12-1971

An Investigation of Some of the Environmental and Edaphic Factors Effecting the Detoxification and Subsequent Degradation of an Herbicide, Butachlor

Douglas D. Baird
University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a dissertation written by Douglas D. Baird entitled "An Investigation of Some of the Environmental and Edaphic Factors Effecting the Detoxification and Subsequent Degradation of an Herbicide, Butachlor." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Plants, Soils, and Insects.

B. S. Pickett, Major Professor

We have read this dissertation and recommend its acceptance:

D. L. Coffey, H. D. Swingle, G. E. Hunt

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

November 23, 1971

To the Graduate Council:

I am submitting herewith a dissertation written by Douglas D. Baird entitled "An Investigation of Some of the Environmental and Edaphic Factors Effecting the Detoxication and Subsequent Degradation of an Herbicide, Butachlor." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy with a major in Agriculture Plant and Soil Science.

Major Professor

We have read this dissertation
and recommend its acceptance:

Homer D. Sprinkle

David L. Coffey

Endue Stroud

Accepted for the Council:

Vice Chancellor for
Graduate Studies and Research

AN INVESTIGATION OF SOME OF THE ENVIRONMENTAL AND EDAPHIC FACTORS EFFECTING THE DETOXICATION AND SUBSEQUENT DEGRADATION OF AN HERBICIDE, BUTACHLOR

A Dissertation

Presented to

The Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

---

by

Douglas D. Baird

December 1971

ACKNOWLEDGEMENT

The author wishes to express his sincere appreciation to Dr. B. S. Pickett, Head, Department of Horticulture, for his support, patience and valuable criticism during the study.

To Drs. D. L. Coffey and H. D. Swingle, Department of Horticulture, and Dr. G. E. Hunt, Department of Botany, for their ideas and evaluation of the manuscript, the author is extremely grateful.

To his family, for their endurance, patience, support and understanding throughout the period of evaluation and preparation, the author is forever indebted.

To Dr. R. P. Upchurch, Agricultural Research and Development Department, Monsanto Company, for his efforts and support in making the research possible and for constructively reviewing the manuscript, the author is indebted.

To Drs. W. A. Darlington and R. C. Briner, Agricultural Research and Development Department, Monsanto Company, for their counsel and advice pertaining to the tracer studies and for reviewing the manuscript, the author is especially appreciative.

To the Agricultural Research and Development Department, Monsanto Company, for supplying the materials and facilities required for the study, the author is thankful.

ABSTRACT

The influence of specific edaphic environmental factors on the detoxication and subsequent degradation of N-Butoxymethyl-2-chloro-2',6'-diethylacetanilide (butachlor) was evaluated under greenhouse and growth chamber conditions. Detoxication of butachlor, as measured by bioassay with barnyardgrass, was significantly enhanced by increasing temperatures to 32 C, making the soil alkaline, flooding, introducing relatively high levels of organic matter and by allowing exposure of more than four weeks. Total degradation of butachlor to CO_2 was enhanced also by high temperatures and length of exposure but was inhibited to a certain extent by flooding. Soil produced metabolites, of which three were soluble in certain organic solvents and five were water soluble, were also influenced by environmental factors, temperature and length of exposure being most significant. Flooding influenced the formation of certain metabolites soluble in organic solvent but did not influence the presence or amounts of water soluble metabolites.

TABLE OF CONTENTS

LIST OF TABLES

TABLE PAGE

TABLE PAGE

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The overall performance of a herbicide is of economic importance and may be governed by many interrelated environmental factors. Much emphasis during recent years has been placed on the correlation of soil and climatic factors with herbicidal activity (70, 91) and to means and methods of application (45). Upchurch et al. (91) measured fourteen factors under field conditions in an effort to correlate soil-environmental relationships with phytotoxic responses. The highest correlation was usually between soil organic matter and plant response.

Losses of herbicides from the soil may be traced to volatilization, chemical assimulation, leaching, photolysis, adsorption and absorption, phytoassimulation and microbial degradation (4, 78, 81, 82, 96). Resistance of herbicides to these degradative processes as well as repeated applications would be expected to result in cumulative persistance with subsequently serious economic implications. However, very rapid losses of a herbicide might lead to failure to control the intended pest and result in economic losses. It has been estimated that for effective and practical weed control, herbicides should persist in phytotoxic concentrations for 1 to 3 months (83).

Microbial degradation provides the major means of detoxification of many classes of herbicides (13, 50, 52, 65, 82, 83). Environmental factors that are conducive to increased microbial activity as increased moisture content (50, 74, 82), temperature (13, 42, 50, 70, 74, 82, 96), organic matter level (42, 50, 82), pH (4, 17, 42, 82, 96) are just a few

of the factors that tend to reduce persistence of many herbicides in the soil. The study of the interactions between herbicides, soil-environmental factors and microflora appears limitless (96).

Butachlor (N-Butoxymethyl-2-chloro-2',6'-diethylacetanilide) has been shown to be a selective preemergence herbicide which controls a wide spectrum of grass and specific broadleaf weeds in transplanted rice grown under flooded conditions and direct drilled seeded rice (7, 28). The use of this compound is not restricted to rice as it may be used in many agronomic and horticultural crops. It is currently marketed by Monsanto Company under the trademark of MACHETE.

The objective of this investigation was to determine the influence of soil moisture, pH, temperature and organic matter on the detoxication rate of butachlor in soil. An attempt was made to determine the influence of moisture and temperature on the soil degradation pathway and metabolic products of butachlor. With both studies, emphasis was placed on the role of microorganisms in detoxication of butachlor.

To facilitate the discussion of herbicides in this text, the common or designated and chemical names of all compounds referred to are summarized in Table 1.

TABLE 1. Common and chemical names of herbicides referred to in the text

Common Name or Designation	Chemical Name
Amitrole	3-amino-s-triazole
Atrazine	2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine
CDAA	N,N-diallyl-2-chloroacetamide
CDEC	2-chloroallyl diethyldithiocarbamate
Chloramben	3-amino-2,5-dichlorobenzoic acid
Chlorpropham	isopropyl-m-chlorocarbanilate
Dalapon	2,2-dichloropropionic acid
Dicamba	3,6-dichloro-o-anisic acid
Diphenamid	N,N-dimethyl-2,2-diphenylacetamide
Diuron	3-(3,4-dichlorophenyl)-1,1-dimethylurea
EPTC	S-ethyl dipropylthiocarbamate
Linuron	3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea
Metobromuron	3-(p-bromophenyl)-1-methoxy-1-methylurea
Monuron	3-(p-chlorophenyl)-1,1-dimethylurea
Neburon	1-butyl-3-(3,4-dichlorophenyl)-1-methylurea
Prometryne	2,4-bis(isopropylamino)-6-(methylthio)-s-triazine
Propachlor	2-chloro-N-isopropylacetanilide
Propanil	3',4'-dichloropropionanilide
Simazine	2-chloro-4,6-bis(ethylamino)-s-triazine
TCA	trichloroacetic acid
Trifluralin	α,α,α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine

Table 1 (continued)

Common Name or Designation	Chemical Name
2,4-D	(2,4-dichlorophenoxy)acetic acid
Vernolate	s-propyl dipropylthiocarbamate

CHAPTER II

REVIEW OF LITERATURE

I. ENVIRONMENTAL INFLUENCES ON PESTICIDE DEGRADATION

Most of the investigations concerned with soil-environmental influences on pesticide decomposition have been under aerobic conditions and degradation of herbicides under anaerobic conditions has not been studied extensively (74). The effective conversion of 1,1,1-Trichloro-2,2-bis (p-chlorophenyl)ethane (DDT), to 1,1-Dichloro-2,2-bis(p-chlorophenyl) ethane (DDD), occurs within three weeks under anaerobic conditions, while more than six months are required under aerobic conditions (34). Furthermore, anaerobic degradation is enhanced by organic matter additions and thus cultural means are available for aiding the degradation of DDT in soils (34). Trifluralin has been shown to be lost from flooded soils very rapidly (74). However, losses from soil under six inches of water did not occur until eight and sixteen weeks after application of neburon and monuron respectively (29). Decomposition of propanil is favored by increased aeration during the initial seven days of exposure (59). The breakdown of picloram (97) is decreased when soil moisture is greater than seventy-seven percent of the moisture holding capacity of the soil. Consideration of anaerobic systems under simulated natural conditions would also encompass herbicidal effects on algae. Loeppky's (61) work indicated that herbicides selective for macroflora in a field might also be selective for microflora. Anaerobic studies of herbicide detoxification or decomposition need not be restricted to those compounds

intended for application to flooded crops. Excessively high rainfall, low spots or poorly drained fields frequently produce anaerobic conditions for a short period of time.

Decomposition of picloram (97), atrazine and diuron (67), dalapon (87), simazine (38), trifluralin (74) and chlorpropham (13) is increased with an increase in temperature in the range of biological reactions. Atrazine decomposition is doubled (76) with each ten degree increase from 10 C to 30 C, while that of diuron is tripled (67). Cool, dry seasons may favor residual of long term herbicides (27, 87). Little or no decomposition of dalapon occurred within two weeks when soil temperatures were less than 15 C, while decomposition was essentially complete at 30 C within two weeks (42). Ogle (70) concluded after studying the influence of several soil factors on the fate of 2,4-D, TCA, chlorpropham and monuron, that in general herbicidal breakdown "was found to be proportional to the temperature as would be expected in microbial decomposition" (70). Of course, loss by volatilization of herbicides such as CDEC,CDAA and EPTC is increased with an increase in temperatures (21, 56, 88).

Soils of high organic matter content have exhibited a high degree of microbial decomposition of dalapon (42, 45), linuron and diphenamid (23), and s-triazines (38, 76). Amitrole degradation is reduced by organic matter and degradation is thought to be largely chemical with only indirect microbial involvement (51). The degradation potential of atrazine decreases with increasing depth and may be directly related to organic matter, microbial population and adsorption (76). The kind and amount of organic matter may also govern degradation (76).

