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FATE OF ACYLANILIDES IN SOILS AND POLYBROMINATED
BIPHENYLS (PBB'S) IN SOILS AND PLANTS

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Sheng-Fu Joseph Chou

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# FATE OF ACYLANILIDES IN SOILS AND POLYBROMINATED BIPHENYLS (PBB'S) IN SOILS AND PLANTS

By

Sheng-Fu Joseph Chou

A DISSERTATION

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## ABSTRACT

# FATE OF ACYLANILIDES IN SOILS AND POLYBROMINATED BIPHENYLS (PBB'S) IN SOILS AND PLANTS

By

Sheng-Fu Joseph Chou

Biodegradation by soil microorganisms accounts for the dissipation of alachlor [2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide], demethoxymethylalachlor (2-chloro-2',6'-diethylacetanilide), and Antor herbicide$^{TM}$ [2-chloro-N-(2',6'-diethylphenyl)-N-methyl(ethylcarboxylate)acetamide] in soil. The half-life of alachlor in several soils was between 7 to 14 days, while the half-lives of demethoxymethylalachlor and Antor were approximately 2.7 and 3.0 days, respectively. Rate of $^{14}CO_2$ production and disappearance of $^{14}C$-alachlor increased with increasing temperatures until temperatures greater than 30$^{o}$C were reached. Organic matter additions to soil failed to increase rates of alachlor degradation. In the field studies alachlor was non-mobile and exhibited half-lives similar to laboratory determined values. The alachlor concentration in soil at yellow nutsedge emergence was approx. 0.2 ppmw. The rate of disappearance of $^{14}C$-ring and $^{14}C$-carbonyl labeled Antor was identical, which suggests that most of the metabolic products from Antor retain the chloroacetyl moiety. I found at least four major alachlor metabolites in hexane-acetone extracts of soil. Two were identified as 2-chloro-2'6'-diethylacetanilide and 1-chloro-acetyl-2,3-dihydro-7-ethylindole.

Most of the added radioactivity in alachlor and Antor could not be recovered as $^{14}CO_2$ or in benzene-isopropanol and hexane-acetone extracts. This unrecovered radioactivity ranged from 86 to 94% after 30 days of

incubation. Most of the alachlor and Antor remained in soil as intermediates which were tightly bound to or incorporated in soil organic matter. However, 51 and 62% of the label were recovered by a humic acid extractant, $Na_4P_2O_7$, from alachlor and Antor amended soils, respectively. The majority of $^{14}C$ label was found in the small molecular weight fraction (mw<500). Autoradiograms showed at least four alachlor and six Antor metabolites in this fraction. All appear to be polar.

The flame retardant, PBB, was found to be comprised of 2,2',4,4' 5,5'-hexabromobiphenyl as the major component, two isomers of pentabromobiphenyl, three additional isomers of hexabromobiphenyl, and two isomers of heptabromobiphenyl. The PBB's were extremely persistent with no isomer showing any consistent disappearance in soil incubations of up to one year. Soil incubation utilizing $^{14}C$-hexa- and heptabromobiphenyls, the two major isomers in PBB, showed that less than 0.2% of the $^{14}C$-PBB was degraded to $^{14}CO_2$ after 12 months. Soils incubated with products of $^{14}C$-PBB which had undergone photodegradation showed only minor conversion to $^{14}CO_2$. Gas chromatographic analysis showed equal, 84%, recovery of PBB in sterilized and non-sterilized soil after one year incubation.

Autoradiograms of corn and soybean seedlings grown in hydroponic solutions showed no translocation of $^{14}C$-PBB (a) from $^{14}C$-PBB treated solutions to plant tops or (b) within the leaf from $^{14}C$-PBB treated spots on the upper leaf surface. A significant portion of the $^{14}C$-PBB associated with the roots was removed when the roots were dipped in acetone. Three root crops (radishes, carrots, and onions) were grown in two soils, one high in clay and organic matter and the other low. The soils were treated with a mixture of fireMaster BP6 (PBB) and $^{14}C$-PBB to achieve final concentrations of 100 ppmw and 100 ppbw. The extent of PBB associated with the roots from the high PBB treatment ranged from approximately 50 to 500 ng/g plant tissue for plants grown in Spinks loamy sand soil and 30 to 120 ng/g plant tissue for plants grown in Brookston clay loam soil. No PBB was found with roots from the lower PBB treatment in Brookston soil.

To my wife, Mei-In, for her encouragement and patience. To my parents and friends, Dr. and Mrs. R. L. Cook, for their moral support.

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# TABLE OF CONTENTS

Page

# LIST OF TABLES

CHAPTER I

Page

CHAPTER II

CHAPTER III

CHAPTER IV

# LIST OF FIGURES

CHAPTER IV

# CHAPTER I

# BIODEGRADATION OF TWO ACYLANILIDE HERBICIDES, ALACHLOR AND ANTOR HERBICIDE$^{TM}$, IN SOILS

## Introduction

Alachlor [2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide] and Antor$^{TM}$ [2-chloro-N-(2',6'-diethylphenyl)-N-methyl(ethylcarboxylate)-acetamide] are preemergence herbicides. Alachlor is now in wide spread use for 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). Antor is a selective herbicide with activity against many annual grasses and some broadleaved weeds. Hercules, Inc., is attempting to obtain registration for use of Antor herbicide on sugar beets, cotton, soybeans, corn, wheat and some vegetable crops.

Despite the importance of the acylanilide class of herbicides little information on degradation and fate of these compounds has been published. Tiedje and Hagedorn (1975) found degradation of alachlor by a common soil fungus, Chaetomium globosum. The products were chloride and four identifiable organic metabolites: 2-chloro-2',6'-diethylacetanilide (DMM or demethoxymethylalachlor); 2,6-diethyl-N-(methoxymethyl)aniline; 2,6-diethylaniline, and 1-chloroacetyl-2,3-dihydro-7-ethylindole. McGahen and Tiedje (1977) also found the degradation of Antor by the same soil fungus and identified five metabolites:

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Antor herbicide is a trademark of Hercules Chemical Corp. Alachlor is the common name of a herbicide marked by Monsanto Chemical Corp. under the trademark of Lasso.

2-chlor-N-(2',6'-diethylphenyl)acetamide, 2-hydroxyl-N-(2'-ethyl-6'-vinylphenyl)-N-methyl(ethylcarboxylate)acetamide, N-methyl(ethylcarboxylate)-2,3-dihydro-7-ethylindole, 2-chloro-N-(2'-ethyl-6'-vinylphenyl)-N-methyl(ethylcarboxylate)acetamide, and N-2',6'-diethylphenyl)-α-(ethylcarboxylate)imine.

The degradation of alachlor by another soil fungus (Smith and Phillips, 1975; Chahal, et al., 1976) and in a model ecosystem has been reported (Yu, et al., 1975), but in neither case were metabolites identified. Hargrove and Merkel (1971) found that acid-catalyzed degradation of alachlor in soil, under conditions of low humidity and high temperature, resulted in the formation of demethoxymethylalachlor. However, this intermediate did not accumulate under more natural soil conditions. Beestman and Deming (1974) reported that microbial degradation was the major route of alachlor dissapation in soil with half-lives ranging from 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 reports for alachlor are consistent with the relatively rapid rate of degradation of other acylanilide herbicides (Bartha, 1971; Chisaka and Kearney, 1970; Kaufman, et al., 1971; Kaufman and Blake, 1973), 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 and Antor degradation in soil and to identify any metabolite intermediates.

# Materials and Methods

## Soil incubations

The degradation of alachlor was studied in Brookston sandy loam (pH, 7.08; OM, 3.38%), Conover silt loam (pH, 7.04; OM, 2.03%), Spinks loamy sand (pH, 6.29; OM, 1.25%), Muscatine loam (pH, 5.24; OM, 5.31%), and Tama clay loam (pH, 5.83; OM, 3.47%) surface soils obtained from plots on Michigan State University experimental farms and an Iowa farm. The soils had never received application of alachlor. The soils were freshly collected and allowed to dry to approximately 12-15% moisture before use.

Fifty grams of soil that had passed through a 2 mm sieve was placed in 250 ml Erlenmeyer flasks. One milliliter of a 100 ppm solution of filter sterilized $^{14}$C-ring labeled alachlor (1.73 mCi/m mole) was distributed dropwise on the soil (equivalent to 1.8 kg/ha). The amended soil in each flask was then moistened by adding 12 ml of sterilized distilled water, sealed and incubated at 25°C for 0, 10, 20, 30, 40, and 50 days. Replicate flasks were sampled at the indicated time periods. This study was repeated in the Spinks, Tama, and Muscatine soils with similar experimental techniques. The half-life ($t_{\frac{1}{2}}$) was calculated as a first order decay constant.

For studies to determine the importance of microbial catalysis in degradation the incubation design was similar but the $^{14}$C-alachlor amended Spinks soil was incubated at 8, 18, 30, and 50°C; or under anaerobic conditions. Other flasks were autoclaved then reinoculated with a 2-ml soil suspension after 1 day, or sterilized with 10 ml propylene oxide. After sterilization 2.5 ml of 100 ppm filter-sterilized

$^{14}$C-alachlor solution (0.02 μCi) was added. In an attempt to stimulate cometabolism, triplicate flasks of Spinks soil were amended with a solution of readily available carbon (0.1% glucose, 0.1% glycine, 0.1% acetate, and 0.1% peptone).

Comparison of the degradation rate of alachlor, demethoxymethylalachlor, and Antor herbicide was conducted in the Brookston soil. Three milliliters of 100 ppm $^{14}$C-ring-labeled alachlor (1.73 mCi/m mole), $^{14}$C-ring-labeled Antor (1.21 mCi/m mole), $^{14}$C-carbonyl-labeled Antor (0.09 mCi/m mole) were distributed dropwise on 30 g soil. Five milliliter of distilled water was used to moisten the soil. Incubation was in the dark at 28$^{\circ}$C for 0, 5, 10, 20, and 30 days. Four flasks of each treatment were extracted after each incubation period.

Respired $^{14}CO_2$ was trapped in 1 ml of 1N NaOH which was contained in a disposable 2 ml beaker suspended above the soils. It was assumed that recovery of the liberated $^{14}CO_2$ in the alkali trap was substantially complete since equivalent levels of $NaH^{14}CO_3$ added to soils showed 95% $^{14}CO_2$ trapped within 8 h.

## Field studies

Alachlor solution was sprayed on 3 x 15 square meter plots at rates 2.24 kg/ha and 4.48 kg/ha. Two adjacent unsprayed plots were used as control. After 0, 2, 7, 14, and 22 days post application, four replicate soil samples were collected from each plot. Each replicate sample was a composite of two surface soil samples, 2 cm deep by 10 cm diameter, taken by a shallow can. To compare field with laboratory degradation, surface soil from the 2.24 kg/ha treated plot was used in laboratory incubations. The field soil was mixed, sieved, placed in Erlenmeyer flasks and moistened as above. Control flasks were sterilized with propylene oxide. Half of the non-sterile flasks were put back in the field and the other half were incubated in the dark in the laboratory at 30°C. Three replicate flasks were sampled on each date.

Fifty days following alachlor application, soil samples were collected by soil probe (3.45 cm ID) from the 4.48 kg/ha plot and divided into 3.8 cm depth increments. Four replicates were used for analysis and each replicate was a composite of four cores.

### Bioassay study

Surface soil samples were collected from control and 4.48 kg/ha alachlor-treated plots 19 days after alachlor application in the manner previously described. Clay pots were filled with 6 cm of control soil. Six yellow nutsedge tubers, collected from the control plot, were placed on the soil surface. The tubers were covered with 2.5 cm of alachlor treated soil. Other assays were of treated soil diluted by 1 part and 2 parts control soil. All pots were placed in the growth chamber under a regime of 12 h fluorescent light (64500 lux) at 29°C and 12 h of dark at 21°C. The pots were located in a randomized design with four replicates. Moisture levels in the soil were maintained by weighing the pots and adding the necessary water daily. After 39 days in the growth chamber (58 days after alachlor applied) when the yellow nutsedge shoots broke through the soil surface in the non-diluted treatment, samples of the top 2.5 cm of soil was collected by a No. 7 stainless steel cork borer. Four replicates each comprised of three composites were used for extraction and GLC analysis of residual alachlor. After 50 days in the growth chamber all shoots were harvested and their dry weights determined.

## Extraction and analyses

Alachlor was 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 shaken with rotary shaker at 250 rpm for 30 min. After decanting the solvent, the second and third extraction were made by shaking solvent and soil at 250 rpm for 30 min prior to decanting. Anhydrous $Na_2SO_4$ was added to the combined extracts to remove water. The combined extracts were concentrated to 50 or 100 ml prior to analysis on Beckman GC-5 gas chromatograph equipped with an electron capture detector. A glass column of 1.83 m by 3 mm ID and containing 1.5% OV-17/1.95% QF-1 on 60/80 mesh Chromosorb Q was used. The inlet, column, and detector temperatures were $220^o$, $200^o$, and $250^oC$, respectively. The carrier gas (He) flow was 85 ml/min.

Soil samples from the bioassay study were extracted by the same procedure except that 1.5 ml distilled water was added in each flask before the extraction. The combined 60-ml benzene-isopropanol extract was condensed to 10 ml prior to analysis.

The field samples were placed into 500 ml Erlenmeyer flasks and 50 ml distilled water added. Three 100-ml portions of benzene-isopropanol (2:1, v/v) were used to extract alachlor following the same methods previously described except that all slurries were shaken on rotary shaker at 250 rpm for 50 min. The combined extracts were concentrated to 20 ml. Two milliliter of each extract was gently blown dry with $N_2$ and the residue redissolved in an appropriate amount of Freon 113 prior to analysis on Perkin-Elmer 900 gas chromatograph equipped with a flame ionization detector. A stainless steel column of 1.8 M by 3 mm O. D.

and containing 3% SE-30 on 60/80 mesh Chromosorb Q was used. The inlet, column, and detector temperatures were 220°, 170°, and 250°C respectively. The carrier gas (He) flow was 30 ml/min.

In studies of comparison of degradation rates all soil samples were extracted with three 40-ml portions of hexane-acetone (9:1, v/v) since I had found that this solvent mixture reduced the amount of soil organic matter extracted yet recovered as much of the acylanilides. The extracts were analyzed on the Beckman GC-5 gas chromatograph equipped with a glass column containing 2% SE-30 on 100/120 mesh Gas-Chrom Q column. The inlet, column, and detector temperature were 230°, 200°, and 270°C respectively. The carrier gas flow was 40 ml/min.

The $^{14}C$-label was radioassayed by a Packard Tri-Carb Liquid Scintillation Spectrometer, Model 3310. One milliliter of the benzene-isopropanol extract was radioassayed in 15 ml of a scintillation solution containing 4 g PPO and 0.5 g POPOP/1 of toluene, while the hexane-acetone extract was radioassayed in 15 ml Bray's solution (1960). The $^{14}CO_2$ trapped in 1 N NaOH was counted in Bray's solution containing 4% Cab-O-Sil. All counts were corrected for quenching by external standardization and machine efficiency.

For the thin-layer chromatographic study of metabolites, the hexane-acetone extracts were concentrated to dryness and redissolved in 0.5 ml chloroform. Samples (10 μl) were spotted on 250 μ pre-coated silica gel 60 F-254 plates (EM Laboratory, Elmsford, NY). TLC plates were developed in benzene-methanol (95:5, v/v) (Yu, et al., 1975) and subsequently examined after autoradiography.

## Results and Discussion

Evidence supporting biodegradation as the major mechanism for alachlor disappearance is summarized in Table 1 and Figure 1. Soil sterilized by two different methods, propylene oxide and autoclaving, showed only a 19% loss of alachlor during incubation compared to a 72% loss in non-sterile soil. Also reinoculation of autoclaved soil caused an increase in degradation rate as would be expected of a microbiological mechanism. Exclusion of $O_2$ also slowed alachlor disappearance as would be expected for most biological reactions though slight anaerobic degradation may have occurred (∿10%). The temperature effect on degradation (Figure 1) yields a pattern expected for biologically and not chemically catalyzed reactions since the rate of both $^{14}CO_2$ production and alachlor disappearance dropped after $30^{o}C$, probably due to denaturation of enzymes. As noted in these and other sterile control treatments in this paper, a low rate of non-biologically catalyzed alachlor disappearance always occurred. Generalizing from these studies, it appears that about 20% of the disappearance is non-biological while 80% is biological.

