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EEE BRANCH REVIEW

DATE:	IN _____	OUT _____	IN _____	OUT _____	IN _____	OUT _____
	FISH & WILDLIFE		ENVIRONMENTAL CHEMISTRY		EFFICACY	
			3/26/76	5/10/76		

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PETITION OR EXP. PERMIT NO. \_\_\_\_\_

DATE DIV. RECEIVED 241-ELE \_\_\_\_\_

DATE OF SUBMISSION 6F1759 \_\_\_\_\_

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TYPE PRODUCT(S): I, D, H, F, N, R, S \_\_\_\_\_

PRODUCT MGR. NO. 3C1D - yes - 2B \_\_\_\_\_

PRODUCT NAME(S) ( ) Plant growth regulator \_\_\_\_\_

COMPANY NAME R. Taylor 25 \_\_\_\_\_

SUBMISSION PURPOSE Cycocel 4L Plant growth regulant \_\_\_\_\_

CHEMICAL & FORMULATION American Cyanamid Company \_\_\_\_\_

Registration for Use on sugarcane

(2-chloroethyl) trimethylammonium chloride

(Cycocel)

- 1.0 Introduction:
- 1.1 Other names: (2-chloroethyl) trimethylammonium chloride, chlormequat chloride, chlorocholine chloride, cycocel. 45.8% active.
- 1.2 Use involved: Plant growth regulator for use in sugarcane.
- 1.3 Other reviews: See reviews of 5/31/74.

2.0 Directions for Use:

- 2.1 To hasten ripening and increase sucrose in sugarcane. Apply Cycocel at 1 gallon in 7 to 10 gallons of water per acre (4.1 lbs a.i./acre) 4 to 10 weeks before harvest.

Apply to sugarcane grown in Hawaii only. Do not apply to crops other than sugarcane. Apply by aircraft. Do not apply to sugarcane to be harvested for seed purposes.

2.2 RINSE/DRAIN AND DISPOSAL PROCEDURE:

- (1) Drain container into spray tank (after normal emptying) in a vertical position for 30 seconds.
- (2) Rinse carefully 3 times with 1 gallon of water for each rinse for a 5 gallon container and drain into spray tank after each rinse.
- (3) Do not reuse container.

DISPOSAL OF PAILS: Preferred disposal - crush and recycle for scrap to a steel melting plant. If preferred disposal cannot be accomplished, container should be crushed and/or buried at an approved dump site according to local and state regulations.

3.0 Discussion of Data

- 3.1 Fate of carbon-14 chlorocholine chloride in soil. Section H, Tab 1.

- 3.1.1 Field Soil Study: (Princeton, N.J.). A sandy loam soil (68 % sand, 24% silt, 8% clay, PH 5.1, OM 3.6% 16.92% 1/3 Bar Water Retention) was treated at a rate of 3 lbs a.i./acre of 14-C chlorocholine chloride in stainless steel cylinders and set in a field. Samples were taken for analyses at periods up to 24 weeks. Soil samples were extracted with 0.3N calcium chloride solution. Extracts and combusted residue were radioassayed by LSC. In a separate experiment using the same soil, the rate of decomposition of parent compound to  $^{14}\text{CO}_2$  was determined in a closed system. The chromatographic behavior of the soil extracts was studied.

Attempts were made to extract the portion of radio-activity that remained after extraction of the soil with calcium chloride solution. Triton X-100 recovered 5% of this activity. Refluxing with 3N hydrochloric acid for 3 hours recovered about 50% of previously unextracted radioactivity along with high concentrations of other organic material. Some of the data are tabulated below:

Table II. Distribution of Radioactivity in Soil at Various Intervals After Treatment With <sup>14</sup>C-Chlorocholine Chloride % Applied Dose

Princeton Sandy Loam Soil												
Depth (in.)	Field Study									Greenhouse Study		
	3 Weeks			6 Weeks			24 Weeks			18 Weeks		
	Ext.	Res.	Total	Ext.	Res.	Total	Ext.	Res.	Total	Ext.	Res.	Total
0-2	73.1	9.1	82.2	6.0	14.0	20.0	0.6	8.0	8.6	1.3	8.2	9.5
2-4	4.0	0.6	4.6	0.8	1.0	1.8	0.2	1.6	1.8	0.5	2.2	2.7
4-6	1.4	0.3	1.7	0.4	0.3	0.7	0.1	1.0	1.1	0.3	1.1	1.4
TOTAL	78.5	10.0	88.5	7.2	15.3	22.5	0.9	10.6	11.5	2.1	11.5	13.6

The percentage recovery of <sup>14</sup>CO<sub>2</sub> from the Princeton soil was 48.68% at 47 days.

TLC analyses of soil extracts were complicated by residual calcium chloride. Analysis of a Princeton soil sample at 6 weeks showed only parent compound to be present. No analysis are given for 24 week samples.