Increasing the soil pH also increases decomposition of dalapon (17, 42, 47), propanil (59), vernolate and amitrole (17). Bailey et al. (4) showed that adsorption of herbicides occurred to the greatest extent on the highly acid montmorillonitic clay as compared to near neutral montmorillonite. Reduction of phytotoxicity of prometryne at pH 4.5 rather than at pH 6.5 has been attributed to adsorption by organic matter additives (95). The distribution of soil microbes as actinomycetes from various depths are associated with neutral pH and moderately high soil moisture (20).

Photolysis has not been identified as a major degradation factor with the majority of herbicides currently in use.

Photolysis has been shown to be important with metobromuron (78), prometryne (55) and is implied with fluorodifen (94) and amiben (62). Sheets (81) states that photolysis reactions occur much more readily in solution than in solid or gaseous states.

In order to determine the influence of the soil microbial population, various techniques have been employed to "sterilize" the media. Hance (36) effectively sterilized soil for atrazine studies by electron beam irradiation at a dosate of 5 m rad. Autoclaving has generally been employed as a means of destroying microbial populations with varying degrees of success. A notable weakness of this system is the lack of residual sterility. Although the effects of autoclaving on soil systems is poorly understood, the known effects include altered structure, increased water-soluble organic matter content, toxin formations, increased soluble salts of Ca, Cu, Mg, Mn, K, Z, P and Al, decreased Fe and NO_3 salts, modified cation exchange capacity and modified water and gas adsorptive capacity (51). Certain biological toxicants as sodium or

potassium azide, sodium arsenite and hydroxylamine have been used as means of maintaining residual sterility (51). Parochetti and Warren (71) studied the behavior of KN_3 in soil and found that volatility was important in the dissipation of KN_3. Soil pH, temperature and soil moisture were the major factors influencing dissipation of KN_3 (71).

Microbial decomposition under laboratory conditions is frequently characterized by three phases: initially a lag phase in which little decomposition occurs; secondarily a very rapid decomposition phase and last a slow but steady decomposition phase (96). The lag phase is generally accepted as the time required for the development of a microbial population capable of metabolizing a specific herbicide. The increase in population is also correlated with the production of enzymes that catalyze the decomposition and these enzymes "are formed from closely related enzymes already present" (96). Environmental factors as well as the specific herbicide will determine the duration and rate of each phase and the definition of each phase is sometimes difficult to determine under field conditions (96).

II. DEGRADATION OF SPECIFIC HERBICIDE CLASSES

A large amount of work has been centered on identification of organisms responsible for degrading phenoxy acetic acid compounds and it has been reviewed and investigated by Audus (3) and Walker (93). The resistance of aromatic herbicides to microorganism degradation is governed by the position of the halogen on the aromatic nucleus and by the linkage and type of aliphatic side chain (1). Dichlorophenol resists destruction when Cl is in the meta position to the phenolic hydroxyl; the position

and not number of halogens governs degradation (65). However, the number of halogens on the aromatic ring will govern degradation of benzoates (65). Correlation of laboratory results with field studies is not always feasible. Quite often exceptionally high amounts of herbicide are used in laboratory studies. Walker and Newman (93) used an equivalent amount of 2240 kg/ha of 2,4-D in perfusion studies as compared to the 2 kg/ha or less used under normal field conditions. In the perfusion tests, the 2240 kg/ha was completely decomposed in 7 to 13 days, while in the field 3 to 6 weeks were required with 2 kg/ha (93). Under field conditions, the "ideal" conditions prevailing in perfusion tests as moisture, aeration and temperature "are not the rule" (93). Conditions of warm temperatures, moist environment and organic soils accelerate phenoxy acetic acid degradation (54).

The s-triazines as a herbicide class have been subjected to numerous investigations with varied results from no microbiological breakdown (65) to identification of a specific organism, Aspergillus fumigatus (46) and others (52). Dehalogenation is advocated as a chemical degradative mechanism for atrazine and microorganisms are not involved (52). N-dealkylation appears to be the major fungal mechanism for degradation and with atrazine, the ethyl group is the major route although some degradation of the isopropyl group occurs (52). Kaufman (52) stated that dealkylation by microorganisms does not insure detoxication and the presence of toxic dealkylated metabolites accounts for inability of bioassay techniques to determine progressive dissipation.

The substituted ureas have been extensively studied and reviewed as to their fate and disappearance from soils (18, 40, 69, 82). Monuron (82) has been shown to be degraded effectively by many bacteria and fungi

under laboratory conditions including Pseudomnas, Aanthomonas, Scarcina, Bacillus, Penicellium and Aspergillus spp. Further work by Murray et al. (69) with the fungi Aspergillus on diuron found that A. niger degraded this urea faster than A. tamarii, which in turn was more effective than A. sydowi. Murray (69) also found that under laboratory conditions, diuron degraded at a faster pace than neburon which was broken down faster than monuron. The rate of disappearance of these ureas was also found by Sheets (80) to be governed by soil moisture, whereby the continuously moist (field capacity) soils produced less phytotoxicity than the alternately wet and dry soil. One of the residues identified from diuron was 3,4-dichloroaniline (18) and Kearney (53) identified 3-chloroaniline as a product of Pseudomonas spp. degradation of chlorpropham. Without the 3-chloro substitution, Arthrobacter and Achromobacter degraded propham to the aniline, carbon dioxide and isopropyl alcohol (16). Propanil (8, 9, 10) has been shown in laboratory studies to be degraded to 3,4-dichloroaniline by a microbial enzyme acylamidase (9). Fusarium solani was identified by Lanzilotta and Palmer (59) as an organism degrading propanil to 3,4-dichloroaniline. They further speculate (60) that "a synthetic compound is susceptible to enzymatic attack because it bears a structural resemblance to some natural product that is sufficient to be mistaken for this substance and used as substrate by the enzyme involved." Lanzilotta and Palmer (60) think that acylamidase is specific to propanil and does not affect other acylanilides as dicryl or karsil because it has specificity to acyl chains of limited length. Karsil, N-(3,4-dichlorophenyl)-2-methylpentanamide, was found to be degraded to 3,4-dichloroaniline by Penicillium spp. with the methylpentanamide moiety being degraded to carbon dioxide, water and acetic acid (79). The

3,4-dichloroaniline product from propanil has been shown by Plimmer et al. (72) to react with soil nitrite to form an intermediate diazonium cation which in turn eventually may form 1,3-bis(3,4-dichlorophenyl)triazine. Tweedy et al. (90) found that bromine substitutions as with metobromuron contrasted to the results of the degradation of chlorine substituted acylanilides. Azobenzine was never detected as has been shown with chloroacylanilide rates up to 1000 times the recommended field rates, but instead degraded to p-bromoacetanilide (90). It was noted also that this work was done at only 10 times the normal metobromuron field rate (90).

A few alpha haloacetamides have been reviewed by Jaworski (54) with discussions of the degradation of 2-Chloro-N,N-diallylacetamide, (CDAA), and 2-Chloro-N-isopropylacetanilide, propachlor. Soil studies of CDAA degradation showed that $^{14}CO_2$ from carbonyl labeled or allyl labeled ^{14}C CDAA evolved about 20 percent of the total ^{14}C trapped. Deming (21) attributes some of the rapid loss of CDAA to volatility. Propachlor is rapidly metabolized by resistant crops to a water-soluble acidic metabolite whereby the chloro group is suspected of being displaced by some nucleophilic endogenous phytosubstrate (54).

Phytotoxic studies with propachlor (5, 86), laboratory and field performance of alachlor (6, 39, 86), and of analogs (11, 43) have indicated the broad spectrum and efficaceous nature of this class of herbicides. Under a wide range of soil-environmental conditions this class has generally exhibited limited duration of soil phytotoxic residues or metabolites. Some environmental and edaphic factors influencing the herbicidal performance of butachlor were described by Baird and Upchurch (7).

In an attempt to find practical as well as a preventive approach to reducing pesticide residues, McClure (66) explored degradation acceleration by the application of nutrient broths to soils in greenhouse bioassay studies. The broth treatments did accelerate the normal degradation rate of most of the herbicides tested. Lanzilotta (59) worked with additions of glucose and yeast extract and found a substantial increase in propanil decomposition, which was reflected in increased cell population rather than in increased activity.

CHAPTER III

METHODS AND MATERIALS

I. BIOASSAY STUDIES

A series of investigations were established during 1970-71 under laboratory and greenhouse conditions. Ray silt loam was utilized in all tests with specific amendments for pH and organic matter studies. Properties of this soil are given in Table 2.

Butachlor was dissolved in acetone and applied at various rates (six to eight levels) to the weighed (150 gms) cover layers in a belt sprayer at a diluent volume of 182 l/ha. Immediately after application, the herbicide was thoroughly incorporated and transferred to individual 10 cm x 22.5 cm 2 mil polyethylene bags. Flooded treatments were established by adding 55 ml of distilled water to each container and all containers were sealed and incubated in illuminated growth chambers (500 f.c. at sample level, 12 hour diurnal period) for specified periods of time. Non-herbicide treated cover layers were also incubated in the same manner and these were used to establish a standard rate response curve after each incubation period. The incubated herbicide substrate treatments were removed from the incubation chambers at regular intervals, allowed to dry to a workable state by evaporation at room temperature (24 hrs at 23 C), applied to pots (9.52 cm sq.) containing 50 seeds each of barnyardgrass (Echinochloa crusgalli L., Beauv.) at a depth of 13 mm and placed in the greenhouse for seventeen days. Subirrigation was employed for all watering requirements. At the end of each forcing period, foliage fresh weights were obtained from both the incubated

TABLE 2. Properties of Ray silt loam

pH.	6.3
Sand.	6.2%
Silt.	83.2%
Clay.	9.6%
Organic matter.	1.0%
Cation exchange capacity.	12.9 meg/100 gms

herbicide substrate samples and the standard samples. The assessment for phytotoxicity was determined by comparing growth reduction values (referred to as GR_{50}) from incubated herbicide response curves with GR_{50} values from the standard response curves as shown by Corbin and Upchurch (17). The GR_{50} value represents a 50 percent growth reduction in foliage fresh weight interpolated from the rate response growth inhibition curve of each replication. The determination of the amount of herbicide detoxified is the differential between the standard and incubated rates at GR_{50}. The percent detoxication was then determined by the following equation:

$$\text{Percent Detoxication} = \left(\frac{\text{treatment } GR_{50} - \text{standard } GR_{50}}{\text{treatment } GR_{50}}\right) \times 100$$

The term detoxication used throughout the text represents loss of phytotoxicity as bioassayed with barnyardgrass. The amount of herbicide detoxified, the GR_{50} value and the percent detoxication were analyzed statistically with the "Analysis of Variance" as described by Snedecor. All bioassay data reported herein were the means of three replications.

