UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MAR 18 2003

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: Review Aerobic Aquatic Metabolism Data for Didecyl dimethyl ammonium chloride (DDAC)

TO: Velma Noble, Product Manager, Team 31
Regulatory Management Branch I
Antimicrobials Division (7510C)

FROM: Srinivas Gowda, Microbiologist/Chemist Srinivas Gowda 3/13/03
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THRU: Jonathan Chen, Acting Team Leader, Team One Jonathan Chen 3/13/03
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Norm Cook, Chief Norman J. Cook 03.18.2003
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DP Barcode: D288350 & D287563
Case: 007280
Submission: S627084
Case Type: Resubmission
Common Name: DDAC, Bardac 22 (2250, 2280)
Chemical Name: Didecyl dimehyl ammonium chloride

EPA Reg. No.: None
MRID No.: 422538-03
Data Submitter: Lonza Inc.
PC Code: 069149
CAS#: 7173-51-5

INTRODUCTION:

Lonza Inc., has submitted the Aerobic Aquatic Metabolism Study data on Didecyl dimethyl ammonium chloride to meet the U.S. Environmental Protection Agency's Environmental Fate Data Requirements published in the Pesticide Assessment Guidelines, Subdivision N, §162-4 for the active ingredient, Didecyl dimethyl ammonium chloride. Also, the Lonza, Inc. has submitted this study conducted on Didecyl dimethyl ammonium chloride to satisfy the data requirement for Didecyl dimethyl ammonium carbonate and Didecyl dimethyl ammonium bicarbonate (Bardac 22C50). Bardac 22C50 (PC Code 69208) is very similar to DDAC. The only difference is the counterion to the quaternary ammonium cation: carbonate/bicarbonate is the counterion for the Bardac 22C50 whereas chloride is the counterion for DDAC. The submitted Aerobic Aquatic Metabolism study has undergone review by Srinivas Gowda of Antimicrobials Division's Risk Assessment and Science Support Branch.

BACKGROUND:

Didecyl dimethyl ammonium chloride (DDAC) is an active ingredient in various wood protection treatments. The study was conducted to determine the rate of degradation of DDAC under aerobic conditions in water plus sediment, identify the major metabolites, and establish the pattern of formation and decline of the metabolites.

The Aerobic Aquatic Metabolism Study entitled "Aerobic Aquatic Metabolism of ^{14}C-Didecyl dimethyl ammonium chloride (^{14}C-DDAC)" U.S. EPA-FIFRA, 40 CFR, Sec. 158.130 Guideline 162-4, by Walter Cranor, Manager, Environmental Fate, ABC Laboratories, Inc., 7200 East ABC Lane, P.O. Box 1097, Columbia, Missouri 65205, ABC Final Report #37008, dated August 6, 1991 (MRID Number 422538-03) has been submitted to the Agency to fulfil the Aerobic Aquatic Metabolism data requirements for the active ingredient, Didecyl dimethyl ammonium chloride.

METHODOLOGY:

The purpose of this study was to investigate the rate of decline of Didecyl dimethyl ammonium chloride under aerobic conditions in water plus sediment obtained from a saltwater harbor, identify the major metabolites, and establish the pattern of formation and decline of the metabolites according to EPA Pesticide Assessment Guidelines, Subdivision N, §162-4.

I. MATERIALS AND METHODS

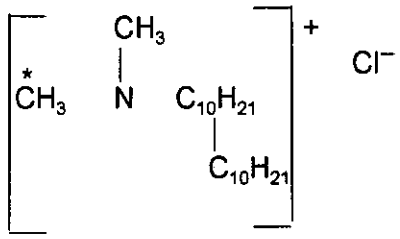
GUIDELINE FOLLOWED: The guidelines followed for this study were U.S. Pesticide Assessment Guidelines, Subdivision N, Environmental Fate: Chemistry Series 162-4.

COMPLIANCE: The study was conducted in compliance with the U.S. EPA GLP Standards. A signed GLP statement was provided which confirmed compliance stating that on exception existed. For the soil characterization data performed at A and L Midwest Laboratories, GLP compliance could not be assured, but that the data was available for review. A Quality Assurance Statement was also provided in the Study Report. No Data Confidentiality statement was provided.

A. **MATERIALS:**

1. **Test Material** ^{14}C-Didecyldimethylammoniumchloride

Chemical Structure:



(* denotes the labeled carbon)

Description: The test substance was a liquid.

Analytical purity: The analytical purity of the test substance was not provided.