Choline chloride (2-ethanol) trimethyl ammonium chloride and Betaine (2-acetic acid) trimethyl ammonium chloride were not detected in the TLC system used although it is not clear whether these compounds would survive the extraction, precipitation and digestion procedures described in the analytical method.

Conclusions:

- (1) Based on evolution of  $^{14}\text{CO}_2$ , half-life of the parent compound in the Princeton sandy loam is about 7 weeks or less. ←
- (2) Only parent compound has been identified by TLC. Material balance data are lacking. The residue remaining after evolution of  $^{14}\text{CO}_2$  has not been chemically identified.
- (3) TLC analyses are given for a 6 week sample. Analysis for longer times are needed.
- (4) Figure (6) is not a TLC autorodiogram as mentioned on Page 83.
- (5) Additional work is needed on identification of the soil metabolites. It should be demonstrated that choline chloride and related compounds would remain intact in the analytical methods used for chlorocholine chloride. It should also be noted that unlabeled degradation products would not be detected by autorodiograms.

3.1.2 Greenhouse soil study: Hawaii clay soil (18% sand, 32% silt, 50% clay, 3.1% O.M., PH 7.1, 1/3 Bar Water Retention 34.88%) was treated with labeled chlorocholine chloride equivalent to 2.42 pounds a.i./acre in test containers 5 inches in diameter and 4 inches deep. Samples were watered with the equipment of about 1 inch of rainfall per week. Some of the data are given below:

Hawaiian Clay Soil (per unit of applied activity)  
Greenhouse Study

Depth (in)	Ext.	3 Weeks		6 Weeks		
		Res.	Total	Ext.	Res.	Total
0-2	3.1	29.0	32.1	1.7	29.7	31.4
2-4	0.1	0.5	0.6	0.1	0.7	0.8
				Plate Beneath Pot		
4-6	0.0	-----	-----	0.0	-----	-----
TOTAL	3.2	29.5	32.7	1.8	30.4	32.2

No attempt was made to identify degradation products.

In a separate study on a Hawaiian soil treated with labeled chlorocholine chloride, 26.26% of applied activity was given off as  $^{14}\text{CO}_2$  in 34 days. No chemical analyses were made to identify other degradation products.

Conclusions:

- (1) Based on soil residues, half-life of the parent compound is less than 3 weeks.
- (2) Rapid binding to the clay soil appears to be taking place with 29.0 and 29.7% of applied activity unextractable at 6 weeks.
- (3) Soil degradation products are not identified and material balance data are lacking. A rough comparison between the separate  $^{14}\text{CO}_2$  studies and other soil metabolism studies indicates that considerable material may not be accounted for.

3.2 Cycocel-A study of its behavior in an Hydrolytic and in a Photolytic Environment: Section H, Tab 5.

3.2.1 Hydrolysis studies on  $^{14}\text{C}$ -Cycocel were carried out at 5 and 50 PPM in sterile water at three temperatures 25°C, 37°C and 45°C and at three PH values/3, 6 and 9) in the absence of light. Samples were taken for radioassay and TLC analysis for periods up to 4 weeks. Some of the data are tabulated below:

Percent on Thin Layer Chromatogram

Fragment	0.5 Weeks					
	25° pH 3		37° pH 6		45° pH 9	
	5 ppm	50 ppm	5 ppm	50 ppm	5 ppm	50 ppm
1. Unknown	2.3	3.7		1.2		
2. CYCOCEL	58.4	56.4	65.8	57.3	71.9	54.1
3. Choline Chloride	39.3	39.9	32.8	41.1	28.1	45.9
4. Origin			1.3	0.4		

	4 Weeks					
	25°		37°		45°	
	pH 3		pH 6		pH 9	
	5 ppm	50 ppm	5 ppm	50 ppm	5 ppm	50 ppm
1. Unknown		2.3		2.0		
2. CYCOCEL		62.2		54.1		51.8
3. Choline Chloride	24.5	35.5	18.3	43.2	21.8	48.2
4. Origin			1.4	0.7		

Conclusions:

(1) Cycocel hydrolyzes to form choline chloride as the only major product. Half-life of parent compound is greater than 4 weeks. Hydrolysis rate is somewhat greater at higher temperature and lower PH levels. Hydrolysis is very rapid initially followed by a much slower rate at 4 weeks.

