Reviewed by: Srinivas Gowda, Microbiologist/Chemist, Team 1 S. Gowda Date 3/13/03

DATA EVALUATION RECORD

STUDY TYPE:	Aerobic Soil Metabolism of Didecyl dimethyl ammonium chloride
DP BARCODE:	D288350 & D287563
PC CODE:	069149
SUBMISSION CODE:	S627084
CASE TYPE:	Resubmission
TEST MATERIAL:	Didecyl dimethyl ammonium chloride
SYNONYMS:	DDAC, Bardac 22 (2250, 2280)
CITATION:	"Aerobic Soil Metabolism of ^{14}C-Didecyl dimethyl ammonium chloride (^{14}C-DDAC)" U.S. EPA-FIFRA, 40 CFR, Sec. 158.130 Guideline 162-1, by Walter Cranor, Manager, Environmental Fate, ABC Laboratories, Inc., 7200 East ABC Lane, P.O. Box 1097, Columbia, Missouri 65205, ABC Final Report #37006, dated August 6, 1991 (MRID Number 422538-01).
SPONSOR:	Lonza Inc.

Data Evaluation Report on the aerobic biotransformation of ^{14}C-Didecyldimethyl-ammoniumchloride (^{14}C-DDAC) in soil

Prepared for:
Office of Pesticide Programs
Antimicrobials Division
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EPA Contract Number: 68-W-01-036
Work Assignment Number: 2-01
Action Number: KSW-03
TAF: 1-4-10
Date: January 23, 2003

Data Requirement:	PMRA DATA CODE:	
	EPA DP Barcode:	D287563
	OECD Data Point:	
	EPA Guideline:	162-1/835.4100

Test material:

Common name:	^{14}C-DDAC
Chemical name:	^{14}C-Didecyldimethylammoniumchloride
IUPAC:	
CAS name:	
CAS No:	7173-51-5
synonyms:	

Primary Reviewer: **Signature:**
{EPA/OECD/PMRA} **Date:**

Secondary Reviewer(s): **Date:**
{EPA/OECD/PMRA}

Company Code:	[for PMRA]
Active Code:	[for PMRA]
Use Site Category:	[for PMRA]
EPA PC Code:	

CITATION:

Study Title:	"Aerobic Soil Metabolism of ^{14}C-Didecyldimethylammoniumchloride (^{14}C-DDAC)"
Year:	August 6, 1991
Author:	Walter Cranor Manager, Environmental Fate
Laboratory Name:	ABC Laboratories, Inc. 7200 East ABC Lane P.O. Box 1097 Columbia, MD 65205
Laboratory Report No.:	37006
Sponsor:	Lonza, Inc. 17-17 Route 208 Fair Lawn, New Jersey 07410

EXECUTIVE SUMMARY:

The biotransformation of radiolabelled didecyldimethylammoniumchloride (DDAC) was studied in a sandy loam soil (pH 6.3, organic carbon 1.8%) collected from Northwood, North Dakota, for 365 days under aerobic conditions in the dark at 25^{0}C and 14.41% moisture (at 1/3 bar atmosphere). The study soil was treated with a sufficient amount of ^{14}C-DDAC to achieve a concentration of 10 ppm. The experiment was conducted in accordance with the Pesticide Assessment Guidelines, Subdivision N, Section 162-1, and in compliance with the U.S. EPA GLP standards. The test system consisted of a standard tall form 3,000 ml resin-pot as the incubation vessel and included vessels for trapping CO_2 and volatile organics. Samples were analysed at 0, 1, 3, 7, 14, 31, 61, 92, 123, 182, and 365 days of incubation. The soil samples were extracted with 30 ml of 80:20 (v:v) dimethylformamide: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 HPLC.

Material balance averaged 100.5 ± 4.1% of the applied amount. The concentration of the parent compound decreased from a mean of 93.7% of the applied amount at day 0, to a mean of 72.85% of the applied amount at the end of the study period. The half-life of ^{14}C-DDAC in aerobic soil was calculated by the Registrant to be 1,048 days. No biotransformation of the parent compound was reported.