Radiochemical purity: The purity stated on the label of the vials containing the test substance (Lot No. 7499-E) was >99%. A 100 mL stock solution was created using the contents of the vials and Millipore water. The radiochemical purity of the stock solution (990 µg/ml) was determined to be 98.0% by a triplicate TLC analysis. Prior to use as a dosing agent, the radiochemical purity of ^{14}C-DDAC was determined to be 98.58 ± 0.04% by TLC analysis.

Specific activity: The specific activity stated on the label of the vials containing the test substance was 9.01 mCi/mmole; whereas, the specific activity provided by the Sponsor in a letter dated September 30, 1987 was 9.14 mCi/mmole. In attempting to resolve the discrepancy, the Sponsor determined that the correct specific activity was actually 9.40 mCi/mmole, based on calculations from data recorded in the laboratory notebook associated with the preparation of the ^{14}C-DDAC. The value used in all calculations performed for this study was 9.01 mCi/mmole. The calculations were not recomputed using 9.40 mCi/mmole because the difference of values is small, the error affects both the dosed and recovered data equally, and the time it would take to correct the extensive amounts of data.

Locations of the radio label: The radio label was located on the methyl carbon.

Storage conditions of test chemicals: The test chemicals were stored in a refrigerator when not in use.

2. **Water collection, storage and properties** See Tables 1, 2, and 3.

Table 1. Description of the Collection and Storage of Water and Sediment Samples

Description	Details
Geographic location:	The water and sediment samples were collected from a pond located near Northwood, North Dakota.
Pesticide use history at the collection site:	Not Provided
Collection procedures:	The water and sediment were collected at the same time in a 5-gallon Nalgene carboy. The container was filled to capacity with water and approximately 6 inches of sediment.
Sampling depth:	Not Provided
Storage conditions:	Sediment The screened sediment was placed in a Rubbermaid dishpan and was covered with approximately 1 inch of pond water. The dishpan was covered loosely with aluminum foil and stored in an environmental chamber at 25°C under dark conditions until analysis. Water The decanted pond water was placed in a 5-gallon wide mouth glass jar and loosely covered with aluminum foil. The jar was stored in an environmental chamber at 25°C under dark conditions until analysis.
Storage length:	Not Provided
Preparation of water and sediment samples:	Sediment After the water was decanted, the sediment was wet-sieved through a 2 mm screen and collected in a Rubbermaid dishpan. Water The water was decanted into a 5-gallon wide mouth glass jar with care taken as not to disturb the sediment. Decantion was halted when the sediment began to mix with the clarified supernatant water layer.

Table 2. Properties of the Water

Property	Details	
Temperature (°C)	25°C (at time of addition to test tubes)	
pH	8.7 (at time of addition to test tubes)	
Redox potential (mv)	Initial	Final
	Not Provided	Not Provided
Oxygen concentration (mg/L)	Initial (at time of addition to test tubes)	Final
	7.8	Not Provided
Dissolved organic carbon (%)	Not Provided	
Hardness ($CaCO_3$)	9.68 gram/gallon	
Electrical conductivity	0.68 mmhos/cm	
Total Dissolved Solids	442 ppm	
Suspended Solids	21 ppm	
Sodium	97 ppm	
Calcium	30 ppm	
Magnesium	22 ppm	
Nitrate	<0.4 ppm	
Sulfate	21 ppm	
Iron	<0.05 ppm	
Manganese	<0.05 ppm	
Chloride	30 ppm	
Fluoride	0.3 ppm	
Biomass (mg microbial C/100 g or CFU or other)	Not Provided (only provided for study sediment)	

Table 3: Properties of the Sediment.

Property	Details
Textural classification	Sandy Loam
% sand	62
% silt	22
% clay	16
pH	8
Organic carbon (%)	1.6
CEC (meq/100 g)	16.3

Redox potential (mv)	Initial			Final	
	Not Provided			Not Provided	
Bulk density (g/cm^3)	1.29				
Moisture at 1/3 atm	19.92				
Biomass (colony forming units per gram (CFU))	Study Day	Bacterial populations in study sediment		Fungal populations in study sediment	
		Control System	Treatment System	Control System	Treatment System
	Initial	9.9×10^5	9.3×10^5	ND	ND
	Final	1.1×10^6	1.5×10^6	$<10^3$	$<10^3$

Notes:
ND = Not Determined

B. EXPERIMENTAL DESIGN:

1. Preliminary experiments:

Method Development:
The use of TLC was investigated during the preliminary study as the principal method for quantification and identification of ^{14}C-DDAC in the definitive study. The TLC methods originally available for analysis of ^{14}C-DDAC did not comply with the metabolism data requirements for pesticide registration. When sediment extracts from the preliminary investigations were analyzed with TLC, and to a lesser extent the ^{14}C-test material stock solution, the parent compound TLC zones tailed back to the origin. A suitable system was developed which employed the use of E.M. Merck, silica gel TLC plates, 60/Kieselguhr F-254, eluted in one dimension by ascending chromotography with a solvent system comprised of 55:20:20:3:2 (v:v:v:v:v) chloroform:isoprooopanol:methanol:water: formic acid. For removal of ^{14}C-residues of DDAC from the sediment, a solvent system of 80:20 N,N-dimethylformide:acetic acid (v:v) was adopted.