The temperature factor was established by incubation in illuminated growth chambers at constant temperatures of 16, 24 and 32 C. Soils autoclaved for 8 hours at 121 C and 1.05 kg per sq. cm. were treated with herbicide and incubated at 32 C to determine microbial influence on detoxication. The influence of soil pH was determined by using four pH levels; 4.5, 5.9, 7.1 and 8.5. Acidic adjustments were accomplished with 1 N H_2SO_4, while basic amendments were made with 1 N NaOH. Organic matter levels were established by thoroughly blending "muck soils" of 78 percent organic matter at three ratios with the silt soil resulting in organic matter levels of 3, 9 and 27 percent.

All studies contained two moisture treatments, flooded and non-flooded (15 percent moisture on an air dried basis), and at least three incubation intervals of two, four and eight weeks. The temperature and sterilization studies also included an incubation period of sixteen weeks. The organic matter and soil pH studies were incubated only at 24 C.

The experiment design of all studies was a randomized block which embraced rate levels, incubation time intervals, moisture regimes and main factor levels. The respective factorials were 8 x 4 x 2 x 3 in the temperature study, 6 x 4 x 2 x 2 in the sterilization study, 6 x 3 x 2 x 4 in the pH study and 6 x 3 x 2 x 3 in the organic matter study. For each experiment, triplicate samples were processed and bioassayed using a single bioassay pot for each sample.

II. RADIO TRACER STUDIES

Ray silt soil as employed with the bioassay studies was also used for the isotopic investigations. The incubation vessels used were patterned after the Biometer Flasks designed by Bartha (8). Modifications were made by utilizing a center well for the trapping solution and by attaching a 100 mm Ascarite filter to the side arm of a 250 ml Kimax suction flask fitted with a rubber diaphragm for sampling with a 13 cm hypodermic needle and syringe. One hundred grams of soil on an air dryed basis was adjusted to 15 percent moisture or flooded with one half an inch "head" of sterilized distilled water. In addition to the moisture factor, sterilization techniques were studied by comparing nonsterilized with steam sterilized soil (121 C at a pressure of 1.05 kg per sq. cm. for 2 hrs), as well as with potassium azide treated soil at 400 ppm as a chemical means of inhibiting microbial activity (57, 71).

^{14}C ring and carbonyl labeled butachlor (0.4 μc/ml and 3.4 μc/ml respectively) were applied to each individual incubator at 24 ppm of butachlor which was equivalent to 4.4 kg/ha on a 13 mm depth basis. Evolved $^{14}CO_2$ was trapped in 10 ml of 1 N NaOH and one ml was sampled on a weekly basis and placed in scintillation vials. Fifteen milliliters of Insta-Gel liquid scintillation solution (manufactured by Packard Instrument Company) was added to each vial and the radioactivity of the $^{14}CO_2$ evolved was determined by liquid scintillation, counting on a Nuclear Chicago Mark I counter for 4 minutes, two counts per sample. Percentage recovery was calculated using a computerized program which converted cpm to dpm from quenching calculations based on external standardization and comparing the dpm in the sample with the dpm of the total original material. All "counts" were corrected for background. Thus, results were expressed as percent ^{14}C of initial radioactivity.

The experimental design of the carbonyl labeled study was a randomized block which included a 3 x 2 x 2 (temperature x moisture x sterilization) factorial with two replications of one flask each. The ring and carbonyl labeled comparison was also a randomized block which included a 2^3 (labels x moisture x sterilization) factorial with two replications of one flask each.

Total CO_2 was also monitored weekly by titration of a 1 ml trapping solution sample with 0.2 N HCL after precipitation with saturated $BaCl_2$ and using 0.1% phenolphthalein as an indicator.

After two, four, eight and eleven weeks of incubation, the treated soils were sampled and organic and aqueous soluble materials were extracted by using the Bligh and Dyer (57) extraction procedure (2 chloroform,

2 methanol, 1.8 water). The two phases were separated, radioactivity counted and the solutions were then concentrated. The chloroform concentrates were spotted on silica gel 20 x 20 cm Thin Layer Chromatography plates (Quantum Industries) and two dimensional chromatographs were developed by using initially isooctane-ether (4:3) and secondarily ether. Chromatograms prepared in this manner were examined with a Baird-Atomic Model 6000 Beta Camera and the percent distribution of the ^{14}C present in each zone was determined by zonal counts per minute.

Aqueous extracts were characterized by paper electrophoresis on a Savant High Voltage Electrophoresis Apparatus with a 30 inch flat plate using 3000 volts and sodium acetate buffer at pH 5.4 and with 2 N acetic acid: 0.6 N formic acid (1:1) at pH 2.2 using Whatman 3 MM paper. The blue dye visual standard was allowed to migrate 23-25 cm from the origin when the chromatograms were removed and dried. Each chromatogram was divided into one cm sections (10 cm sections towards the cathode and 25 cm sections toward the anode from the origin) and placed in scintillation vials. Elution of each section was accomplished with 2 ml of 1:1 Ethanol-water eluant oscillating for 30 minutes prior to the addition of 15 ml of Insta-Gel liquid scintillation solution. Four minute counts were made, the dpm recovered and the percent of distribution determined. Percentage of each major metabolite was determined and the metabolite identified by distance from the origin in relationship to the standard dyes.

CHAPTER IV

RESULTS AND DISCUSSION

I. BIOASSAY INVESTIGATIONS

A series of investigations to determine the rate of detoxication of butachlor as influenced by various environmental and edaphic factors revealed that conditions pertinent and conducive to microbial development also enhanced detoxication. Bioassay is one of the methods currently employed to determine detoxication. It is generally recognized as being pertinent because the concentrations of the toxicant are similar to those employed in the field. In all the bioassay tests used, it was necessary to employ a rather extensive concentration range of butachlor in order to be able to establish a relative value from each of several types of responses investigated. Within the course of each investigation, the role of the herbicide concentration became quite evident as related to implied microbial detoxication. The detoxication rate appeared proportional to the toxicant concentration. At low toxicant concentrations, the capacity of the microbes to detoxify is quite large, while as the concentrations of the herbicide increases, the capacity of the microbes for detoxication may approach saturation as the rate decreases.

Effect of Temperature, Moisture and Exposure Duration

The influence of incubation temperature, lengths of exposure and moisture on the detoxication as indicated by barnyardgrass bioassay is shown in Table 3. A concentration range of butachlor from 2240 to 8.7 kg/ha x 10^{-3} was utilized to determine preemergence inhibition of foliage

TABLE 3. Influence of temperature and moisture during various incubation periods on preemergence activity of butachlor on barnyardgrass in Ray silt loam

Incubation Period	Temp. During Incuba.	Moisture Regime*	Percent Inhibition of Foliage Fresh Weight								
			Rate of butachlor - kg/ha x 10^{-3}								
			8.7	17.5	35	70	140	280	560	1120	2240
2 weeks	16C	NF	5	20	39	70	98	100	100	100	100
		F	15	22	41	66	79	97	100	100	100
	24C	NF	0	4	6	49	95	98	99	100	100
		F	0	5	15	21	75	97	99	100	100
	32C	NF	0	0	12	45	91	97	99	100	100
		F	0	0	35	54	69	85	98	99	100
4 weeks	16C	NF	10	16	25	35	95	97	100	100	100
		F	13	23	30	22	79	95	98	100	100
	24C	NF	0	0	30	42	40	72	94	100	100
		F	0	27	33	42	59	79	97	100	100
	32C	NF	0	0	19	29	54	63	93	99	100
		F	0	0	20	35	69	85	91	97	100
8 weeks	16C	NF	0	0	0	13	73	89	99	100	100
		F	0	0	0	4	38	67	98	100	100
	24C	NF	0	0	0	0	0	4	79	99	100
		F	0	0	0	0	0	11	36	92	100
	32C	NF	0	0	0	0	1	17	48	91	100
		F	0	0	0	0	0	5	22	70	93
16 weeks	16C	NF	0	0	0	0	14	35	98	100	100
		F	0	0	0	0	8	17	93	100	100
	24C	NF	0	0	0	0	0	20	54	98	100
		F	0	0	0	0	0	0	19	37	100
	32C	NF	0	0	0	0	0	0	15	45	98
		F	0	0	0	0	0	0	20	30	72

* NF = nonflooded, F = flooded

fresh weight at each temperature, moisture level and incubation period. This concentration range appeared quite extravagent for determining the GR_{50} value initially as reflected by the two week incubation results. However, as incubation time was increased, the higher concentrations employed became more valuable and critical evaluations of detoxication could still be assessed as long as sixteen weeks after initiation.

Preconditioning the soil to various temperatures, moisture levels or incubation periods prior to herbicide application did not significantly influence preemergence activity when the herbicide was immediately exposed to the bioassay species. This series of evaluations represented the standards for the test (Table 4).

An analysis of the effect of incubation duration and temperature (Table 5) revealed several important relationships. The amount of butachlor detoxified was related to both incubation duration and temperature (Figure 1). A period of eight weeks was required to significantly detoxify butachlor at 24 or 32 C. The amount detoxified was increased significantly with an increase in temperature only after eight weeks of incubation. At the lowest temperature of 16 C, significant differences in the amount detoxified did not occur until sixteen weeks. Thus there appears to be a function of temperature relative to time in detoxifying butachlor. The data indicate that the higher the temperature (32 C), the shorter the time required for substantial detoxication whereas the cooler the temperature the longer the time required for similar detoxication. Burnside et al. (12) found that temperature was one of the most important factors in regulating simazine detoxication. Further work by Harris (38) indicated that the rate of conversion of simazine to hydroxysimazine was enhanced by increasing the temperature, and the detoxication

TABLE 4. Preemergence activity of butachlor controls during specific periods on barnyardgrass in Ray silt loam

Rate of butachlor kg/ha x 10^{-3}	Percent Inhibition of Foliage Fresh Weight during Specific Control Periods				
	2 wks.	4 wks.	8 wks.	16 wks.	Period Avg.
560	100	100	100	100	100
280	100	100	100	100	100
140	98	98	99	96	98
70	94	86	94	93	92
35	61	73	59	62	64
17.5	4	15	10	12	10
8.7	4	11	0	0	4

TABLE 5. Influence of incubation temperature and duration on detoxication of butachlor in Ray silt loam*

Incubation Period	Temperature	kg/ha x 10^{-3} Detoxified	kg/ha x 10^{-3} GR_{50}
2 weeks	16C	19a	50a
	24C	43a	84a
	32C	57a	89a
4 weeks	16C	58a	88a
	24C	131ab	162ab
	32C	148ab	176ab
8 weeks	16C	127ab	161ab
	24C	519b	553b
	32C	764c	798c
16 weeks	16C	333b	364b
	24C	894c	925c
	32C	1419d	1433d

* Values followed by the same letter in the same column do not differ at the .05 level (Duncan's Multiple Range).

