral feed
Duration of test:	95 to 96 days
Doses/concentration levels:	100, 500, 1000, 4000 and 8000 ppm
Sex:	Male and female
Exposure period:	95 days (males) and 96 days (females)
Frequency of treatment:	7 days/week
Control group and treatment:	Yes, concurrent, no treatment, fed basal diet
Postexposure observation period:	None
Statistical methods:	Parametric variables were compared for the dose and control groups using Levene's test for homogeneity of variance, by analysis of variance and by separate variance or pooled variance t-tests. Non-parametric data were analyzed by the Kruskal-Wallis test or by the Wilcoxon rank sum test as modified by Mann-Whitney. Frequency data were compared using Fisher's exact tests.
Remarks:	Dietary concentrations of ADBAC were based on the percent active ingredient. Observations for mortality were made twice daily. Detailed clinical observations were performed once each week and observations for overt signs were made on all other days. Body weights and food

consumption data were collected weekly for all animals. Ophthalmic examinations were performed prior to final sacrifice. Blood samples were collected from 10 animals/sex/group when possible for clinical chemistry and hematology analyses. All surviving rats were examined for gross pathologic changes. Histopathologic examinations were performed on selected tissues from 10 randomly selected animals/group from all dose groups.

Results

NOAEL (NOEL):	NOAEL = 1000 ppm (approximately 70 mg/kg/day)
LOAEL (LOEL):	LOAEL < 4000 ppm
Actual dose received:	6, 31 and 62 mg/kg/day for males in the 100, 500 and 1000 ppm groups, respectively 8, 38 and 77 mg/kg/day for females in the 100, 500 and 1000 ppm groups, respectively Mean intake for both sexes was approximately 7, 35, and 70 mg/kg/day, respectively. Due to mortality in the 4000 and 8000 ppm groups, actual doses could not be calculated.
Toxic response/effects:	Described below
Statistical results:	See below
Remarks:	The steep dose response from 100% mortality in the 4000 ppm group to minimal or no effects in the 1000 ppm group precluded a definition of an adequate LOAEL in this study. No animals in the 8000 ppm group survived past day 8 of treatment. Only three male and four female rats survived to terminal sacrifice from the 4000 ppm group. Clinical signs of toxicity, decreased food consumption and body weights, gross necropsy findings (principally ileus consisting of distended fluid and gas-filled viscera) and histopathologic effects (related to the gastro-intestinal changes) were observed for animals in the 4000 and 8000 ppm groups. Except for a slight trend toward decreased food consumption and body weight for males in the 1000 ppm group, no treatment-related findings in any in-life, clinical pathology, gross pathology, organ weights, or histopathology evaluations were observed in males or females from any other dose group.

Conclusions The oral NOAEL for ADBAC was 1000 ppm in the diet (approximately 70 mg/kg/day).

Remarks: The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability (Klimisch):	1A
Remarks:	Reliable without restriction; guideline study.

References

Van Miller, J. P. and E. V. Weaver. 1988. Ninety-Day Dietary Toxicity Study with Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) in Rats. Unpublished report number 51-503. Bushy Run Research Center, Export, PA, U. S.

5.4 REPEATED DOSE TOXICITY

Test Substance

Identity:	Alkyl dimethyl benzyl ammonium chloride (CAS RN 68424-85-1; Alkyl (C12-16) dimethylbenzylammonium chloride. An incorrect CAS RN, 68391-01-5, was included in the study report.)
Purity:	81.09% ethanol/water solution

Method

Method/Guideline followed:	U. S. EPA FIFRA 82-3
Test type:	Dermal
GLP:	Yes
Year:	1989
Species:	Rat
Strain:	Sprague-Dawley CD®
Route of administration:	Dermal
Duration of test:	13 weeks
Doses/concentration levels:	2, 6 and 20 mg/kg
Sex:	Male and female
Exposure period:	6 hours/day
Frequency of treatment:	5 days/week
Control group and treatment:	Yes, 2 ml/kg water
Postexposure observation period:	None
Statistical methods:	Parametric variables were compared for the dose and control groups using Levene's test for homogeneity of variance, by analysis of variance and by separate variance or pooled variance t-tests. Non-parametric data were analyzed by the Kruskal-Wallis test or by the Wilcoxon rank sum test as modified by Mann-Whitney. Frequency data were compared using Fisher's exact tests.
Remarks:	The dose levels were selected based on a range-finding study that indicated the test material was irritating to the skin above the high dose of 20 mg/kg/day. All solution concentrations of ADBAC were corrected for percent active ingredient. The dorsal area of the trunk of each animal was clipped prior to dose administration. The dosing solution was applied directly to the back. Following application, the entire application site was covered with a sterile gauze pad and the rats were wrapped using Vetrap® Bandaging Tape for approximately six hours. After removal of the wraps, the application site was rinsed with water and the area blotted dry. Observations for mortality were made twice daily. Detailed clinical observations, including observation of the dosing sites, were performed once each week and observations for overt signs were made

on all other days. Body weights and food consumption data were collected weekly for all animals. Ophthalmic examinations were performed prior to final sacrifice. Blood samples were collected from all surviving animals just prior to sacrifice for clinical chemistry and hematology analyses. All surviving rats were examined for gross pathologic changes. Histopathologic examinations were performed on selected tissues from 10 randomly selected animals/group from all dose groups.

Results

NOAEL (NOEL):	NOAEL = 20 mg/kg
LOAEL (LOEL):	LOAEL > 20 mg/kg
Actual dose received:	2, 6 and 20 mg/kg
Toxic response/effects:	Described below.
Statistical results:	See below
Remarks:	No systemic toxicity, as measured by clinical signs, food consumption, body weights or weight gain, ophthalmic changes, hematology or clinical chemistry measurements, gross pathology or histopathology. Slight local irritation (hyperkeratosis) was observed for males in all groups (including controls) and for females in all treatment groups.

Conclusions The NOAEL for systemic toxicity was determined to be greater than 20 mg/kg (dose limited by irritation).

Remarks: The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability (Klimisch):	1A
Remarks:	Reliable without restriction; guideline study.

References

Gill, M. W. and C. L. Wagner. 1990. Ninety-Day Subchronic Dermal Toxicity Study with Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) in Rats. Unpublished report number 52-623. Bushy Run Research Center, Export, PA, U. S.

5.4 REPEATED DOSE TOXICITY

Test Substance

Identity:	Alkyl dimethyl benzyl ammonium chloride (CAS RN 68424-85-1; Alkyl (C12-16) dimethylbenzylammonium chloride)
Purity:	81.09% ethanol/water solution

Method

Method/Guideline followed:	No guideline (range-finding for chronic study)
Test type:	Oral
GLP:	Yes
Year:	1991
Species:	Dog
Strain:	Beagle
Route of administration:	Oral feed
Duration of test:	8 weeks
Doses/concentration levels:	400, 800, 1200 and 1600 ppm
Sex:	Male and female
Exposure period:	8 weeks
Frequency of treatment:	7 days/week
Control group and treatment:	Yes, concurrent, no treatment
Postexposure observation period:	None
Statistical methods:	No statistics performed
Remarks:	This study was conducted in order to choose dose levels for the one year chronic study. Dietary concentrations were based on percent active ingredient. Two dogs per sex per dose group were assigned to the study. Dogs were observed for overt signs of toxicity and mortality at least twice daily. Detailed clinical observations, body weights and food consumption were recorded weekly. A physical examination was conducted on all animals prior to study termination. Hematological and clinical chemistry evaluations were conducted on all animals prior to study initiation and termination. After at least 56 days of treatment, all animals were euthanized and received a thorough postmortem examination. The adrenals, brain, heart, kidneys, liver, ovaries, pituitary, testes with epididymides and thyroid/parathyroid were weighed. Microscopic examinations were performed on the above organs (except brain) and on the bone (rib), lung with bronchi, lymph nodes, pancreas, spleen and thymus.

Prior to the start of the 8-week study, two dosing methods studies were conducted:
Two-week palatability study design: This study was conducted to determine the maximum dose of ADBAC that can be administered in the diet to dogs. ADBAC was administered in the diet at concentrations of 0, 400, 1200, 2400 and 4000 ppm active ingredient for two weeks. One male and one female dog was evaluated at each concentration. Observations were conducted at least twice daily to assess mortality and overt signs of toxicity. Detailed observations and body weights were recorded weekly. Food consumption data were collected daily. After the termination of the treatment period, the dogs were returned to the control diet and retained for use in a subsequent gavage study.
Two-week gavage study design: ADBAC was administered by gavage (as an aqueous slurry mixed with basal diet) at daily dosage levels of 0, 10, 30, 60 and 100 mg active ingredient/kg/day for two weeks. One-half of the total daily dose of ADBAC was administered in the morning and the other half was administered in the afternoon. One male and one female dog were evaluated at each dosage level. Observations were conducted at least twice daily to assess mortality and overt signs of toxicity. Detailed observations and body weights were recorded weekly. Food consumption data were recorded daily. Dogs were euthanized and discarded following two weeks of treatment.