Effect of Didecyldimethylammoniumchloride on Sediment Microbial Populations:
A 14-day preliminary study was conducted to assess if the indigenous sediment microorganisms would be adversely affected by the test material. A 10 ml portion of pond water was added to each of 24 culture tubes containing 5.4 grams of sediment at 40.3% moisture. Six sets of four tubes were dosed at 0, 1, 3, 10, 30, and 100 ppm by the addition of 439 µl of aqueous non-radiolabeled DDAC solutions at 0.0 µg/ml, 11.4 µg/ml, 34.2 µg/ml, 114 µg/ml, 342 µg/ml and 1.14 mg/ml, respectively. The resulting dosed flooded sediment samples were incubated in the dark at 25 ± 1°C until used for microbial plate count analysis at 0, 1, 7, and 14 days after dosing.

Determination of Sediment Moisture Content and Preparation of Study Samples:
Three samples of wet study sediment, weighing approximately 8 grams each, were dried for 4 hours at approximately 100°C in a vacuum oven. The percent moisture in the samples (i.e., the weight loss observed upon drying) was determined to be 26.8%.

Preliminary Study:
A 14-day preliminary study was performed prior to the initiation of the current study to evaluate the proposed experimental design and to determine an approximate half-life of ^{14}C-DDAC. A 100 µL aliquot of ^{14}C-DDAC stock solution (990 µg/mL) was added to three of six test tubes to serve as treatment samples. Two of the dosed tubes and two of the control tubes were placed in a metabolism system in the environmental chamber under dark conditions at 25 ± 1°C. The remaining two tubes served as the day-0 analytical samples. Sediment, water, and carbon dioxide and volatile traps were sampled and analyzed at 0, 7, and 14 days after dosing. From the results, it was determined that there was no evidence to indicate that any degradation had taken place during the two-week preliminary metabolism study, indicating that the design of the definitive study was appropriate.

2. **Experimental conditions:** See Table 4.

Table 4. Study Design

Criteria		Details
Duration of the test		365 days
Water:		The test water, obtained from a pond located near Northwood, North Dakota, was not filtered prior to use.
Amount of sediment and water per treatment		Each culture tube contained 13.855 ± 1 gram (10 gram dry weight) of sediment and 20 ml of water.
Sediment/water ratio		Not Reported
Application rates		The sediment was treated with ^{14}C-DDAC to achieve a nominal concentration of 10 mg/L by adding a 137-µl aliquot of the purified ^{14}C-DDAC dosing solution at 732 µg/ml to the sample tubes.
Control conditions, if used (present differences from other treatments, i.e., sterile/non-sterile, experimental conditions)		Controls were exposed to the same conditions as treated samples except that they were not dosed with the test substance.
No. of replications	Control, if used:	34
	Treatments:	34
Test apparatus (Type/material/volume)		The incubation vessel used was a standard tall form 3000-ml resin-pot. All ground lass joints were treated with sealing wax to preserve the integrity of the closed system. The metabolism vessels were contained within an environmental control chamber maintained with dark conditions at a regulated temperature of 25°C.

Table 4. Study Design (continued)

Criteria		Details		
Details of traps for CO_2 and volatile organics, if any		A compressed air supply was used to flush ^{14}C-volatiles from the test system. The system operated under positive pressure with an air-flow rate of approximately 50 ml/minute. The effluent was passes through a 250-ml ethylene glycol trapping solution and then a 250 ml 1.0 N sulfuric acid trapping solution. Continuing on, the effluent passed through two 250 ml 1.0 N KOH trapping solutions. A secondary purpose for this procedure was to replenish the air in the system and thus maintain aerobic conditions.		
If no traps were used, is the system closed/open		N/A		
Identity and concentration of co-solvent		No co-solvent was identified in the Study Report..		
Test material application	Volume of the test solution used/treatment	A 137-µl aliquot of the purified ^{14}C-DDAC dosing solution at 732 µg/ml was added to each of the treated culture tubes.		
	Application method (eg: mixed/not mixed etc.)	Following dosing, all samples were vortexed.		
Any indication of the test material adsorbing to the walls of the test apparatus		This issue was not addressed in the Study.		
Microbial biomass/microbial population in the sediment of the control		Study Day	Bacterial Populations in Sediment (CFU)	Fungal Populations in Sediment (CFU)
		Initial (Day-0)	9.9×10^5	Not Determined
		Final (12-Month)	1.1×10^6	$<10^3$
Microbial biomass/microbial population in the sediment of the treated		Study Day	Bacterial Populations in Sediment (CFU)	Fungal Populations in Sediment (CFU)
		Initial (Day-0)	9.3×10^5	Not Determined
		Final (12-Month)	1.5×10^6	$<10^3$
Microbial biomass/microbial population in the water of the control and treated		Not Provided		
Experimental conditions:	Temperature	25°C		
	Continuous darkness (Yes/No)	Yes		
Other details, if any		---		