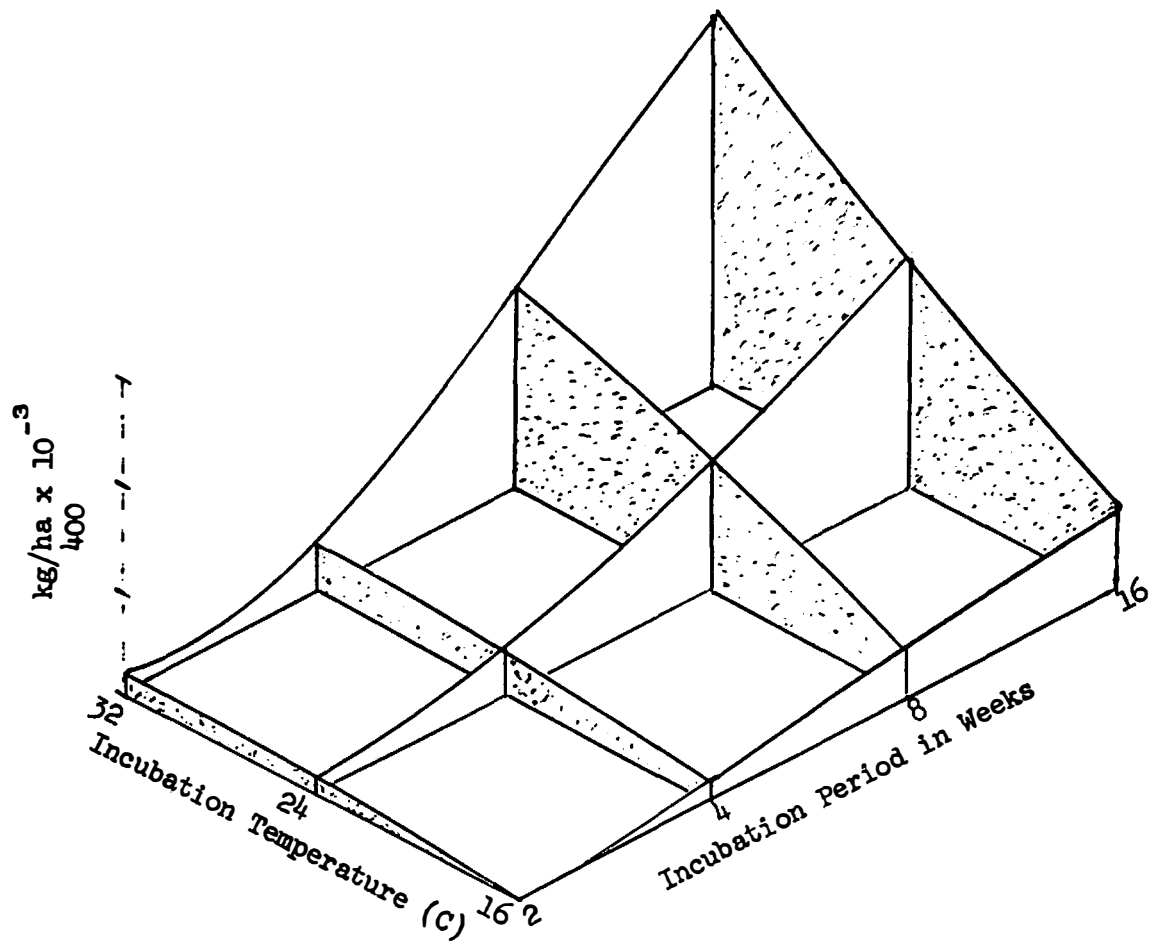


Figure 1. Kg/ha x 10^{-3} of butachlor detoxified as influenced by incubation temperature and duration.

of atrazine is also accelerated by high temperatures (38). It has been generally accepted that 2-hydroxy derivatives of s-triazines in the soil are generated by a non-biological hydrolysis mechanism (38). Thus it is apparent that temperature is an important factor in either microbial or chemical detoxication.

There is a significant difference in detoxication at the various temperatures with non-flooded moisture levels and between 16 C and 24 C under flooded conditions (Table 6). Moisture influenced the amount of butachlor detoxified to a greater extent at 24 C than at 16 or 32 C. Although not at a significant level, there was a definite tendency towards greater detoxication under flooded conditions and this factor would imply shorter residual under tropical and subtropical cultural systems. The triazines are detoxified at faster pace in aqueous solutions than in "moist" soil (38) at different temperatures.

The matter of increased volatilization with increased temperature during the detoxication study was not considered important due to the method of incubation of samples in sealed polyethylene bags.

Autoclaving Influence on Herbicide Detoxication

Microbial activity appears to be one means of substantial detoxication of butachlor, although perhaps not the only means. Autoclaving (Table 7) of the soil media prior to herbicide application did not result in appreciable differences when the herbicide was immediately exposed to the bioassay species; i.e., standard response. However, after an incubation period of the herbicide prior to bioassay exposure, there was a comparatively substantial difference in detoxified amounts with nonautoclaving at incubation periods from two to eight weeks. There was also

TABLE 6. Detoxication of butachlor as influenced by incubation moisture and temperature in Ray silt loam*

Moisture Regime	Incubation Temperature	Percent Detoxication	kg/ha x 10^{-3} Detoxified
Nonflood	16C	63 a	114 a
	24C	75 b	265 b
	32C	83 c	496 c
Flood	16C	70 ab	154 ab
	24C	84 c	528 c
	32C	86 c	698 c

* Values followed by the same letter in the same column do not differ at the .05 level (Duncan's Multiple Range).

TABLE 7. Influence of sterilization and incubation periods on pre-emergence activity and detoxication of butachlor*

		kg/ha x 10^{-3} butachlor	
		Rate Req. of Standards for GR_{50}	Amount Detoxified
Autoclaved soil	2 weeks	32.5a	113a
	4 weeks	33.4a	153ab
	8 weeks	32.8a	174ab
Nonautoclaved soil	2 weeks	32.7a	244b
	4 weeks	31.2a	425c
	8 weeks	29.1a	768d

* Values followed by the same letter in the same column do not differ at the .05 level (Duncan's Multiple Range).

apparently a trend, although non-significant, of an increase in detoxication over time with autoclaved treatments. Whether this was due to microbial activity or to chemical degradation is strictly speculative. However, the lack of residual sterility with autoclaved material is recognized and the possibility of this factor contributing towards the increasing trend is acknowledged. With time the relative increase of the detoxified amounts is relative constant with the autoclaved samples (21 kg/ha x 10^{-3}) while with time there was an increasing amount (180-340 kg/ha x 10^{-3}) of the nonautoclaved samples. This would support the proposition that microbial detoxication is of primary importance and nonbiological detoxication of secondary importance.

Flooded or nonflooded moisture levels within each sterilization treatment did not influence the standard response (Table 8) indicating prior preconditioning by autoclaving and moisture levels did not influence subsequent butachlor activity. However, moisture levels during incubation of butachlor prior to exposure to the bioassay species did not significantly influence detoxication. Under non-autoclaved conditions, there was a substantial increase in detoxication with flooding while under autoclaved conditions the difference was not significant.

Detoxication as Influenced by Organic Matter, Moisture and Length of Exposure

Soil organic matter has often been identified as a soil factor influencing herbicidal behavior. An increase in soil organic matter has been known to increase microbial population and/or organic matter treatments may be interpreted as a possible reduction in residue level as a result of a particular rate of microbial degradation. Organic matter

TABLE 8. Influence of steam sterilization and moisture regimes on pre-emergence activity and detoxication of butachlor*

Soil Treatment	Moisture Regime	Rate Req. of Standards for GR_{30}	Amount Detoxified
Autoclaved soil	Non-flood	32.7a	107a
	Flood	33.2a	198a
Nonautoclaved soil	Non-flood	31.2a	331b
	Flood	30.9a	627c

tends to increase the granular and porous structure of soil media and the change in structure would be expected to increase microbial activity and in turn aid in detoxication (66).

Preconditioning factors (Table 9) influencing the activity of butachlor as a preemergence herbicide appeared to be primarily organic matter content and secondarily flooding over a long period of time (8 weeks) at a high organic matter level. Incubation of butachlor at the various organic matter levels (Table 10), moisture regimes and exposure time required substantial herbicide concentration ranges in order to achieve a bioassay response. Organic matter adsorption was likely the rate determination factor.

This adsorption was clearly evident with the controls when the GR_{50} level was determined (Table 11). With an increase in organic matter content above 9 percent, there was a required increase in concentration of butachlor to achieve a relative or prescribed amount of activity. In conjunction with the high organic matter influence, the greater the moisture level (flooding), the greater the amount of butachlor required for control.

Significant detoxication was an apparent function of organic matter as with an increase in O.M. levels, there was an increase in the amount detoxified. Although not at a significant level, there appeared to be a tendency to increased detoxication with an increase in moisture level.