The greater quantity of $^{14}C$ extracted than alachlor extracted (GLC analysis) shown in both Table 1 and Figure 1 suggests that alachlor metabolites were also extracted.

The half-lives ($t_{\frac{1}{2}}$) of alachlor in several soils are reported in Table 2. They ranged between 7 to 14 days which is in agreement with the 2 to 14 day values reported by Beestman and Deming (1974). The slightly slower rate of degradation for experiment II in Spinks soil may have been due to the lower temperature and/or the more inactive state of the microflora due to energy depletion in soils collected in summer.

Table 1. Evidence for biological degradation of alachlor in soil; effect of sterilization and anaerobic conditions on recovery of $^{14}C$-alachlor after incubation in Spinks soil.

| Treatment | Incubation temp. ($^{o}C$) | Incubation time (day) | % of original recovered[a] | |
|---|---|---|---|---|
| | | | Alachlor by GLC | $^{14}C$ extracted |
| None | - | 0 | 94.6 | 97.8 |
| None | 30 | 13 | 28.5 | 45.1 |
| Propylene oxide | - | 0 | 90.6 | 95.3 |
| Propylene oxide | 30 | 13 | 82.5 | 86.6 |
| Autoclaved | - | 0 | 95.2 | 97.3 |
| Autoclaved | 30 | 13 | 80.1 | 84.7 |
| Autoclaved, reinoculated | 30 | 13 | 45.1 | 61.6 |
| Anaerobic | 24-27 | 13 | 70.2 | 81.5 |

[a] Mean of three or four replicates.

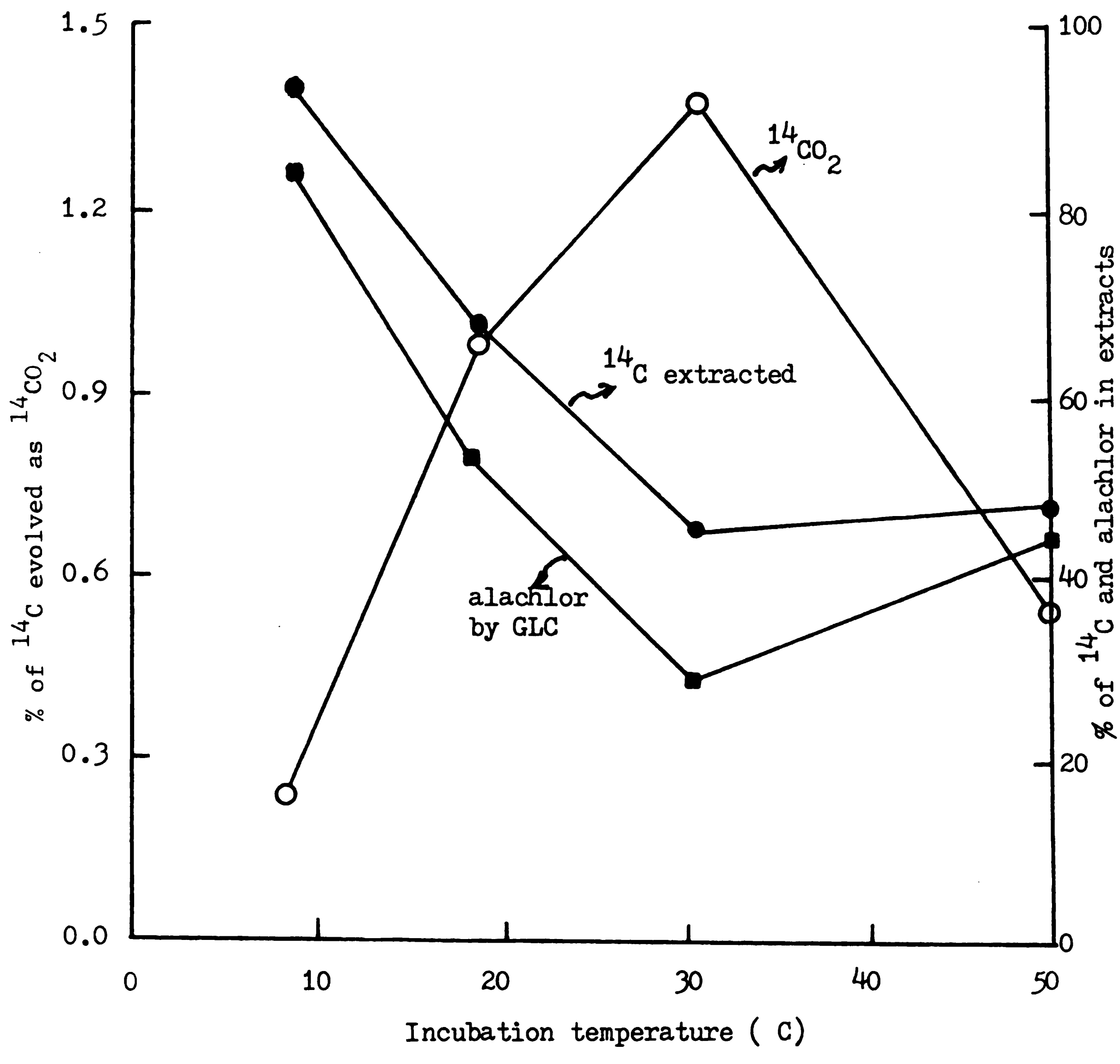


Figure 1. Effect of incubation temperature on recovery of $^{14}$C from alachlor as $^{14}$CO$_2$ and in benzene-isopropanol extracts after 13 days incubation in Spinks soil.

Table 2. Half-lives of alachlor in several Michigan and Iowa soils.

| Soil and sample description | Date soil collected | Conc. in soil (ppmw) | Incubation temp. ($^{o}C$) | Half-life (days) |
|---|---|---|---|---|
| Conover | Feb. 1973 | 2.0 | 25 | 10.4 |
| Spinks, I | Feb. 1973 | 2.0 | 25 | 7.4 |
| Spinks, II | Sept. 1973 | 5.0 | 18 | 12.5 |
| Tama (Iowa) | Oct. 1973 | 5.0 | 18 | 11.9 |
| Muscatine (Iowa) | Oct. 1973 | 5.0 | 18 | 13.6 |
| Brookston I | Feb. 1973 | 2.0 | 25 | 8.5 |
| Brookston II | July, 1976 | 10.0 | 28 | 8.0 |
| Brookston, lab, flask | May, 1974 | 8.85[a] | 30 | 8.4 |
| Brookston, field, flask | May, 1974 | 8.85[a] | V[b] | 9.2 |
| Brookston, field, 2.24 kg/ha | May-Jun., 1974 | 8.60[a] | V | 10.5 |
| Brookston, field, 4.48 kg/ha | May-Jun., 1974 | 13.20[a] | V | 12.7 |

[a] Concentration assuming distribution to 2 cm depth.

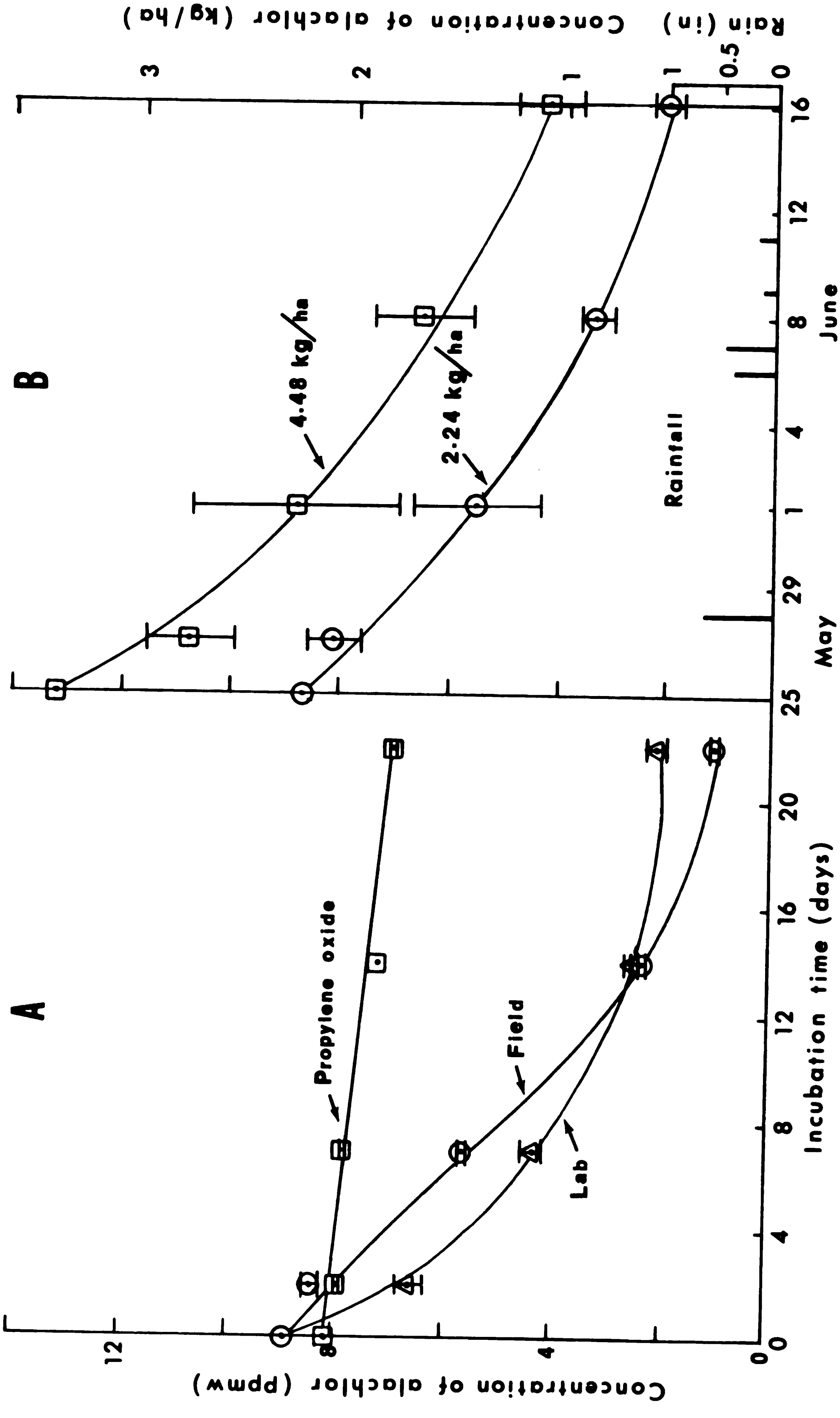
[b] Variable temperature in the field.

The latter mechanism has been reported to be important to rates of EDTA biodegradation (Tiedje, 1977).

In previous experiments I could not enrich organisms using alachlor as the sole carbon source. Since biodegradation does occur in soil and by consitutive enzymes in a soil fungus (McGahen, 1977), a cometabolic degratory mechanism seemed the likely explanation for soil degradation. However, organic matter additions to Spinks soil failed to increase rates of alachlor degradation. After 20 days of incubation only 0.13% of the original $^{14}C$-alachlor was evolved as $^{14}CO_2$ when available carbon was added compared to 2.6% in non-amended soil. These carbon substrates apparently allowed rapid growth of microorganisms that were incapable of degrading alachlor. A similar result was observed when glucose additions to Spinks soil failed to increase the rate of glyphosate degradation (Moshier, 1977). Tiedje and Hagedorn (1975) had found that the presence of carbon in the medium did not enhance the rate of alachlor degradation by resting cells of *Chaetomium globosum*.

The losses of alachlor in field studies are shown in Figure 2B. The degradation of alachlor in both treatments followed the same pattern and rate. By the 22nd day 2.02 ppmw and 4.18 ppmw of alachlor were recovered from 2.24 kg/ha and 4.48 kg/ha treated plots, respectively. The half-lives were 10.5 to 12.7 days, respectively (Table 2). This confirms that a rapid rate of degradation also occurred in the field though it was slightly slower than for laboratory conditions. This was probably due to the more restrictive moisture and temperature conditions in the field. In any case the general similarity between field and laboratory results prove the validity of the flask incubation for assessing degradation in soils.

Figure 2. Alachlor remaining in Brookston soil after incubation under field or laboratory conditions. A-Field treated soil incubated in flasks in lab, field or sterilized. B-Field soils treated with two concentrations of herbicide and rainfall they experienced.


A
B
Propylene oxide
Field
Lab
Concentration of alachlor (ppmw)
12
8
4
0
Incubation time (days)
0
4
8
12
16
20
4.48 kg/ha
2.24 kg/ha
Rainfall
Concentration of alachlor (kg/ha)
3
2
1
Rain (in)
1
0.5
0
25
29
1
4
8
12
16
May
June

Evidence for absence of extensive leaching of alachlor in field soils is shown in Figure 3. After this 50 day period during which 8 cm of rain fell, 92.1% of the alachlor was recovered from the top 3 cm of soil.

Because the data indicated rapid alachlor degradation, the minimum effective soil concentration was determined by bioassay and GLC analysis. The results are summarized in Table 3. Sixty-nine days after alachlor application the dry weight of yellow nutsedge shoots was only 20.7 mg for treated soil, compared to the control pots with 10 times this biomass. At 58 days when the yellow nutsedge shoots began to emerge, I found only 0.22 ppmw alachlor remained in the soil. At this low concentration the inhibition was probably released so that the shoots of yellow nutsedge started to elongate. Armstrong et al. (1973) found that an alachlor concentration of $3.7 \times 10^{-6}$ M (1 ppm) still effectively inhibited yellow nutsedge growth in petri dish culture. However, alachlor does not inhibit sprouting of yellow nutsedge tubers. Knowing the minimum effective concentration and the half-life of degradation one should be able to predict the approximate date of weed emergence, or alternatively to adjust the concentration applied to achieve the desired length of inhibition. In this field study this calculation would predict 5½ half-lives or 60 days, which was about when weed emergence in the field was noted.

The losses of $^{14}$C-ring labeled alachlor and demethoxymethylalachlor (a postulated alachlor metabolite), and $^{14}$C-ring and $^{14}$C-carbonyl labeled Antor herbicide following incubation in soil are shown in Table 4. For all three chemicals, the quantity of $^{14}CO_2$ produced was minor relative to decomposition of the substrate. The $^{14}$C extracted was either the same as (for Antor) or slightly greater than (for alachlor and DMM) the

Figure 3. Distribution of alachlor in Brookston soil profile 50 days after spray application of the herbicide to the soil surface.