3. **Aerobic conditions:**

Metabolism vessels were connected to a compressed air supply to obtain aerobic conditions. The system operated under positive pressure with an air-flow rate of approximately 50 ml/minute. Redox potentials or other evidence of aerobic conditions were not provided.

4. **Sampling:** See Table 5.

Table 5. Sampling Details

Criteria	Details
Sampling intervals	Sampling of the sediment, water, and volatiles was conducted on days 0,1, 3, 7, 14, 31, 61, 92, 123, 182, 273, and 365 after dosing. Sampling of volatiles was also conducted on days 151, 212, 243, 304, and 335 after dosing.
Sampling method	Access into the vessel was achieved through the use of a rubber expansion plug fitted into the large center hole of the resin-pot lid. Samples were obtained from individual pre-weighed 25-mm by 150-mm culture tubes containing 10.000 ± 0.001 g dry basis of flooded sediment withdrawn from the vessel through the large center hole of the resin-pot lid.
Method of collection of CO_2 and volatile organic compounds	CO_2 and volatile organic compounds were collected through the sampling of the ethylene glycol, sulfuric acid, and KOH trapping solutions.
Sampling intervals/times for: 1) sterility check, if sterile controls are used: 2) aerobicity:	 Not Provided Not Provided
Sample storage before analysis	Not Provided
Other observations, if any	N/A

C. ANALYTICAL METHODS:

1. Separation of the sediment and water:

The flooded sediment samples were removed from the metabolism vessel and centrifuged for approximately ten minutes. Following centrifugation, the water was decanted.

2. Extraction/clean up/concentration methods:

Extractable residues from sediment samples
Sample extraction was performed by adding 30 mL of 80:20 (v:v) dimethylformamide:acetic acid to the sediment sample and shaking for 1 hour. The solution was then centrifuged for 10 minutes. The extract was decanted into a 100 mL graduated mixing cylinder and the procedure was repeated twice. The subsequent extracts were combined to give a composite extract, which was adjusted with extraction solvent to a final volume of 100 mL.

Three 1.00-ml portions of the composite extracts were taken for LSC analysis. The composite extract was then transferred into a 4-ounce amber bottle and frozen until analysis of ^{14}C-DDAC by TLC or HPLC.

Non-extractable residues from sediment samples
A soil sample from the 12-month sample point was used to analyze non-extractable residue. This sample (9.850 g) was previously analyzed to contain 0.989 ppm of bound ^{14}C-DDAC equivalents (10.61% of dose). The sample was transferred into a 250-mL flask along with 50 mL of 80:20 (v:v) DMF:acetic acid and the mixture was vigorously boiled under reflux for three hours. A 10 gram control sample was spiked with ^{14}C-DDAC and processed concurrently to assess any degradation attributed to the process. After the extraction mixtures cooled to room temperature, the samples were transferred to culture tubes and centrifuged and the extracts were decanted. Then, the sediments were rinsed with two 20 mL aliquots of extraction solvent. The combined extract and solvent rinses were adjusted to a final volume of 100 mL and analyzed by LSC then HPLC. The sediment samples were allowed to air dry and were analyzed by combustion analysis to determine the levels of ^{14}C-activity remaining on the sediment after extraction under harsh conditions. Samples were combusted in triplicate and were performed on a Packard 306 Tricarb oxidizer. Following combustion, ^{14}C-activity was measured by LSC analysis.

Following storage for several days, the extract was noted to contain suspended solids. A 10-ml aliquot of the extract was filtered through a 0.45 µ filter to remove these materials. The filtered materials were then analyzed by HPLC.