Extractable ^{14}C-residues decreased from a mean of 98.55% of the applied amount at day 0 to a mean of 74.87% of the applied amount at the end of study period. Non-extractable ^{14}C-residues increased from a mean of 1.45% of the applied amount at day 0 to a mean of 17.74% of the applied at the end of the incubation period. At study termination, an average of 1.95% of the applied radioactivity was present as volatile organics. CO_2 was not present in any significant amount.

Results Synopsis:

Soil type:	Sandy loam
Half-life:	1,048 days
Major transformation products:	None reported
Minor transformation products:	None reported

Study Acceptability: This study is classified acceptable and mostly satisfies the guideline requirements for an aerobic biotransformation study in soil. The deficiencies and points of concern are noted in this study review.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The guidelines followed for this study were U.S. Pesticide Assessment Guidelines, Subdivision N, Environmental Fate: Chemistry Series 162-1.

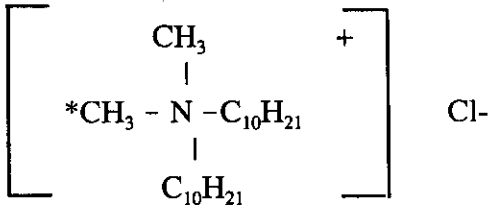
COMPLIANCE: The study was conducted in compliance with the U.S. EPA GLP Standards. A signed GLP statement was provided which confirmed compliance stating that one exception existed. For the soil characterization data performed at A and L Midwest Laboratories, GLP compliance could not be assured, but the data was available for review. A Quality Assurance Statement and a Data Confidentiality Statement were also provided in the Study Report.

A. MATERIALS

1. Test Material:

Chemical: ^{14}C-Didecyldimethylammoniumchloride

Chemical Structure:



(* denotes the labeled carbon)

Description: The test substance was described as a liquid.

Radiochemical purity: The radiochemical purity stated on the vials containing the test substance was >99%. A 100 ml stock solution was created using the contents of the vials and Millipure water. The radiochemical purity of the stock solution (990 µg/ml) was determined to be 90.0% by a triplicate TLC analysis.

Lot/Batch No.: 7499-E

Specific activity: The specific activity stated on the vial label was 9.01 mCi/mmol.

Locations of the radio label:	The radio label was located on the methyl carbon.
Storage conditions of test chemicals:	The two vials and the primary stock solution was stored in the refrigerator.
Physico-chemical properties of ^{14}C-DDAC:	Water solubility, vapor pressure/volatility, UV absorption, pK_a, K_{ow}/log K_{ow}, and stability of the compound at room temperature were not provided in the Study Report.

2. Soil Characteristics:

Soil collection and storage:	The soil used in this study was collected from an agricultural field in Norwood, North Dakota, and supplied by an independent contractor. The soil was air-dried and then sieved through a 2 mm mesh screen upon receipt by ABC Laboratories, Inc. A representative subsample of approximately 100 g was collected and shipped to A and L Midwest Agricultural Laboratories, Inc. for soil characterization.
Soil Properties:	See Table 1.

Table 1: Properties of the soils

Property	Soil
Soil texture	sandy loam
% sand	78
% silt	10
% clay	12
pH	6.3
Organic carbon (%)	1.8
CEC (meq/100 g)	0.7
Moisture at 1/3 atm (%)	14.41
Bulk density (g/cm^3)	1.23

B. EXPERIMENTAL CONDITIONS

1. Preliminary experiments:

Methods Development:
During a preliminary study, the use of TLC was investigated as the principal method for quantification and identification of ^{14}C-DDAC. A suitable system was developed using both silica gel and reverse phase systems. The method employs the use of E.M. Merck, silica gel TLC plates, 60/Kieselguhr F-254, eluted in one dimension by ascending chromatography with a solvent system comprised of 55:20:20:3:2 (v:v:v:v:v) chloroform:isopropanol:methanol:water: formic acid. For the extraction procedure, a solvent system of (80:20) N,N-dimethylformamide (DMF):acetic acid (v:v) was adopted for the removal of ^{14}C-residues from study soil.