Results

NOAEL (NOEL): NOAEL: (8-week study) = 800 ppm (approximately 31 mg/kg/day)

LOAEL (LOEL): LOAEL: (8-week study) = 1200 ppm (approximately 48 mg/kg/day)

Actual dose received:
Males
400 ppm = 15.4 mg/kg/day
800 ppm = 27.4 mg/kg/day
1200 ppm = 45.5 mg/kg/day
1600 ppm = 71.4 mg/kg/day
Females
400 ppm = 22.0 mg/kg/day
800 ppm = 34.3 mg/kg/day
1200 ppm = 50.7 mg/kg/day
1600 ppm = 51.5 mg/kg/day

	The mean intake for both sexes was approximately 18.7, 30.8, 48.1, and 61.4 mg/kg/day for the 400, 800, 1200, and 1600 ppm groups, respectively. Note: The report table incorrectly cited the compound received for the 1200 and 1600 ppm groups as 37.9 and 44.6 mg/kg/day, respectively for the males and 42.3 and 32.2 mg/kg/day, respectively for the females.
Toxic response/effects:	Described below
Statistical results:	No statistics conducted
Remarks:	8-Week Dietary Study: All animals survived to study termination. Reductions in body weight gains for males and females were noted in the 1600 ppm group. Similar but less pronounced reductions in body weight gains were noted for males and females in the 1200 ppm group. No changes in food consumption were observed. Cholesterol values for male and female dogs were reduced in the 1200 and 1600 ppm dose groups. Based on the results of this study, a dietary concentration of 1200 ppm was selected as the high dietary concentration for the chronic toxicity study (IRDC Project ID 638-004), since the effects seen at this level were expected to produce sufficient toxicity to satisfy but not to exceed the criteria for a maximum tolerated dietary concentration. Further, the effects observed at 1600 ppm were considered to be too severe to be used in a chronic exposure situation. Low and intermediate dietary concentrations of 120 and 400 ppm were selected as increments of the high dietary concentration and were expected to provide no-effect level(s) and/or an intermediate level of effect in the chronic study. Two-week palatability study results: All animals survived to study termination. There was an increase in the frequency and severity of diarrhea throughout the study for the dogs in the 2400 and 4000 ppm dose groups relative to that in the control group. Decreased food consumption and body weight losses also occurred for the dogs in the 240 and 4000 ppm groups. No treatment-related effects were observed for the dogs in the 400 and 1200 ppm groups. Based on the results of this study, ADBAC dietary concentrations of $\geq$ 2400 ppm exceed the level of palatability in the beagle dog. Two-week gavage study results: All animals survived to study termination. There was an increase in frequency and severity of emesis, ptyalism, diarrhea and soft stool for the dogs in the 60 and 100 mg/kg/day dose groups relative to that in the control group. Slight increases in these finding were also present at the 10 and 30 mg/kg/day dose levels.

Decreases in food consumption or body weight were observed for animals in the 60 and 100 mg/kg/day dose groups and transient decreases in food consumption were observed for females in the 30 and 100 mg/kg/day dose groups. Based on the results of this study, 60 mg/kg/day of ADBAC administered by gavage as an aqueous slurry likely exceeds the dosage that could be tolerated in a 90-day repeated administration study.

Conclusions The NOAEL for ADBAC in this 8-week dog study was 800 ppm (approximately 31 mg/kg/day).

Remarks: The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability (Klimisch): 1A

Remarks: Reliable without restriction; guideline study.

References

Goldenthal, E. I. 1994. Evaluation of ADBAC in a Eight-Week Dietary Toxicity Study in Dogs. Unpublished report number 638-003. International Research and Development Corp., Mattawan, MI, U. S.

Goldenthal, E. I. 1993. Evaluation of ADBAC in a Two-Week Palatability Study in Dogs. Unpublished report number 638-001. International Research and Development Corp., Mattawan, MI, U. S.

Goldenthal, E. I. 1994. Evaluation of ADBAC in a Two-Week Gavage Study in Dogs. Unpublished report number 638-002. International Research and Development Corp., Mattawan, MI, U. S.

5.4 REPEATED DOSE TOXICITY

Test Substance

Identity:	Alkyl dimethyl benzyl ammonium chloride (CAS RN 68424-85-1; Alkyl (C12-16) dimethylbenzylammonium chloride)
Purity:	81.09% ethanol/water solution

Method

Method/Guideline followed:	U. S. EPA FIFRA 83-1(b)
Test type:	Oral
GLP:	Yes
Year:	1993
Species:	Dog
Strain:	Beagle
Route of administration:	Oral feed
Duration of test:	1 year
Doses/concentration levels:	120, 400 and 1200 ppm
Sex:	Male and female
Exposure period:	1 year
Frequency of treatment:	7 days/week
Control group and treatment:	Yes, concurrent, no treatment
Postexposure observation period:	None
Statistical methods:	Body weights, food consumption, clinical pathology laboratory values and organ weights were analyzed using one-way analysis of variance (ANOVA) followed by a Bartlett's test for homogeneity of variance (if appropriate). If Bartlett's test was not significant, a Dunnett's t-test was used for the pairwise comparisons; otherwise, the Welch t-test with a Bonferroni correction was used. When non-parametric statistical procedures were required, the rank transformation methods described by Conover and Iman were used.
Remarks:	ADBAC was offered in the diet for a period of one year to groups of four male and four female beagle dogs at concentrations based on active ingredients of 0, 120, 400 and 1200 ppm ADBAC. Control dogs received the basal diet. Dogs were offered free access to the treated or control diets during a three-hour period each day. Observations were conducted at least twice daily to assess mortality and signs of overt toxicity. Detailed observations, body weights and food consumption were recorded weekly. A physical examination was conducted during the pretest period, at 3, 6 and 9 months of study and prior to study termination. An ophthalmologic examination was conducted during the pretest period and prior to study

termination. Clinical pathology evaluations were conducted on all animals prior to study initiation and at 6 and 12 months of study. At study termination, a thorough post-mortem examination was conducted on all dogs. A complete set of all major tissues and organs were harvested and selected organs were weighed. Protocol-specified tissues were processed histologically and microscopic examinations were conducted.

Results

NOAEL (NOEL):	NOAEL = 400 ppm (approximately 14 mg/kg/day)
LOAEL (LOEL):	LOAEL = 1200 ppm (approximately 35 mg/kg/day)
Actual dose received:	<u>Males</u> 120 ppm = 3.79 mg/kg/day 400 ppm = 13.1 mg/kg/day 1200 ppm = 33.8 mg/kg/day <u>Females</u> 120 ppm = 3.67 mg/kg/day 400 ppm = 14.6 mg/kg/day 1200 ppm = 38.6 mg/kg/day The mean intake for both sexes was approximately 3.73, 13.8 and 35.2 mg/kg/day for the 120, 400 and 1200 ppm groups, respectively.
Toxic response/effects:	Described below
Statistical results:	See below
Remarks:	No test substance-related clinical signs were observed. Decreased body weight and body weight gain were observed for males and females in the 1200 ppm group. Food consumption was decreased throughout the study for males in the 1200 ppm group and questionable, treatment-related decreases in food consumption were observed for females in the 1200 ppm group. No treatment-related changes in body weight or food consumption were observed in males or females in the 120 or 400 ppm groups. Ophthalmologic and physical examination findings revealed no test substance-related effects. Cholesterol levels were decreased in males and females in the 1200 ppm group. There were no other treatment-related biochemistry changes observed. No treatment-related effects were observed in hematology, urinalysis, macroscopic and microscopic pathology examinations or organ weight data.

Conclusions The NOAEL for ADBAC in this chronic dog study was 400 ppm (approximately 14 mg/kg/day).

Remarks: The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability (Klimisch): 1A

Remarks: Reliable without restriction; guideline study.

References

Goldenthal, E. I. 1994. Evaluation of ADBAC in a One-Year Chronic Dietary Toxicity Study in Dogs. Unpublished report number 638-004. International Research and Development Corp., Mattawan, MI, U. S.

5.4 REPEATED DOSE TOXICITY

Test Substance

Identity:	Alkyl dimethyl benzyl ammonium chloride (CAS RN 68424-85-1; Alkyl (C12-16) dimethylbenzylammonium chloride. An incorrect CAS RN, 68391-01-5, was included in the study report.)
Purity:	81.09% ethanol/water solution

Method

Method/Guideline followed:	U. S. EPA FIFRA 83-2
Test type:	Oral
GLP:	Yes
Year:	1988-1989
Species:	Mouse
Strain:	CD-1®
Route of administration:	Oral feed
Duration of test:	Approximately 78 weeks
Doses/concentration levels:	100, 500 and 1500 ppm
Sex:	Male and female
Exposure period:	78 weeks
Frequency of treatment:	7 days/week
Control group and treatment:	Yes, two concurrent control groups fed basal diet
Postexposure observation period:	None
Statistical methods:	Parametric variables were compared for the dose and control groups using Levene's test for homogeneity of variance, by analysis of variance and by pooled variance t-tests. Non-parametric data were analyzed by the Kruskal-Wallis test or by the Wilcoxon rank sum test as modified by Mann-Whitney. Frequency data were compared using Fisher's exact tests.
Remarks:	Animals were observed for mortality twice daily. Detailed clinical observations, including palpitations, were performed weekly. Observations for overt clinical signs were made once daily during treatment except on days of detailed observations. Body weights and food consumption were measured weekly for the first 14 weeks of the study and every other week thereafter. Hematology measurements were made for 10 animals per sex from the high and control dose groups during week 52 and all dose groups at study termination during week 79. All surviving animals were subjected to a complete gross necropsy examination during week 79. All tissues, including reproductive tissues, were examined histologically from the control and high dose groups. In addition, the lungs, liver,

kidneys, and all gross lesions were processed and examined histologically from all animals in the other dose groups.
Dose range-finding screen study design: A dose range-finding study was performed to provide information on the potential toxicity of ADBAC in the diet and to aid in the selection of a maximum tolerated dose for a chronic toxicity study in mice. CD-1® mice (15/sex/group) were exposed to ADBAC in the diet at concentrations of 0, 2000 or 3000 ppm for 14 days. Evaluations for clinical signs of toxicity, food consumption, body weight and gross pathology findings were made.