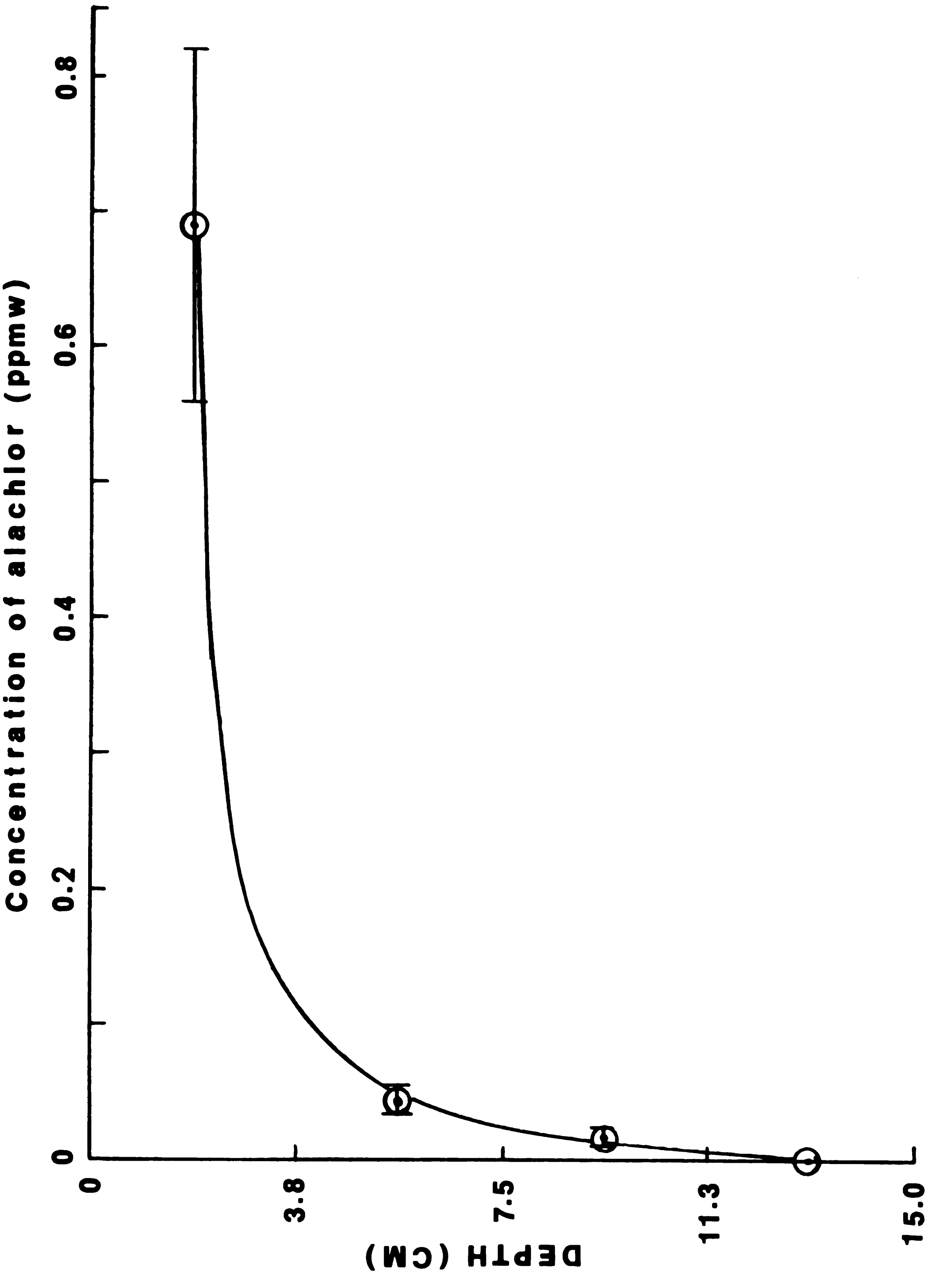

Concentration of alachlor (ppmw)
0
0.2
0.4
0.6
0.8
DEPTH (CM)
0
3.8
7.5
11.3
15.0

Table 3. Bioassay in yellow nutsedge tubers on alachlor amended soil.

| Treatments | Weight of shoot at 69 days | Alachlor at time of shoot elongation by GLC | Shoot emergence |
|---|---|---|---|
| | (mg)[a] | (ppmw) | (days since treatment) |
| Control | 285 ± 117.1 | | ND[b] |
| ½ treated soil + ½ control soil | 61.8 ± 20.9 | ND | ND |
| 2/3 treated soil + 1/3 control soil | 49.2 ± 9.4 | ND | ND |
| Treated soil | 20.6 ± 9.7 | 0.22 ± 0.02 | 58 |

[a] All treatments were significantly different from each other (<0.05).

[b] ND = not determined.

Table 4. Percent of added $^{14}C$ recovered as $^{14}CO_2$ and in hexane-acetone extracts after incubation in Brookston soil.[a]

| Incubation time | Ring-labeled alachlor | | | Ring-labeled DMM | | | Ring-labeled Antor | | | Carbonyl-labeled Antor | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $^{14}CO_2$ | $^{14}C$-extracted | GLC[b] | $^{14}CO_2$ | $^{14}C$-extracted | GLC | $^{14}CO_2$ | $^{14}C$-extracted | GLC | $^{14}CO_2$ | $^{14}C$-extracted | GLC |
| (day) | (% of total added)[c] | | | | | | | | | | | |
| 0 | 0 | 99.9 | 98.1 | 0 | 98.5 | 96.3 | 0 | 99.3 | 98.8 | 0 | 99.4 | 99.0 |
| 5 | 0.3 | 70.4 | 64.7 | 0.4 | 44.8 | 34.2 | 0.28 | 39.6 | 37.9 | 0.8 | 38.4 | 36.2 |
| 10 | 0.5 | 51.8 | 42.1 | 1.50 | 18.4 | 5.3 | 0.4 | 7.7 | 7.1 | 1.1 | 8.0 | 6.1 |
| 20 | 1.3 | 25.2 | 14.1 | 2.8 | 12.5 | 2.2 | 1.5 | 2.3 | 1.4 | 3.4 | 2.2 | 1.0 |
| 30 | 2.2 | 15.2 | 4.8 | 4.0 | 11.2 | 1.4 | 2.1 | 2.2 | 0.9 | 4.4 | 2.0 | 0.5 |
| 30-sterile | 0.1 | 90.7 | 88.3 | 0.0 | 91.1 | 89.1 | 0.1 | 93.2 | 91.3 | 0.1 | 91.9 | 90.4 |
| Half-life | | | 8.0 | | | 2.7 | | | 3.0 | | | 2.9 |

[a] Mean of four replicates.

[b] Percent of substrate recovered from hexane-acetone extract by GC analyses.

[c] Substrate added was 300 µg/30 g soil.

quantity of parent compound recovered by GLC. This indicates that most of the metabolites were not extractable. All parent compounds had virtually disappeared after 30 days incubation.

The short half-life of DMM (2.7 days) compared to that for alachlor (8.0 days) explains why large quantities of this possible intermediate would not accumulate. The large quantity of this compound found by other investigators (Hargrove and Merkle, 1971) must have occurred because the high temperature ($46^{o}C$) severely inhibited biological activities.

The degradation rate of Antor was approximately three times that of alachlor. The $^{14}C$ extracted and the parent substrate recovered were identical for the $^{14}C$-ring and $^{14}C$-carbonyl-labeled Antor at each sampling date. This suggests that most of the metabolic products of Antor retain the chloroacetyl side chain.

The $^{14}C$ in extracts in excess of the parent herbicide (Table 1, 4 and Figure 1) was thought to be due to the presence of metabolites. Hexane-acetone extracts of soil were examined by TLC-autoradiography for the presence metabolites. The autoradiogram shown in Figure 4 revealed at least four metabolites, two of which could be identified as (a) 2-chloro-2',6'-diethylaniline (DMM) and (b) 1-chloroacetyl-2,3-dihydro-7-ethylindole. Identification was based on identical mobilities to the fungal produced standards on TLC and by GLC. Since it is possible that the indoline ring closed in the heated GC inlet, the indoline peak was collected from the effluent of the GLC columns and run on TLC beside the uninjected material. The mobilities were identical indicating no alternation in structure by GLC conditions. This material separated by GLC was also used as the standard on TLC to locate the indoline spot.

Figure 4. Autoradiogram of alachlor metabolites from hexane-acetone extract. 1 - alachlor metabolites produced by *Chaetomium*; 2 - alachlor standard; 3 - demethoxymethyl alachlor standard; 4, 5, 6, and 7 - hexane-acetone extracts after 0, 10, 20, and 30 days of incubation in Brookston soil, respectively. Identified spots: a - demethoxymethyl-alachlor; b - 1-chloroacetyl-2,3-dihydro-7-ethylindole; c - alachlor.

The two identified products appear to be the only soil metabolites incommon with fungal metabolites. The fungus produces at least three additional products not found in soil, while the soil produces two not formed by the fungus.

No metabolites of Antor could be found by similar TLC analyses. This is consistent with finding no $^{14}C$ extracted in excess of that attributable to the herbicide (Table 4).

Polar metabolites of both alachlor and Antor could be recovered from soil by using the humic acid extractant, 0.1 M $Na_4P_2O_7$. The binding of these intermediates to soil organic matter and their characterization are discussed elsewhere (Chapter II).

# Literature Cited

1. Armstrong, F. F., W. F. Meggitt, and D. Penner. 1973. Yellow nutsedge control with alachlor. Weed Sci. 21:354-357.

2. Bartha, R. 1971. Fate of herbicide-derived chloroaniline in soil. J. Agri. Food Chem. 19:385-387.

3. Beestman, G. B., and J. M. Deming. 1974. Dissipation of acetanilide herbicides from soils. Agron. J. 66:308-311.

4. Bray, G. A. 1960. A simple efficient liquid scintillation counter. Anal. Biochem. 1:279-285.

5. Chahal, D. S., I. S. Bans, and S. L. Chopra. 1976. Degradation of alachlor [2-chloro-N-(methoxymethyl)-2',6'-diethylacetanilide] by soil fungi. Plant and Soil. 45:689-692.

6. Chisaka, H. and P. C. Kearney. 1970. Metabolism of propanil in soils. J. Agri. Food Chem. 18:854-858.

7. Hargrove, R. S. and M. G. Merkle. 1971. The loss of alachlor from soil. Weed Sci. 19:652-654.

8. Kaufman, D. D., J. R. Plimmer, and J. Iwan. 1971. Microbial degradation of propachlor. Amer. Chem. Soc. Abst. 162nd Meeting, Washington, D. C. A-21.

9. Kaufman, D. D. and J. Blake. 1973. Microbial degradation of several acetamide, acylanilide, carbamate, toluidine and urea pesticides. Soil Biol. Biochem. 5:297-308.

10. McGahen, L. L. and J. M. Tiedje. 1977. Degradation of two new acylanilide herbicides, Antor and Dual by the soil fungus, Chaetomium globosum. submitted to J. Agri. Food Chem.

11. McGahen, L. L. 1977. Metabolism of two new acylanilide herbicides, Antor [2-chloro-N-(2',6'-diethylphenyl)-N-methyl(ethylcarboxylate)-acetamide] and Dual [2-chloro-N-(2'-ethyl-6'-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide] by the soil fungus, Chaetomium globosum. M.S. thesis, Michigan State University, E. Lansing, Mich. pp. 39.

12. Moshier, L. J. 1977. Factors affecting N-(phosphoromethyl)glycine (Glyphosate) activity in turfgrass and alfalfa [Medicago sativa (1)] Seedling environments and degradation in the soil. Ph.D. thesis, Michigan State Univ., E. Lansing, Mich. pp. 85.

13. Smith, A. E. and D. V. Phillips. 1975. Degradation of alachlor by *Rhizoctonia solani*. Agron. J. 67, 347-349.

14. Taylor, R. 1972. Degradation of alachlor [2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide] in soils and by microorganisms. M.S. thesis, Michigan State Univ., E. Lansing, Mich. pp. 25.

15. Tiedje, J. M. and M. L. Hagedorn. 1975. Degradation of alachlor by a soil fungus, *Chaetomium globosum*. J. Agri. Food Chem. 23, 77-81.

16. Tiedje, J. M. 1977. Influence of environmental parameters on EDTA biodegradation in soils and sediments. J. Environ. Quality. 6, 21-25.

17. Wax, L. M., E. W. Stoller, F. W. Slife, and R. N. Anderson. 1972. Yellow nutsedge control in soybeans. Weed Sci. 20, 194-200.

18. Yu, C. C., G. M. Booth, D. J. Hansen, and J. R. Larsen. 1975. Fate of alachlor and propachlor in a Model ecosystem. J. Agri. Food Chem. 23, 877-879.

# CHAPTER II

# CHARACTERIZATION OF POLAR AND HUMIC-BOUND SOIL METABOLITES OF ALACHLOR AND ANTOR HERBICIDE™

## Introduction

Acylanilide herbicides are rapidly degraded by microorganisms in soil but their aromatic portion is persistent. Chou and Tiedje (1975) found that the amount of $^{14}CO_2$ evolved from $^{14}C$-ring labeled alachlor in soils averaged only 4.1% of the original label after 50 days of incubation. After this incubation 82% of the added label could not be extracted from soil with benzene-isopropanol or accounted for as $^{14}CO_2$. Similar results were observed for Antor. This suggests that polar and/or bound metabolites are the major fate of acylanilide herbicides in soils. Hsu and Bartha (1974a, 1974b, 1976) have shown that binding of acylanilide-derived residues with soil organic matter does occur. However, information of this type has not been published for the N-alkyl substituted acylanilides. Because most of the herbicide material could not be recovered, this study was undertaken to characterize the fate of the metabolite. Evidence for polar and bound intermediates is presented.

# Materials and Methods

## Substrates

Alachlor was obtained from City Chemical Co. and was purified as described by Tiedje and Hagedorn (1975). The final purity was 99.9% as determined by GLC. Uniformly $^{14}$C-ring-labeled alachlor (specific activity, 1.73 mCi/m mole) was supplied by Monsanto Chemical Co., St. Louis, Mo. The label purity was 99.7% by thin-layer chromatography.

2-chloro-2',6'-diethylacentanilide (demethoxymethylalachlor) was prepared by hydrolysis of alachlor in 5 N HCl according to the procedure of Hargrove and Merkle (1971). The purity of the final product was 99.9% by GLC (Tiedje and Hagedorn, 1975). $^{14}$C-ring-labeled demethoxymethylalachlor was prepared from $^{14}$C-ring-labeled alachlor in the same manner, the specific activity was 0.05 mCi/m mole.

Antor was obtained as a formulation from Hercules, Inc., Wilmington, Dela., and was purified by the same method used for alachlor. This procedure was repeated until the Antor purity was 99.9% by GLC. $^{14}$C-ring- (specific activity 1.21 mCi/m mole) and $^{14}$C-carbonyl-labeled Antor (0.09 mCi/m mole) were also provided by Hercules, Inc. The carbonyl label was located in the chloroacetyl moiety.

$^{14}$C-alachlor and $^{14}$C-Antor metabolites were prepared by incubating these substrates with resting cells of Chaetomium globosum as previously described (Tiedje and Hagedorn, 1975; McGahen and Tiedje, 1977). The incubation was long enough so that most of the substrate was metabolized. The metabolites were extracted with $CHCl_3$, the solvent removed, and the metabolites redissolved in water-ethanol (9:1, v/v). The activity of this alachlor metabolite solution used for binding experiments was 6 nCi/ml; this solution was filter sterilized before use. Metabolites used as TLC standards were extracted in $CHCl_3$ and concentrated prior to use.

Soil incubation

Incubation studies were conducted in 250 ml Erlenmeyer flasks which contained 30 g of soil that had passed through a 2 mm sieve. The soils were stored in large sealed jars at 4°C and never allowed to air-dry before use. These herbicide materials were dissolved in water solution (usually 100 ppm) and distributed dropwise on the soil surface. Final substrate concentrations were 5 to 10 μg/g soil. Distilled water was added as necessary to moisten the soil to within 60 to 80% of water holding capacity. All soils were incubated sealed, in the dark at 28°C. The respired $^{14}CO_2$ was trapped in 1 ml 1 N NaOH which was contained in a disposable 2 ml beaker suspended above the soils.

Incubations were terminated by extraction of the soil with either benzene-isopropanol (2:1, v/v) or hexane-acetone (9:1, v/v) (Chapter I). Three successive extractions were used as this was found to entirely remove the substrate.

The effectiveness of more rigorous extractants at removing residual $^{14}C$ materials was examined using soils which had been extracted with benzene-isopropanol. The following extractants were used: $H_2O$, p-dioxane, dimethylformamide (DMF), tetrahydrofuran (THF)-ethanol (3:1, v/v), 0.1 M Na-EDTA, 0.1 M $Na_4P_2O_7$ (pH=7.0) and 0.5 N NaOH. All soil samples were shaken in the extractant for 24 h on a rotary shaker. An additional set of soil samples was extracted by refluxing with $CHCl_3$ for 24 h. The soil was removed by centrifugation or filtration through Whatman No. 1 filter paper. The soluble $^{14}C$ was assayed by liquid scintillation counting (Chapter I). For extractants that removed colored humic material, the solution was combusted by wet oxidation (Allison, et al., 1965), the $^{14}CO_2$ trapped and counted. The $^{14}C$ remaining in soil after extraction was determined by the above combustion method on 5 g of air-dried soil. Four replicate samples were used for each extractant.

### Molecular weight fractionation of $^{14}C$-metabolites

Brookston soil incubated with $^{14}C$-alachlor and $^{14}C$-Antor for 30 days was extracted with hexane-acetone to remove non-polar substrate and metabolites, and re-extracted with $Na_4P_2O_7$ to remove polar and bound metabolites. The latter solution was fractionated according to molecular weight by ultrafiltration using an Amicon Diaflo cell, Model 52. The membranes (with their nominal molecular weight cut offs) and the sequences of use were: XM-300 (300,000 mw), XM-50 (50,000 mw), UM-2 (1,000 mw), and UM-05 (500 mw). The procedure employed was similar to that of Ogura (1974) and Wheeler (1976) who successfully used the method to fractionate dissolved organic matter in estuarine waters. The Diaflo cell was operated at the lowest pressure necessary to achieve the desired flow of 0.4 and 0.1 ml/min. Fifty milliliters of the pyrophosphate extract was fractionated until approximately 3 ml remained above the filter. Two 10 ml portions of distilled water were used to wash the concentrate above each filter. The filtrate and concentrate were assayed by liquid scintillation counting to determine the total $^{14}C$ in each fraction.

