

BIODEGRADATION OF ALACHLOR [2-CHLORO-2',6'-DIETHYL-N-(METHOXYMETHYL) ACETANILIDE] IN SOILS

Thesis for the Degree of M. S. MICHIGAN STATE UNIVERSITY SHENG-FU J. CHOU 1974



#### ABSTRACT

### BIODEGRADATION OF ALACHLOR [2-CHLORO-2',6'-DIETHYL-N-(METHOXYMETHYL)ACETANILIDE] IN SOILS

By

Sheng-Fu J. Chou

Biodegradation by soil microorganisms accounts for the dissipation of 2-chloro-2',6'-diethy1-N-(methoxymethy1) acetanilide (alachlor) and its possible breakdown product, 2-chloro-2',6'-diethyl acetanilide (demethoxymethyl alachlor) in soil. The half-life of alachlor in three soils was 8 to 16 days, while the half-life of demethoxymethyl alachlor was approximately 2 days. The amount of <sup>14</sup>CO<sub>2</sub> evolved from <sup>14</sup>C-ring labeled alachlor in soils averaged only 4.1% of the original label after 50 days of incubation. After this period of incubation an average of 82.1% of the added label could not be extracted with benzene: isopropanol or accounted for as  $14_{CO_2}$ . However, more than 49% of the added radioactivity after 60 days incubation could be recovered by weak alkali extraction; 17.0% fractioned with humic acid and 31.9% with fulvic acid. Gas chromatographic analysis of the solvent extracts did not show any metabolite peaks during 50 and 60 day incubation periods. Alachlor appears to biodegrade rapidly in soil but

# Sheng-Fu J. Chou

the resulting metabolites appear to be bound to soil organic matter and to degrade slowly.

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## **BIODEGRADATION OF ALACHLOR**

# [2-CHLORO-2',6'-DIETHYL-<u>N</u>-(METHOXYMETHYL)ACETANILIDE]

## IN SOILS

By

Sheng-Fu J. Chou

## A THESIS

Submitted to Michigan State University in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

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To my parents, brothers, sisters, and wife, whose memory gave me the inspiration to finish this work.

To Dr. and Mrs. R. L. Cook and Professor M. H. Wu, whose wisdom enlightened me.

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# TABLE OF CONTENTS

I	Page
INTRODUCTION	1
MATERIALS AND METHODS	3
Soils	3
Incubation systems	3
Solvent extraction of soils	4
Analyses	5
Alachlor-humic substances interaction	6
Chemicals	6
RESULTS AND DISCUSSION	9
REFERENCES	28

# LIST OF TABLES

Table		Page
1	Properties of the soils used in the experiments	3
2	Alachlor remaining in three soils as determined by gas chromatography	10
3	Half-lives of alachlor in several soils	11
4	Percent of original radioactivity as $^{14}CO_2$ and in benzene:isopropanol extracts from three soils amended with $^{14}C$ -alachlor	17
5	Distribution of radioactivity following incubation of $^{14}$ C-alachlor in Spinks soil	21

.

# LIST OF FIGURES

Figure				Page
1	Scheme for alkali extraction and subsequent fractionation of soil organic matter com-			-
	ponents	•	•	7
2	Progressive loss of alachlor in three soils as analyzed by gas chromatography	•	•	13
3	Alachlor and demethoxymethyl alachlor remaining after incubation in autoclaved and non-autoclaved soil	•	•	15
4	<sup>14</sup> CO <sub>2</sub> evolution from <sup>14</sup> C-ring labeled alachlor in soils	•	•	19
5	Progressive loss of benzene-isopropanol extractable radioactivity from three soils.	•	•	23