Effects of DDAC on Soil Microbial Populations:
A 14-day preliminary experiment was conducted to determine if the indigenous soil microorganisms would be adversely affected by the test material. 5.1 g of study soil were added to 24 culture tubes and 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 samples were incubated in the dark at 25 ± 1°C until used for microbial plate count analysis, which was conducted at each dose level at 0, 1, 7, and 14 days after dosing.

Determination of Soil Moisture Content:
Three soil samples, each weighing approximately 5 grams, were dried for 4 hours at approximately 100°C in a vacuum oven. The weight loss due to drying was taken as the percent moisture in the sample. The samples were found to contain 6.63% moisture.

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 aerobic half-life of ^{14}C-DDAC. Six samples of soil were placed into culture tubes and dosed with a 100 µl aliquot of ^{14}C-DDAC stock solution (990 µg/ml). Soil, plus carbon dioxide and volatile traps, were sampling 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 2.

Table 2: Experimental design

Parameter		Soil
Duration of the test		365 days
Soil condition (Air dried/fresh)		air dried
Soil (g/replicate)		10.638 ± 0.001
Test concentrations (mg a.i./kg soil) and equivalent g a.i./ha		9.766 mg ^{14}C-DDAC/kg soil
Control conditions		Controls were exposed to the same conditions as treated samples except that they were not dosed with the test substance.
No. of Replication	Controls	34
	Treatments	34
Test apparatus (Type/material/volume)		The incubation vessel was a standard tall form 3,000 ml resin-pot. Soil samples were placed into 25 mm by 150 mm culture tubes.

8

Table 2: Experimental design

Parameter		Soil	
Details of traps for CO_2 and organic volatile, if any.		The effluent was passed though a 250 ml ethylene glycol trapping solution, then a 250 ml 1.0 N sulfuric acid trapping solution, and finally, two 250 ml 1.0 N KOH trapping solutions.	
If no traps were used, is the system closed/open?		N/A	
Identity and concentration of co-solvent		No co-solvent identified	
Test material application	volume of test solution used/treatment	137 µL	
	application method (eg: applied on surface, homogeneous mixing etc.)	Not specified	
	Is the co-solvent evaporated:	Not specified	
Microbial biomass/microbial population of control soil (colony forming units/g)		initial	final
		2.4×10^6	8.4×10^5
Microbial biomass/microbial population of treated soil, if provided (colony forming units/g)		initial	final
		2.3×10^6	1.3×10^6
Any indication of the test material adsorbing to the walls of the test apparatus		Not specified	
Experimental conditions	Temperature (°C)	24-26°C	
	Moisture content	Soil moisture levels were maintained between 70% and 75% of field moisture capacity.	
	Moisture maintenance method	Deionized water was added to tubes as needed to keep soil moisture levels within the range specified.	
	Continuous darkness (Yes/No):	Yes	
Other details, if any			

3. Aerobic conditions: To maintain aerobic conditions, a compressed air supply was used to flush ^{14}C-volatiles from the test system which was operated under positive pressure with an air flow rate of approximately 50 ml/minute. The Study Report did not provide any evidence that aerobic conditions were maintained throughout the experiment.