Residues from water samples
The water samples were adjusted to volume and triplicate 1.0-ml aliquots were taken for LSC analysis. Prior to analysis of the 6, 9, and 12 month samples for ^{14}C-DDAC, the aliquots were evaporated to dryness and redissolved in methanol. This concentration step was conducted to increase the level of 14C-radioactivity available for TLC analysis. All other water samples were analyzed for ^{14}C-DDAC by co-chromatography TLC without additional pre-treatment.

Residues from volatile samples
For the analysis of volatiles, 1 mL aliquots of the trapping solutions were analyzed by duplicate LSC to determine the amount of ^{14}C-radioactivity. The presence of $^{14}CO_2$ was confirmed in composite mixtures of the first KOH trapping solutions using barium chloride which produces the insoluble precipitant barium carbonate. The composites were analyzed in triplicate 1.0-mL aliquots by LSC to determine the amount of ^{14}C-radioactivity. Then, a portion of barium chloride was added to a 10 mL aliquot of each composite. After the composites underwent vortex mixing and centrifuging, triplicate 1.0 mL aliquots were taken of each supernatant for LSC analysis and the reductions of soluble ^{14}C-activity provided measures of $^{14}CO_2$ present.

3. Total ^{14}C measurement:

Total ^{14}C is taken to be the summation of total volatile ^{14}C-activity, total extractable ^{14}C-activity, total aqueous ^{14}C-activity, and total ^{14}C-non-extractable residues.

Measurements of radioactivity were made by LSC analysis using a benchtop microprocessor-controlled spectrometer (Beckman Model 3801 Liquid Scintillation Counting System). Liquid samples were pipetted into scintillation vials where they received aliquots of scintillation fluid (Beckman Ready Gel MP®). All samples were counted for 5 minutes or to a 2 sigma (95%) confidence level using a single label dpm data calculation program.

4. Derivatization Method:

Derivatization methods were not used.

5. Identification and quantification of parent compound:

The water samples and the extractable residues from the sediment samples were analyzed using TLC for the quantification and identification of ^{14}C-DDAC. Due to poor ^{14}C-recovery, the 6-, 9-, and 12-month extractable residues were re-analyzed using HPLC. HPLC was also used to analyze the non-extractable residue extracts from the 12-month sediment sample.

Thin Layer Chromatography (TLC)
The TLC system was developed at ABC Laboratories. Merck, silica gel TLC plates, 60/Kieselguhr F-254, were employed for all TLC work. All TLC analyses were accomplished with overspotting of ^{14}C-DDAC nonradiolabeled analytical standard. All plates were developed by ascending chromatography to a distance of 15 cm using a solvent system composed of 55:20:20:3:2 (v:v:v:v:v) chloroform:isopropanol:methanol:water:formic acid. TLC zones corresponding to ^{14}C-DDAC were located as a dark spot by irradiation with longwave ultraviolet light by scanning for ^{14}C-radioactivity using a Radiomatic® RTLC Multi-Scanner and by formation of autoradiograms.

High Performance Liquid Chromatography (HPLC)
The HPLC method was developed at ABC Laboratories. A Shimadzu LC-6A HPLC pump fitted with a Rheodyne 7125 injector and an Alltech RP18/cation mixed mode 100Å, 7-μ HPLC column was used for the analysis of sediment extract samples taken from the 6-month, 9-month and 12-month samples points. A flow rate of 1.5 mL/minute was used with a gradient system using methanol and 0.025 M KH_2PO_4 buffered at pH 4 with 0.005 M tetrabutylammonium dihydrogenphosphate (TBAP). The gradient system used is as follows:

Time in Minutes	Percent Methanol	Percent 0.025 M KH_2PO_4 Buffered at pH 4 with 0.005 M TBAP
0	0	100
20	100	0
25	0	100
30	0	100

6. Identification and quantification of transformation products:

According to the Study Report, the parent compound, ^{14}C-DDAC, was the only distinct TLC zone observed having ^{14}C activity. Additionally, fractions other than ^{14}C-DDAC observed from HPLC analyses could not be attributed to the presence of any unique degradation product. As such, transformation products were not identified or quantified.

7. Detection limits (LOD, LOQ) for the parent compound:

The limits of detection are directly related to the sensitivity of the counting. Twice the background is used as the limit of detection. Table 6 presents the detection limits and analytical sensitivities obtained.

Table 6. Detection Limits for the Parent Compound

Type of sample	Typical background (dpm)	Limit of detection (µg)	Equivalent residue level (ppm)
Bound residues	50	≤0.001	0.005
Aqueous and Extractable residues	30	≤0.001	0.005
Volatile residues and $^{14}CO_2$	75	≤0.001	0.004 to 0.002
HPLC analysis	30	≤0.001	0.005

8. Detection limits (LOD, LOQ) for the transformation products (indicate the criteria/reference, if provided):

Transformation products were not identified and quantified.