### INTRODUCTION

It has been estimated that every year the foliage and soils of the United States are doused with approximately one billion pounds of synthetic organic pesticides. In this process more than 800 substances are used in different registered formulations that now exceed 60,000 in number (Minter et al., 1969). Several synthetic weed killers are constructed around an anilide moiety or one of its chlorinate chemical cousins. These herbicides include a relatively new class, acylanilides, as well as older types, phenylcarbamates and phenylureas, and they comprise a significant proportion of all agricultural chemicals now in use. Alachlor [2-chloro-2',6'-diethyl-N-(methoxymethyl) acetanilide], an acylanilide preemergence herbicide, has recently become popular for the control of annual grasses, redroot pigweed (Amaranthus retroflexus L.) and yellow nutsedge (Cyperus esculentus L.) in corn (Zea mays L.) and soybeans [Glycine max (L.) Merr.] (Armstrong et al., 1973; Wax et al., 1972). Though considerable work has been reported on the degradation and fate of other acylanilide herbicides in soils (Bartha, 1971; Bartha and Pramer, 1970; Chisaka and Kearney, 1970; Kaufman and Blake, 1973; Kaufman et al., 1971), little information of this type has been published for alachlor.

Hargrove and Merkle (1971) found that chemical degradation of alachlor in soil, under conditions of low humidity and high temperature, resulted in the formation of 2-chloro-2',6'-diethyl acetanilide. However, this intermediate did not accumulate under more natural soil conditions. Beestman and Deming (1972) have reported that microbial degradation was the major route of alachlor degradation in soil with half-lives ranging between 2 to 14 days for several soils. Taylor (1972) found that alachlor degradation in soil was not accompanied by mineralization of the aromatic portion of the herbicide. These findings for alachlor are consistent with the relatively rapid rate of degradation of other acylanilide herbicides (Bartha and Pramer, 1970; Chisaka and Kearney, 1970; Kaufman and Blake, 1973; Kaufman et al., 1971), though in several cases degradation was not complete (Bartha, 1971; Chisaka and Kearney, 1970).

This investigation was initiated to determine the rate and extent of alachlor degradation in soil and to characterize any residues from intermediates which may occur.

## MATERIALS AND METHODS

## Soils

The degradation of alachlor was studied in Brookston sandy loam, Conover sandy loam, and Spinks sandy loam surface soils obtained from plots on Michigan State University experimental farms. The soils had never received applications of alachlor. The soil properties are shown in Table 1. The soils were freshly collected and allowed to dry to approximately 15% moisture before use.

		Organic	Mechan	ical ana	lysis
Soil type	pН	matter	sand	silt	clay
			(%)	(%)	(%)
Brookston sandy loam	7.1	3.38	54.2	33.0	12.8
Conover sandy loam	7.0	2.03	69.0	20.6	10.4
Spinks sandy loam	6.3	1.25	65.0	29.8	5.2

Table 1. Properties of the soils used in the experiments

#### Incubation systems

Fifty grams of soil that had passed through a 2 mm sieve were placed in 250 ml Erlenmeyer flasks. A portion of Brookston soil was autoclaved for three 30 min periods every other day to provide the sterile soil. Sterility was confirmed at the end of the incubation by the absence of growth when soil was inoculated onto nutrient agar. One milliliter of a 100 ppmw solution of filter sterilized  $^{14}$ C-ring labeled alachlor (0.01 µCi) was distributed dropwise on the unautoclaved soil in each flask (equivalent to 4.5 kg alachlor/ha). In the experiments with 2-chloro-2',6'-diethyl acetanilide (demethoxymethyl alachlor) and in experiments with autoclaved soil, 2.5 ml of a 100 ppmw solution of substrate was used. The amended soil in each flask was then moistened by adding 12 ml of sterilized distilled water, sealed and incubated at 25 C. Replicate flasks were sampled at the indicated time periods for the following analyses.

Respired  ${}^{14}\text{CO}_2$  was trapped in 1 ml of 1 N NaOH which was contained in a disposable 2 ml beaker suspended above the soils. It is assumed that recovery of the liberated  ${}^{14}\text{CO}_2$  in the alkali trap was substantially complete since equivalent levels of NaH ${}^{14}\text{CO}_3$  added to the soils showed 95%  ${}^{14}\text{CO}_2$  trapped within 8 hr.