### Thin-layer chromatographic study of metabolites

The last fraction from the Diaflo filtration (mw< 500) was extracted with three 50-ml portions of diethyl ether. Anhydrous $Na_2SO_4$ was added to the combined ether extracts to remove water. The extracts were concentrated to 0.5 ml and 10 μl was spotted on a 250 μ pre-coated silica gel 60 F-254 plate (EM laboratory, Elmsford, NY). Alachlor and Antor metabolites produced by Chaetomium globosum were also spotted on the plates as standards. The sodium pyrophosphate extract from a $^{14}C$-demethoxymethylalachlor amended soil was also extracted with ether and analyzed on the same plate. TLC plates were developed in a non-polar solvent system--benzene-methanol (95:5, v/v) (Yu, et al., 1975) and a polar solvent system--1-butanol-glacial acetic acid-water (12:3:5, v/v) (Armstrong, et al., 1973). After development plates were examined for $^{14}C$ spots by autoradiography with Kodak No-Screen X-ray film.

## Binding of alachlor metabolites to soil and soil organic matter

$^{14}C$-alachlor metabolites and $^{14}C$-alachlor were incubated with soil and soil organic matter fractions to determine their binding capacity. For the soils experiments 30 g of propylene oxide sterilized Spinks and Brookston soils was incubated with 3 ml of alachlor metabolites. After the indicated incubation periods the soils were extracted with three 40 ml portions of hexane-acetone. The $^{14}C$ extracted was counted by liquid scintillation counting.

Humic acid was obtained by shaking Brookston soil with 0.1 M $Na_4P_2O_7$ (pH 7.0) under $N_2$ for 24 h, centrifuging out the soil, adding HCl until pH 2 was reached, and centrifuging to recover the humic acid pellet. The pellet was washed three times with distilled water and the humic acid freeze-dried. The humic powder was added to a flask and 1 ml of $^{14}C$-metabolite and $^{14}C$-alachlor solutions added. The flasks were flushed with $N_2$, sealed and incubated. After the indicated incubation periods the flasks were extracted with four 20-ml portions of hexane-acetone (8:2, v/v). The extracted $^{14}C$ was counted as above.

The humic-fulvic complex was collected as above except not fractionated by pH precipitation. Salts were removed by dialysis against water containing Dowex 50W-X8 H-form. Four days later 30 ml of the humic complex was placed in a flask and 2 ml of $^{14}C$-metabolites and -alachlor were added. The flasks were flushed with $N_2$, sealed and incubated in an anaerobic glove box. After incubation the flasks were extracted with three 50-ml portions of diethyl ether and the $^{14}C$ extracted counted as above. All data from binding experiments are means of triplicate flasks.

## Results and Discussion

The mass balance of $^{14}C$-alachlor products at different stages of decomposition is shown in Table 1. Very little of the $^{14}C$ was respired to $CO_2$ and only minor quantities could be recovered by organic solvent extraction. The major portion of the label, 86% at 30 days, required a more rigorous extractant for removal or could not be removed at all. The growth in the unaccounted for fraction relative to other fractions was greatest in the later stages of incubation, indicating that substrate and intermediate products were being slowly converted to the resistant soil humin fraction.

Three successive extractions with benzene-isopropanol 1 h after addition to soil was found to remove >97% of alachlor and demethoxymethylalachlor and was assumed to remove other hydrophobic intermediates as well. Following this exhaustive extraction other polar and more rigorous extractants were used (Table 2). Water, which should remove polar intermediates, recovered only 10% of the residual $^{14}C$. DMF and dioxane, which should not extensively alter soil organic matter though do extract

Table 1. Percent of added radioactivity recovered from $^{14}C$-alachlor after incubation in Brookston soil[a].

| Incubation time | Evolved as $^{14}CO_2$ | Hexane-acetone extract | $Na_4P_2O_7$ extract[b] | Unaccounted for |
|---|---|---|---|---|
| (days) | | (%) | | |
| 0 | 0.0 | 98.4 | - | 1.6 |
| 10 | 0.5 | 48.0 | 39.0 | 12.5 |
| 20 | 1.1 | 23.9 | 51.5 | 23.5 |
| 30 | 1.4 | 12.4 | 51.3 | 34.9 |

[a] 250 µg alachlor/30 µg soil; data are the means of four replications.

[b] pH of extracting solution was 11.5.

Table 2. Percent of added radioactivity recovered from $^{14}C$-alachlor and $^{14}C$-demethoxymethylalachlor after 20 days incubation in Spinks soil.

| Substrate[a] | As $^{14}CO_2$ | In benzene-isopropanol extract[b] | In soil residue | | | Total radioactivity recovered |
|---|---|---|---|---|---|---|
| | | | Treatment | Extracted | Combusted[b] | |
| Alachlor | 2.5 | 23.3 | NaOH (0.5 N)[c] | 45.4 | 23.7 | 94.8 |
| | 2.5 | 19.0 | $Na_4P_2O_7$ (0.1 M)[c] | 37.4 | 36.1 | 94.2 |
| | 2.5 | 23.5 | Na-EDTA (0.1 N)[d] | 22.0 | 45.7 | 93.6 |
| | 2.4 | 21.5 | THF-ethanol[d] | 19.9 | 49.1 | 92.9 |
| | 2.5 | 21.1 | DMF[d] | 23.3 | 31.1 | 78.3 |
| | 2.5 | 21.3 | p-dioxane[d] | 14.7 | 44.6 | 83.2 |
| | 2.4 | 21.5 | $CHCl_3$reflux[d] | 17.1 | 48.8 | 89.8 |
| | 2.5 | 21.6 | $H_2O$[d] | 10.1 | 53.2 | 87.3 |
| Demethoxymethyl alachlor | 7.5 | 11.1 | DMF[d] | 15.6 | 58.3 | 92.5 |
| | 7.8 | 12.6 | $Na_4P_2O_7$ (0.1 M)[c] | 35.3 | 37.3 | 93.0 |

[a] 250 µg substrate/50 µg soil; data are the means of four replications.

[b] Three successive extractions prior to extraction of soil residue.

[c] Residual soil organic carbon was combusted by wet oxidation and the $^{14}CO_2$ trapped and radioassayed.

[d] Extracts combusted to $CO_2$ and $^{14}CO_2$ radioassayed; the efficiency of combustion was 95.8%.

[e] Extracts directly assayed by liquid scintillation spectrometer.

a portion of it, extracted additional $^{14}C$. THF and EDTA which can alter the structure of organic matter was no more effective than DMF in removing residual $^{14}C$. The humus extractants, $Na_4P_2O_7$ and NaOH, recovered the most $^{14}C$. Because of the high pH of the NaOH extraction, substantial chemical alteration of the humus is thought to occur. These changes are far less severe with the pyrophosphate extractant if buffered at pH 7.0 and carried out under an inert atmosphere. In addition I found that the NaOH solution decomposed about 50% of added alachlor under the extractive regime while pyrophosphate had no effect. Thus, pyrophosphate was used as the humus extractant in subsequent studies.

The inability of water and other polar solvents to remove much of the $^{14}C$ indicates that most of the metabolites were bound to organic matter. This binding to organic matter is also shown by the significant quantities of $^{14}C$ removed by the humus extractant (pyrophosphate) and the large quantity remaining after this extraction, presumably in the humin fraction. The presence of label in the humin fraction was confirmed by recovery of $^{14}C$ as $^{14}CO_2$ by combustion of the soil.

Demethoxymethylalachlor, the alachlor metabolite (Chapter I), was converted somewhat more readily to $^{14}CO_2$ though most of the products were associated with the organic matter, as was the case for the parent herbicide. This similar fate is consistent with DMM being an important alachlor intermediate in soil. Antor, a structurally related acylanilide herbicide, had a similar fate. As shown in Table 3, little $^{14}C$ was recovered as $^{14}CO_2$ and by the organic solvent extraction while most appeared bound to organic matter.

If herbicide metabolites were bound to organic matter the $^{14}C$ material extracted with humus could be expected to be associated with

Table 3. Distribution of $^{14}C$ following 30 days of incubation of $^{14}C$-alachlor and $^{14}C$-Antor herbicide in Brookston soil.

| | | | Soil residue extracted by alkali treatment[a] | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Fractionation of $Na_4P_2O_7$ extract by mol wt. | | | | |
| Substrate[a] | As $^{14}CO_2$ | Hexane-acetone extract | $Na_4P_2O_7$ extract | >300,000 | 300,000 to 50,000 | 50,000 to 1000 | 1000 to 500 | <500 |
| | (%) | | | | | | | |
| Alachlor (ring) | 1.4 | 12.4 | 51.3 | 2.7 | 1.6 | 2.6 | 2.2 | 35.8 |
| Antor (ring) | 2.2 | 2.2 | 62.7 | 1.3 | 1.0 | 1.9 | 2.8 | 50.2 |
| Antor (carbonyl) | 4.4 | 2.0 | 62.4 | 2.4 | 1.2 | 2.2 | 3.6 | 47.2 |

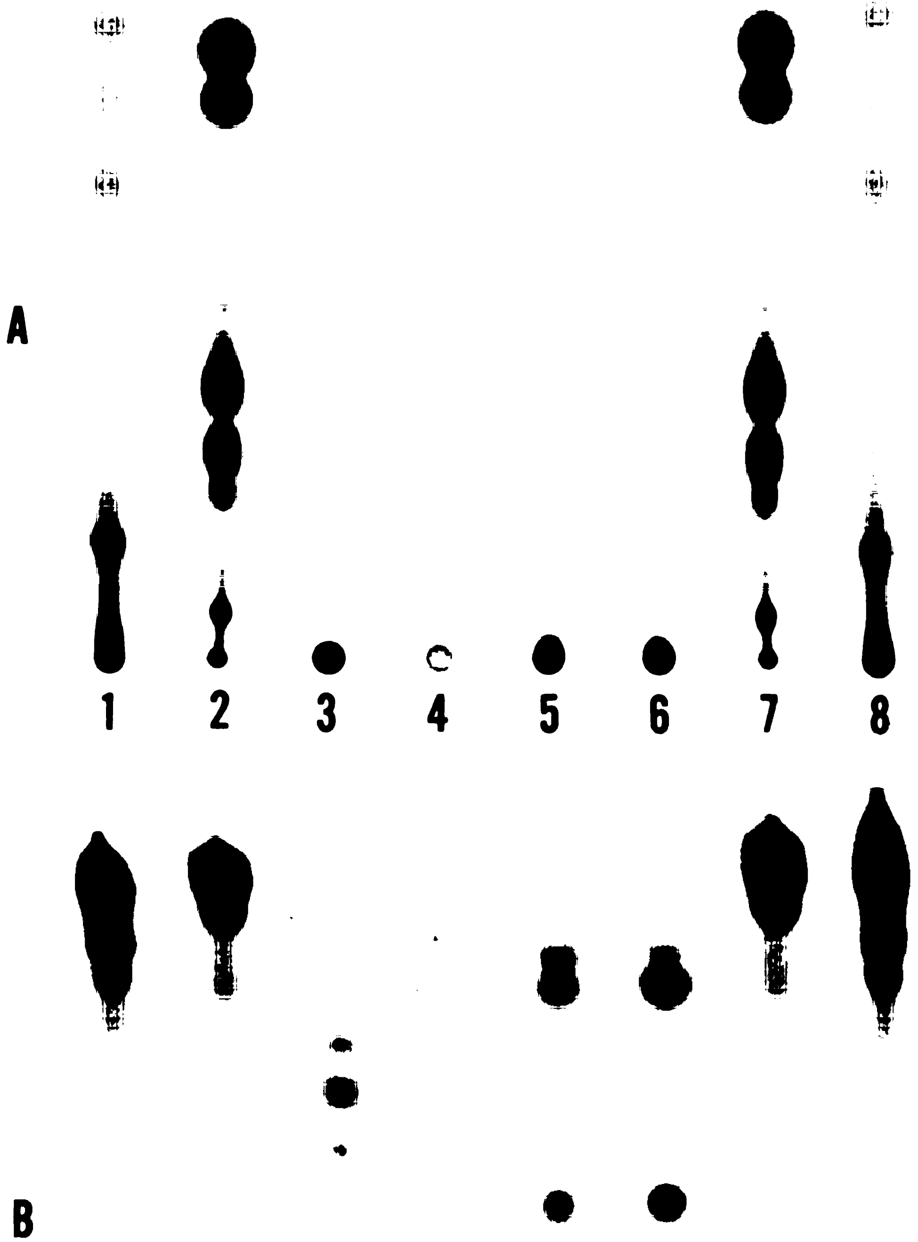
[a] 300 μg substrate/30 g soil; data are means of triplicate flasks.

larger molecular weight organic matter. Though most of the $^{14}C$ passed through the 500 molecular weight exclusion filter (Table 3), 8 to 10% was excluded by the smallest molecular weight filter. The somewhat continuous distribution of $^{14}C$ with molecular weight is the type of distribution that would also be expected for soil organic matter.

The small molecular weight $^{14}C$ materials (<500 mw) were examined by GLC and TLC. The former showed no products that would run on GLC (3% SE-30 column, Chapter I). The autoradiogram of the $^{14}C$ products separated by TLC is shown in Figure 1. The products did not move from the origin in a non-polar solvent system (Figure 1A), while most of the fungal produced metabolites did. These results show that none of the substrate remained in this extraction, thus confirming the completeness of the initial solvent extraction and that the substrates were metabolized to other products. The $^{14}C$ products were separated with the polar solvent system (Figure 1B). None of the products match the fungal metabolites. Alachlor and demethoxymethylalachlor have one product in common while the former shows two other products. None of these products were the same as those found in solvent extracts of soil (Chapter I). The Antor products from the $^{14}C$ ring and carbonyl label are the same, indicating retention of the chloroacetyl moiety in the metabolites. Three major and three minor Antor products were observed.

The above chromatographic characteristics indicate that these products are polar. Because of their small molecular weight they are probably not bound or conjugated to other organic material. Since their quantity is greater than that recoverable by water extraction, they were probably either masked by the organic matter or bound but released by the pyrophosphate extraction.

Figure 1. Autoradiograms of alachlor and Antor metabolites found in sodium pyrophosphate extracts of soil. A, non-polar solvent system; B, polar solvent system. 1 & 8, fungal metabolites of alachlor; 2 & 7, fungal metabolites of Antor. Pyrophosphate extracts of soil after 30 days incubation of: 3, $^{14}$C-alachlor; 4, $^{14}$C-demethoxymethylalachlor; 5, $^{14}$C-ring labeled Antor; 6, $^{14}$C-carbonyl labeled Antor.


A
1
2
3
4
5
6
7
8
B

## Binding of fungal alachlor metabolites to soil

The binding to soil of solvent extractable alachlor metabolites produced by C. globosum is shown in Table 4. The loss in extractability of $^{14}C$ metabolites was noted immediately, 0 time (within 1 h), and increased to less than 1/2 of the quantity added at 4 days. Because my previous results implied that organic matter was the soil component responsible for binding, similar extractability studies were done after exposure of the metabolites to humic acid powder (Table 5) and to humic and fulvic acid solution (Table 6). The metabolites but not alachlor were bound to the humic materials. The more rapid binding by the solution was probably due to the greater reactivity of the freshly prepared solution compared to the stored humic powder. Both materials, however, showed the degree of binding noted in soil (Table 4). Thus some nonpolar as well as polar metabolites may be bound to 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 derivative 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-diethylaniline, has been shown by Tiedje and Hagedorn (1975) to be an intermediate of alachlor degradation by a soil fungus. It is not known if degradation of the acylanilides proceeded this far in soil, however. Propachlor which is more similar to alachlor than propanil because of the N-alkyl substituent, was reported to show very little mineralization of the $^{14}C$-carbonyl group in soil (Kaufman et al., 1971). This suggests that aniline was not formed rapidly from this

Table 4. Percent of $^{14}C$ that could be extracted from sterilized Spinks and Brookston soils to which $^{14}C$-alachlor metabolites produced by fungi had been added.

| | $^{14}C$ recovered in hexane-acetone extract[a] | | |
|---|---|---|---|
| Incubation time | Control (no soil) | Spinks soil | Brookston soil |
| (day) | (%) | | |
| 0 | 97.1 ± 0.3 | 76.8 ± 0.3 | 73.7 ± 1.4 |
| 2 | 97.6 ± 0.4 | 55.6 ± 0.4 | 55.9 ± 1.8 |
| 4 | 97.1 ± 0.2 | 48.3 ± 0.7 | 48.7 ± 0.3 |

[a] Each value is the mean of three replications.