Results

NOAEL (NOEL):	NOEL = 500 ppm (approximately 82 mg/kg/day) for toxicity The NOEL for carcinogenicity was > 1500 ppm (approximately 259 mg/kg/day)
LOAEL (LOEL):	LOAEL = 1500 ppm (approximately 259 mg/kg/day) for toxicity
Actual dose received:	0, 15, 73 and 229 mg/kg/day for male mice 0, 18, 92 and 289 mg/kg/day for female mice The mean intake for both sexes was approximately 16, 82, and 259 mg/kg/day for the 100, 500, and 1500 ppm groups, respectively.
Toxic response/effects:	Described below
Statistical results:	The 1500 ppm group of both the male and female mice showed statistically significantly lower mean body weights and body weight gains that were evident by the end of the first week of treatment.
Remarks:	No treatment-related mortality, clinical signs of toxicity, increases in palpable masse, changes in food consumption, changes in hematological parameters, differences in organ weights, observations at necropsy or differences in histopathologic findings were observed. Treatment-related findings included depressed body weights and body weight gains in males and females in the 1500 ppm group throughout the study. Dose range-finding screen results: The doses corresponded to approximate mean intake levels of 410 and 679 mg/kg/day for males and 523 and 824 mg/kg/day for females of the 2000 and 3000 ppm groups, respectively. Mortality occurred in both sexes from the 3000 ppm dose group (2 animals/sex). Clinical signs of toxicity observed in both sexes from the 3000 ppm group included emaciation, dehydration, unkempt appearance, pallor and hunched posture. Only one male from the 200 ppm dose

group was observed with treatment-related clinical signs. A dose-related loss of body weight was observed in both sexes from the animals from the 2000 and 300 ppm dose groups. Gross pathology findings (principally ileus consisting of distended fluid-filled and gas-filled cecum), were observed in approximately 80% of the male mice and 90% of the females from the 3000 ppm dose group. Treatment-related findings seen at necropsy for the animals from the 2000 ppm group was limited to dilatation and distension of the cecum in approximately 30% of the male and female mice. Based on the weight loss and ileus observed in the animals from the 2000 ppm dose group, this level is considered above the acceptable dietary concentration for long-term exposure to ADBAC.

Conclusions The test substance was not considered to be carcinogenic in this strain of mice under the conditions of this study. (Author of report**)**

Remarks: The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability (Klimisch): 1A

Remarks: Reliable without restriction; guideline study.

References

Gill, M. W., S. J. Hermansky and C. L. Wagner. 1991. Chronic Dietary Oncogenicity Study with Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) in Mice. Unpublished report number53-515. Bushy Run Research Center, Export, PA, U. S.

Weaver, E. W. and J. P. Van Miller. 1988. Two-Week Dose Range Finding Screen with Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) in Mice. Unpublished report number 51-514. Bushy Run Research Center, Export, PA, U. S.

5.4 REPEATED DOSE TOXICITY

Test Substance

Identity:	Alkyl dimethyl benzyl ammonium chloride (CAS RN 68424-85-1; Alkyl (C12-16) dimethylbenzylammonium chloride. An incorrect CAS RN, 68391-01-5, was included in the study report.)
Purity:	81.09% ethanol/water solution

Method

Method/Guideline followed:	U.S. EPA FIFRA 83-5
Test type:	Oral
GLP:	Yes
Year:	1988-1990
Species:	Rat
Strain:	Sprague-Dawley CD®
Route of administration:	Oral feed
Duration of test:	2 years
Doses/concentration levels:	300, 1000 and 2000 ppm
Sex:	Male and female
Exposure period:	104 weeks
Frequency of treatment:	7 days/week
Control group and treatment:	Yes, two concurrent control groups fed basal diet
Postexposure observation period:	None
Statistical methods:	Parametric variables were compared for the dose and control groups using Leven's test for homogeneity of variance, by analysis of variance and by separate variance or pooled variance t-tests. Non-parametric data were analyzed by the Kruskal-Wallis test or by the Wilcoxon rank sum test as modified by Mann-Whitney. Frequency data were compared using Fisher's exact tests.
Remarks:	Animals were observed for mortality twice daily. Detailed clinical observations, including palpitations, were performed weekly. Observations for overt clinical signs were made once daily during treatment except on days of detailed observations. Body weights and food consumption were measured weekly for the first 14 weeks of the study and every other week thereafter. Ophthalmic examinations were performed for all animals prior to the start of the study and prior to final sacrifice. Hematology, clinical chemistry and urinalysis measurements were conduced on 15 animals/sex/group at 26, 52, 78 and 104 weeks of study. When possible the same animals were used at each interval. All animals were fasted prior to bleeding or sacrifice. For urine collections, the rats were placed in metabolism cages for 24 hours with access to the appropriate dosed feed and

water. All surviving animals were subjected to a complete gross necropsy examination following the 104-week treatment period. Tissues, including reproductive tissues, were examined histologically from the control and high dose group. In addition, the lungs, liver, kidneys, and all gross lesions were processed and examined histologically from all animals in the other dose groups.
Dose range-finding screen study design: A dose range-finding study was performed to provide information on the potential toxicity of ADBAC in the diet and to aid in the selection of a maximum tolerated dose for the dietary toxicity/oncogenicity study. Sprague-Dawley CD® rats (15/sex/group) were exposed to ADBAC in the diet at concentrations of 0, 2000 or 3000 ppm for 14 days. Evaluations for clinical signs of toxicity, food consumption, body weight and gross pathology findings were made.

Results

NOAEL (NOEL):	NOAEL = 1000 ppm (approximately 50 mg/kg/day) for toxicity The NOAEL for carcinogenicity is > 2000 ppm (approximately 102 mg/kg/day)
LOAEL (LOEL):	LOAEL = 2000 ppm (approximately 102 mg/kg/day) for toxicity
Actual dose received:	0, 13, 44 and 88 mg/kg/day for male rats 0, 17, 57 and 116 mg/kg/day for female rats The mean intake for both sexes was approximately 15, 50, and 102 mg/kg/day for the 300, 1000, and 2000 ppm groups, respectively.
Toxic response/effects:	Described below
Statistical results:	See below
Remarks:	An increased incidence of loose feces was noted in the male rats in all groups treated with ADBAC. Based upon previous 14-day and 90-day dietary studies with ADBAC, the increased incidence of loose feces in this study was considered potentially treatment-related, but was not considered biologically significant due to the lack of a dose response relationship in incidence, the infrequent nature of the observation throughout all dose groups, and the lack of histological changes in the digestive tract. There were no other clinical signs observed in male rats considered to be treatment-related. No clinical signs observed in the female rats were considered to be related to treatment with ADBAC. Treatment-related effects on body weight and food consumption was seen in both male and female rats in

the 2000 ppm group. The mean absolute body weights of the 2000 ppm group male and female rats were statistically significantly decreased at most measurements period from Week 1 to Week 26 (male) and Week 1 to 60 (female) and, while not consistently statistically significant, remained decreased throughout the study. On a percentage basis, the differences from the control ranged between 4 -5 % in males and 6 - 9% in females from Weeks 13 - 104. There also appeared to be a depression in food consumption in the male rats in the 1000 ppm treatment group during the first few months of the study. However, because of the small and transient nature of this finding, no toxicological significance was attributed to it. No treatment related effects were observed in survival, the type or incidence of palpable masses, clinical pathology, organ weights, gross and microscopic anatomic pathology or ophthalmology.
Dose range-finding screen results: The doses corresponded to approximate mean intake levels of 165 and 213 mg/kg/day for males and 167 and 218 mg/kg/day for females of the 2000 and 3000 ppm groups, respectively. No mortality occurred in either sex. Clinical signs of toxicity (loose feces), decreased food consumption and body weights, and gross pathology findings (principally ileus consisting of distended fluid- and gas-filled viscera) were observed for animals in the 300 ppm dose group. An initial trend toward decreased food consumption and body weight gain was observed for males in the 200 ppm dose group. This effect was assumed to be related to diet aversion. Females at the 200 ppm dose had decreased food consumption only. Treatment-related finding for the animals from the 200 ppm group of both sexes at necropsy was limited to dilatation and distension of the cecum in approximately one half of the animals.

Conclusions The test substance was not carcinogenic in this strain of rats under the conditions of this study. (Author of report).