### Solvent extraction of soils

Alachlor and demethoxymethyl alachlor were extracted from the soil with three 50 ml portions of benzene:isopropanol (2:1, v/v). For the first extraction, the solvent-soil mixture was allowed to stand in the flask overnight and then was shaken on a rotary shaker at 150 rpm for 30 min. After decanting the solvent the second and third extractions were made by shaking solvent and soil at 150 rpm for 30 min prior

to decanting. Anhydrous  $Na_2SO_4$  was added to the solvent to remove water. The combined extracts were concentrated to 50 or 100 ml prior to analysis.

#### Analyses

All samples were diluted to appropriate volume and analyzed on a Beckman GC-5 gas chromatograph equipped with an electron capture detector. A glass column of 1.83 m by 3 mm I.D. and containing 1.5% OV-17 / 1.95% QF-1 on 60/80 chromosorb Q was used. The column, inlet, and detector temperatures were 200, 220, and 250 C, respectively. The carrier gas (He) flow was 85 ml/min. The retention time and minimum sensitivity for alachlor were 3.3 min and 0.01  $\mu$ g, respectively, and for demethoxymethyl alachlor they were 1.9 min and 0.025  $\mu$ g, respectively. Fifty grams of each soil without added chemicals were also extracted as a control; no interfering peaks were found.

The <sup>14</sup>C-label was assayed by liquid scintillation counting using a Packard Tri-Carb Liquid Scintillation Spectrometer, Model 8310. One milliliter of the benzene: isopropanol extract was counted in 15 ml of a scintillation solution containing 4 g PPO, 0.5 g POPOP/liter of toluene. The <sup>14</sup>CO<sub>2</sub> trapped in 1 N NaOH was counted in Bray's solution (1960) containing 4% Cab-O-Sil. All counts were corrected for quenching by external standardization and for machine efficiency. All reported data are the averages of two replicates.

## Alachlor-humic substances interaction

Either 2.5 ml (experiment III) or 5.0 ml (experiment II) of 100 ppmw  $^{14}$ C-ring labeled alachlor (0.025 and 0.05  $\mu$ Ci, respectively) were added to 50 g of Spinks soil. Treated samples were incubated and extracted at 0, 10, 14, 20, 40 and 60 days by the benzene:isopropanol extraction procedure previously described. After this solvent extraction, the soils were shaken in 50 ml 0.5 N NaOH for 24 hr. This alkali extract was fractioned according to the scheme shown in Figure 1. Each fraction was assayed for radioactivity.

Several fulvic acid fractions were extracted with three 50 ml portions diethyl ether, the extract dried with anhydrous Na<sub>2</sub>SO<sub>4</sub> and analyzed by the gas chromatographic procedure previously described except that stainless steel columns, 6 ft by 1/8 O.D., containing 5% SE-30 and 4% SE-30 / 6% QF-1 on 60/80 chromosorb Q were used. These ether extracts were also examined for <sup>14</sup>C compounds by thinlayer chromatography and subsequent radioautography. Each sample was concentrated to 0.5 ml before 50 µl was spotted on a 250 µ silica gel H layer. The chromatogram was developed in petroleum ether : chloroform : 95 percent ethanol (7:2:1, v/v/v).

### Chemicals

Analytical grade alachlor and uniformly <sup>14</sup>C-ring labeled alachlor were supplied by the Monsanto Chemical



\*Fractions assayed for  $1^4$ C content

Figure 1. Scheme for alkali extraction and subsequent fractionation of soil organic matter components. Co., St. Louis, Mo. The label purity was determined to be 99.7% by thin-layer chromatography. Demethoxymethyl alachlor was prepared by hydrolysis in 5 N HCl according to the method of Hargrove and Merkle (1971). Nanograde benzene and isopropanol were purchased from Burdick and Jackson Laboratories, Muskegon, MI.

### **RESULTS AND DISCUSSION**

The loss of alachlor in three soils is shown in Table 2 and Figure 2. By the 10th day, more than 50% of added alachlor was gone in the Brookston and Spinks soils, while almost 50% disappeared in Conover soil. By the 50th day, only 1.4% and 1.5% of the original amount was recovered from Brookston and Spinks soils, respectively, and 2.8% from the Conover soil. This indicates a rapid and similar rate of degradation of alachlor in the three soils. The half-life of alachlor in these three soils ranged between 8 and 16 days (Table 3) over three experiments, which is in agreement with the values reported by Beestman and Deming (1972).The slightly slower rate of degradation for experiment III may have been due to the more deteriorated state of the microflora in late summer sample and lower incubation temperature.