Incubation periods exerted significant degrees of influence on activity and detoxication (Table 12, Figure 2). Relatively little difference in activity of the standard was noted with prior conditioning over time at the two lowest organic matter levels. However, when prior conditioning

TABLE 9. Influence of organic matter and moisture levels during various incubation periods prior to butachlor applications as expressed by subsequent preemergence activity on barnyardgrass in Ray silt loam*

Incubation Period	Organic Matter Content	Moisture Regime	Percent Inhibition Foliage Fresh Weight Rate kg/ha x 10^{-3} butachlor					
			70	140	280	560	1120	2240
2 weeks	3	Nonflood	57	72 ab	93 ab	99 a	100	--
		Flood	47	78 ab	96 a	99 a	100	--
	9	Nonflood	--	32 c	81 b	85 b	99	100
		Flood	--	54 b	93 ab	99 a	99	100
	27	Nonflood	--	--	--	70 c	89	99
		Flood	--	--	--	73 bc	92	98
4 weeks	3	Nonflood	48	66 ab	96 a	97 ab	100	--
		Flood	46	76 ab	93 ab	100 a	100	--
	9	Nonflood	--	65 b	79 b	99 a	100	100
		Flood	--	52 b	92 ab	97 ab	100	100
	27	Nonflood	--	--	--	82 bc	95	100
		Flood	--	--	--	87 ab	95	98
8 weeks	3	Nonflood	69	85 a	95 ab	100 a	100	--
		Flood	45	79 ab	93 ab	98 ab	100	--
	9	Nonflood	--	64 b	81 b	97 ab	98	100
		Flood	--	51 b	73 b	95 ab	99	100
	27	Nonflood	--	--	--	70 c	82	98
		Flood	--	--	--	52 d	69	95

* Values followed by the same letter in the same column do not differ at the .05 level (Duncan's Multiple Range).

TABLE 10. Influence of incubation of butachlor in soil of different organic matter and moisture levels as expressed by subsequent preemergence activity on Echinochloa crusgalli in Ray silt loam

Incubation Period	Organic Matter Content	Moisture Regime	Percent Inhibition Foliage Fresh Weight Rate kg/ha x 10^{-3} butachlor					
			70	140	280	560	1120	2240
2 weeks	3	Nonflood	32	69	96	96	100	--
		Flood	11	61	92	99	100	--
	9	Nonflood	--	32	81	85	99	100
		Flood	--	26	83	88	99	100
	27	Nonflood	--	--	--	55	77	98
		Flood	--	--	--	48	81	98
4 weeks	3	Nonflood	19	65	96	97	100	--
		Flood	5	69	97	98	100	--
	9	Nonflood	--	30	88	96	100	100
		Flood	--	25	80	90	98	100
	27	Nonflood	--	--	--	63	79	100
		Flood	--	--	--	57	91	99
8 weeks	3	Nonflood	11	33	77	87	99	--
		Flood	9	17	62	84	95	--
	9	Nonflood	--	4	7	32	90	100
		Flood	--	11	29	39	84	99
	27	Nonflood	--	--	--	27	59	90
		Flood	--	--	--	21	29	88

TABLE 11. Influence of organic matter and moisture levels on performance and detoxication of butachlor*

Moisture Regime	Percent Organic Matter Content	butachlor kg/ha x 10^{-3}	
		Control Standard GR_{50}	Amount Detoxified
Nonflood	3	64 a	81 a
"	9	124 a	244 b
"	27	395 b	311 bc
Flood	3	77 a	89 a
"	9	129 a	212 ab
"	27	428 c	474 c

* Values followed by the same letter in the same column do not differ at the .05 level (Duncan's Multiple Range).

TABLE 12. Influence of organic matter and incubation periods on preemergence activity and detoxication of butachlor*

Incubation Period	Percent Organic Matter Content	butachlor kg/ha x 10^{-3} Control Standard GR_{50}	Amount Detoxified
2 weeks	3	71a	37a
"	9	113a	70a
"	27	405b	181a
4 weeks	3	74a	50a
"	9	106a	91a
"	27	326b	158a
8 weeks	3	66a	168a
"	9	134a	521b
"	27	511c	838c

* Values followed by the same letter in the same column do not differ at the .05 level (Duncan's Multiple Range).

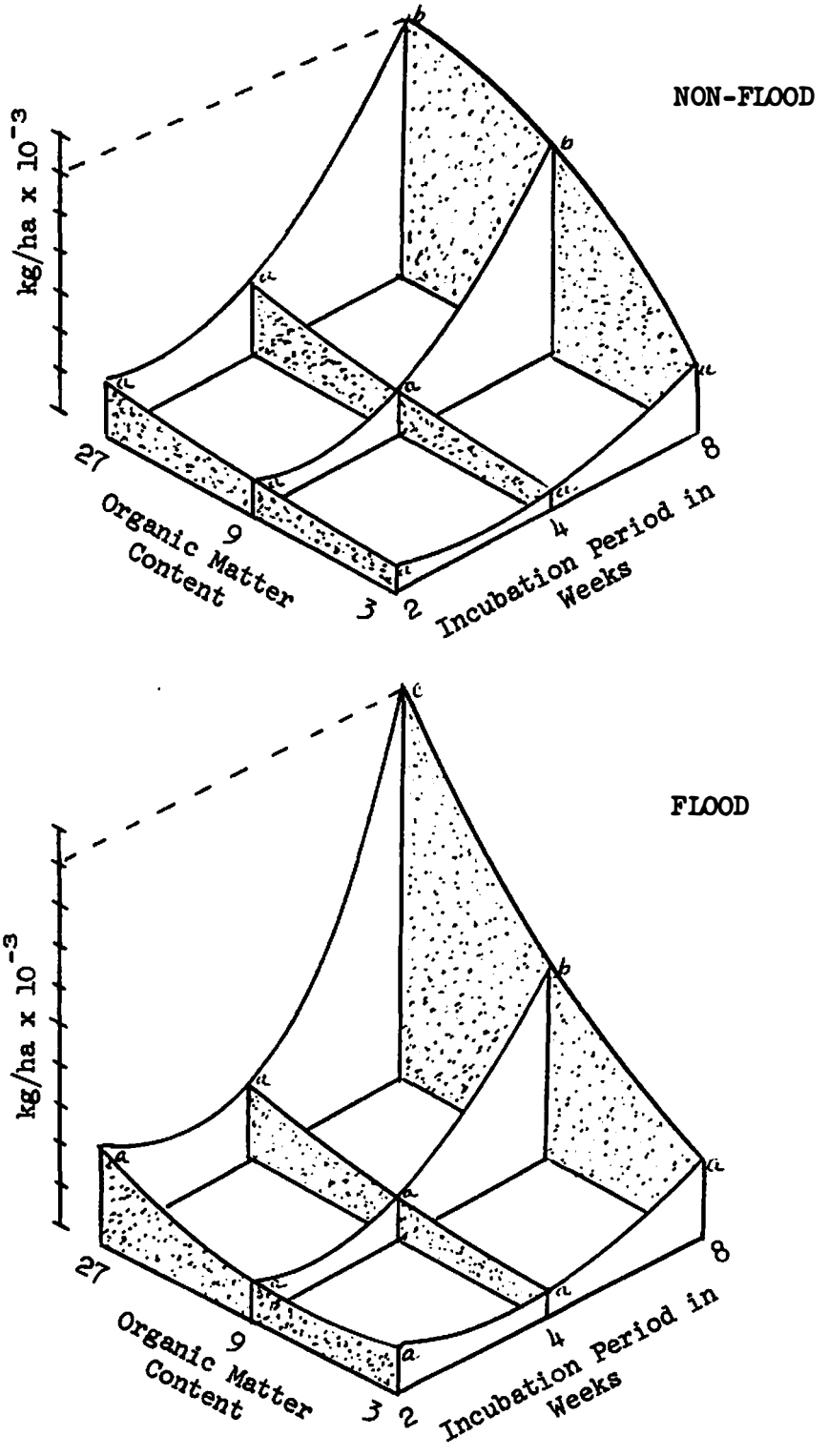


Figure 2. Influence of organic matter and incubation period at different moisture levels on the detoxication of butachlor.

at the high organic matter level over time, there appeared to be a significant difference in response after four weeks with less activity at eight weeks.

This effect was evident when the amount of butachlor detoxified was calculated. Only after eight weeks was the detoxified amount significantly different and the difference was apparent at all organic matter levels. This time effect could be considered as the lag period and at least four weeks were required for microbial adjustments and increases to exhibit differences at all organic matter levels. Conjugations with various organic acids would be more apparent over time and with an increase in organic matter levels, and thus would reflect detoxication. Thus metabolism by active and presumably large microbial populations in high organic matter soils might in fact offset the effects of adsorption upon availability in the soil solution and the consequential reduced inactivation (82).

Moisture level differences (Figure 2) were not evident until eight weeks and only at the high organic matter level did flooding enhance detoxication. This may reflect an increase in soluble amounts, in a specific organism or group of organisms, or organic matter itself may be a catalytic agent in de-alkylation or hydrolysis of butachlor. Kaufman *et al.* (51) working with amitrole found that degradation was less in muck soil than in sandy or silty clay loam, even though total CO_2 evolved was greater in muck soils. Kaufman also did not observe a lag period with amitrole and he concludes degradation of amitrole was largely a chemical process with indirect involvement of soil microbes.

Influence of Soil pH, Moisture and Length of Exposure on Detoxication

The response of butachlor to preconditioning of the media by varying pH, moisture and incubation periods on barnyardgrass is shown in Table 13. Rates of from 3.7 to 302 kg/ha x 10^{-3} were utilized in establishing a standard response as well as to determine the effect of preconditioning.

The effect of soil pH alone (Figure 3) indicates a rather substantial influence on preemergence activity of butachlor. At threshold concentration levels or less (33.6 kg/ha x 10^{-3}), the greatest degree of preemergence activity was achieved at pH 5.9 and the least activity at pH 7.1 and 8.5. At rates of butachlor greater than this threshold level as 100.9 kg/ha x 10^{-3}, the pH effect was nullified. When the GR_{50} level was calculated (Table 14), the amount of butachlor required at pH 5.9 was one half of the amount at pH 4.5 and one third the amounts at pH 7.1 and 8.5.

When butachlor was incubated at the various pH levels, moisture regimes and for prescribed durations, the degree of subsequent control of barnyardgrass (Table 15) was also influenced as reflected by the percentage inhibition of foliage fresh weight. The reduction of foliage fresh weight inhibition by various pH treatments also reflected the rate and amount of detoxication (Table 16). The greatest amount of butachlor detoxified occurred at pH 7.1 and 8.5, and the amount at pH 8.5 increased with time. The amount also increased from incubation at two weeks to four weeks at pH 7.1. At the end of the eight week cycle, there was significantly more butachlor detoxified at pH 8.5 than at any other pH level tested. The apparent lag period in detoxication was generally evident for a two or four week period depending on the soil pH level.