Remarks: The endpoint has been adequately characterized (ADBAC Joint Venture)

Data Quality

Reliability (Klimisch):	1A
Remarks:	Reliable without restriction; guideline study.

References

Gill, M.W., S. J. Hermansky and C. L. Wagner. 1991. Chronic dietary toxicity/oncogenicity study with alkyl dimethyl benzyl ammonium chloride (ADBAC) in rats. Bushy Run Research Center, Export, PA, U.S. Unpublished report (No. 53-543).

Weaver, E. W. and J. P. Van Miller. 1988. Two-week dose range finding screen with alkyl dimethyl benzyl ammonium chloride (ADBAC) in rats. Bushy Run Research Center, Export, PA, U.S. Unpublished report (No. 51-513).

5.5 GENETIC TOXICITY *IN VITRO*

Test Substance

Identity:	Barquat MB-50 (CAS RN 68424-85-1; Alkyl (C12-16) dimethylbenzylammonium chloride)
Purity:	The purity of the a.s. is typically >93%; the a.s. is supplied in aqueous/alcohol solution of 50% or 80% a.s. Doses are based on a.s. (i.e. corrected for the dilution in alcohol/water)

Method

Method/Guideline followed:	Directive 92/69/EEC, B.14, OPPTS Harmonised Guideline
Type:	Ames
System of testing:	Bacterial
GLP:	Yes
Year:	2001
Species/Strain:	*Salmonella typhimurium* TA1535, TA1537, TA102, TA98 and TA100
Metabolic activation:	With and without Aroclor 1254-induced rat liver S9
Concentrations tested:	Cytotoxicity: 0.15 – 5000 µg/plate Genotoxicity: 0.15 – 50 µg/plate (+/- S9 activation)
Statistical methods:	None
Remarks:	9-Aminoacridine (9AA), mitocycin (MMC) and nitroquinoline-oxide (4NQO) were used as the control in the presence of metabolic activation. N-ethyl-N-nitro-N-nitrosoguanidine (ENNG), 2-aminoanthracene (2AA), benzo(a)pyrene (BP) and 1,8-dihydroxyanthraquinone (DANTHRON) were used as the control in the absence of metabolic activation. Untreated test culture was used as the negative control. Dimethyl sulphoxide served as the vehicle control. The genotoxicity test was carried out twice.

Results

Result:	The test substance was toxic at concentrations of 15 µg/plate and above in both the presence and absence of metabolic activation. No genotoxic effects were recorded.
Cytotoxic concentration:	Toxic at 15.0 µg/ml and higher with and without activation
Genotoxic effects:	Negative
Statistical results:	None
Remarks:	The test substance caused a visible reduction in the growth of the bacterial lawn to all of the tester strains both with and without S9-mix beginning at 15 µg/plate.

Conclusions	ADBAC was not mutagenic with or without metabolic activation in this assay.
Remarks:	The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability (Klimisch):	1A
Remarks:	Reliable without restriction; guideline study. Key study

References

Thompson, P.W. (2001) LZ1392 (Alkyl(C10-C18) (Dimethylbenzylammonium Chloride): Reverse mutation assay "Ames Test" using *Salmonella typhimurium*. Project No. 102/367. Safepharm Laboratories Limited, Derby, UK. (Unpublished)

5.5 GENETIC TOXICITY *IN VITRO*

Test Substance

Identity:	Barquat MB-50 (CAS RN 68424-85-1; Alkyl (C12-16) dimethylbenzylammonium chloride)
Purity:	The purity of the a.s. is typically >93% ADBAC; the a.s. is supplied in aqueous/alcohol solution of 50% or 80% a.s. Doses are based on a.s. (i.e. corrected for the dilution in alcohol/water)

Method

Method/Guideline followed:	OECD Test Guideline No. 473, Directive 92/69/EEC B10
Type:	Chromosomal aberration
System of testing:	Human lymphocytes
GLP:	Yes
Year:	2001
Species/Strain:	Human lymphocytes
Metabolic activation:	With and without Aroclor 1254-induced rat liver S9
Concentrations tested:	Test 1 and 2: 2 μg/ml to 24 μg/ml (with S9 activation) Test 1: 2 μg/ml to 24 μg/ml (without S9 activation) Test 2: 1 μg/ml to 16 μg/ml (without S9 activation)
Statistical methods:	None
Remarks:	Cyclophosphamide (CP) was the positive control in the presence of metabolic activation. Mitomycin C (MMC) was the positive control in the absence of metabolic activation. Media served as the negative control and Eagle's minimal essential medium with HEPES buffer (MEM) was the vehicle control. A range-finding cytotoxicity assay was performed prior to the chromosomal aberration assay using the following concentrations: 19.5 to 5000 μg/ml. Dose levels selected for the mutation assays covered nontoxic and slightly toxic doses. Duplicate cell cultures of both non-activation and S9 metabolic activation assays were evaluated. An independent repeat test was included.

Results

Result:	The test substance was considered negative for chromosomal aberrations in human lymphocytes in vitro under the S9 metabolic activation and nonactivation conditions of the assay. There was no indication of chromosomal ploidy changes in cultures exposed to the test substance in either the presence or absence of S9 mix. Mutant frequencies of all cultures treated with the test

	material were within the acceptable range for background mutant frequencies. In all of the assays, no culture had a mutant frequency that was statistically elevated over background levels.
Cytotoxic concentration:	Slightly toxic at 20 μg/ml with S9 activation in test 1. Toxic at 16 μg/ml without S9 activation in test 1 and toxic at 20 μg/ml without S9 activation in test 2.
Genotoxic effects:	Negative
Statistical results:	None
Remarks:	

Conclusions Cytotoxicity observed.
No genotoxicity observed

Remarks: The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability (Klimisch): 1A

Remarks: Reliable without restriction; guideline study. Key study

References

Durward, R. (2001) LZ1392 (Alkyl (C10-C18) dimethylbenzylammonium Chloride): Chromosomal aberrations assay in human lymphocytes *in vitro*. Project No. 102/366. Safepharm Laboratories Limited, Derby, UK. (Unpublished)

5.5 GENETIC TOXICITY *IN VITRO*

Test Substance

Identity:	Alkyl dimethyl benzyl ammonium chloride (CAS RN 68424-85-1; Alkyl (C12-16) dimethylbenzylammonium chloride)
Purity:	80% ethanol/water solution

Method

Method/Guideline followed:	U. S. EPA FIFRA 84-4
Type:	Mammalian cell forward mutation assay (CHO/HGPRT gene mutation)
System of testing:	Nonbacterial
GLP:	Yes
Year:	1988
Species/Strain:	Female Chinese hamster ovary cells (CHO)
Metabolic activation:	With and without Aroclor 1254-induced rat liver S9
Concentrations tested:	1.0 μg/ml to 20.0 μg/ml without S9 activation 1.0 μg/ml to 40.0 μg/ml with S9 activation
Statistical methods:	Kastenbaum-Bowman test
Remarks:	The test substance was soluble in sterile deionized water up to 50.0 mg/ml. 5-Bromo-2'-deoxyuridine (BrdU) was used as the positive control in the assays without metabolic activation. 3-Methycholanthrene (MCA) was used as the positive control in the assays with S9 metabolic activation. A range-finding cytotoxicity assay was performed prior to the mutation assay. Dose levels selected for the first trial of the mutation assays covered nontoxic and highly toxic doses. Two independent nonactivation and S9 metabolic activation assays were performed. The following are criteria for a positive response: 1) A dose-related or toxicity–related increase in mutant frequency. It is desirable to obtain this relation for at least three doses. 2) If an increase in mutant frequency is observed for a single dose near the highest testable toxicity and the number of mutant colonies is more than twice the value needed to indicate a significant response, the test substance generally will be considered mutagenic.

Results

Result:	ADBAC was considered negative for inducing forward mutations at the HGPRT locus in CHO cells under the S9 metabolic activation and nonactivation conditions of the assay.

Cytotoxic concentration:	Completely toxic at 20.0 µg/ml and higher without activation and completely toxic at 50.0 µg/ml and higher with activation.
Genotoxic effects:	Negative
Statistical results:	Described below
Remarks:	Mutant frequencies of all cultures treated with the test material were within the acceptable range for background mutant frequencies (0 to 15 x 10^{-6}). In all of the assays, no culture had a mutant frequency that was statistically elevated over background levels and greater than 15 x 10^{-6}. Three toxic cultures in one nonactivation trial did achieve statistical significance; however, the significant mutant frequencies were within the acceptable background range (< 15 x 10^{-6}) and were not confirmed at equitoxic dose levels in the independent nonactivation trial. The significant cultures in that one trial were apparently due to normal assay variation.

Conclusions ADBAC was not mutagenic with or without metabolic activation in this assay.

Remarks: The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability (Klimisch): 1A

Remarks: Reliable without restriction; guideline study.

References

Young, R. R. 1989. Mutagenicity Test on Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) in the CHO/HGPRT Forward Mutation Assay. Unpublished report number 10238-0-435. Hazleton Laboratories America, Inc., Vienna, VA, U. S.