4. Supplementary experiments: No supplementary experiments were conducted.

5. Sampling: See Table 3.

Table 3. Sampling details

Parameters	Details
Sampling intervals	Samples were collected on day 0, 1, 3, 7, 14, 31, 61, 92, 123, 182, 273, and 365 days after dosing
Sampling method for soil samples	Samples were obtained from individual preweighed 25-mm by 150-mm culture tubes containing 10.000 ± 0.001 g dry basis of soil withdrawn from the vessel through a large center hole of the resin-pot lid
Method of collection of CO_2 and volatile organic compounds	CO_2 and volatile compounds were trapped using ethylene glycol solution, sulfuric acid solution, and KOH trapping solution
Sampling intervals/times for:	
sterility check:	Not mentioned
moisture content:	Checked at 14 days, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 months after dosing
redox potential/other:	Not mentioned
Sample storage before analysis	Not mentioned
Other observations, if any	

C. ANALYTICAL METHODS

1. Extraction/clean up/concentration methods:

Sample extraction was performed by adding 30 ml of 80:20 (v:v) dimethylformamide(DMF): acetic acid 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.

2. Non-extractable residue determination:

A soil sample from the 12-month sampling point was used to analyze non-extractable residue. This sample (9.922 g) was previously analyzed to contain 1.423 ppm ^{14}C-DDAC equivalents. 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 control samples was spiked with ^{14}C-DDAC and processed concurrently to ensure no loss due to the extraction process. After the extraction mixtures cooled to room temperature, the samples were transferred to culture tubes and centrifuged and the extracts were decanted. The soils were rinsed with two 20 ml aliquots of extraction solvent and the combined extract and solvent rinses were adjusted to a final volume of 100 ml and analyzed by LSC analysis. The sediment samples were allowed to air dry and were analyzed by combustion analysis to determine the levels of ^{14}C-activity remaining.

3. Total ^{14}C measurement:

Total ^{14}C was reported to be the summation of total volatile ^{14}C-activity, total extractable ^{14}C-activity, and total ^{14}C-non-extractable residues. The analysis methods for total extractable and total non-extractable residues was provided above. 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. 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.

Measurements of radioactivity were made 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.

3. Derivatization method, if used:

Not used.

4. Identification and quantification of parent compound:

TLC and HPLC were both used for the quantification and identification of ^{14}C-DDAC during the metabolism study and in addition, HPLC was used as a confirmational analytical method for levels of ^{14}C-DDAC quantified by 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 DDAC nonradiolabeled analytical standard. 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.

For HPLC, a Shimadzu LC-6A HPLC pump fitted with a Rheodyne 7-μ HPLC column was used for the analysis of soil 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 0.0125 M KH_2PO_4 buffered at pH 4 with 0.005 M tetrabutylammonium dihydrogenphosphate (TBAP).

5. Identification and quantification of transformation products:

Transformation products were not identified and quantified.

6. 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 taken as the limit of detection. The limit of detection reported in the Study Report for bound residues, extractable residues, volatile residues and CO_2, and HPLC analysis was $\leq$0.001 μg.

7. Detection limits (LOD, LOQ) for the transformation products:

Transformation products were not identified and quantified.

II. RESULTS AND DISCUSSION:

A. TEST CONDITIONS: Soil moisture levels were maintained between 70% and 75% of field moisture capacity for all dosed and control study soil samples. The temperature data showed that the 24-hour average temperature was considered suitable to maintain viable soil microorganisms. Aerobicity was not mentioned in the Study Report.

B. MATERIAL BALANCE: Total recovery of radiolabelled material ranged from 90.2 to 105.2% of the applied amount. Mean overall recovery was 100.5 ± 4.1% of the applied amount. Table 5 provides biotransformation as a percentage of applied radioactivity for extractable residues, non-extractable residues, organics, and total ^{14}C recovery.