3.2.2 Photolysis in distilled water and as Thin Film on Galss.

<sup>14</sup>C Cycocel at 5.1 PPM in distilled water was pipetted into Vycor flasks. Flasks were sealed with parafilm and placed in direct sunlight (Asheville N.C.). In a second test <sup>14</sup>C Cycocel was deposited as a thin film on the inside of Vycor test tubes and exposed to direct sunlight. Samples were taken for both tests up to 12 days, for radioassay and TLC analyses. Some of the data are tabulated below:

Distribution of <sup>14</sup>C-CYCOCEL Degradation Products After Exposure to Sunlight (% on Thin-Layer Chromatogram)

Thin-Film on Glass

	1 Day		12 Days	
	Sample	Blank	Sample	Blank
Unknown	2.1	2.1	14.6	1.3
CYCOCEL	24.6	26.3	14.3	19.6
Choline Chloride	72.6	71.0	67.9	77.3
Origin	0.7	0.6	1.4	1.7

In Distilled Water

	1 Day		12 Days	
	Sample	Blank	Sample	Blank
Unknown	3.0	4.3	1.8	1.5
CYCOCEL	29.6	23.6	4.8	30.4
Choline Chloride	66.8	71.3	92.9	67.6
Origin	0.6	0.8	0.6	0.5

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Conclusions:

(1) Cycocel converted rapidly to choline chloride in exposed as well as 'dark' control samples on glass surfaces and in aqueous solution.

(2) Photodegradation studies on soil are needed.

3.3 Leaching characteristics of 14C-Cycocel in various soil types under laboratory conditions. Section H, Tab 4.

Cycocel, randomly labeled in the 1 and 2 position of the ethyl group was applied at 3.0 lb a.i./A incorporated in an agricultural sand, a sandy loam, a clay loam and a silt loam. Twelve inch high columns were treated with 20 acre-inches of water at a rate not exceeding one acre-inch of water per hour to the prewetted column. Leachate and soil increments were radioassayed. No chemical analyses were performed. Test soil characteristics and some of the test data are given below:

	<u>Agricultural Sand</u>	<u>Sandy Loam</u>	<u>Silt Loam</u>	<u>Clay Loam</u>
Organic Matter %:	0.26	3.6	5.2	3.2
Sand Content, %:	96	68	24	22
Silt Content, %:	3	24	56	33
Clay Content, %:	1	8	20	45
Water Retention (%) at 1.3 Bar:	2.04	16.9	30.7	30.4
pH	4.9	5.1	6.7	6.6
Cation Exchange Capacity: mc/100 g	1.6	6.1	9.0	12.5
Nitrate:	-----	36.0	5.0	29.0

Distribution of Activity in soil + Leachate following application of 20 acre inches of water

	<u>Agricultural Sand</u>	<u>Sandy Loam</u>	<u>Silt Loam</u>	<u>Clay Loam</u>
0 - 3"	75	97	103	102
3 - 6"	14	11	1.6	2.7
6 - 9"	2.6	1.0	0.5	0.8
9 - 12"	0.4	1.0	<0.1	0.5
Total in Soil	<u>92</u>	<u>110</u>	<u>105</u>	<u>106</u>
Leachate	<u>&lt;0.1</u>	<u>&lt;0.1</u>	<u>&lt;0.1</u>	<u>&lt;0.1</u>
Total Recovery, %	92	110	105	106

Conclusions:

(1) Cycocel and/or radiolabeled degradation products leached moderately in the agricultural sand tested but did not leach significantly in sandy loam, silt loam or clay loam. 

3.4 Leaching characteristics of 14C cycocel and its degradation products following aging in clay loam soil under laboratory conditions. Section H, Tab 3.

Cycocel randomly labeled in the 1 and 2 positions was incorporated into clay loam soil and allowed to age for 30 days at which time 69% of the applied activity remained. The treated soil was overlaid on a packed column of clay loam 12 inches high and 3 inches indiameter. The prewetted column was treated with 0.5 acre-inches of water daily for 45 days. Leachate and three inch segments were assayed for 14-C activity. Original application rate was approximately 1.5 lb a.i./A. At 45 days, 79% of the applied activity was found in the top 3 inches of soil 2% in the 3-6" segment and 1% in the 6-9" inch segment. A total of 2.5% of applied activity was found in the leachate. Degradation products were not identified. About 15% of applied activity was unaccounted for at the end of the test.

Conclusions:

(1) Cycocel aged in clay loam did not leach strongly under conditions of the test.

(2) Leaching of degradation products was not determined since chemical analyses were not performed.

3.5 Runoff Characteristics of 14C-Cycocel applied to clay loam soil under greenhouse conditions. Section H, Tab 2.

Radiolabeled Cycocel was applied at a rate equivalent to 1.3 lb. a.i./A to a Hawaii clay loam soil packed into a laboratory apparatus to produce a bed 12 inches wide by 36 inches long by 3 inches deep inclined at a slope of 8°. The pesticide was applied to the upper one-third of the bed. The entire soil area was subjected to artificial rainfall at 1, 3 and 7 day intervals after treatment for a total of approximately 3 acre-inches of simulated rainfall. Soil, runoff water, and sediment were assayed radiochemically for <sup>14</sup>C activity. Following application of artificial rainfall, the treated soil retained 84.4% of the applied activity soil below the treated area 9.3% and runoff and leachate 9.7%. Recovery of applied activity was therefore 103.44%

Conclusions:

(1) Significant runoff of Cycocel (9.7% of applied occurred) under test conditions at approximately one-third recommended dosage.