As seen in Figure 3, the degradation of both chemicals in autoclaved soil was minor. Extractable demethoxymethyl alachlor declined only 5.5% after 20 days of incubation in autoclaved soil compared with a loss of 97.8% in the nonautoclaved soil. Similarly, alachlor decreased only 14.4% after 50 days incubation in autoclaved soil compared with 92.8% degradation in non-autoclaved soil. These results show that microorganisms are the major agents of

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Ę			Alachlor r	erovered.		
	Brook	ston	Cono	ver	Spii	ıks
	(bu)	(	(bu)	(	(bd)	(
	94.7 93.7	94.2	94.4 94.9	94.7	98.5 98.2	98.3
	44.1 38.0	41.1	53.2 58.3	55.7	30.7 30.2	30.5
	13.2 12.5	12.8	39.9 43.7	41.3	2 <b>4.</b> 5 24.0	24.2
	<b>4.</b> 3 <b>4.</b> 2	4.2	10.9 12.2	11.5	11.4 11.0	11.2
	1.4 1.4	1.4	2.7 2.9	2.8	1.4 1.6	1.5

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Table

Experiment	Replicates	Soils	Date soil	collected	Conc. in soil (ppmw)	Incubation temperature (°C)	Half-life (days) <sup>a</sup>
I	2	Brookston	Feb. 1	1973	2.0	25	6
	2	Conover	Feb. 1	1973	2.0	25	11
	2	Spinks	Feb. 1	1973	2.0	25	ø
II	2	Spinks	Feb. 1	1973	10.0	22	10
III	4	Spinks	Sept.	1973	5.0	18	16
α							

<sup>a</sup>Average of replicates; variability among replicates  $< \pm 0.4$  days.

Figure 2. Progressive loss of alachlor in three soils as analyzed by gas chromatography.

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Brookston	00
Conover	ΔΔ
Spinks	00

...

![](_page_22_Figure_0.jpeg)

Figure 2

Figure 3. Alachlor and demethoxymethyl alachlor remaining after incubation in autoclaved and non-autoclaved soil.

00	Alachlor	
ΔΔ	Demethoxymethy1	alachlor

•

![](_page_24_Figure_0.jpeg)

Figure 3

decomposition of both alachlor and demethoxymethyl alachlor in soils.

The loss of demethoxymethyl alachlor from Spinks soil (Figure 3) followed the same loss pattern as alachlor except that demethoxymethyl alachlor was degraded at a much faster rate. The half-life was approximately 2 days, a rate of disappearance four times greater than for alachlor. This suggests that it is unlikely that demethoxymethyl alachlor would ever accumulate in soils except in unusual circumstances where biological activities are severely inhibited; this could explain the occurrence of this product in a previous report (Hargrove and Merkle, 1971).

The data did not show demethoxymethyl alachlor to be an intermediate since it was never recovered from alachlor amended soil. Nevertheless, the more rapid rate of decomposition of this chemical than for alachlor is consistent with its remaining a candidate for an intermediate. In separate studies on fungal metabolism of alachlor, demethoxymethyl alachlor has been shown to be an intermediate (Tiedje and Hagedorn, unpublished data).

Based on the recovery of the <sup>14</sup>C of alachlor as <sup>14</sup>CO<sub>2</sub>, however, the rapid loss of alachlor in three soils was not accompanied by complete respiration of the herbicide (Table 4 and Figure 4). After 50 days of incubation, an average of only 4.1% of the initial radioactivity was trapped in the NaOH. At this rate of metabolism it would take more than 3 years before complete mineralization of

Tncubation	Brook	ston	Cono	ver	Sp	inks
time	14C02	Extracts	14C02	Extracts	14c02	Extracts
(days)	(	(	(	(	(	(
0	0.0	90.5	0.0	88.5	0.0	95.8
10	2.1	30.0	1.3	43.4	1.4	32.8
20	2.6	26.2	1.4	28.6	1.9	22.0
30	3.3	15.6	2.8	15.4	2.1	13.8
50	3.7	14.4	5.1	14.0	3.6	12.9
<sup>a</sup> After	50 days the	sterile soil s	showed 0.01\$	14 <sub>C</sub> in the Na	OH trap.	