Table 5. Loss in extractability of alachlor and alachlor metabolites following incubation with humic acid powder.

| Incubation Time | $^{14}$C-extracted[a] Alachlor | $^{14}$C-extracted[a] Metabolites |
|---|---|---|
| (day) | (%) | (%) |
| 0 | 94.9 | 96.1 |
| 2 | - | 73.9 |
| 3 | 93.6 | - |
| 6 | - | 58.2 |
| 10 | 92.9 | - |

[a] Mean of triplicate determinations.

Table 6. Loss in extractability of alachlor and alachlor metabolites following incubation with freshly extracted humic and fulvic acid.

|  | $^{14}C$ extracted[a] |  |
|---|---|---|
| Incubation time | Alachlor | Metabolites |
| (day) | (%) |  |
| 0 | 90.7 | 66.6 |
| 3 | 90.3 | 65.6 |
| 6 | 85.9 | 57.9 |

[a] Mean of triplicate determinations.

herbicide. My results with $^{14}C$-carbonyl-Antor also indicated that soil metabolism did not reach the aniline stage. Thus, aniline binding may not be a generally important mechanism to acylanilide fate in soil.

The large number of complex metabolites, many showing secondary ring closure, found as fungal products of alachlor, Antor and metolachlor (Tiedje and Hagedorn, 1975; McGahen and Tiedje, 1977) may be significant intermediates in soil. Most of these retain the chloroacetyl moiety and would be expected to be persistent. Also the precursors of the ring closure are reactive and could be expected to covalently bond to soil organic matter. This could explain why these compounds could not be recovered by solvent extraction.

The N-alkyl substituted acylanilides all seem to show a similar fate in soil. Very little of the herbicide is metabolized to $CO_2$, only minor quantities are converted to non-polar extractable intermediates and major quantities are bound to organic matter. Some polar metabolites are in this latter fraction but these appear to be only a minor portion. Thus it appears that these herbicides are rather rapidly degraded to components that cannot be distinguished from soil humus. The slow rate of $^{14}CO_2$ production is consistent with what is expected for turnover of humus. Extrapolations based on following $^{14}CO_2$ production from alachlor for 70 days suggest a period of 3 to 4 years before most of the $^{14}C$ label would be mineralized. Because the metabolites are so intimately associated with humus, environmental hazards would not be expected.

# Literature Cited

1. Allison, L. E., W. B. Bollen, and C. D. Modie. 1965. Total carbon. In Methods of Soil Analysis, C. A. Black (ed.), Amer. Soc. Agron. Inc., Madison, Wisc., pp-1346-1366.

2. Armstrong, T. F., W. F. Meggitt, and D. Penner. 1973. Adsorption, translocation, and metabolism of alachlor by yellow nutsedge. Weed Sci. 21:357-359.

3. Bartha, R. 1971. Fate of herbicide-derived chloroaniline in soil. J. Agri. Food Chem. 19:385-387.

4. Chou, S. F. and J. M. Tiedje. 1975. Interaction of alachlor biodegradation intermediates with soil organic matter. Agron. Abst. 127.

5. Hargrove, R. S. and M. G. Merkle. 1971. The loss of alachlor from soil. Weed Sci. 19:652-654.

6. Hsu, T. S. and R. Bartha. 1974a. Interaction of pesticide-derived chloroaniline residues with soil organic matter. Soil Sci. 116:444-452.

7. Hsu, T. S. and R. Bartha. 1974b. Biodegradation of chloroaniline-humus complexes in soil and in culture solution. Soil Sci. 118:213-220.

8. Hsu, T. S. and R. Bartha. 1976. Hydrolyzable and non-hydrolyzable 3,4-dichloroaniline-humus complexes and thier respective rates of biodegradation. J. Agri. Food Chem. 24:118-122.

9. Kaufman, D. D., J. R. Plimmer, and J. Iwan. 1971. Microbial degradation of propachlor. Amer. Chem. Soc. Abst. 162nd Meeting, Washington, D. C. A-21.

10. McGahen, L. L. and J. M. Tiedje. 1977. Degradation of two new acylanilide herbicides, Antor and Dual by the soil fungus, Chaetomium globosum. Submitted to J. Agri. Food Chem.

11. Ogura, N. 1974. Molecular weight fractionation of dissolved organic matter in coastal seawater by ultrafiltration. Mar. Biol. 24:305-312.

12. Tiedje, J. M. and M. L. Hagedorn. 1975. Degradation of alachlor by a soil fungus, Chaetomium globosum. J. Agri. Food Chem. 23:77-81.

13. Yu, C. C., G. M. Booth, D. J. Hansen, and J. R. Larsen. 1975. Fate of alachlor and propachlor in a model ecosystem. J. Agri. Food Chem. 23:877-879.

14. Wheeler, J. R. 1976. Fractionation by molecular weight of organic substances in Georgia coastal water. Limnology and Oceanography. 21:846-852.

# CHAPTER III

# CHARACTERIZATION OF POLYBROMINATED BIPHENYLS AND THEIR PERSISTENCE IN SOIL

## Introduction

In 1973 an industrial flame retardant (fireMaster BP-6, manufactured by Michigan Chemical Corporation, St. Louis, Michigan) was accidentally added to livestock feed in place of magnesium oxide (Carter, 1976; Isleib and Whitehead, 1975; Robertson and Chynoweth, 1975). The addition of this chemical, which is a mixture of several polybrominated biphenyl (PBB) isomers, into livestock rations had a catastrophic impact on Michigan's livestock industry and resulted in thousands of farm animals killed and buried. Altogether 15,942 cattle, 2,700 swine, 1,200 sheep, and 1.5 million domestic fowl had been killed and buried by May 3, 1975 (Robertson and Chynoweth, 1975). This incident has been termed the most costly and disastrous accidental contamination ever to occur in United States agriculture (Isleib and Whitehead, 1975). The most tragic aspect, however, was the extensive contamination of farm families from eating their PBB contaminated products, the consequences of which will probably not be known for some time.

Most of the attention has been focused on the effects of PBB on animals and humans. However, information on the contamination of other ecosystem components such as plants, soils, water, and air is needed to evaluate the fate of PBB's that have entered soils by contaminated manure, milk, carcasses, and from the manufacturing plant. This study reports the chemical characterization of fireMaster BP-6 and the persistence of PBB in soils.

## Materials and Methods

### PBB Substrates

$^{14}$C-PBB (lot 872-244) was synthesized and purified according to our specifications by New England Nuclear Corp., Boston, Mass. The product contained the two major isomers of fireMaster BP-6, approximately 65% 2,2'4,4',5,5'-hexabromobiphenyl and 35% 2,2',3,4,4',5,5'-heptabromobiphenyl (Moore et al., 1977). Identity was confirmed by finding identical retention times to standards run on Dexsil and SE-30 gas chromatographic columns. No other components were detected by gas chromatography. The specific activity was 9.34 mCi/m mole. This $^{14}$C-PBB was mixed 1:1 with fireMaster BP-6, lot 6244A, in ethanol to achieve a final concentration of 21 ppm; this solution was added to soil.

Since PBB is readily photodegraded by sunlight, products from UV photolysis of PBB were also added to soil. The photolyzed $^{14}$C-PBB solution was prepared by irradiating a mixture of 50 ppm fireMaster BP-6 and 9.4 ppm of $^{14}$C-PBB in hexane for 30 min a Hanovia photolysis vessel (Ace Glass Incorporated, Vineland, N.J.). The exposed solution turned light yellow and showed some precipitate. The clear fraction was decanted and concentrated to 25 ml. This photolyzed material was diluted in ethanol (14 x) to achieve a radioactive concentration similar to the non-photolyzed sample prior to addition to soil.

### Soil Incubations

The susceptibility of PBB's to biodegradation was evaluated using Brookston sandy loam and Spinks loamy sand soils which were freshly collected from the University Experimental Farms. Twenty-five grams of soil which had passed through a 2 mm sieve was placed in a 125 ml

Erlenmeyer flask. The soil was not allowed to air dry. One milliliter containing 100 μg of PBB in acetone was distributed in microdrops over the soil surface. The soil in each flask was then moistened with 5 ml of basal salt solution. Half of the flasks received additional organic matter in the basal salts solution in an attempt to stimulate cometabolism. This amendment per flask was 0.2% glucose, 0.2% peptone, 0.2% Na acetate, and 0.05% m-bromobenzoic acid per gram fresh weight of soil. The flasks were closed with a rubber stopper and incubated in the dark at 28 C, except for a set of Brookston soils with the organic amendment which was incubated at room temperature in an anaerobic glove box (Eh $<$ 0.05 mv). Three flasks were analyzed for each treatment after 0, 6, 12, and 24 weeks of incubation.

In the second part of this study twenty-five grams of Brookston sandy loam soil which had passed through a 2 mm sieve was placed in a 250 ml Erlenmeyer flask. Half of the flasks were sterilized as follows prior to addition of the two substrates. Each of thse flasks received 2 ml water and, 1 h later, 3 ml of propylene oxide, which was distributed dropwise on the soil surface. The flasks were covered with foam plugs and placed in a hood for 3 days to allow the sterilant to volatilize. Sterilization was confirmed by inoculating a loopfull soil on a trypticase agar slant and finding no growth after one week of incubation.

Sterilized and non-sterilized flasks received either 1 ml of the filter sterilized $^{14}$C-PBB solution or 1 ml of the UV-irradiated $^{14}$C-PBB solution distributed dropwise on the soil. Three milliliters of sterilized distilled water was used to moisten each soil. All flasks were then sealed with a sterilized rubber stopper. A 2 ml sterilized plastic beaker which contained 1 ml 1N NaOH was suspended above the soil to trap respired $^{14}CO_2$. All samples were incubated in the dark at 28 ± 1° C for 0, 3, 6, 9, or 12 months. The $^{14}CO_2$ traps were counted periodically as described elsewhere (Tiedje and Mason, 1974). Four flasks of each treatment were extracted after each incubation period. The data presented are the means of four replicates.

## Analyses

PBB was extracted from soil with three 30-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 250 rpm for 30 min. After decanting the solvent, the second and third extractions were made by shaking solvent and soil at 250 rpm for 30 min prior to decanting. The extracts were combined and two 20-ml portions of deionized distilled water were added to remove the isopropanol. Anhydrous $Na_2SO_4$ was added to the solvent to remove remaining water.

For comparison of the PBB extraction efficiency of solvent systems, 50 g of soil from a previous greenhouse study which was amended 100 ppbw PBB's (Jacobs et al. 1976) was placed in 250 ml Erlenmeyer flasks and moistened with 11 ml distilled water. The solvent systems tested were three 50-ml portions each of benzene-isopropanol (2:1, v/v), hexane-acetone (9:1, v/v), and benzene-hexane (1:1, v/v).

In the second part of the study $^{14}C$-PBB was extracted from soil with three 40-ml portions of hexane-acetone (9:1 v/v) since this reduced the amount of soil organic matter extracted (Table 1). Before extraction the soil samples were vibrated on a mini-shaker to ensure moisture uniformity. The extraction procedure was the same as used previously

Table 1. Recovery of six major PBB isomers from soil by using different solvents

| Solvent | Recovery of PBB, percent of original amendment[a] | | | | | |
|---|---|---|---|---|---|---|
| | 5 BrI | 5 BrII | 6 BrI | 6 BrII | 6 BrIII | 7 Br |
| Benzene-isopropanol (2:1, v/v) | 88.7 | 91.6 | 87.8 | 80.3 | 77.6 | 91.6 |
| Hexane-acetone (9:1, v/v) | 95.0 | 98.0 | 99.3 | 87.3 | 98.2 | 97.6 |
| Benzene-Hexane (1:1, v/v) | 29.4 | 33.5 | 43.0 | 26.1 | 22.4 | 44.3 |

[a] mean of triplicates.

except for the first extraction, in which the soil-solvent mixture was now allowed to stand in the flask for 1 h.

The $^{14}C$ was assayed by liquid scintillation counting. One milliliter of concentrated hexane-acetone extract was counted in 15 ml Bray's solution (Bray, 1960). The $^{14}CO_2$ trapped in 1 N NaOH was counted in Bray's solution containing 4% Cab-O-Sil. All counts were corrected for quenching by external standardization and for machine efficiency.

Concentrated extracts were analyzed on a Beckman GC-5 gas chromatograph equipped with an electron capture detector and a 2% Dexsil 300 gc column; the column and conditions have described by Filonow et al. (1976). A glass column, 1.83 m x 3 mm ID, containing 2% SE-30 on 100/120 mesh Gas-Chrom Q and operated at 265 C with a carrier flow of 40 ml/min was used for the analyses of samples from the second part of the experiment and greenhouse soil samples as it was faster and provided slightly greater separations (Figure 1). A stainless steel column of 3% SE-30 on 100/120 mesh Cromosorb W, 1.83 m x 2.3 mm ID, was used in Perkin Elmer 900 gas chromatograph for detection of components in fireMaster BP-6 by flame ionization; the column was programmed from 150 to 260 C at 8 degree/min.

The mass spectra were obtained with an LKB 9000 gas chromatograph-mass spectrometer with an ionizing voltage of 70 eV. The glc column was 3% SP-2100 on Supelcoport, 1.83 m x 3 mm ID, and programmed from 200 to 300 C at 5 degree/ min. The proton nmr was done on a Varian DA60 and the C-13 nmr was done on a Varian CFT-20 instrument with $CDCl_3$ as the solvent; the sample analyzed was the 6 Br(I) peak purified from fireMaster BP-6 (Filonow et al., 1976).

For the thin-layer chromatography study of PBB components, the hexane-acetone extracts were concentrated to 0.5 ml; 10 μl was spotted on 250 μ pre-coated Kieselguhr G plates (Analtech, Inc.) pretreated with parafin according to the method of DeVos and Peet (1971). The precoated plate was soaked in petroleum ether (b.p. 40-60°C) containing 8% of liquid parafin until the adsorbent layer was saturated with the solvent. The plates were developed in parafin saturated acetonitrile-acetone-methanol-water (20:9:20:1, v/v). After development the $^{14}C$ on the plates was localized by autoradiography.

### Pure Culture Studies

Studies were done with two biphenyl degrading bacteria, *Nocardia sp*. (NCIB 10603) and *Pseudomonas sp*. (NCIB 10643), for which the biphenyl catabolic pathway is known to see if they could metabolize any of the six major PBB isomers when present in a biphenyl growth medium (Baxter et al., 1974).

## Results and Discussion

The fireMaster BP-6 product was found to be a mixture of at least eighteen different components which could be separated and detected by flame ionization gas chromatography on a 3% SE-30 column. However, 98% of the product is accounted for the six major peaks shown in Figure 1 (peaks 1 to 6). The quantity of each component varies somewhat with each batch, but the hexabromobiphenyl isomer I (peak 3) was always the largest and a heptabromobiphenyl isomer (peak 6) the second largest in the samples examined.

The peaks were identified from gc-mass spectra (Figure 2) to be isomers of pentabromobiphenyl (M+ 544), hexabromobiphenyl (M+ 622) and

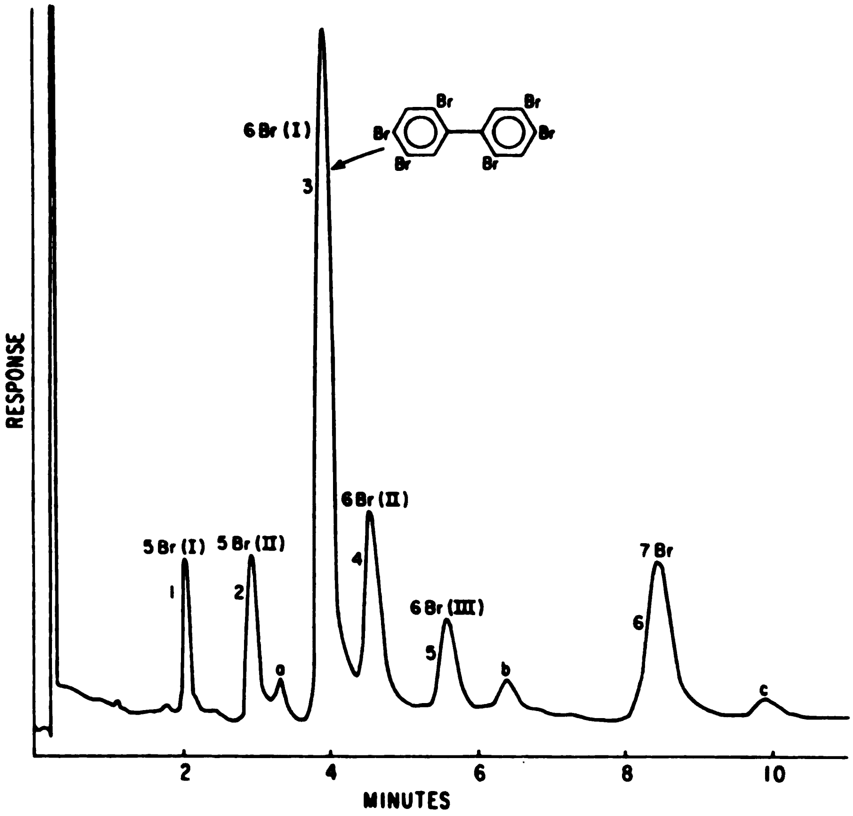


Figure 1. Gas chromatogram showing the six major components of PBB (peaks 1 to 6) and their identity, and the three minor components (peaks a to c). The major component has the isomeric structure shown.