Table 5: Biotransformation of ^{14}C-DDAC, expressed as percentage of applied radioactivity (9.766 μg ^{14}C-DDAC equivalents/g of soil), in sandy loam soil under aerobic conditions

Sampling times (days)	0	1	3	7	14	31	61	92	123	182	273	365
Compound	Percent of applied radioactivity											
Total extractable residues	97.76	99.34	92.16	93.33	90.03	87.49	83.99	86.28	83.57	78.98	79.69	77.59	78.8	69.42	76.03	73.71
Non-extractable residues	1.62	1.28	7.84	11.81	12.33	14.12	17.77	16.59	20.29	24.35	20.29	25.42	20.09	20.9	20.91	14.57
Volatiles																
Et[a]	0.00	0.00	0.01	0.01	0.01	0.01	0.02	0.02	0.05	0.09	0.09	0.14	0.27	0.48	0.68	0.68
H[b]	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
K1[c]	0.00	0.00	0.00	0.00	0.00	0.02	0.13	0.13	0.29	0.43	0.43	0.56	0.75	1.03	1.27	1.27
K2[d]	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	0.01	0.01	0.01	0.03	0.15	0.15	0.34	0.52	0.52	0.70	1.02	1.52	1.95	1.95
Total % recovery	99.38	100.6	100	105.2	102.4	101.7	101.9	103	104.2	103.9	100.5	103.7	99.91	91.84	98.89	90.23

a Ethylene Glycol Trapping Solution
b Sulfuric Acid Trapping Solution
c First KOH Trapping Solution
d Second KOH Trapping Solution

C. TRANSFORMATION OF PARENT COMPOUND:

The concentration of the parent compound decreased from a mean of 93.7% of the applied amount at day 0 to a mean of 72.85% of the applied amount at the end of the study period. However, no biotransformation of the parent compound was reported.

1. Half-life:

The Registrant's calculated half-life for ^{14}C-DDAC, based on a first-order degradation, was 1,048 days. Versar also performed calculations based on the information provided in the report. Versar calculated both the percent recovered as ^{14}C-DDAC and the percent of dose recovered using the *u*g/g values provided for extractable ^{14}C residues, ^{14}C-DDAC, and the applied dose. Using the percent recovered as ^{14}C-DDAC and the percent of dose recovered, Versar then calculated a half-life of 1,050 days.

2. Transformation products:

Transformation products were not reported in the Study Report.

3. Extractable and Non-Extractable Residues:

Extractable ^{14}C-residues decreased from a mean of 98.55% of the applied amount at day 0 to a mean of 74.87% of the applied amount at study termination. Non-extractable ^{14}C-residues increased from a mean of 1.45% of the applied amount at day 0 to a mean of 17.74% of the applied amount at the end of incubation period.

4. Volatilization:

At the end of the study, an average of 1.95% of the applied radioactivity was present as volatile organics. CO_2 was not present in any significant amounts.

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.

D. SUPPLEMENTARY EXPERIMENT-RESULTS:

No supplementary experiments were conducted.

III. STUDY DEFICIENCIES:

The following study deficiencies were noted:

- The pH of the test substance was not provided.
- A description of the field soil sampling collection and subsequent storage was not provided in the Study Report. No information on whether the soil had been previously treated with pesticides was given.
- The treatment rate was not provided. The guidelines state that the treatment rate should be at the highest proposed field use rate; however, the field use rate was not provided and could not be obtained by Versar. It is also unclear as to how the dosing solution was applied.
- The Study Report states that control samples were analyzed, however the results are not provided.
- The Study Report did not provide any data to show that aerobic conditions were maintained throughout the experiment (e.g., redox potential).

IV. REVIEWER'S COMMENTS:

The following points of concern were noted:

- On Table IV, the Registrant lists the "% Recovered" for Day 0-1 as 89.1%, however, when Versar performed the calculations, using the "dpm analyzed" (2759) and the "dpm Recovered" (2549) provided in the Study Report, the "% Recovered" was found to be 92.4%.
- For the quality control samples (Table V), it was unclear how the Registrant calculated the "% Recovered as ^{14}C-DDAC". Versar was unable to verify those values presented.
- The raw data were not included in the Study Report and therefore, the values presented could not be verified.

V. REFERENCES:

The study report provided the following reference on determining minimum quantifiable limit (MQL):

Currie, Lloyd A., "Limits of Qualitative Detection and Quantitative Determination", Analytical Chemistry, Vol. 40, No. 3, March 1968, pp. 586-593.