Percent of original radioactivity as 14 from three soils amended with 140-stocktora Table 4.

Figure 4.  $^{14}CO_2$  evolution from  $^{14}C$ -ring labeled alachlor in soils.

Brookston	00
Conover	ΔΔ
Spinks	00

![](_page_28_Figure_0.jpeg)

Figure 4

the herbicide residue would occur. The majority of the radioactivity in the benzene:isopropanol extracts (Table 4 and Figure 5) was accounted for by unmetabolized alachlor since the quantity of alachlor in these extracts as determined by gas chromatography (Table 2) was similar to the observed radioactivity. After 50 days, however, the radioactivity of the extract ranged from 12.9 to 14.4% of the original addition in contrast with 1.4 to 2.9% alachlor at this time. Nevertheless, no other products could be detected by gas chromatography in these or in earlier extracts.

It is significant that most of the added radioactivity in alachlor could not be recovered as  $14_{CO_2}$  or in benzene: isopropanol extracts. This unrecovered radioactivity ranged from 80.8 to 83.5% of the added label after 50 days incubation for experiment I and 63.8% after 60 days incubation for experiment III. An indication of the fate of the unrecovered <sup>14</sup>C from alachlor is shown by the data in Table 5. In experiments II and III, even though the rate of degradation was much slower for experiment III (Table 3), the distribution of the <sup>14</sup>C label among the various fractions (Table 5) was similar at a same stage of alachlor degradation in the two experiments. In experiment III 63.8% of the label was not recovered in the benzene: isopropanol extract nor as  $14_{CO_2}$  though 48.9% was recovered by subsequent alkali extraction. In both experiments the majority of the alkali soluble label fractionated as fulvic acid (approximately 31%) whereas 17% fractioned as

Table 5.	Distribution	of	radioactivity	following	incubation	of	14 <sub>C-alachlor</sub>	in	Spinks
	1:00								
	TTOS								

			Soil residues	s extract	ed by alkali	treatment	
	Benzene:		In supernate separation of acid	after E humic	In humic	Extracted from humic	Total radio-
Incubation	isopropanol extract	14 <sub>C02</sub>	Extracted with ether	Aqueous layer	acid pre- cipitate	acid with ether	activity recovered
(days)	(	(	(	(	(	(	( 8 )
Experiment 14	II 30.7	1.7	26.2	3.2	17.0	17.3	79.1
Experiment			C		i,	Ę	
0 0	98.0(88.8) 77 3(58 6)	0.00	10 1	ND 1 3	ND 3 6	ND A Q	98.U
20	58.6(34.4)	0.24	16.0	2.9	6.5 0		84.2
40	39.4(6.2)	0.28	22.7	6.8	11.5	12.1	80.6
60	35.8(2.9)	0.35	( 0.008) 26.1	5.8	17.0	15.3	84.7
p0	6.2(0.02)	0.00	( 0.000) 59.7(30.0)	) 4.3	16.4	ND	86.6
aAve	srage of two rep	licates;	expressed as	percent	of added <sup>14</sup> C	in each frac	tion.

 $^{\mathrm{b}}$ Percent of added alachlor found in fraction by gas chromatography.

<sup>c</sup>ND = not determined

<sup>d</sup>Soil samples were extracted by alkali prior to extraction with benzene:isopropanol.

Figure 5. Progressive loss of benzene-isopropanol extractable radioactivity from three soils.

Brookston	00
Conover	ΔΔ
Spinks	00

.

![](_page_32_Figure_0.jpeg)

Figure 5

humic acid. Total recovery of radioactivity averaged only 79.1 and 84.7% of the initial label after 14 and 60 days incubation, respectively. Incomplete recovery of the label in the alkali extract may be due to the association of the alachlor residue with insoluble humin which is not removed by a single extraction with 0.5 N NaOH.