TABLE 13. Influence of soil treatment prior to preemergence application of butachlor as expressed by percent inhibition of foliage fresh weight of barnyardgrass*

Incubation Period	Soil pH	Moisture Regime	Percent Inhibition of Foliage Fresh Wt. Rate kg/ha x 10^{-3} butachlor 3.7	11	33	100
2 weeks	4.5	Nonflood	4 ab	31 b	72 ab	98 ab
		Flood	10 ab	29 b	72 ab	98 ab
	5.9	Nonflood	14 ab	45 bc	75 ab	95 ab
		Flood	19 ab	52 bc	79 ab	97 ab
	7.1	Nonflood	15 ab	16 ab	61 b	99 a
		Flood	1 a	3 ab	56 b	99 a
	8.5	Nonflood	3 ab	2 a	57 b	97 ab
		Flood	9 ab	5 ab	62 b	99 a
4 weeks	4.5	Nonflood	7 ab	26 ab	79 ab	95 ab
		Flood	7 ab	23 ab	71 ab	91 b
	5.9	Nonflood	18 b	61 c	80 ab	94 ab
		Flood	21 b	56 c	87 a	95 ab
	7.1	Nonflood	0 a	14 ab	55 b	82 c
		Flood	0 a	3 ab	54 b	88 bc
	8.5	Nonflood	0 a	1 a	59 b	94 ab
		Flood	0 a	9 ab	54 b	95 ab
8 weeks	4.5	Nonflood	11 ab	20 ab	74 ab	98 ab
		Flood	16 ab	31 b	77 ab	96 ab
	5.9	Nonflood	17 b	54 bc	84 a	99 a
		Flood	19 b	59 c	79 ab	96 ab
	7.1	Nonflood	1 a	10 ab	59 b	94 ab
		Flood	6 ab	14 ab	53 b	93 ab
	8.5	Nonflood	2 ab	15 ab	59 b	91 b
		Flood	4 ab	3 ab	56 b	88 bc

* Values followed by the same letter in the same column do not differ at the .05 level (Duncan's Multiple Range).

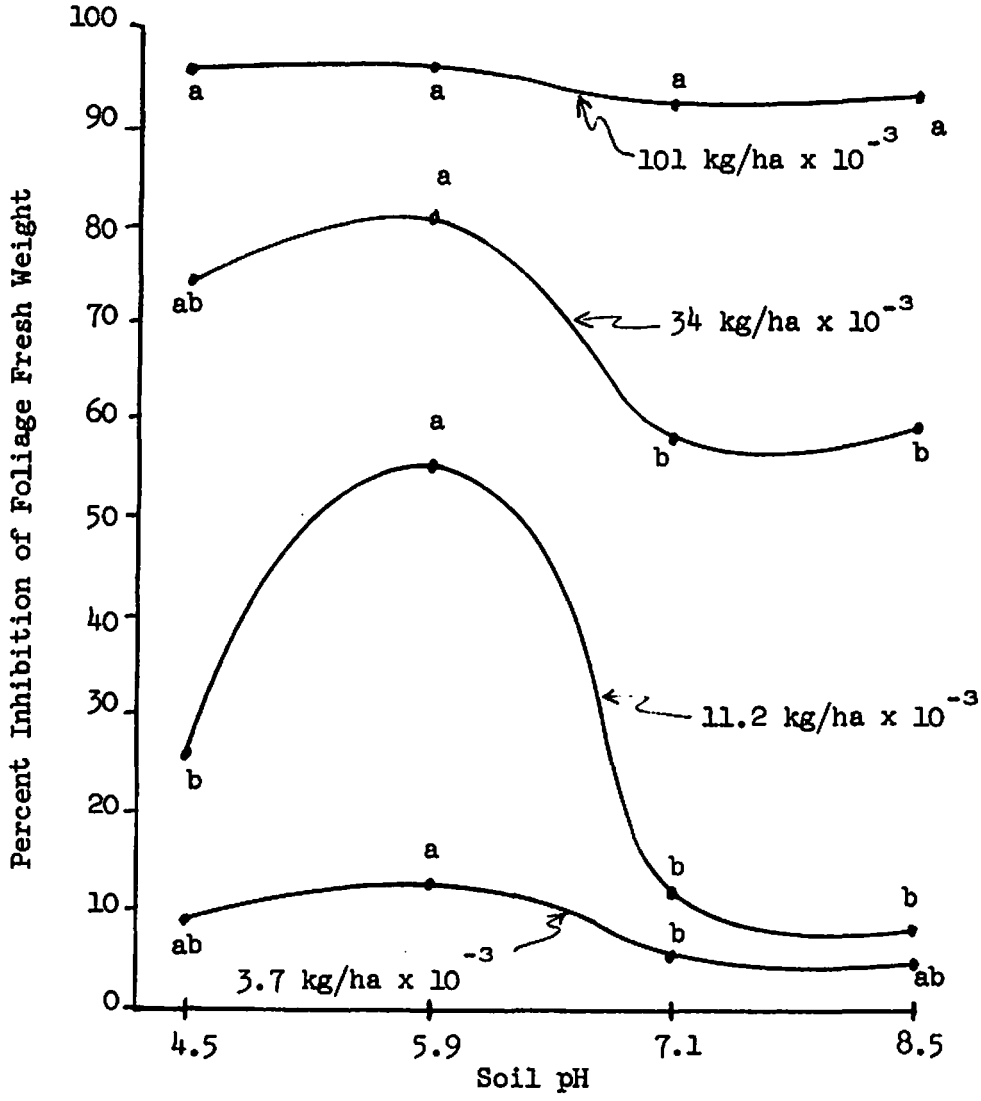


Figure 3. Effect of soil pH on preemergence activity of butachlor at various rates on barnyardgrass in Ray silt loam.

TABLE 14. Influence of soil pH on preemergence activity of butachlor on barnyardgrass in Ray silt loam soil*

Soil pH	kg/ha x 10^{-3} required of butachlor for GR_{50} on barnyardgrass
4.5	22.0 b
5.9	10.6 a
7.1	31.1 c
8.5	31.6 c

* Values followed by the same letter do not differ at the .05 level (Duncan's Multiple Range).

TABLE 15. Influence of soil pH and moisture levels during various incubation periods on the subsequent preemergence activity of butachlor on barnyardgrass in Ray silt loam soil as expressed by percent inhibition of foliage fresh weight

Soil pH	Rate kg/ha x 10^{-3}	Percent Inhibition					
		2 weeks		4 weeks		8 weeks	
		Nonflood	Flood	Nonflood	Flood	Nonflood	Flood
4.5	302	100	100	100	100	100	95
	100	97	96	83	69	62	54
	33	69	68	60	39	47	21
	11	41	49	38	7	6	10
	4	0	9	3	2	4	1
5.9	302	100	100	100	85	97	90
	100	98	97	79	68	87	68
	33	84	50	64	52	59	39
	11	56	20	48	10	47	14
	4	15	18	10	0	11	8
7.1	302	100	97	93	52	94	39
	100	88	43	41	0	24	22
	33	55	4	9	0	4	14
	11	10	0	0	0	0	10
	4	3	0	0	0	0	10
8.5	302	100	100	90	54	66	23
	100	66	39	33	4	4	7
	33	10	6	6	0	8	6
	11	7	3	0	0	11	4
	4	2	1	0	0	8	3

TABLE 16. Influence of soil pH and incubation periods on preemergence activity and detoxication of butachlor in Ray silt loam soil*

Soil pH	Incubation Period	kg/ha x 10^{-3} butachlor Control Standard GR_{50}	Amount Detoxified
4.5	2 weeks	21.2 b	5 a
"	4 "	22.4 bc	15 ab
"	8 "	21.9 bc	54 ab
5.9	2 weeks	10.9 ab	9 a
"	4 "	11.1 ab	26 ab
"	8 "	9.7 a	41 ab
7.1	2 weeks	28.1 bc	48 ab
"	4 "	35.0 c	179 c
"	8 "	30.9 bc	225 c
8.5	2 weeks	32.5 c	76 b
"	4 "	30.8 bc	200 c
"	8 "	31.4 c	321 d

* Values followed by the same letter in the same column do not differ at the .05 level (Duncan's Multiple Range).

When moisture was a factor with soil pH detoxication (Table 17), then only at pH 7.1 and 8.5 was there any significant difference in the amount detoxified. At these levels, flooding substantially increased the amount of herbicide detoxified.

A discussion of the effect of media pH on detoxication must embrace consideration of pH influences on microbial species, populations and activity, influence on the ionic character of the toxicant and the media colloids, the cation exchange capacity and the adsorption-dissociation relationships.

Low acidic soil pH has generally been known to favor fungi development due to lack of competition from bacteria or actinomycetes, whereas soil pH ranges of neutral to alkaline favor bacterial and actinomycetes development. The actinomycetes are usually inferior in numbers to bacteria; however, they constitute a very important and physiologically active group capable of degrading numerous organic molecules (20). The most frequently isolated member of the group belongs to the genus Streptomyces. Davies (20), in a soil study, determined that various Streptomyces species were increased in numbers with neutral or alkaline soil in comparison to acidic soil and where the soil moisture was relatively high at 6 percent level.