5.5 GENETIC TOXICITY *IN VITRO*

Test Substance

Identity:	Alkyl dimethyl benzyl ammonium chloride (CAS RN 68424-85-1; Alkyl (C12-16) dimethylbenzylammonium chloride)
Purity:	80% ethanol/water solution

Method

Method/Guideline followed:	OECD-ISBN 92-64-12221-4, Protocol 482, 1986 and U. S. EPA FIFRA and TSCA – Health Effects Test Guidelines, 40 CFR 798.5550, Final Rule 1986.
Type:	Unscheduled DNA synthesis assay (UDS)
System of testing:	Nonbacterial
GLP:	Yes
Year:	1988
Species/Strain:	Male rat primary hepatocyte
Metabolic activation:	None
Concentrations tested:	Ranging from 10.60 to 0.053 μg/ml
Statistical methods:	See assay evaluation criteria below
Remarks:	A preliminary assessment of toxicity was conducted to select dose levels for the UDS assay. For the UDS assay, male rat hepatocytes were treated with the test substance at concentrations ranging from 10.6 μg/ml to 0.053 μg/ml. Seven treatments from 8.50 to 0.319 μg/ml were selected for autoradiography; however, 8.50 μg/ml was not selected for UDS analysis due to rounded cellular morphology, which precluded analysis of nuclear grains. Six treatments ranging from 6.37 to 0.319 μg/ml were selected for analysis of nuclear labeling in order to determine the possible induction of UDS by the test substance. The solvent was sterile deionized water and was used as the concurrent negative control. The test substance was soluble in water. The positive control, 2-acetylaminofluorene, was not toxic in this assay. The criteria for a positive response were: 1) An increase in the mean net nuclear grain count of six grains per nucleus above the concurrent negative control, 2) an increase in the percent of nuclei having six or more net grains to at least 10% above the concurrent negative control value and/or 3) the percent of nuclei with 20 or more grains to reach or exceed 2% of the analyzed population.

Results

Result:	Concentrations below 8.50 µg/ml were nontoxic and resembled the solvent controls in cellular morphology. Six treatments ranging from 6.37 µg/ml to 0.319 µg/ml were selected for analysis of nuclear labeling. The analyzed treatments were, at most, weakly toxic (98.5% to 117.1% survival), but the higher concentrations demonstrated morphological changes indicative of a toxic response. None of the criteria used to indicate UDS were approached by the chemical treatments and no dose-related response was observed.
Cytotoxic concentration:	Lethal at concentrations exceeding 8.50 µg/ml and excessively toxic at 8.50 µg/ml (68.7% survival). No overt toxicity was observed at 6.37 ug/ml (98.5% survival), but the morphologies of the cells still were slightly different from the solvent control cells.
Genotoxic effects:	Inactive
Statistical results:	See above
Remarks:	

Conclusions The test substance, ADBAC, did not induce significant changes in the nuclear labeling of rat primary hepatocytes for an applied concentration range of 6.37 to 0.319 µg/ml. The test substance was therefore considered inactive in the Rat Primary Hepatocyte UDS Assay. (Author of report)

Remarks: The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability (Klimisch):	1A
Remarks:	Reliable without restriction; guideline study. NOTE: An independent repeat assay was conducted as a separate study (McKeon, 1992).

References

Cifone, M. A. 1989. Mutagenicity Test on Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) in the Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay and 1992 Addendum. Unpublished report number 10238-0-447. Hazleton Laboratories America, Inc., Vienna, VA, U. S.

McKeon, M. E. 1992. Genotoxicity test on Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) in the Assay for Unscheduled DNA Synthesis in Rat Liver Primary Cell Cultures. Unpublished report number 14778-0-447. Hazleton Washington, Inc., Vienna, VA, U. S.

5.5 GENETIC TOXICITY *IN VITRO*

Test Substance

Identity:	Alkyl dimethyl benzyl ammonium chloride (CAS RN 68424-85-1; Alkyl (C12-16) dimethylbenzylammonium chloride)
Purity:	80% ethanol/water solution

Method

Method/Guideline followed:	U. S. EPA FIFRA 84-4
Type:	Unscheduled DNA synthesis assay (UDS)
System of testing:	Nonbacterial
GLP:	Yes
Year:	1991
Species/Strain:	Female rat primary hepatocyte
Metabolic activation:	None
Concentrations tested:	Ranging from 11.8 to 0.538 μg/ml
Statistical methods:	See assay evaluation criteria below
Remarks:	The solvent utilized for this test was sterile deionized water and was used as the solvent control. The 2-Acetylaminofluorene was prepared in dimethylsulfoxide and was used as the positive control. A range of 14 concentrations of ADBAC (11.8 to 0.538 μg/ml) in deionized water were applied initially to the cells. The test substance was soluble in media at all concentrations tested. A viable cell count was then obtained 20.5 hours after initiation of treatments. Six concentrations were chosen for analysis of nuclear labeling, starting with the highest dose that resulted in a sufficient number of survivors with intact morphologies (6.46 μg/ml) and proceeding to successively lower doses. The criteria for a positive response were: 1) An increase in the mean net nuclear grain count to at least five grains per nucleus above the concurrent solvent control value, and/or; 2) an increase in the percent of nuclei having five or more net grains such that the percentage of these nuclei in test cultures is at least 10% above the percentage observed in the solvent control cultures.

Results

Result:	Treatments of 11.8 to 7.00 μg/ml were not analyzed for nuclear labeling due to high toxicity. The 4.31 ug/ml dose level and lower doses were nontoxic. Six treatments from 6.46 to 0.538 μg/ml covered a good range of toxicity (62.7% to 99.8% survival) and were selected for analysis of nuclear labeling. None of the criteria used to indicate UDS

	were approached by any of the analyzed treatments and no dose-related response was observed.
Cytotoxic concentration:	7.00 µg/ml (53.6%)
Genotoxic effects:	Inactive
Statistical results:	See above
Remarks:	Heavily-labeled nuclei (blackened with numerous grains) represent cells undergoing DNA replication as opposed to DNA repair. The number present in this study was low and did not interfere with the assay. Only 35 cells (or 0.32%) among the 11,000 cells screened in the entire assay were heavily labeled.
Conclusions	The test substance, ADBAC, did not induce significant changes in the nuclear labeling of rat primary hepatocytes for an applied concentration range of 6.46 to 0.538 µg/ml. The test substance was therefore considered inactive in the Rat Primary Hepatocyte UDS Assay. (Author of report).
Remarks:	The endpoint has been adequately characterized (ADBAC Joint Venture).
Data Quality	
Reliability (Klimisch):	1A
Remarks:	Reliable without restriction; guideline study. NOTE: this study was conducted as an independent repeat of a previous study (Cifone, 1989)
References	Cifone, M. A. 1989. Mutagenicity Test on Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) in the Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay and 1992 Addendum. Unpublished report number 10238-0-447. Hazleton Laboratories America, Inc., Vienna, VA, U. S. McKeon, M. E. 1992. Genotoxicity test on Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) in the Assay for Unscheduled DNA Synthesis in Rat Liver Primary Cell Cultures. Unpublished report number14778-0-447. Hazleton Washington, Inc., Vienna, VA, U. S.

5.6 GENETIC TOXICITY *IN VIVO*

Test Substance

Identity:	Hyamine 3500 (CAS RN 68424-85-1; Alkyl (C12-16) dimethylbenzylammonium chloride)
Purity:	80.2% ethanol/water solution

Method

Method/Guideline followed:	OECD 474
Type:	Cytogenetic assay (*in vivo* micronucleus assay)
GLP:	Yes
Year:	1985
Species:	Mouse
Strain:	NMRI
Sex:	Male and female
Route of administration:	Oral (gavage)
Doses/concentration levels:	0 and 400 mg/kg
Exposure period:	24-, 48- and 72-hour intervals
Statistical methods:	Differences were assessed by a one-way analysis of variance. In the case of polychromatic erythrocytes (PCE); % of total PCE + normochromatic erythrocytes (NCE) was determined and evaluated on the values observed. For the mean micronuclei (MN) per thousand PCE, the test was done on computed rank values transformed to normal scores according to Blom's method.
Remarks:	In a preliminary test, seven groups of one to six mice per group were dosed with the test substance at dose levels ranging from 400 to 5000 mg/kg/day to determine the maximum tolerated dose. Based the survival of the mice in this preliminary test, 400 mg/kg was chosen as the maximum tolerated dose for the micronucleus test. The micronucleus test consisted of five groups of five mice/sex/group: one negative control treated with distilled water; one positive control treated with 30 mg cyclophosphamide/kg; and three groups treated with the test substance at 400 mg/kg. The dose volume administered to the mice was 1 ml/100 g. Test group animals were sacrificed 24, 48 or 72 hours after treatment. The laboratory's historical control data indicated that the number of micronuclei counted in blood marrow smears of control animals 24, 48 and 72 hours after treatment was almost equal; therefore, all control animals were sacrificed at 24 hours. Mice were not fasted before administration of test substance.

Results

PCE/NCE ratio:

Dose Group	Percent PCE
Negative control	45.0
Positive control	35.6
24-hour test	39.5*
48-hour test	41.3*
72-hour test	37.8*

* = significantly different from the negative control group

Genotoxic effects: Negative

Dose Group	Mean micronuclei (per 1000 PCE)
Negative control	1.0
Positive control	44.1
24-hour test	0.8
48-hour test	1.1
72-hour test	1.0

NOAEL (NOEL): NOAEL = 400 mg/kg

Statistical results: There were no statistically significant differences in the mean MN of the test group compared to the negative control group. See above for percent PCE statistical results.