In experiment III, the percent of <sup>14</sup>C in the benzene: isopropanol extract was significantly higher than the percent of alachlor found in the fraction by gas chromatography (Table 5). This suggests that the benzene: isopropanol extracts contained metabolites though none could be conclusively resolved by gas chromatography.

Most of the label in the humic and fulvic acid fractions was extracted into ether (Table 5). None of this extracted label was alachlor as determined by gas chromatography; one small peak with approximately the same retention time as demethoxymethyl alachlor was present. Radioautograms of thin layer chromatograms of the same samples showed no alachlor spot ( $R_f$ =0.60). The only spot had an  $R_f$  of 0.06 and tailed to the origin. The limited movement and tailing of the spot suggests that the <sup>14</sup>C was at least moving with, if not complexed to, other soil organic materials. If the latter were the case, this would explain the absence of any major peaks in the gas chromatographic analysis.

Though alachlor was not altered in the absence of soil in the alkali extraction procedure, approximately one-half of the alkali extracted label was not recovered as alachlor

at zero time (Table 5, last row). This suggests that the alkali catalyzed some binding of alachlor to soil so that it could not be recovered by ether extraction. This finding may be of significance in interpreting the data from the incubation experiments in Table 5 if the prior benzene: isopropanol extraction did not remove all of the The data on efficiency of alachlor extraction alachlor. from soils (Table 6) shows that benzene: isopropanol is the superior extractant and suggests that it recovered most of the free and weakly bound alachlor leaving little of the herbicide for alkali catalyzed reaction with soil organic matter. In any case, the absence of any alachlor in the 40 and 60 day ether fractions (Table 5) would probably not have occurred if only alkali catalysis were responsible since it left major amounts of alachlor in the fraction.

The recovery of the <sup>14</sup>C label after alkali treatment, which is known to solubilize organic matter, suggests that alachlor intermediates are chemically bonded to soil organic matter. Bartha (1971) has reported that propanil (3', 4'dichloropropionanilide) metabolism in soil resulted in 73% of the ring carbon being bound to soil. A portion of this label could be recovered after alkali treatment and was identified to be the aniline derivated of propanil. Intermediates with free amide groups, such as the anilines, could be expected to react with soil organic matter. One such intermediate from alachlor, 2,6-diethyl aniline, has been shown by Tiedje and Hagedorn (unpublished data) to be an intermediate of alachlor degradation by a soil fungus.

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Solvent	Extracted (%)	Additional <sup>14</sup> C extracted by benzene:isopro- panol (%)	Total ex- tracted(%)
Benzene:isopropanol	99.5		99.5
Water	74.6	14.7	89.3
10% NaCl	60.7	30.6	91.4
0.1 N HC1	52.7	33.4	86.1
0.5 N NaOH	79.4	6.1	85.5

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Table 6. Extraction properties of  $^{14}$ C-alachlor at zero time

Propachlor (2-chloro-<u>N</u>-isopropylacetanilide), which is more similar to alachlor than propanil, because of the <u>N</u>alkyl substituent, was reported to show very little mineralization of the <sup>14</sup>C-carbonyl group in soil (Kaufman *et al.*, 1971). This suggests that aniline was not formed rapidly from this herbicide. In the present study precursors of the anilines could also contribute to the soil bound intermediates; however, they may have been hydrolyzed to anilines during the alkali treatment.

Though biodegradation cannot be definitely proven from the present study, it is the most reasonable explanation. The following findings suggest that alachlor was biodegraded and not bound to soil organic matter by chemical or physical reactions: (1) alachlor disappearance did not occur in autoclaved soil, but did occur in non-autoclaved soil; (2) the disappearance of alachlor in non-autoclaved soil was probably not due to inability to extract bound alachlor since benzene: isopropanol was shown to be a superior extractant and because total recovery of alachlor was achieved from the non-autoclaved zero time sample; (3) the alachlor disappearance curve did not fit the pattern of an adsorption reaction which normally would show a very rapid disappearance of the reactant: (4) the  $^{14}$ Clabel recovered in the ether extracts of the fulvic acid fraction was not alachlor as was shown by gas and thin layer chromatography.

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![](_page_39_Picture_0.jpeg)