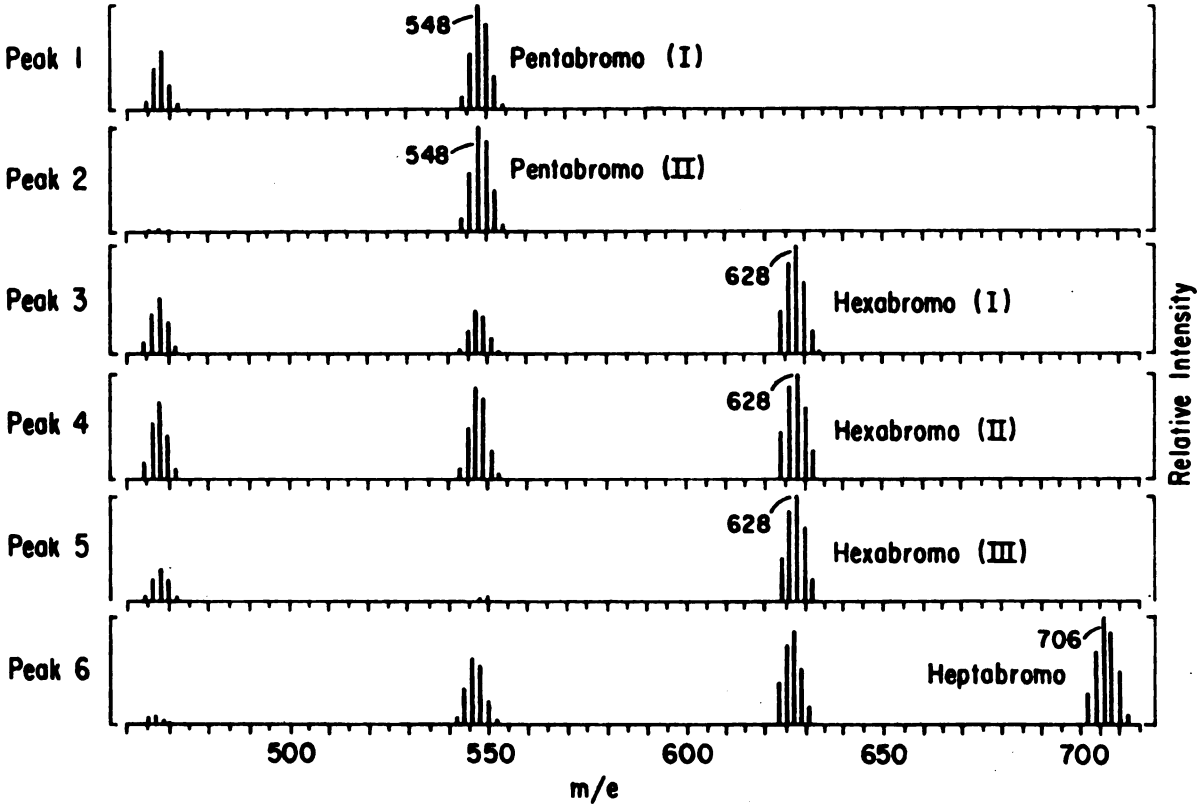


Figure 2. Mass spectra of the six major components of PBB; m/e of base peak is identified.

heptabromobiphenyl (M+ 700). The major component, 6 Br(I), was previously known to be a hexabromobiphenyl from gc-ms analysis of PBB's, but mass spectrometry provides no structural information. It can be seen from Figure 3 that the proton nmr spectrum shows the presence of two distinct peaks of equal intensity; thus the major isomer has a symmetrical structure. The major component was identified by carbon-13 nmr to be 2,2',4,4',5,5'-hexabromobiphenyl (Figure 1) on the following evidence: δc, ppm from TMS: C-1, 140.3, biphenyl linkage; C-2, 122.4, 2 Br; C-3, 136.7, 2 H; C-4, 125.9, 2 Br; C-5, 123.8, 2 Br; C-6, 134.8, 2 H (Figure 4). The hydrogen shifts were confirmed by a Gated Decoupling. The three minor components which are shown in the glc trace in Figure 1 but which were not quantitated in these studies were identified by gc-mass spectrometry as follows: a, hexabromobiphenyl; b, heptabromobiphenyl; and c, unknown but suspected to be octabromobiphenyl.

The recovery of PBB components after incubation in Brookston soil is shown in Table 2. It is clear that the PBB persisted for one-half year in soil with only slight disappearance of three of the components and no disappearance of the other three. The same observation of persistence holds for incubations with added available organic matter, under anaerobic conditions, and for Spinks soil as is summarized in Table 3. Since Spinks soil is more coarse textured and contains less organic matter may account for the inactivity of this soil for PBB degradation. The persistence of these PBB compounds is consistent with the evidence reported for PCB compounds which shows that the more heavily chlorinated members (penta or greater) are resistant to degradation though many lesser chlorinated components are metabolized (Ahmed and Focht, 1973; Baxter _et al._, 1975; Tucker _et al._, 1975).

Figure 3. 60 MHz proton NMR spectrum of the major isomer (6 BrI) of fireMaster BP-6.

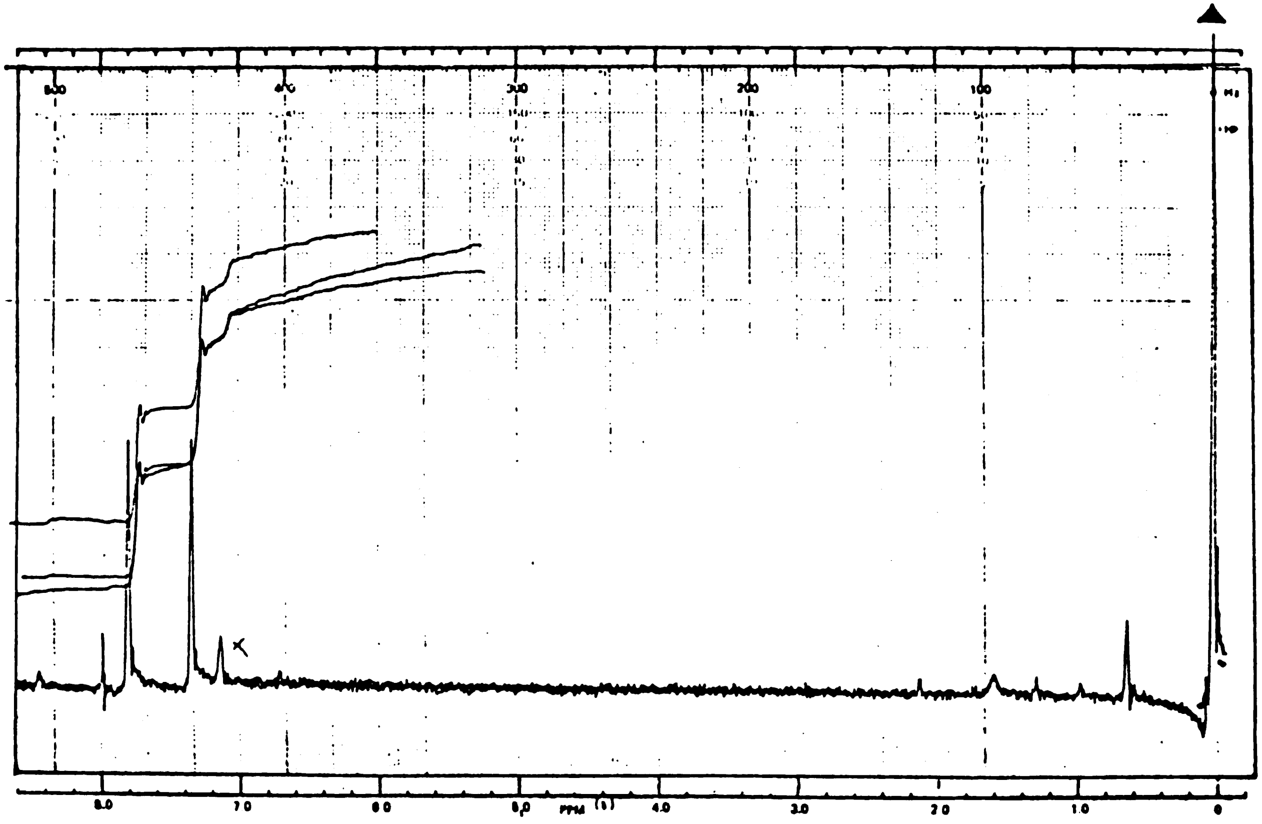

500
400
300
200
100
8.0
7.0
6.0
5.0
PPM (1)
4.0
3.0
2.0
1.0
0

Figure 4. C-13 NMR spectrum of the major isomer (6 BrI) of fireMaster BP-6.

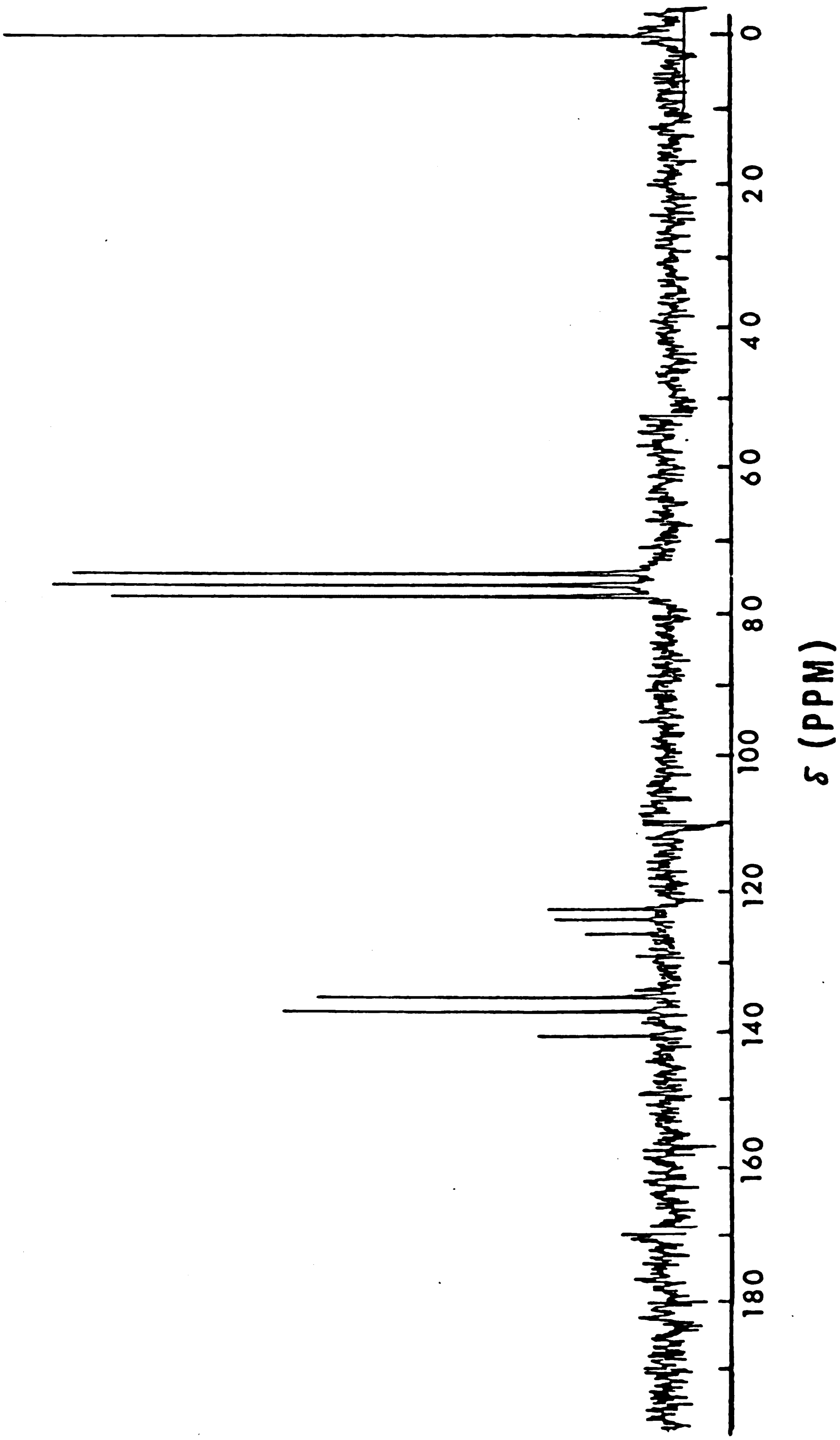

0
20
40
60
80
100
120
140
160
180
δ (PPM)

Table 2. Recovery of six major PBB isomers after incubation of 4 ppm in Brookston soil.

| Incubation time (weeks) | Recovery of PBB, percent of original amendment* | | | | | |
|---|---|---|---|---|---|---|
| | 5 Br I | 5 Br II | 6 Br I | 6 Br II | 6 Br III | 7 Br |
| Glassware | $70.3^{a}$ | $88.0^{a}$ | $85.7^{a}$ | $83.1^{a}$ | $71.0^{a}$ | $73.1^{a}$ |
| 0 | $74.4^{a}$ | $87.0^{a}$ | $83.2^{a}$ | $82.5^{a}$ | $74.2^{a}$ | $76.7^{a}$ |
| 6 | $70.0^{a}$ | $69.3^{b}$ | $82.0^{a}$ | $76.2^{ab}$ | $74.5^{a}$ | $75.7^{a}$ |
| 12 | $72.0^{a}$ | $65.6^{b}$ | $67.2^{b}$ | $71.0^{bc}$ | $69.8^{a}$ | $78.0^{a}$ |
| 24 | $67.5^{a}$ | $65.0^{b}$ | $66.7^{b}$ | $69.3^{c}$ | $70.8^{a}$ | $76.3^{a}$ |

*a,b,c = Ranges of equivalence within columsn at P (.05).

Table 3. F ratios for comparison of recoveries of 6 PBB isomers at zero time and after 24 weeks of incubation.

| Treatment | 5 Br I | 5 Br II | 6 Br I | 6 Br II | 6 Br III | 7 Br |
|---|---|---|---|---|---|---|
| Brookston soil | (60.26)*** | (58.33)*** | (43.87)*** | (31.37)*** | (0.21) | (0.01) |
| Brookston, anaerobic + OM[†] | ( 4.18) | (50.93)*** | ( 1.16) | ( 2.56) | (24.25)*** | (6.75)* |
| Brookston + OM[†] | ( 2.24) | (44.25)*** | ( 0.07) | ( 6.18)* | (48.96)*** | (0.52) |
| Spinks soil | ( 1.47) | ( 0.02) | ( 0.43) | ( 0.09) | ( 0.10) | (6.13)* |

* Disappearance significant at P (.10).

** Disappearance significant at P (.05).

*** Disappearance significant at P (.01).

[†] Amendment of organic matter.

A difficulty in these studies is the lack of precision due to variability in quantity recovered among replications. This does not appear to be due to technique since ratios between peaks vary in a random manner among the extracted solutions. It is also evident from Table 2 that a substantial loss occurred in the glassware in the absence of soil. These analytical difficulties are also apparent in other reports (Babish et al., 1975; Gutenmann and Liske, 1975). Given these difficulties, one can only interpret the highly significant and consistent trends in the data. Only the disappearance of the second pentabromobiphenyl isomer (5 BrII) in the Brookston soil (Table 3) fulfills this condition. Whether this disappearance is due to microbial degradation, sorption, or masking was not resolved although some biodegradation seems possible since only a specific isomer showed a decrease and there is precedence for microbial metabolism of certain dichloro substituted aromatic rings of PCB compounds (Ahmed and Focht, 1973; Baxter et al., 1975). It is feasible that certain dibromo substituted aromatic rings of pentabromobiphenyls could be attacked since biphenyl oxidizing bacteria require an adjacent 2-3 position to form the first biphenyl metabolite cis-2,3-dihydro-2,3-dihydroxybiphenyl (Gibson et al., 1973).