Remarks: One mouse in the 72-hour test substance dose group was found dead on day 3 after treatment. The PCE count was reduced in the test groups and in the positive control group, which indicated that the dosage of 400 mg/kg was sufficiently high to affect erythropoiesis. The number of micronuclei in the polychromatic erythrocytes was similar in the negative control group and the test group.

Conclusions Under these experimental conditions the test substance can be designated as nonmutagenic in the micronucleus test. (Author of report)

Remarks: The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability (Klimisch):	1A
Remarks:	Reliable without restriction; guideline study.

References

Kallesen, T. 1985. Assessment of the Mutagenic Activity of Hyamine 3500 in the Mouse Micronucleus Test. Unpublished report number 10753. SCANTOX Biological Laboratory Ltd., Denmark.

5.8 TOXICITY TO REPRODUCTION

Test Substance

Identity:	Alkyl dimethyl benzyl ammonium chloride (CAS RN 68424-85-1; Alkyl (C12-16) dimethylbenzylammonium chloride. An incorrect CAS RN, 68391-91-5, was included in the study report.)
Purity:	81.09% ethanol/water solution

Method

Method/Guideline followed:	U. S. EPA FIFRA 83-4
Type:	Two-generation reproduction study
GLP:	Yes
Year:	1989
Species:	Rat
Strain:	Sprague-Dawley CD®
Route of administration:	Oral feed
Doses/concentration levels:	300, 1000 and 2000 ppm
Sex:	Male and female
Control group and treatment:	Yes, concurrent, no treatment
Frequency of treatment:	7 days/week
Duration of test:	F_0: 19 weeks (from 1st prebreed dose to last F_0 sacrifice) F_1: 24 weeks (from 1st F_1 wean to last F_1 sacrifice) F_2: to weaning
Premating exposure period for males:	F_0: 10 weeks; F_1: 10 to 14 weeks (beginning with 1st weaning)
Premating exposure period for females:	F_0: 10 weeks; F_1: 10 to 14 weeks (beginning with 1st weaning)
Statistical methods:	The results of the quantitative continuous variables (e.g., body weights, food consumption, etc.) were compared for the three treatment groups and one control group by use of Levene's test for equal variances, analysis of variance and (pooled or separate variance) t-test. Non-parametric data were statistically evaluated using the Kruskall-Wallis test followed by the Mann-Whitney U test for pairwise comparisons when appropriate. Frequency data were compared using the Fisher's exact test.
Remarks:	Rats were exposed to dietary concentrations (corrected for active ingredient) of 0, 300, 1000 or 2000 ppm. A total of 28 male and 28 female rats were evaluated at each dose level. After a 10-week pre-breed period rats were mated (one male to one female) for three weeks to produce the F1

generation. Exposures continued through mating, gestation, parturition and lactation. At weaning, 28 F1 weanlings/sex/group were selected to produce the F2 generation, and were then exposed to the same dietary concentrations of ADBAC as their parents for 10 weeks. After their pre-breed exposure, F1 animals were paired as described above. All procedures during mating, gestation, and lactation of the F1 parents and selected F2 weanlings were performed as described above. All F0 and F1 parental animals were necropsied and examined for gross lesions; selected reproductive tissues from the high dose and control groups were examined histologically as were other tissues with gross lesions. Ten F1 and F2 weanlings/sex/group were randomly selected and examined for gross lesions. Remaining nonselected F1 and F2 pups at weaning were euthanized and discarded after the necropsy of the selected pups.

Results

NOEL:

Parental = 1000 ppm (approximately 73 mg/kg/day)
Offspring = 1000 ppm (approximately 73 mg/kg/day)
Reproduction NOEL ≥ 2000 ppm (approximately 145 mg/kg/day)

Actual dose received:

Mean test substance consumed (mg/kg/day) calculated for F0 and F1 adult animals during the prebreed exposure period:

Dose Group	F0 Males	F0 Females	F1 Males	F1 Females
300 ppm	20.7	25.5	19.1	24.8
1000 ppm	68.2	81.3	62.5	78.5
2000 ppm	134.7	164.7	125.4	157.1

Mean consumption for the males and females throughout the study was 22.5, 72.6 and 145.5 for the 300, 1000 and 2000 ppm groups, respectively.

F_0 data:

F_0 10-Week Premating Exposure: Males exhibited no reduction in body weight. Females at 2000 ppm had reductions in body weight for weeks 5, 6, 9 and 10 of treatment. Body weight gain was reduced at 2000 ppm for one week during the prebreed

	treatment. Food consumption in F_0 females at 2000 ppm was reduced for the first four exposure weeks. F_0 Female Gestation and Lactation Period: Reproductive parameters were unaffected by treatment. Females in the 2000 ppm group showed significant reductions in body weights on day 0 of gestation, but no body weight gain reductions. Body weight gains throughout lactation and body weights on lactation day 21 were increased in the females in the 2000 ppm group. Food consumption during gestation and lactation was unaffected by test substance treatment. F_0 Postmortem: No treatment-related lesions were observed in the necropsy of F_0 males and females. No treatment-related lesions were observed in the histopathologic examination of selected organs from F_0 males and females at 2000 ppm.
F_1 data:	F_1 10-Week Prebreed Exposure: F_1 males at 2000 ppm exhibited no reduction in body weights but did have reduced weight gain in the second treatment week. Food consumption was reduced for F_1 males at 2000 ppm for two of the 10 treatment weeks. There were no significant effects on F_1 females. F_1 Female Gestation and Lactation Period: Reproductive parameters were unaffected by treatment. Maternal body weights at 2000 ppm were unaffected during the gestational and lactational periods. Gestational food consumption was reduced for days 7 - 11 and 14 - 17 at 2000 ppm. F_1 Postmortem: No treatment-related lesions were observed in the necropsy of F_1 males and females. There were no treatment-related lesions observed in the histopathologic examination of selected organs from the 2000 ppm group. There were no apparent treatment-related deaths of adult animals on study.
Offspring toxicity:	F_1 Litters: Pups exhibited reduced body weights per litter on days 21 (weaning) and 28 (postweaning) at 2000 ppm. F_1 pup body weight gains were reduced for lactation days 14 - 21 and 21 - 28 (postweaning). No effects of treatment on postnatal deaths (postnatal days 0 - 28) were observed. No treatment-related lesions were observed in the

necropsy of F_1 pups that died during lactation or of randomly selected F_1 pups (10/sex/dose).
F_2 Litters: Pup body weights per litter were reduced at 2000 ppm at postnatal day 28 (postweaning). Pup weight gains per litter also were reduced at 2000 ppm for lactational days 14 - 21 (preweaning) and for days 21 - 28 (postweaning). Perinatal deaths and lactational survival were unaffected by treatment. There were no treatment-related lesions observed in the necropsy of F_2 pups that died during lactation or of randomly selected F_2 pups (10/sex/group).

Statistical results: See above

Remarks: Exposure of CD® rats to the test substance in the diet for two generations resulted in parental toxicity at the target dose level of 2000 ppm: perinatal toxicity was concomitant with parental toxicity, being well-defined at 2000 ppm. There were no treatment-related reproductive effects observed in this study. The A/D ratio (the dose level at which there were no observable effects in adults/the dose level at which there were no observable effects on offspring) is 1 (1000 ppm/1000 ppm) indicating no increased risk to the offspring in the absence of indications of adult toxicity.

Conclusions No reproductive toxicity was observed for ADBAC in this study.

Remarks: The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability: 1A

Remarks: Reliable without restriction; guideline study.

References

Neeper-Bradley, T. L. 1990. Two-Generation Study in Sprague-Dawley (CD®) Rats with Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) Administered in the Diet. Unpublished report number 52-524. Bushy Run Research Center, Export, PA, U. S.