II. RESULTS AND DISCUSSION:

A. TEST CONDITIONS:

Records of the test conditions maintained throughout the study, such as aerobicity and temperature, were not provided in the Study Report. However, the Study Report states that during the 365 days of incubation, the 24-hour average temperature was between 24 and 26°C. The Study Report provided the microbial activity of bacterial populations in the sediment for the initial day (day 0), day 31 (1-month), day 92 (3-month), and the final day (day 365). Additionally, final microbial activity was provided for fungal populations in the sediment. These results are provided in Table 7. From the day 0 to day 365, the bacteria population increased from 9.9 x 10^5 to1.1 x 10^6 colony forming units (CFU) per gram in the control system and from

9.3×10^5 to 1.5×10^6 CFU per gram in the treatment system. The final fungal population was $<10^3$ in both systems. Based on these results, it appears that the microbial populations were biologically active throughout the study.

Table 7. Microbial Activity in the Control and Treatment Systems

Study Day	Bacterial populations in study sediment (CFU per gram)		Fungal populations in study sediment (CFU per gram)	
	Control System	Treatment System	Control System	Treatment System
Day 0 (Initial)	9.9×10^5	9.3×10^5	ND	ND
1-Month	5.8×10^4	5.2×10^4	ND	ND
3-Months	1.1×10^6	8.9×10^5	ND	ND
12-Months (Final)	1.1×10^6	1.5×10^6	$<10^3$	$<10^3$

B. MATERIAL BALANCE:

Table 8 reports biotransformation as a percentage of applied radioactivity for extractable residues, non-extractable residues, volatile residues, aqueous residues, and total ^{14}C recovery. Total recovery of radiolabeled material ranged from 97.5 to 107.3 % of the applied amount. Mean total recovery was 105.0 ± 2.8 % of the applied amount. For sediment, water, and air, overall mean recovery was 101.9 (93.0 extractable and 8.9 non-extractable), 1.5, and 1.5%, respectively.

Table 8: Biotransformation of ^{14}C-DDAC, Expressed as Percentage of Applied Radioactivity (9.326 µg ^{14}C-DDAC Equivalents/g of Soil), in Sandy Loam Soil Under Aerobic Conditions

Compound	Sampling times (days)																			
	0	0 (Mean)	1	3	7	14	31	31 (Mean)	61	92	92 (Mean)	123	182	273	365	365 (Mean)	Overall Mean
	Percent of Applied Radioactivity [a]																
Total Extractable Residues	87.6	92.4	90.0	94.2	96.1	87.3	95.4	96.7	95.1	95.9	95.2	96.7	97.2	96.9	95.8	96.8	82.2	90.2	89.6	89.9	93.0 ± 4.4
Non-Extractable Residues	7.7	7.9	7.8	10.4	6.3	18.1	8.4	6.9	7.5	7.2	9.2	6.4	6.4	6.4	5.6	6.2	13.0	10.6	12.0	11.3	8.9 ± 3.3
Volatiles [b,c,d]																					
Et	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
H	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
K_1	0.00	0.00	0.00	0.00	0.00	0.01	0.09	0.62	0.62	0.62	1.39	1.90	1.90	1.90	2.33	2.92	3.59	4.51	4.51	4.51	
K_2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
Total	0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.62	0.62	0.62	1.39	1.90	1.90	1.90	2.33	2.92	3.59	4.51	4.51	4.51	1.5 ± 1.6
Total Aqueous Residues	2.3	2.2	2.2	1.1	3.8	2.0	2.0	2.1	2.7	2.4	0.7	0.9	0.9	0.9	0.7	0.7	0.7	0.8	1.1	1.0	1.5 ± 0.9
Total % Recovery	97.5	102.5	100.0	105.7	106.2	107.3	106.0	106.2	106.0	106.1	106.5	105.9	106.4	106.2	104.4	106.7	99.5	106.1	107.3	106.7	105.0 ±

a Initial Measured Dose = 9.326 µg ^{14}C-DDAC equivalents/g of sediment

b Individual values for volatiles may be less than minimum quantifiable limit, whereas, summation of all observed values are reported.

c Volatiles collected at days between sample points are included with the following analysis days.

d Et = Ethylene Glycol Trapping Solution
H = Sulfuric Acid Trapping Solution
K1 = First KOH Trapping Solution
K2 = Second KOH Trapping Solution

C. TRANSFORMATION OF PARENT COMPOUND:

Through TLC and HPLC analyses, the quantity of the parent compound (^{14}C-DDAC) in the sediment extracts and water was determined. These results, reported as a percent of radioactive dose applied, are shown in Table 9. The concentration of ^{14}C-DDAC in water decreased from a mean of 2.19% of the applied amount at day 0 to a mean of 0.74% of the applied amount at study termination. For sediment, the mean concentration of ^{14}C-DDAC at day 0 was 86.0% of the applied amount and at study termination was 87.8% of the applied amount. No biotransformation of the parent compound was reported.