3.6 Uptake of Radioactivity by Corn Plants Growing in Soil Treated with <sup>14</sup>C Chlorocholine Chloride. Section H, Page 73.

A Princeton soil (the same as used in the soil metabolism tests) was treated with Cycocel at 3 P.P.M. The test container was maintained in the greenhouse and watered with the equivalent of 2 inches of rain per week. After 14 weeks of aging, sweet corn seeds were planted. Plant samples were taken periodically by cutting the stem at soil level. Plant material was homogenized and extracted with methanol. Extract and combusted plant residue were radioassayed.

TLC analysis of methanol extract (4 week sample) gave only one spot coincident with parent compound. Some of the data we tabulated below:

Table V. Residual Radioactivity in Corn Plants Grown in Princeton Soil Treated 18-Weeks With <sup>14</sup>C-Chlorocholine Chloride (in PPM Equivalents of Chlorocholine Chloride)

	<i>Weeks after planting</i>			
	1	2	3	4
Methanol Extract	0.08	0.27	0.36	0.47
Marc	0.01	0.02	0.04	0.05
TOTAL	0.09	0.29	0.40	0.52

Conclusions:

- (1) Cycocel is taken up readily into corn plants with concentration increasing with time to 0.52 P.P.M. at 4 weeks after planting.
- (2) Parent compounds was the only material chemically identified from plant extract by TLC.

3.7 Fate of <sup>14</sup>C Chlorocholine Chloride in Sugarcane Grown in Hawaii. Section DIC, Tab I.

Radiolabeled chlorocholine chloride was applied to mature sugarcane plants in Hawaii. Samples were harvested up to 8 weeks after application. Adhering green leaves and tops and stalks (millable cane) were analyzed separately. Chemical and radioanalysis showed that essentially all of the radioactivity was due to parent compound at 8 weeks after treatment. Since this was a field experiment no attempt was made to maintain material balance.

Conclusions:

- (1) Radiolabeled cycocel did not significantly degrade to other radiolabeled material or sugar cane leaves and stalks.
- (2) Apparently no attempt was made to identify possible unlabeled degradation products.

3.8 A Study of (2-chloroethyl) trimethylammonium chloride in Rat. Section DIC, Tab 2.

Radiolabeled chlorocholine chloride when administered orally to a rat was 95.6% eliminated in the urine over a period of 46.5 hours. Respiration gases during the period accounted for 0.44% of the applied dosage. Feces tissue residues accounted for the remaining activity with total recovery of 98.71%. Parent chlorocholine chloride was the only radiolabeled material detected in the urine by chromatography.

Conclusions:

- (1) Chlorocholine chloride is essentially unmetabolized in rat and is rapidly eliminated intact in the urine.

4.0 Conclusions:

4.1 Cycocel hydrolyzes at a rapid initial rate to choline chloride and photodegrades to the same product in aqueous solution. It degrades fairly rapidly in soil/binds to clay soils. Soil degradation products are not identified. Cycocel leaches moderately in a sandy soil but not in soils with higher clay content. Intact Cycocel is taken up into corn plants from treated soil. It is essentially unmetabolized in rot and does not metabolize to other radiolabeled products on sugarcane plant surfaces.

4.2 Rotational crop data would not be needed for this use on sugarcane in Hawaii only.

5.0 Recommendations:

5.1 We cannot concur with the proposed use on sugarcane due to data deficiencies.

5.2 In addition to the studies already submitted, the following type data will be needed:

*Andly state  
that the  
chemical  
cannot be  
used*

(a) Anaerobic soil metabolism data.

(b) Field persistence data.

(c) Effects of soil microorganisms on the active ingredient.

(d) Effects of the active ingredient on soil microorganisms.

(e) Photodegradation on soil surfaces.

(f) Fish residue accumulation studies. *Catfish + Bluegill*

5.3 The following comments are made on the studies already submitted:

5.3.1 In the soil degradation studies, additional work is needed on identification of the degradation products or metabolites. It is noted that no attempt was made to identify unlabeled products.

It should also be demonstrated that choline chloride or related products would remain intact in the extraction and analytical methods used.

Studies should be done on typical Hawaii sugarcane soils and continued to 90% loss of parent compound and until patterns of accumulation and decline of products are established. Material balance data should be included.

All environmental chemistry data as required by Section 3 of the regulations must be either submitted or referenced prior to registration.

If other new uses of the active ingredient are proposed, additional environmental chemistry data may be required.

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