In pure culture studies, no PBB metabolites were found in biphenyl medium after 21 days of incubation. Apparently these two microorganisms can not degrade fireMaster BP-6.

In the second part of the study the recovery of the two major PBB isomers (hexa- and heptabromobiphenyls) after various periods of incubation in Brookston sterile and non-sterile soil is shown in Table 4. Whether analyzed by $^{14}C$ or gas chromatography the data clearly show no detectable biodegradation after 1 year. It is also striking that the

Table 4. Recovery of the two major isomers (hexa- and heptabromobiphenyls) after incubation of 0.71 ppmw in Brookston sandy loam soil.

| | | % of the original amount of PBB recovered[a] | | | | |
|---|---|---|---|---|---|---|
| Analysis | sterilized | 0 month | 3 months | 6 months | 9 months | 12 months |
| $^{14}C$ extracted | yes | 93.3[b] | 86.8 | 88.5 | 85.5 | 84.7 |
| | no | 92.7 | 85.3 | 87.3 | 84.8 | 84.3 |
| Hexa isomer by GLC | yes | 91.5[c] | 87.0 | 89.5 | 83.8 | 85.6 |
| | no | 90.9 | 86.2 | 85.4 | 87.8 | 84.9 |
| Hepta isomer by GLC | yes | 89.6[d] | 85.3 | 87.1 | 82.2 | 83.9 |
| | no | 89.1 | 82.9 | 86.2 | 82.1 | 83.8 |

[a] Each value is the mean of four reps.

[b] % of total $^{14}C$ recovered.

[c] % of the hexabromobiphenyl isomer recovered by GLC analysis.

[d] % of the heptabromobiphenyl isomer recovered by GLC analysis.

$^{14}C$ and GLC analysis of each peak showed virtually identical quantities on each date. GLC data of the recovery of non-$^{14}C$ labelled isomers is shown in Table 5. The only possible evidence for degradation is for the 5 BrI isomer, which does not confirm the previous suggestion which indicated that only the 5 BrII isomer might have been subject to slight biodegradation. Of interest is the significant loss in extractability with time of all isomers (Table 4 & 5) in the sterile as well as non-sterile treatments.

Total $^{14}C$ collected in 1N NaOH is shown in Table 6. Though slightly more label was trapped from non-sterilized soil, the amount of additional label volatilized in the viable treatment was insignificant. Soils incubated with photodegradation products of $^{14}C$-PBB showed enhanced though still only minor conversion to $^{14}CO_2$. Products created by the photo-decomposition of PBB by UV-irradiation are apparently more volatile than non-irradiated PBB as shown by the increase in $^{14}C$ collected from sterilized soils, especially at the first sampling. The microbial activity which occurred in the non-sterilized treatments appeared to increase the amount of $^{14}C$ volatilized, suggesting that some of the $^{14}C$-degradation products of UV-irradiation may be metabolized. PBB irradiated by UV-light forms lower brominated biphenyls (Ruzo and Zabik, 1975).

The extractability of the $^{14}C$ photodegraded PBB is shown in Table 7. Much of the added material was not extracted, in marked contrast to PBB (Table 2). Apparently the photodegraded products are more reactive with the soil organic matter thereby preventing their extraction. The early loss of extractability (0 and 3 months) is supportive of this explanation.

Table 5. Recovery of PBB isomers after incubation of 0.4 ppmw in Brookston soil.

| Incubation time (months) | Percent of PBB isomers of original amendment recovered[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 5 Br I | | 5 Br II | | 6 Br II | | 6 Br III | |
| | S | N-S[c] | S | N-S | S | N-S | S | N-S |
| 0 | 87.1 | 86.3 | 89.4 | 88.0 | 83.4 | 84.6 | 79.4 | 80.7 |
| 3 | 80.3 | 79.2 | 78.2 | 76.3 | 80.5 | 80.0 | 74.7 | 73.7 |
| 6 | 77.4 | 79.5 | 75.7 | 73.7 | 81.3 | 80.6 | 71.3 | 70.6 |
| 9 | 78.9 | 75.4 | 76.1 | 74.5 | 82.5 | 79.3 | 72.1 | 70.2 |
| 12 | 77.6 | 71.4 | 72.7 | 70.3 | 79.9 | 78.5 | 70.7 | 71.4 |

[a] Each value is the mean of four replications.

[b] S = sterilized soil.

[c] N-S = non-sterilized soil.

Table 6. Percent of original $^{14}C$ evolved as $^{14}CO_2$ from $^{14}C$-PBB amended Brookston soil.

| Treatment | Substrate | % of original $^{14}C$ trapped in NaOH[a] | | | |
|---|---|---|---|---|---|
| | | 3 months | 6 months | 9 months | 12 months |
| Sterilized | $^{14}C$-PBB | 0.03 | 0.07 | 0.10 | 0.12 |
| Non-sterilized | $^{14}C$-PBB | 0.04 | 0.08 | 0.14 | 0.17 |
| Sterilized | UV-$^{14}C$-PBB[b] | 4.43 | 5.72 | 6.17 | 6.76 |
| Non-sterilized | UV-$^{14}C$-PBB | 6.24 | 7.20 | 7.96 | 9.94 |

[a] Each value is the mean of four replications.

[b] UV exposed $^{14}C$-PBB.

Table 7. Percent of added $^{14}C$-extracted from UV-irradiated $^{14}C$-PBB amended Brookston soil.

| | % of $^{14}C$ recovered in hexane-acetone extracts[a] | | | | |
|---|---|---|---|---|---|
| Treatment | 0 month | 3 months | 6 months | 9 months | 12 months |
| Sterilized | 76.1 | 36.5 | 34.1 | 32.9 | 30.6 |
| Non-sterilized | 72.1 | 35.4 | 32.0 | 30.6 | 28.9 |

[a] Each value is the mean of four replications.

Since partially degraded PBB would not yield $^{14}CO_2$, we also examined soil extracts for other $^{14}C$ products by TLC-autoradiography (Figure 5). The TLC system used was shown to separate the isomers of fireMaster (detection by UV). No intermediates of PBB degradation could be found. From the autoradiogram it is apparent that the two $^{14}C$-PBB isomers were the only $^{14}C$ products in soil. The autoradiogram of UV-treated $^{14}C$-PBB extract from soil is shown in Figure 6. It also showed little difference between the original amendment and the extract from the soil incubation. The marked change in the PBB isomers due to the UV irradiation is clearly shown by this figure. Virtually none of the original isomers remains. Most of the label is at the solvent front, the position where lesser brominated forms would be expected to run. The label at the origin and the streaking indicates that some of the $^{14}C$-PBB products may have complexed with the soil organic matter. This is consistent with the low efficiency of extraction of these photolyzed products.

## Conclusion

The potential hazards from PBB-contaminated soils are low since PBB's are not taken up by plants (Chapter IV) or leached to ground water (Filonow, *et al.*, 1976) at concentrations expected to be present, and they are probably not volatilized due to their low vapor pressure ($5.2 \times 10^{-8}$ mm Hg at 25C, Jacobs, *et al.*, 1976); however, they may remain in the soils for many years because of their resistence to degradation. Only low levels of contamination are expected in soils on most of the exposed farms because of dilution, as we have found for the farms examined (Jacobs *et al.*, 1977). In certain rare and localized situations

Figure 5. Autoradiogram of TLC plate showing $^{14}$C-PBB standard and $^{14}$C in extracts after incubation in soil. "1 and 6" $^{14}$C-PBB standard "2 and 3" extracts from sterilized soil after 6 and 12 months incubation, respectively, "4 and 5" extracts from non-sterilized soil after 6 and 12 months incubation, respectively.

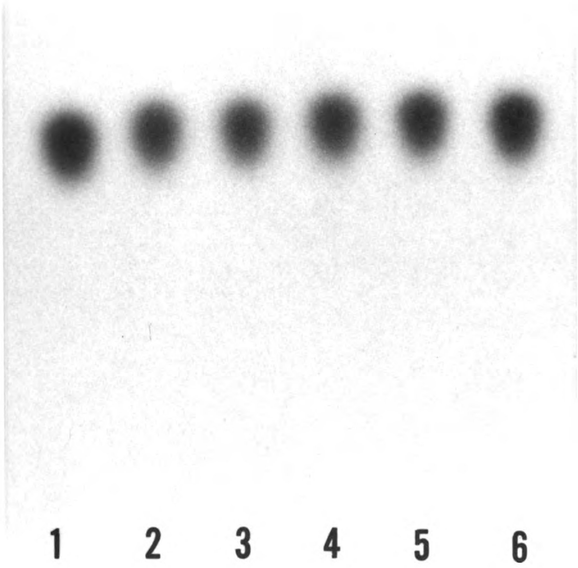
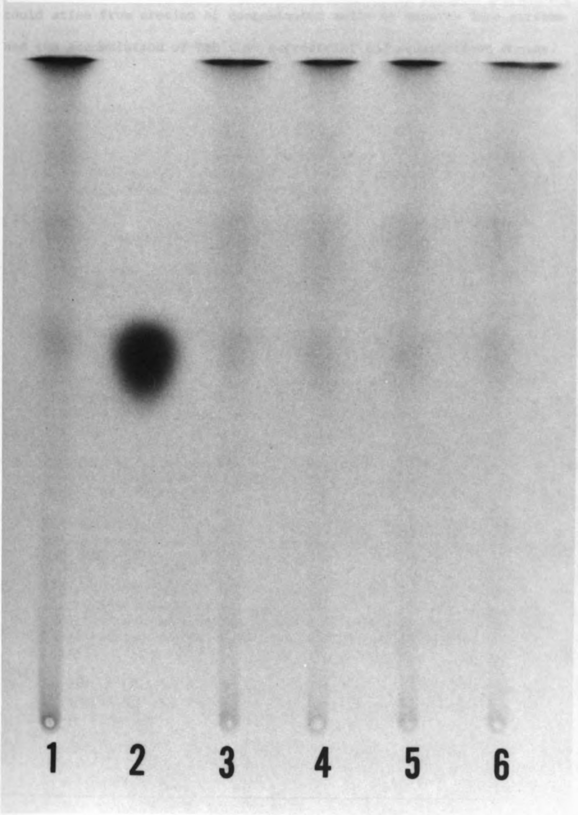

1
2
3
4
5
6

Figure 6. Autoradiogram of TLC plate showing $^{14}$C-PBB standard, UV-irradiated $^{14}$C-PBB and $^{14}$C in extracts after incubation in soil. "1" UV-irradiated $^{14}$C-PBB standard, "2" $^{14}$C-PBB standard, "3 and 4" extracts from sterilized soil after 6 and 12 months incubation, respectively, "5 and 6" extracts from non-sterilized soil after 6 and 12 months incubation, respectively.


1
2
3
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where high level contamination may have occurred, potential concern could arise from erosion of contaminated soils or manures into streams and the accumulation of PBB's in terrestrial and aquatic food chains.

# Literature Cited

1. Ahmed, M., and D. D. Focht. Oxidation of polychlorinated biphenyls by Achromobacter PCB. Bull. Environ. Contam. Toxicol. 10, 70 (1973).

2. Babish, J. G. W. H. Gutenmann, and G. S. Stoewsant. Polybrominated biphenyls: Tissue distribution and effect on hepatic microsomal enzymes in Japanese quail. J. Agri. Food Chem. 23, 879 (1975).

3. Baxter, R. A., P. E. Gilbert, R. A. Lidgett, J. H. Mainprize, and H. A. Vodden. The degradation of polychlorinated biphenyls by microorganisms. Science of the Total Environment. 4, 53 (1975).

4. Bray, G. A. A simple efficient Liquid Scintillation Counter. Anal. Biochem. 1:279 (1960).

5. Carter, L. J. Michigan's PBB Incident: Chemical Mix-up Leads to Disaster. Science. 192, 240 (1976).

6. De Vos, R. H. and E. W. Peet. Thin-layer chromatography of polychlorinated biphenyls. Bull. Environ. Contam. Toxicol. 6, 164 (1971).

7. Filonow, A. B., L. W. Jacobs, and M. M. Mortland. Fate of polybrominated biphenyls (PBB's) in soils. Retention of hexabromobiphenyl in four Michigan soils. J. Agri. Food Chem. 24, 1201 (1976).

8. Gibson, D. T., R. L. Roberts, M. C. Wells, and V. M. Kobal. Oxidation of biphenyl by Beijerinckia species. Biochem. Biophys. Res. Comm. 50, 211 (1973).

9. Gutenmann, W. H., and D. J. Lisk. Tissue storage and excretion in milk of polybrominated biphenyls in ruminants. J. Agri. Food Chem. 23, 1005 (1975).

10. Isleib, D. R., and G. L. Whitehead. Polybrominated biphenyls: An Agricultural Incident and its Consequences. I. The Agricultural effect of exposure. IXth annual conf. on Trace Substances in Environmental Health, Univ. of Missouri, Columbia, MO, June 10, (1975).

11. Jacobs, L. W., S. F. Chou, and J. M. Tiedje. Fate of polybrominated biphenyls (PBB's) in soils. Persistence and plant uptake. J. Agri. Food Chem. 24, 1198 (1976).

12. Jacobs, L. W., S. F. Chou, and J. M. Tiedje. Environmental implications of PBB's in soils and waters. National PBB Workshop, Michigan State University, Oct. 24-25, 1977.

13. Moore, R. W., J. V. O'Conner, nd S. D. Aust. Idenitrification of a major component of polybrominated biphenyl as 2,2'3,4,4',5,5'-heptabromobiphenyl. Submitted for publication (1977).

14. Robertson, L. W., and D. P. Chynoweth. Anather halogenated hydrocarbon. Environ. 17, 25 (1975).

15. Ruzo, L. O., and M. J. Zabik. Polyhalogenated biphenyls: photolysis of hexabromo and hexachlorobiphenyl in methanol solution. Bull. Environ. Contam. Toxicol. 13, 181 (1975).

16. Tucker, E. S., V. W. Saeger, and O. Hicks. Activated sludge primary biodegradation of polychlorinated biphenyls. Bull. Environ. Contam. Toxicol. 14, 705 (1975).

# CHAPTER IV

# PLANT UPTAKE OF POLYBROMINATED BIPHENYLS (PBB'S)

## Introduction

PBB have entered Michigan soils from manures of farm animals fed PBB, from disposal of milk, carcasses and other produce, and from effluent and dust discharges of the PBB manufacturing plant. Since most insoluble halogenated hydrocarbons are not taken up or translocated by plants, one would predict PBB to behave similarly. However, because of the importance of this conclusion to the quality of future Michigan food, I felt it prudent to thoroughly investigate this question.

In my preliminary greenhouse studies I had shown no PBB in tops of orchard grass and carrots grown in soils amended with high levels of PBB (Jacobs et al., 1976). I did, however, find traces (20-40 ppb) of PBB associated with carrot roots. I also found no detectable PBB in plants collected from the most highly contaminated Michigan fields (Jacobs et al., 1977).

This study was undertaken to evaluate PBB translocation from roots and leaves using $^{14}$C-PBB and to more thoroughly evaluate the association of PBB with root crops, carrots, radishes and onions.

## Materials and Methods

Corn and soybean seedlings were grown in a coarse sand-vermiculite mixture with Hoagland's solution. At 3 weeks of age the seedlings were removed, the adhering particles washed off the roots, and the plants placed in Hoagland's no. 1 hydroponic solution. For root uptake studies, $^{14}$C-PBB (hexa- and heptabromobiphenyl isomers only, see Jacobs et al., 1977) was added to the hydroponic solution to achieve a final PBB concentration of 100 ppb (1.42 μCi/L). To determine translocation from leaves, a 0.5 μl water drop containing 17 ng $^{14}$C-PBB (0.25 μCi) was spotted on the upper surface of a mature leaf. In the first trial, plants were exposed to PBB for 3 and 7 days, while in the second trial, plants were exposed for 4 and 8 days.