Table 9. Concentration of ^{14}C-DDAC in Water and Sediment

Days After Application	Concentration of ^{14}C-DDAC (% of applied dose)		
	Sediment	Water	Total
0	82.9	2.2	85.1
0	89.1	2.18	91.3
0 (Mean)	86	2.19	88.2
1	88.5	1.05	89.6
3	92	3.56	95.6
7	79.5	1.86	81.4
14	91.3	1.88	93.2
31	88.2	1.89	90.1
31	90.3	2.45	92.8
31 (Mean)	89.3	2.17	91.4
61	95.2	0.57	95.8
92	92.6	0.91	93.5
92	93.6	0.78	94.4
92 (Mean)	93.1	0.85	93.9
123	86.7	0.69	87.4
182	89.9	0.37	90.3
182	95.9	0.37	96.3
182 (Mean)	92.9	0.37	93.3
273	81.2	0.44	81.6
365	88.5	0.65	89.2
365	87.1	0.83	87.9
365 (Mean)	87.8	0.74	88.5
Overall Mean	88.62	1.36	89.98

1. Initial Dose = 9.326 µg ^{14}C-DDAC equivalents/g of sediment.
2. All samples were analyzed by TLC except for the 182-, 273-, and 365-day samples which were analyzed by HPLC.

1. **Half-life:**

Based on first-order degradation, the Registrant calculated a half-life of 8,365 days for ^{14}C-DDAC in the entire test system (i.e. extractable sediment residues and water residues). Using first-order degradation, the Agency also calculated the half-life of ^{14}C-DDAC in the entire test system and also in just the water test system and the sediment test system. As shown in Table 10, Agency calculated a half-life of 8,366 days in the entire test system, 180 days in the water test system, and 22,706 days on the sediment test system.

Table 10. Half-lives of ^{14}C-DDAC

Test System	First-order half-life		
	Half-life (days)	Regression equation	R^2
water	180	$y = ^{-} 0.00385x + 0.47$	0.0406
sediment	22706	$y = ^{-} 0.000031x + 4.49$	0.0053
entire system	8366	$y = ^{-} 0.000083x + 4.511$	0.4529

2. **Transformation Products:**

Transformation products were not reported in the Study Report.

3. **Extractable and Non-Extractable Residues:**

Extractable [^{14}C] residues in sediment decreased from 90.0% at day 0 to 89.9% of the applied amount at the end of incubation period. Non-extractable [^{14}C] residues in sediment increased from 7.8 % at day 0 to 11.3 % of the applied amount at the end of incubation period.

4. **Volatilization:**

[^{14}C] volatiles rose from 0.0% of the applied radioactivity at day 0 to 4.51% of the applied radioactivity at the end of the incubation period. All of the [^{14}C] activity observed was determined to be CO_2, as the [^{14}C] activity collected was precipitated as barium carbonate by the addition of barium chloride to composites of the trapping solutions collected during the study.

5. **Transformation Pathway:**

The biotransformation pathway was not provided in the Study Report. The Registrant reported that transformation products were not present in any significant amounts.

III. SUMMARY OF DATA:

The aerobic biotransformation of didecyldimethylammoniumchloride (DDAC) was studied in a pond water/sediment system (water - pH of 8.7; sediment - pH of 8, sandy loam texture, organic carbon content of 1.6%) from Northwood, North Dakota for 365 days in dark at 25°C. The sediment was treated with a sufficient amount of ^{14}C-DDAC to achieve a nominal concentration of 10 parts per million (ppm). Each culture tube contained approximately 10 gram dry weight of sediment and 20 ml of water. The experiment was conducted in accordance with the Environmental Protection Agency, Pesticide Assessment Guidelines, Subdivision N, Section 162-4. The test system consisted of a standard tall form 3000-mL resin-pot as the metabolism vessel. Traps were attached for the collection of carbon dioxide (CO_2) and volatile organic compounds. Samples were analyzed at 0, 1, 3, 7, 14, 31, 61, 92, 123, 182, 273, and 365 days of incubation. Before analysis by thin layer chromatography (TLC), the 6-, 9-, and 12-month water samples were concentrated by evaporating aliquots to dryness and redissolving in methanol. The other water samples were not concentrated prior to analysis. The sediment samples were extracted with 30 ml of 80:20 (v:v) dimethylfomamide (DMF):acetic acid and then shaken for 1 hour, followed by centrifugation for 10 minutes. Quantification and identification of the ^{14}C-DDAC residues was performed using TLC and/or high performance liquid chromatography (HLPC).