The magnitude of the adsorption of organic compounds is governed by the pH of the total system, both the solution and surface phases, the cation exchange capacity, the chemical character of the compound and its water solubility. Adsorption and cation exchange capacity of soils high in organic matter is high; and of the clay minerals, the order of magnitude decreases from montmorillonite to illite to kaolinite, due

TABLE 17. Influence of soil pH and moisture levels on preemergence activity and detoxication of butachlor in Ray silt loam soil*

Soil pH	Moisture Regime	kg/ha x 10^{-3} butachlor: Control Standard GR_{50}	kg/ha x 10^{-3} butachlor: Amount Detoxified
4.5	Nonflood	21.8 a	14 a
"	Flood	22.6 a	34 a
5.9	Nonflood	9.9 a	12 a
"	Flood	11.3 a	38 a
7.1	Nonflood	30.6 a	79 a
"	Flood	32.8 a	222 c
8.5	Nonflood	31.0 a	138 b
"	Flood	32.1 a	260 c

* Values followed by the same letter in the same column do not differ at the .05 level (Duncan's Multiple Range).

to the respective decrease in cation exchange sites. With montmorillonite clays, adsorption is greatest with acidic pH than basic, adsorption of basic organic compounds are dependent more on surface acidity than solution pH, while the converse is true of acidic organic compounds (4). The surface pH of montmorillonite is 3-4 pH units lower than the suspension pH (4). With organic matter, Corbin and Upchurch (17) related the increase in cation exchange capacity with increases in alkalinity "within limits". They further relate the compound charge with exchange capacity, implicating detoxication regulation. Positive charged herbicides would be more readily adsorbed and "presumably might be less available to microbes", while anionic herbicides would be repelled by the negatively charged colloids and should be available for detoxication (17). The relative detoxication increase of butachlor at the alkaline pH range plus the increased amount under flooding would substantiate the ionic repulsion theory (van der Waals forces). However, there are several mechanisms of adsorption, and the specie mechanisms and stage of compound detoxication would be expected to have certain relationships. For compounds which are basic in chemical character and contain one N-H group, adsorption can occur by an important adsorption mechanism known as hydrogen bonding (4). Adsorption of an N-dealkylated butachlor could occur by formation of a hydrogen bond between the amino group and the oxygen of the colloid surface. Such a reaction is the prime mechanism of adsorption of basic organic compounds and is reported to di-ethylamine and aliphatic amines (4). Adsorption of aniline is either by protonation at or near the particle surface and by base saturation due to dissociation of the proton in residual water

on the clay surface and subsequent protonation (4). The carbonyl oxygen would be another hydrogen bonding site. Adsorption of acidic compounds is primarily due to van der Waals forces and to proton association-hydrogen bonding from carboxyl groups.

Evidence of a Lag Period

The factors held relatively constant throughout all the bioassay tests were the moisture levels and the incubation period. During the course of each of the various tests, it became evident that the time factor was of increasing importance in assessing the detoxication rate or amount (Table 18). The moisture level also appeared to consistently influence detoxication at a relatively uniform and predictable pace. Although the amounts detoxified within each test were different due undoubtedly to other test variables, the response to moisture and incubation periods were quite similar. The lag period was quite evident through the initial four weeks of incubation and detoxication increased from four to eight weeks (and to sixteen weeks with the temperature test). Flooding for a period of eight weeks also consistently increased the detoxified amount of butachlor after the first four week lag period. This flooding factor undoubtedly influenced the total amount in a soluble state in relation to adsorption and subsequently microbial detoxication. The consistent evidence of lag period supports the theory of microbial detoxication as a primary mechanism with butachlor.

One can conclude from these sets of experiments that the temperature, pH, organic matter and moisture level of the soil environment has a significant effect on the intrinsic capacity of microbes to assume a pertinent role in the partial or total inactivation of butachlor.

TABLE 18. Influence of moisture levels and incubation periods on detoxication of butachlor during various greenhouse tests*

Moisture Regime	Incubation Period	kg/ha x 10^{-3} detoxified		
		Temperature Test	Sterilization Test	pH Test
Nonflood	2 weeks	66 a	138 a	15 a
"	4 "	137 a	185 a	62 ab
"	8 "	385 b	333 b	106 b
"	16 "	708 c	-	-
Flood	2 weeks	81 a	239 ab	53 ab
"	4 "	146 a	392 b	148 b
"	8 "	622 c	604 c	214 c
"	16 "	1105 d	-	-

* Values followed by the same letter in the same column do not differ at the .05 level (Duncan's Multiple Range).

II. TRACER STUDIES

Recovery of Ring and Carbonyl Labeled Butachlor as $^{14}CO_2$ as Influenced by Environmental Factors

The recovery of radioactivity from butachlor ^{14}C treated soils as $^{14}CO_2$ is shown in Table 19 on a weekly, cumulative basis. The maximum amount of $^{14}CO_2$ evolved of the total carbonyl labeled butachlor applied was 13 percent at the end of 11 weeks at 32 C under non-flooded conditions, while only 4.5 percent of the ring label was recovered as $^{14}CO_2$ in eleven weeks. Less of both labeled materials was recovered as $^{14}CO_2$ from flooding, autoclaving, KN_3 treatments of 400 ppm and temperatures of 24 and 16 C.

The amount of $^{14}CO_2$ evolved from carbonyl labeled butachlor on a per week basis (Figure 4) increased until the fifth week of incubation, then there was a varied and slight decrease for the next six weeks. The most significant increases and decreases in amounts of $^{14}CO_2$ evolved was at 32 C.

The percentage of the total amount of carbonyl labeled butachlor that was degraded and measured as $^{14}CO_2$ as a function of temperature and time is shown in Table 20. There was no difference in the amount of $^{14}CO_2$ evolved during the eleven weeks of incubation at the cool temperature of 16 C. As the temperature was increased to 24 and 32 C, then significant differences were observed after six and eleven weeks of incubation.

When incubation moisture levels and duration are considered (Table 21), there is a significant difference in the amount of $^{14}CO_2$ evolved under nonflooded levels at seven and eleven weeks. Flooding

TABLE 19. Percent ^{14}C of total amount of carbonyl or ring labeled butachlor applied recovered as $^{14}CO_2$ on a cumulative basis at three incubation temperatures, moisture levels and with various sterilization methods

Temperature C	Moisture*	Sterilization Method	Percent of Total ^{14}C Applied Recovered as $^{14}CO_2$										
			1st Week	2nd Week	3rd Week	4th Week	5th Week	6th Week	7th Week	8th Week	9th Week	10th Week	11th Week
16	NF	-	0.52	0.65	0.80	1.25	1.70	2.03	2.38	2.68	2.90	3.17	3.22
	"	Autoclave	0.18	0.24	0.26	0.28	0.32	0.35	0.38	0.40	0.43	0.44	0.46
	"	KN_3	0.09	0.15	0.18	0.22	0.26	0.30	0.34	0.36	0.40	0.42	0.45
	Fl	-	0.90	1.11	1.31	1.41	1.52	1.64	1.72	1.78	1.83	1.88	1.91
	"	Autoclave	0.12	0.15	0.18	0.20	0.22	0.24	0.26	0.28	0.29	0.33	0.32
	"	KN_3	0.07	0.12	0.18	0.20	0.24	0.27	0.30	0.32	0.35	0.36	0.39
24	NF	-	0.57	0.77	1.27	2.19	2.88	3.67	4.48	5.23	6.22	6.96	7.78
	"	Autoclave	0.14	0.16	0.25	0.31	0.38	0.45	0.50	0.56	0.64	0.81	1.33
	"	KN_3	0.18	0.21	0.28	0.31	0.49	0.53	0.58	0.61	0.70	0.77	0.98
	Fl	-	1.31	1.57	1.80	2.00	2.17	2.32	2.48	2.58	2.72	2.83	2.96
	"	Autoclave	0.14	0.18	0.24	0.26	0.49	0.61	0.66	0.68	0.76	0.80	0.84
	"	KN_3	0.24	0.35	0.44	0.61	0.86	1.02	1.14	1.20	1.36	1.51	1.57
32	NF	-	0.88	1.94	3.15	4.78	6.52	8.01	9.42	10.38	11.42	12.31	13.14
	"	Autoclave	0.36	0.46	0.57	0.72	0.88	1.06	1.21	1.28	1.39	1.48	1.54
	"	KN_3	0.27	0.39	0.51	0.58	0.68	0.81	0.95	1.04	1.15	1.21	1.30
	Fl	-	1.47	1.90	2.32	2.64	2.90	3.13	3.33	3.46	3.68	4.20	4.34
	"	Autoclave	0.21	0.28	0.35	0.47	0.57	0.68	0.78	0.88	1.16	1.76	2.32
	"	KN_3	0.38	0.62	0.99	1.35	1.68	1.86	2.02	2.14	2.26	2.38	2.01
	NF**	-	0.17	0.32	0.63	0.99	1.52	2.07	2.64	3.08	3.62	4.06	4.54
	"	Autoclave	0.15	0.20	0.46	0.60	0.76	0.91	1.04	1.12	1.21	1.28	1.36
	"	KN_3	0.14	0.18	0.26	0.30	0.41	0.45	0.48	0.50	0.52	0.54	0.58
	Fl**	-	0.20	0.34	0.49	0.64	0.71	0.80	0.88	0.94	0.01	0.08	0.14
	"	Autoclave	0.18	0.32	0.46	0.58	0.68	0.73	0.84	0.92	1.01	1.08	1.12
	"	KN_3	0.17	0.30	0.39	0.49	0.60	0.68	0.78	0.85	0.92	0.98	1.05