After the indicated exposure periods the plants were removed from the hydroponic solutions and the roots were washed by dipping them into distilled water. In the second trial, part of the plant roots that were exposed to $^{14}$C-PBB were quickly dipped five times into an acetone bath and then washed with distilled water. All plants were then immediately frozen with crushed dry ice, freeze dried, and later examined by autoradiography.

Radishes, carrots, and onions were grown in the greenhouse on Brookston clay loam (3.27 % org. C) and Spinks loamy sand (1.31 % org. C) soils amended with a mixture of 100 ppmw of fireMaster BP6 and 50 ppbw $^{14}$C-PBB (0.68 μCi/k soil). The soil was prepared by mixing 4.9 g of fireMaster BP6 and 2.35 mg $^{14}$C-PBB (33.1 μCi) in 100 ml acetone with 300 g of air-dried soil in a 500 ml brown glass bottle. The soil was gently blown with $N_2$ to remove excess acetone then mixed in a twin shell dry blender overnight at low speed. The PBB-treated soil was diluted with 48.7 kg of untreated soil in a mixer overnight to achieve the desired concentration of 100 ppmw. Fourty-eight grams of 100 ppmw soil was further diluted with 48 kg of untreated soil to achieve the 100 ppbw concentration. From my previous experience this procedure gave a very satisfactory distribution of PBB in soil.

PBB amended soil (5 kg) was placed in a plastic container lined with a polyethylene bag. The radish, carrot, and onion seeds were planted 1.25 cm below the soil surface and nutrient solutions were added as needed. The moisture levels of the soils were maintained at 15 and 10% for the Brookston and Spinks soils, respectively, by weighing the container and adding the required amount of distilled water daily. After 6, 9, and 10 weeks the radishes, carrots, and onions, respectively, were carefully removed from the container. The tubers or bulbs were washed with tap water then dried with paper towels. A few of the plants grown on the 100 ppmw PBB-treated soils were immediately frozen with liquid nitrogen, freeze-dried, and later examined by autoradiography.

Plant roots were cut into small pieces and extracted with hexane-acetone (1:1 v/v) in a Waring blender using a glass mixing container. Three successive 100 ml portions of hexane-acetone were used to extract each plant sample and then combined. The hexane-acetone phase was separated, dried with anhydrous $Na_2SO_4$, and finally concentrated to 10 ml on rotary flash-evaporator. This concentrate was passed through a Florisil column to remove interfering components by eluting with 200 ml benzene-hexane (1:1, v/v). The eluent was concentrated to 10 ml for gas chromatographic analysis.

Concentrated extracts from the above samples were analyzed on a Beckman GC-5 gas chromatograph equipped with an electron capture detector (Filonow et al., 1976) and a 2% SE-30 on 100/120 mesh Gas-Chrom Q column operated at 250°C with a carrier flow of 40 ml/min.

## Results and Discussion

The hydroponic study and greenhouse experiment were designed to favor the greatest uptake of PBB. Corn and soybeans were selected for the hydroponic studies since both are major crops in Michigan. The results of the $^{14}C$ uptake studies are shown in Figures 1, 2, 3, and 4. Autoradiograms of corn and soybean seedlings grown in the presence of $^{14}C$-PBB showed no translocation of PBB's. PBB was found concentrated at the roots, but no PBB was translocated to the plant tops. Due to the insolubility of PBB in water, I expected that the PBB would be primarily associated with the root surface. A significant portion of the $^{14}C$-PBB was removed when the roots were dipped in acetone (see Figure 2 and 4) as shown by the lighter autoradiograms. The autoradiograms also showed no movement of PBB within the leaf from the site of $^{14}C$-PBB application.

Three root crops, radishes, carrots, and onions, were grown in two soils which differed greatly in organic matter and clay content. The extent of PBB uptake by roots was determined by autoradiography and by gas chromatographic analysis. No PBB uptake was shown on autoradiograms, however I did find trace amounts of PBB by gas chromatography associated with the roots (Table 1), because of the greater sensitivity of the latter method. The trace amounts of PBB found on the roots were probably associated with root surface. Iwata and Gunther (1974) found 97% of PCB residues in carrot roots in the peel. This observation has also been found for DDT and other organochlorine pesticides in soil in which carrots were grown.

Carrot roots showed more PBB than radish or onion (Table 1). The plants grown on the sandier, low organic matter Spinks soil showed higher PBB uptake than ones grown on the Brookston soil which had more clay and organic matter. This finding is consistent with the report of Filonow *et al.*, (1976) who found that the adsorption of hexabromobiphenyl increased with increasing soil organic matter.

From these results plus my previous results of greenhouse (Jacobs, et al. 1976) and field studies (Jacobs et al., 1977) in which I found no PBB in plant tops, I conclude that PBB will not be transferred from contaminated soil to plant tops. Thus, recontamination of animals from feeds grown on contaminated soil will not likely occur from this source. Although root crops from very highly contaminated soil might contain traces of PBB. much of this PBB could probably be removed by peeling.

Figure 1. Soybean plants (top) and autoradiographs (bottom). A, 7 days after leaves were treated with $^{14}$C-PBB's. B and C are after root exposure to $^{14}$C-PBB's for 3 and 7 days, respectively.

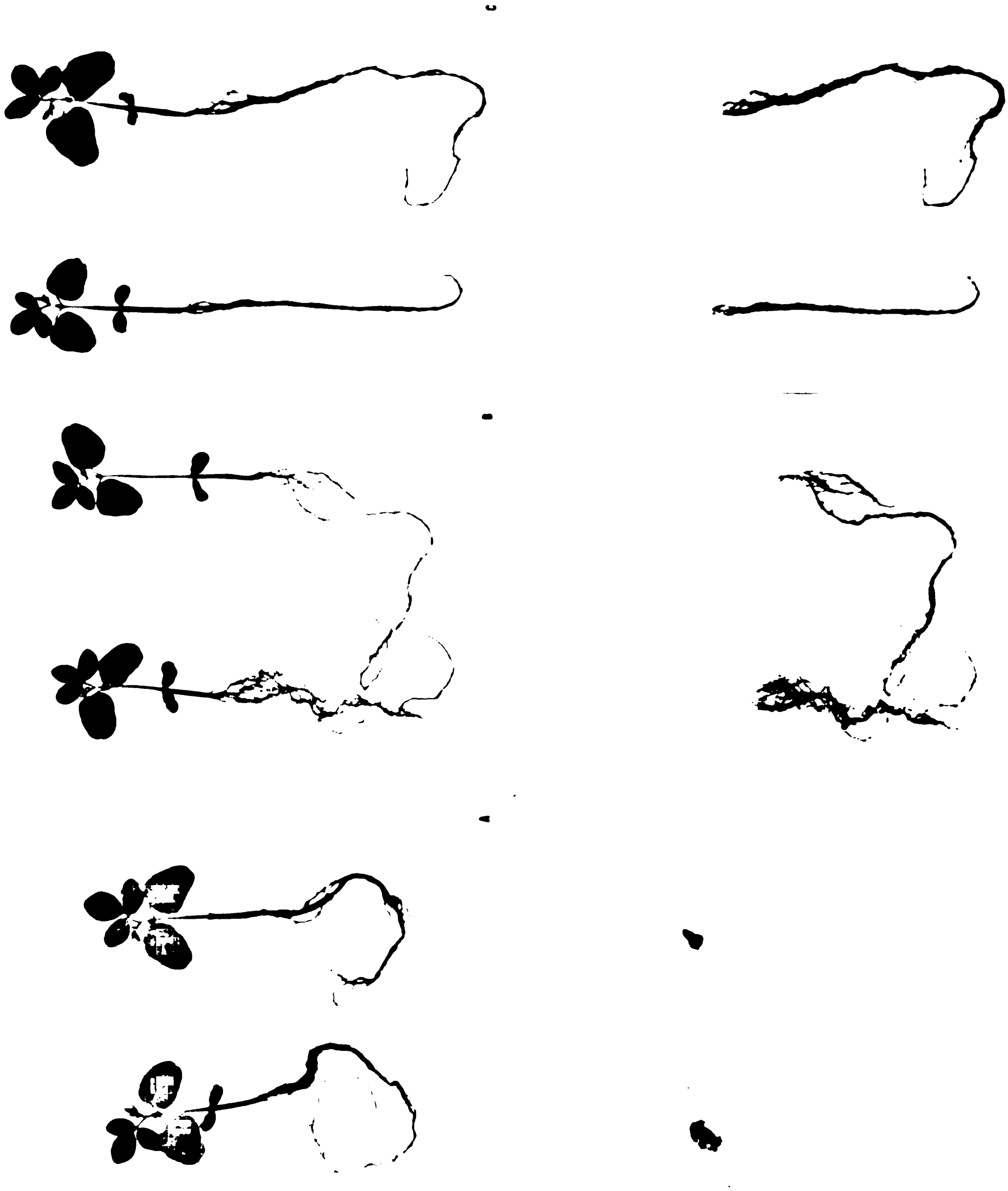

Figure 2. Soybean plants (top) and autoradiographs (bottom). A and B are after root exposure to $^{14}C$-PBB's for 4 and 8 days, respectively. C is identical to A except that roots were dipped in acetone prior to autoradiography. D, 8 days after leaves were treated with $^{14}C$-PBB's.


A
B
C
D

Figure 3. Corn plants (top) and autoradiographs (bottom). A, 7 days after leaves were treated with $^{14}$C-PBB's. B and C are after root exposure to $^{14}$C-PBB's for 3 and 7 days, respectively.

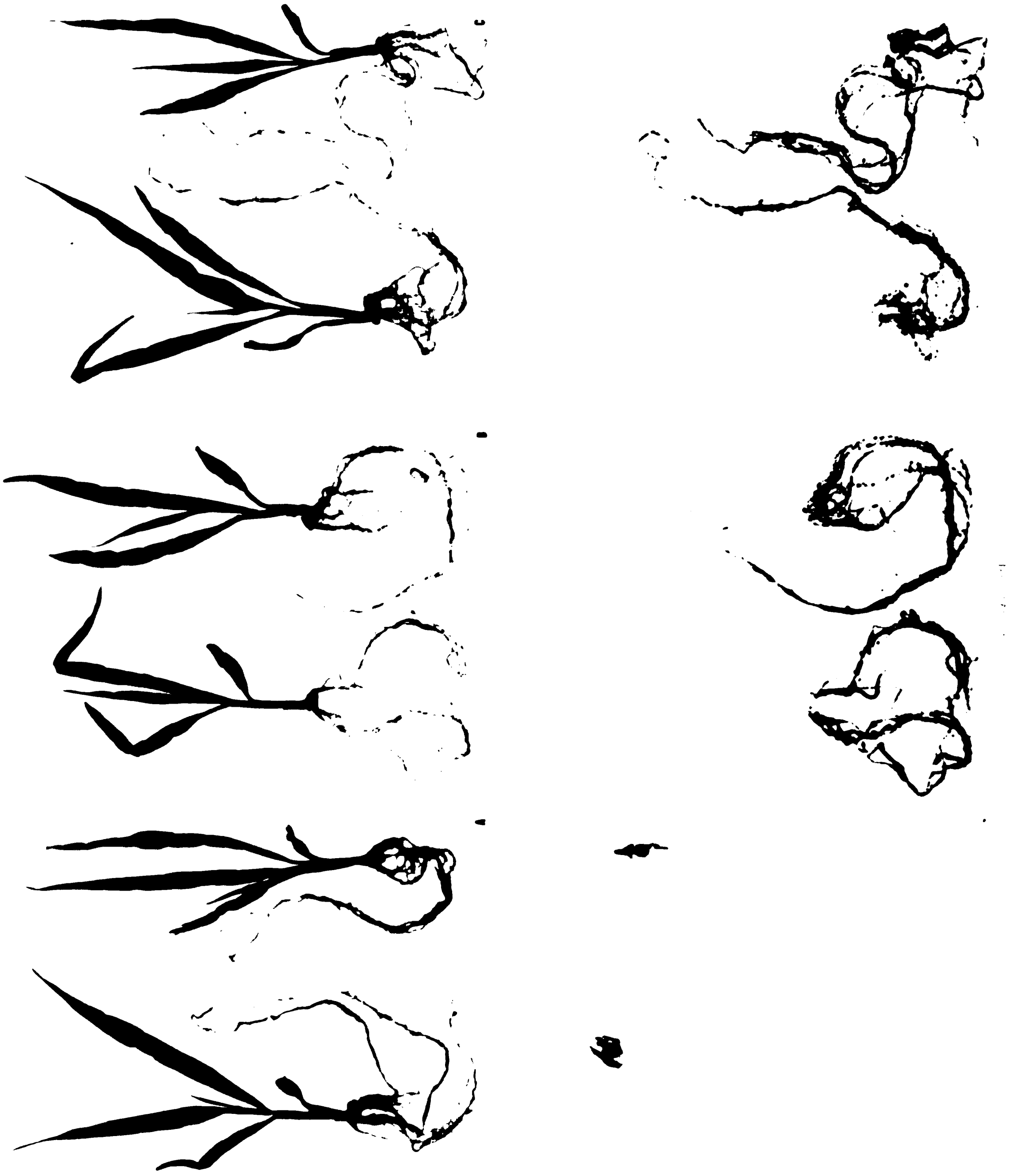

Figure 4. Corn plants (top) and autoradiographs (bottom). A and B are after root exposure to $^{14}C$-PBB's for 4 and 8 days, respectively. C is identical to A except that roots were dipped in acetone prior to autoradiography. D, 8 days after leaves were treated with $^{14}C$-PBB's.

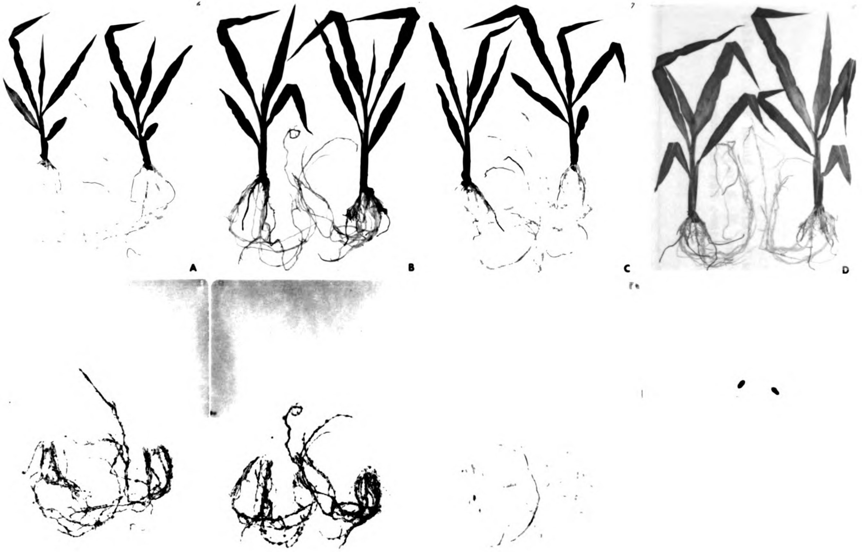
A
B
C
D

Table 1. PBB found associated with radish, carrot and onion roots after 6, 9, and 10 weeks, respectively, of growth in PBB contaminated soil.

| Soil type | PBB added (ppm) | PBB in plant roots, ng/g* | | |
|---|---|---|---|---|
| | | Radishes | Carrots | Onions |
| Spinks loamy sand | 0.1 | 7.2 | 20.4 | nd[†] |
| | 100 | 48.7 | 535 | 62.8 |
| Brookston clay loam | 0.1 | nd | nd | nd |
| | 100 | 43.7 | 117 | 33.8 |

* On wet weight basis, each value is the mean of three replications.

[†] nd = not detectable.

# Literature Cited

1. Filonow, A. B., L. W. Jacobs, and M. M. Mortland. Fate of polybrominated biphenyls (PBB's) in soils. Retention of hexabromobiphenyl in four Michigan soils. J. Agri. Food Chem. 24, 1201 (1976).

2. Iwata, Y., F. A. Gunther, and W. E. Westlake. Uptake of PCB (Arochlor 1254) from soil by carrots under field conditions. Bull. Environ. Contam. Toxicol. 11, 523 (1974).

3. Jacobs, L. W., S. F. Chou, and J. M. Tiedje. Fate of polybrominated biphenyls (PBB's) in soils. Persistence and plant uptake. J. Agri. Food Chem. 24, 1198 (1976).

4. Jacobs, L. W., S. F. Chou, and J. M. Tiedje. Environmental implications of PBB's in soils and waters. National PBB Workshop, Michigan State University, Oct. 24-25, 1977.