5.9 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Test Substance

Identity:	Alkyl dimethyl benzyl ammonium chloride (CAS RN 68424-85-1; Alkyl (C12-16) dimethylbenzylammonium chloride. An incorrect CAS RN, 68391-01-5, was included in the study report.)
Purity:	81.09% ethanol/water solution

Method

Method/Guideline followed:	U. S. EPA FIFRA 83-3
GLP:	Yes
Year:	1991
Species:	Rat
Strain:	Sprague-Dawley CD®
Route of administration:	Oral gavage
Doses/concentration levels:	10, 30 and 100 mg/kg/day
Sex:	Female
Exposure period:	Days 6 through 15 of gestation
Frequency of treatment:	7 days/week
Control group and treatment:	Yes, 5 ml/kg Milli®-Q water
Duration of test:	Day 21 of gestation
Statistical methods:	Quantitative continuous variables were compared for the three treatment groups and the control group by use of Levene's test for equality of variances, analysis of variance (ANOVA), and t-tests. Nonparametric data were evaluated using the Kruskal-Wallis test, followed by the Mann-Whitney U test when appropriate. Incidence data were compared using the Fisher's Exact Test.
Remarks:	Three groups of 25 timed-pregnant female rats each were dosed with ADBAC at concentrations of 10, 30 and 100 mg/kg/day by oral gavage on gestation days (GD) 6 through 15. A control group of 25 timed-pregnant rats received water. Administered dose volumes of water or aqueous solutions of ADBAC were based on the individual dam's body weight on GD 6 and a constant volume of 5 ml/kg/day. Concentrations were adjusted for percent active ingredient. Throughout the study, (GD 0 - 21), female rats were observed for mortality twice daily, clinical signs of toxicity daily (twice daily during dosing) and maternal body weights and food consumption were measured at varying intervals. At scheduled sacrifice on GD 21, a gross necropsy was performed on all dams. In addition, the dams were evaluated for body weight, liver and gravid uterine weight, number of corpora lutea, and number and status of implantation sites. All live fetuses

were dissected from the uterus weighed and examined for external malformation and variations and gender determinations. Approximately one-half of the live fetuses in each litter were examined for thoracic and abdominal visceral abnormalities. These fetuses were decapitated and their heads were fixed in Bouin's solution for examination of craniofacial structures by serial sectioning. Intact fetuses (approximately one-half) were processed for skeletal staining with alizarin red S and examined for skeletal malformations and variations.

Range-finding study design: A range-finding study was conducted to determine dose levels for the definitive developmental toxicity study. Five groups of five timed-pregnant female rats each were dosed with ADBAC at concentrations of 25, 50, 100, 200 or 400 mg/kg/day by oral gavage on gestation days (GD) 6 through 15. A control group of five timed-pregnant female rats received water. Administered dose volumes of water or aqueous solutions of ADBAC were based on the individual dam's body weight on GD 6 and a constant volume of 5 ml/kg/day. Concentrations were adjusted for percent active ingredient. Throughout the study, (GD 0 - 21), female rats were observed for mortality twice daily, clinical signs of toxicity daily (twice daily during dosing) and maternal body weights and food consumption were measured at varying intervals. At scheduled sacrifice on GD 21, a gross necropsy was performed on all dams. In addition, the dams were evaluated for body weight, liver and gravid uterine weight, number of corpora lutea and number and status of implantation sites. All live fetuses were dissected from the uterus, counted, sexed, weighed, examined for external malformation and variations and then euthanized and discarded.

Results

Maternal toxicity NOEL:	10 mg/kg
Developmental toxicity NOEL:	> 100 mg/kg
Actual dose received:	0, 10, 30 and 100 mg/kg
Maternal data:	Treatment-related clinical signs included perioral wetness in 67% of dams in the 100 mg/kg group. Audible respiration during and subsequent to the treatment period also was observed in three dams in the 100 mg/kg group. One dam in this group exhibited dehydration, unkempt appearance, loose feces, urine stains and perioral wetness. Audible respiration was observed in two dams in the 30 mg/kg group. One of these dams also exhibited urine

	stains, gasping, perinasal encrustation, loose feces and perioral wetness. No treatment-related clinical signs were observed in animals in the 10 mg/kg group during or subsequent to treatment. Food consumption was reduced for days 6 - 9 of gestation in the 30 and 100 mg/kg groups. There were no effects of treatment on gestational body weight and weight gain, corrected body weight or gravid uterine weight. Pregnancy rate was equivalent among groups and ranged from 84 to 100%. Twenty-one to 25 live litters were available for evaluation from each group. There were no statistically significant differences between treated and control animals in gestational parameters (including total number of implantations, number of viable and nonviable implants per litter).
Fetal data:	There were no statistically significant differences between treated and control fetal body weights per litter or in the incidences of external, visceral or skeletal malformations or variations.
Statistical results:	See above
Remarks:	Range-finding study results: Doses of 200 and 400 mg/kg/day resulted in 100% mortality early in the treatment regimen. The pregnancy rate ranged from 60-100%. All surviving pregnant females had one or more live fetuses at scheduled sacrifice. Various clinical signs of toxicity were observed in the females prior to death and included ataxia, hypoactivity, urogenital area wetness, audible respiration, loose feces, perioral wetness and perioral encrustation. One characteristic clinical sign, perioral wetness, also was observed at 100 mg/kg/day. Thus, 100 mg/kg/day was selected as high dose level as some maternal toxicity was likely to be observed in the definitive study. No maternal toxicity was observed at 50 mg/kg/day or below in the range-finding study; therefore, the two lower doses of 30 and 10 mg/kg/day were chosen on a half log scale below the high dose level.

Conclusions No developmental toxicity was observed for ADBAC in this study.

Remarks: The endpoint has been adequately characterized. (ADBAC Joint Venture)

Data Quality

Reliability (Klimisch): 1A

Remarks: Reliable without restriction; guideline study.

References

Neeper-Bradley, T. L. 1992. Developmental Toxicity Evaluation II of Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) Administered by Gavage to CD® Rats. Unpublished report number 91N0031. Bushy Run Research Center, Export, PA, U. S.

Chun, J. S. and L. C. Fisher. 1993. Developmental Toxicity Dose Range-Finding Study of Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) Administered by Gavage to CD® Rats. Unpublished report number 54-613. Bushy Run Research Center, Export, PA, U. S.

Myers, R. C. 1994. Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) Quat 80%: Five-Day Peroral Toxicity Study in the Female Rabbit. Unpublished report number 54-568. Bushy Run Research Center, Export, PA, U. S.

5.9 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Test Substance

Identity:	Alkyl dimethyl benzyl ammonium chloride (CAS RN 68424-85-1; Alkyl (C12-16) dimethylbenzylammonium chloride. An incorrect CAS RN, 68391-01-5, was included in the study report.)
Purity:	81.09% ethanol/water solution

Method

Method/Guideline followed:	U. S. EPA FIFRA 83-3
GLP:	Yes
Year:	1991
Species:	Rabbit
Strain:	New Zealand White
Route of administration:	Oral gavage
Doses/concentration levels:	1, 3 and 9 mg/kg/day
Sex:	Female
Exposure period:	Days 6 through 18 of gestation
Frequency of treatment:	7 days/week
Control group and treatment:	Yes, 2 ml/kg Milli®-Q water
Duration of test:	Day 29 of gestation
Statistical methods:	Quantitative continuous variables were compared for the three treatment groups and the control group by use of Levene's test for equality of variances, analysis of variance (ANOVA), and t-tests. Nonparametric data were evaluated using the Kruskal-Wallis test, followed by the Mann-Whitney U test when appropriate. Incidence data were compared using the Fisher's Exact Test.
Remarks:	All rabbits were naturally mated at the testing facility. Three groups of 16 timed-pregnant female rabbits each were dosed with ADBAC at concentrations of 1, 3 and 9 mg/kg/day by oral gavage on gestation days (GD) 6 through 18. A control group of 16 timed-pregnant rabbits received water. Administered dose volumes of water or aqueous solutions of ADBAC were based on the individual doe's body weight on GD 6 and a constant volume of 2 ml/kg/day. Concentrations were adjusted for percent active ingredient. Throughout the study, (GD 0 - 29), female rabbits were observed for mortality twice daily, clinical signs of toxicity daily (twice daily during dosing) and maternal body weights and food consumption were measured at varying intervals. At scheduled sacrifice on GD 29, a necropsy was performed on all does. In addition, the does were evaluated for body weight, liver and gravid uterine weight, number of corpora lutea, and number and

status of implantation sites. All live fetuses were dissected from the uterus weighed and examined for external and thoracic and abdominal visceral malformation and variations. Approximately one-half of the fetuses were examined for craniofacial malformations by serial sectioning. All fetuses were processed for skeletal staining with alizarin red S and examined for skeletal malformations and variations.

Developmental Toxicity Range-Finding Study Design: A range-finding study was conducted to determine dose levels for the definitive developmental toxicity study. Five groups of five timed-pregnant female rabbits each were dosed with ADBAC at concentrations of 1, 3, 10, 30, or 60 mg/kg/day by oral gavage on gestation days (GD) 6 through 18. A control group of five timed-pregnant female rabbits received water. Administered dose volumes of water or aqueous solutions of ADBAC were based on the individual doe's body weight on GD 6 and a constant volume of 2 ml/kg/day. Concentrations were adjusted for percent active ingredient. Throughout the study, (GD 0 - 29), female rabbits were observed for mortality twice daily, clinical signs of toxicity daily (twice daily during dosing) and maternal body weights and food consumption were measured at varying intervals. At scheduled sacrifice on GD 29, a gross necropsy was performed on all does. In addition, the does were evaluated for body weight, liver and gravid uterine weight, number of corpora lutea and number and status of implantation sites. All live fetuses were dissected from the uterus, counted, weighed, examined for external malformation and variations and then euthanized and discarded.

Five-Day Peroral Toxicity Study Design: Prior to initiation of the developmental toxicity range-finding study, this probe study was performed to evaluate the short-term repeated dose toxicity of ADBAC. Two non-fasted female rabbits (non-pregnant) per group were given repeated peroral doses (once daily for five consecutive days) of ADBAC dissolved in distilled water at concentrations of 3, 10, 30 and 100 mg/kg. Animals were observed frequently for signs of toxicity following each daily dose. Body weights were recorded just prior to each day's dose and before sacrifice on the eighth day of the study. A necropsy was performed on all rabbits that died or were sacrificed.