The material balance averaged 105 ± 2.8 % of the applied amount for the yearlong study. The mean total recovery of the radiolabelled material applied was 1.5 ± 0.9% for water and 101.9 % for sediment (93.0 ± 4.4% for extractable residues and 8.9 ± 3.3% non-extractable residues). The concentration of ^{14}C-DDAC in water decreased from 2.20 % at day 0, to 0.83% of the applied at study termination. The concentration of ^{14}C-DDAC in the sediment increased from 86.0% at day 0 to 87.8% of the applied, at the end of the study period. At test termination, 101% of the applied radioactivity was partitioned from water to sediment. No transformation products were detected in the water or sediment.

Extractable [^{14}C]residues in sediment decreased from an average of 90.0 % at day 0, to 89.9 % of the applied amount at the end of incubation period. Non-extractable [^{14}C]residues in sediment increased from 7.8% at day 0, to 11.3% of the applied amount, at study termination. At the end of the study, 4.5 % of the recovered radioactivity was present as volatiles. The volatiles were determined to be ^{14}C-carbon dioxide via precipitation with barium chloride.

As determined by the Agency, the half-lives of ^{14}C-DDAC in water, sediment, and in the entire system were 180 days, 22,706 days (60.5 years), and 8,366 days (22.9 years), respectively. The Registrant calculated a half-life of 8,365 days for ^{14}C-DDAC in the entire system. The Registrant did not calculate separate half-lives for ^{14}C-DDAC in the water and sediment systems.

Results Synopsis:

Test system used:	The water and sediment used was collected from a pond in Northwood, North Dakota.
Half-life in water:	180 days
Half-life in sediment:	22,706 days
Half-life in the entire system:	8,366 days
Major transformation products:	None
Minor transformation products:	None

Study Acceptability:

This study is classified acceptable and satisfies the guideline requirements for an aerobic biotransformation study in a water/sediment system.

RASSB's CONCLUSIONS AND RECOMMENDATIONS:

Risk Assessment and Science Support Branch (RASSB) concludes that the submitted Aerobic Aquatic Metabolism study on Didecyl dimethyl ammonium chloride (DDAC) reflects the Guidelines specified by the U.S. Environmental Protection Agency's Environmental Fate Data Requirements published in Pesticide Assessment Guidelines, Subdivision N, § 162-4 and the findings/conclusions are scientifically sound.

The material balance averaged 105 ± 2.8% of the applied amount for the year-long study. The mean total recovery of the radiolabelled material applied was 1.5 ± 0.9% for water and 101.9 % for sediment (93.0 ± 4.4% for extractable residues and 8.9 ± 3.3% non-extractable residues). The concentration of ^{14}C-DDAC in water decreased from 2.20% at day 0, to 0.83% of the applied at study termination. The concentration of ^{14}C-DDAC in the sediment increased from 86.0% at day 0 to 87.8% of the applied, at the end of the study period. At test termination, 101% of the applied radioactivity was partitioned from water to sediment. No transformation products were detected in the water or sediment.

Extractable [^{14}C]residues in sediment decreased from an average of 90.0% at day 0, to 89.9% of the applied amount at the end of incubation period. Non-extractable [^{14}C]residues in sediment increased from 7.8% at day 0, to 11.3% of the applied amount, at study termination. At the end of the study, 4.5% of the recovered radioactivity was present as volatiles. The volatiles were determined to be ^{14}C-carbon dioxide via precipitation with barium chloride.

The half-lives based on first-order degradations of ^{14}C-DDAC in water, sediment, and in the entire system were 180 days, 22,706 days (60.5 years), and 8,366 days (22.9 years), respectively. No biotransformation of the parent compound was reported. The study demonstrates that ^{14}C-DDAC is stable to microbial degradation and this conclusion is supported by the absence of significant $^{14}CO_2$ or isolated degradation products after 12 months.

RASSB recommends that the submitted Aerobic Aquatic Metabolism study under the MRID number 422538-03 be accepted to satisfy the Aerobic Aquatic Metabolism data requirements for the active ingredient, Didecyl dimethyl ammonium chloride, provided that the registrant submits the following additional data/information to complete the study report:

1. How the aerobic conditions were assured and maintained.

cc: Srinivas Gowda/RASSB/AD

Chemical File(069149)/AD