Results

Maternal toxicity NOEL:	3 mg/kg
Developmental toxicity NOEL:	> 9 mg/kg
Actual dose received:	0, 1, 3 and 9 mg/kg/day
Maternal data:	Hypoactivity, and labored or audible respiration were observed in animals in the 9 mg/kg group. No evidence of maternal toxicity was observed in animals in the 1 or 3 mg/kg groups.
Fetal data:	Fetal examinations indicated no evidence of developmental toxicity in any treatment group. No treatment-related differences were observed in mean fetal body weight or incidences of external, visceral or skeletal malformations or variations.
Statistical results:	See above
Remarks:	Range-finding study results: Doses of 60 and 30 mg/kg/day resulted in 100% and 40% mortality, respectively early in the treatment regimen. Various clinical signs of toxicity were observed in females from the 60 and 30 mg/kg/day groups prior to death and included hypoactivity, paralysis, prostration, cold extremities, audible and slow respiration, loose feces and perioral wetness. Audible respiration, body weight loss and reduced food consumption were observed in the majority of surviving does in the 30 mg/kg/day group. Mean body weight losses, reduced food consumption and audible respiration were also observed in the 10 mg/kg/day group. With a larger sample size in the definitive study and the variability of responses in rabbits, there was concern that a dose of 10 mg/kg/day would result in an unacceptable level of mortality; therefore, the dose of 9 mg/kg/day was selected as the high dose level for the definitive study. A dose of 1 mg/kg/day was selected as the low dose as it was expected to be a no observable effect level. Five-Day Peroral Toxicity Study Design: Both rabbits in the 100 mg/kg group died (one after three and one after five days of dosing). Signs of toxicity first appeared after the third dose and included sluggishness, labored breathing, convulsions and abdominal distention. There was also a progressive loss of body weight. At necropsy, finding included hemorrhaged intestines, red trachea, red esophagus, red and mottled lungs and red patches on one stomach lining. One rabbit in the 30 mg/kg group died one day after the third dose. This animal exhibited spasms, gasping and prostration after the third dose and had a distended abdomen at the time of death. The surviving rabbit did not show signs of toxicity until the day of

sacrifice when it exhibited labored breathing and moderate weight loss. The necropsy of both rabbits revealed red lungs and trachea. Both rabbits administered 10 mg/kg of ADBAC survived but had inconsistent weight loss and gross pathologic findings included red lungs, red patches on the trachea and reddened esophagus. There were not deaths or signs of toxic effect in either rabbit in the 10 mg/kg group. At necropsy, one animal had red patches on the trachea.

Conclusions No developmental toxicity was observed for ADBAC in this study.

Remarks: The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability (Klimisch): 1A

Remarks: Reliable without restriction; guideline study.

References

Neeper-Bradley, T. L. and M. F. Kubena. 1992. Developmental Toxicity Evaluation of Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) Administered by Gavage to New Zealand White Rabbits. Unpublished report number 91N0032. Bushy Run Research Center, Export, PA, U. S.

Chun, J. S. and T. L. Neeper-Bradley. 1993. Developmental Toxicity Dose Range-Finding Study of Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) Administered by Gavage to New Zealand White rabbits. Unpublished report number 54-603. Bushy Run Research Center, Export, PA, U. S.

5.9 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Test Substance

Identity:	BTC 471 (CAS RN 68391-01-5; Alkyl (C12-18) dimethylbenzylammonium chloride)
Purity:	50% w/w a.s.

Method

Method/Guideline followed:	Methods used were comparable to OECD Guideline 414
GLP:	No
Year:	1977
Species:	Rat
Strain:	Wistar derived albino
Route of administration:	Oral gavage
Doses/concentration levels:	0, 5, 15 and 50 mg/kg/day
Sex:	Female
Exposure period:	Days 6 through 15 of gestation
Frequency of treatment:	Once daily during exposure period
Control group and treatment:	Yes, concurrent positive (aspirin, 250 mg/kg/day) and negative
Duration of test:	Day 20 of gestation
Statistical methods:	Confidence Belts for proportions with a confidence coefficient of 0.95 was used to compare the experimental and control groups. If the significance was not clearly definable, the probability of the occurrence was determined by the computation of exact probabilities.
Remarks:	The study was carried out in a manner similar to OECD Guideline 414. 22-37 pregnant rats/group were treated with Alkyl(C12-18)dimethylethylbenzylammonium Chloride at concentrations of 0, 5, 15 and 50 mg/kg/day or aspirin at 250 mg/kg/day. The dams were sacrificed at day 20 and the foetuses were examined for visceral and skeletal variations and malformations.

Results

Maternal toxicity NOEL:	15 mg/kg/day
Developmental toxicity NOEL:	> 50 mg/kg/day
Actual dose received:	0, 5, 15 and 50 mg/kg/day
Maternal data:	There were no clinical signs reported. There were 3 deaths of dames in the 50 mg/kg/day test group. There were no effects of treatment on gestational body weight. Effects on gestation were not noted in any treatment group. Twelve females in the positive control group resorbed their uterine contents.

Fetal data:	There were no treatment-related effects related to bodyweight. Decreased weights were observed in the positive control group. There were no malformations or gross finding at necropsy. There were no treatment-related variations or malformations with respect to skeletal or visceral findings.
Statistical results:	Not stated
Remarks:	No developmental toxicity including teratogenicity was observed at any dosage employed, as compared to the negative control group. There were no treatment-related effects on the body weight or reproductive parameters. Increased mortality was observed at 50 mg/kg/day; however, this finding was not statistically significant.There were no treatment-related effects on foetal body weight or visceral/skeletal findings.In the positive control group, increased fetal resorption, decreased fetal weight and increase incidences of skeletal and soft tissue variations were observed.

Conclusions

No developmental toxicity, including teratogenicity was observed at any dosage employed. The "no observable effect level" (NOEL) for maternal toxicity was 15 mg/kg/day; the NOEL for developmental toxicity was > 50 mg/kg/day.

Remarks: The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability (Klimisch): 2C

Remarks: Reliable with restrictions

References

Knickerbocker, M and Stevens, K.R. (1977) Teratologic evaluation of BTC-471 in Rats. Food and Drug Research Laboratories, Inc., Waverly, NY, USA. Project No: 5130b (unpublished).

5.9 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Test Substance

Identity:	Benzyldimethylstearylammonium chloride (CAS RN 122-19-0; Benzyldimethyloctadecylammonium chloride)
Purity:	Not stated

Method

Method/Guideline followed:	Not stated
GLP:	Not stated
Year:	1981
Species:	Rat
Strain:	CFY Sprague-Dawley
Route of administration:	Dermal
Doses/concentration levels:	1.6, 3.3 and 6.6%
Sex:	Female
Exposure period:	Days 6 through 15 of gestation
Frequency of treatment:	7 days/week
Control group and treatment:	Yes, concurrent (treatment not stated)
Duration of test:	Day 20 of gestation
Statistical methods:	Not stated
Remarks:	Groups of 20 mated female rats were exposed by dermal application to 0, 1.6, 3.3 and 6.6% (w/v) of the test substance in distilled water at a dosage volume of 0.5 ml/rat. The test substance was applied with a syringe and massaged into the saved area (4 x 4 cm) of skin in the scapular region for not more than 1 minute. The material was left on the skin and was neither occluded nor removed by washing. The number of pregnant dams ranged from 10 to 20 per group. All animals were observed for signs of systemic and local reactions. Body weights, food and water consumption were recorded at regular intervals throughout the study. On day 20 of pregnancy, dams were killed, litter values determined, and fetuses subsequently examined for skeletal and visceral abnormalities.

Results

Maternal toxicity NOEL:	NOAEL = 6.6% (Local irritation, but no systemic toxicity observed)
Developmental toxicity NOEL:	6.6%
Actual dose received:	Not stated
Maternal data:	There were no systemic signs of reaction, no deaths or treatment-related macroscopic pathology changes in internal organs. A dose-related increase in the local irritation was recorded in terms of animals affected and

	degree of erythema and edema. The initial reaction was evident within a day of the first administration, reaching a peak around the mid-point of the dosing period, thereafter stabilizing or declining. Stabilization was frequently associated with scab formation. There were no marked or consistent dose-related differences in weight gain or food and water consumption.
Fetal data:	Litter values, assessed by litter size, post-implantation loss, litter and mean fetal weights and the embryonic and fetal development were not affected by treatment. There were no significant differences from concurrent control values with respect to the incidence of malformations or anomalies seen in the litters or fetuses.
Statistical results:	Not stated
Remarks:	

Conclusions No developmental toxicity was observed for the test substance in this study.

Remarks: The endpoint has been adequately characterized (ADBAC Joint Venture).

Data Quality

Reliability (Klimisch): 2A

Remarks: Reliable with restrictions; acceptable, well-documented publication which meets basic scientific principles.

References

Palmer. A. K., A. M. Bottomley, J. A. Edwards and R. Clark. 1983. Absence of Embryotoxic Effects in Rats with Three Quaternary Ammonium Compounds (Cationic Surfactants). Toxicology 26:313 - 315.