* NF = Non-flooded, Fl = flooded

** Ring label only

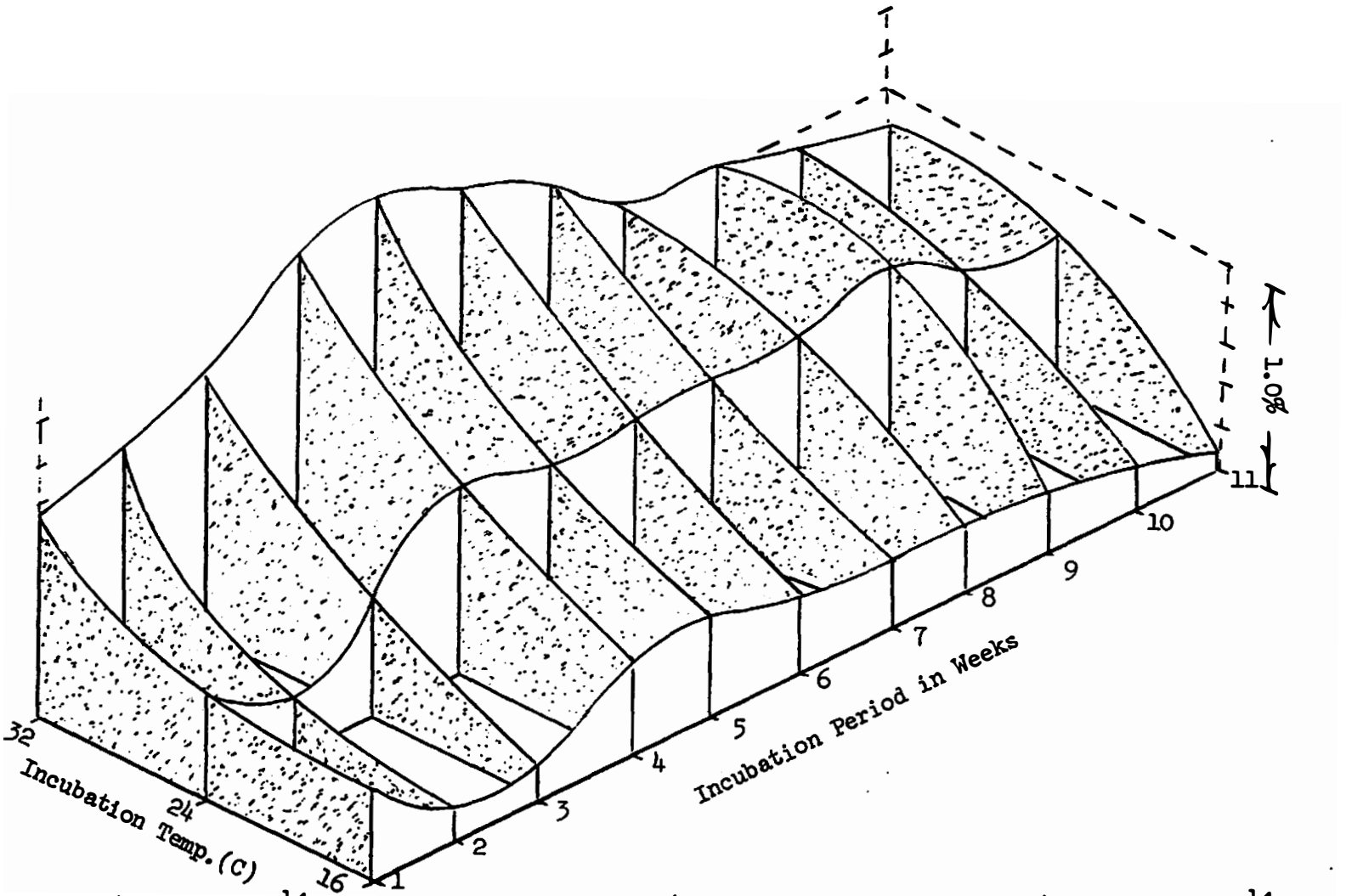


Figure 4. Percent ^{14}C of total amount applied (carbonyl labeled butachlor) recovered as $^{14}CO_2$ on a weekly basis at three incubation temperatures under non-flooded moisture conditions.

TABLE 20. Influence of incubation temperature and duration on degradation of carbonyl labeled butachlor to $^{14}CO_2$ on a weekly cumulative basis in Ray silt loam*

Incubation Period	Percent of Total ^{14}C Recovered as $^{14}CO_2$		
	16 C	24 C	32 C
1 week	0.32 a	0.43 ab	0.59 ab
2 weeks	0.40 ab	0.54 ab	0.94 ab
3 "	0.48 ab	0.74 ab	1.32 ab
4 "	0.59 ab	0.95 ab	1.76 bc
5 "	0.71 ab	1.21 ab	2.20 bc
6 "	0.81 ab	1.43 b	2.59 c
7 "	0.89 ab	1.65 bc	2.95 cd
8 "	0.95 ab	1.81 bc	3.20 cd
9 "	1.03 ab	2.07 bc	3.51 dc
10 "	1.10 ab	2.29 bc	3.89 dc
11 "	1.12 ab	2.57 c	4.17 e

* Values followed by the same letter do not differ at the .05 level (Duncan's Multiple Range).

TABLE 21. Influence of moisture level and duration during incubation on the degradation of carbonyl labeled butachlor to $^{14}CO_2$ on a cumulative weekly basis in Ray silt loam*

Incubation Period	Percent of Total ^{14}C Recovered as $^{14}CO_2$	
	Nonflood	Flood
1 week	0.36 a	0.54 ab
2 weeks	0.55 ab	0.69 ab
3 "	0.83 ab	0.87 ab
4 "	1.18 ab	1.02 ab
5 "	1.56 bc	1.18 ab
6 "	1.91 bc	1.31 b
7 "	2.25 c	1.41 bc
8 "	2.49 cd	1.48 bc
9 "	2.80 cd	1.60 bc
10 "	3.07 cd	1.78 bc
11 "	3.35 d	1.89 bc

* Values followed by the same letter do not differ at the .05 level (Duncan's Multiple Range).

appears to inhibit the degradation of carbonyl labeled butachlor in comparison to nonflooding. The difference between moisture levels did not become significant until an incubation temperature of 32 C was maintained (Table 22). Within each moisture regime, the differences due to temperature are apparent at each level under nonflooded conditions, while under flooded environment, the difference of $^{14}CO_2$ is only between 16 and 32 C.

Sterilization treatments of autoclaving and KN_3 (Table 23) significantly inhibited $^{14}CO_2$ evolution of carbonyl labeled butachlor treated soils at both moisture levels. There was no difference between sterilization methods during the eleven weeks of testing, although it was observed that there was a greater tendency for an increase in $^{14}CO_2$ with the flooded KN_3 treatment than with the nonflooded.

Degradation of the ring labeled butachlor was significantly slower than the carbonyl labeled butachlor (Table 24). Significant differences in the percentage of the total amount of radioactivity applied that was recovered as $^{14}CO_2$ was apparent after three weeks. In turn, this difference was also apparent for each following week through to the eleventh week.

The degradation of the carbonyl labeled butachlor was also significantly greater and faster under both moisture levels than the ring label (Table 25). There was essentially no difference between labels as to the amount evolved as $^{14}CO_2$ with the different sterilization techniques (Table 26).

The resistance of the phenyl ring to degradation by microbes is well known. Recent work by Chisaka and Kearney (15) with propanil,

TABLE 22. Moisture levels and incubation temperatures influencing the production of $^{14}CO_2$ from carbonyl labeled butachlor in Ray silt loam soil*

Temperature	Percent of Total ^{14}C Recovered as $^{14}CO_2$	
	Nonflood	Flood
16 C	0.85 a	0.68 a
24 C	1.62 b	1.24 ab
32 C	3.09 c	1.84 b

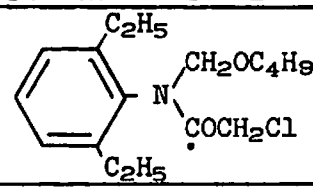
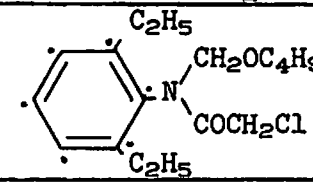
* Values followed by the same letter do not differ at the .05 level (Duncan's Multiple Range).

TABLE 23. Effect of soil media sterilization treatments at the two incubation moisture levels on the degradation of carbonyl labeled butachlor

Sterilization Method	Percent Total ^{14}C Recovered as $^{14}CO_2$	
	Nonflood	Flood
Nonsterile	4.41 a	2.28 b
Autoclaved	0.62 c	0.53 c
KN_3	0.54 c	0.95 c

* Values followed by the same level do not differ at the .05 level (Duncan's Multiple Range).

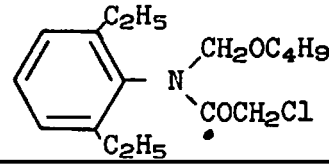
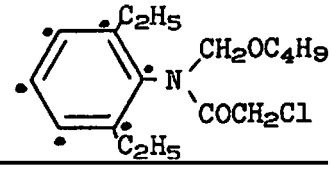
TABLE 24. Comparison of degradation of the two labeled butachlor treatments as a function of time in Ray silt loam*

Incubation Period in Weeks	Percent of Total ^{14}C Recovered as $^{14}CO_2$ 	
1	0.60 ab	0.16 a
2	0.94 ab	0.29 ab
3	1.32 bc	0.45 ab
4	1.76 bc	0.60 ab
5	2.20 c	0.78 ab
6	2.59 cd	0.94 ab
7	2.95 cd	1.10 b
8	3.20 d	1.23 bc
9	3.51 dc	1.38 bc
10	3.89 dc	1.51 bc
11	4.17 c	1.63 bc

* Values followed by the same letter do not differ at the .05 level (Duncan's Multiple Range).

• Indicates position of ^{14}C label

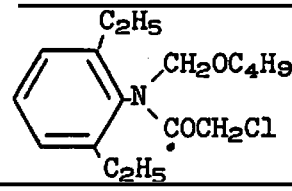
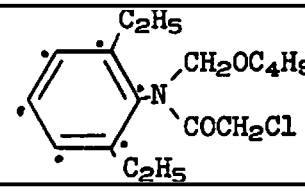
TABLE 25. Comparison of percent ^{14}C of the total labeled amount of butachlor applied recovered as $^{14}CO_2$ in Ray silt loam under two moisture levels*

Moisture Regime	Percent of Total ^{14}C Recovered as $^{14}CO_2$	
		
Nonflood	3.09 a	1.12 bc
Flood	1.84 b	0.71 c

* Values followed by the same letter do not differ at the .05 level (Duncan's Multiple Range).

• Indicates position of ^{14}C label

TABLE 26. Influence of sterilization techniques on degradation of butachlor*

Sterilization Method	Percent of Total ^{14}C Recovered as $^{14}CO_2$	
		
Nonsterilized	5.24 a	1.45 b
Autoclaved	0.92 bc	0.77 bc
KN_3	1.23 bc	0.52 c

* Values followed by the same letter do not differ at the .05 level (Duncan's Multiple Range).

• Indicates position of ^{14}C label.

3',4'-dichloropropionanilide, showed that more than 60 percent of the initial carbonyl ^{14}C added to soil was evolved as $^{14}CO_2$ within five days. However, $^{14}CO_2$ evolution from ring labeled propanil was very much slower and amounted to less than 3 percent after twenty-five days. Both of these anilines, propanil and butachlor, have been evaluated with labeling of the ring or carbonyl group and reports are not available on the fate of alkyl labeling and subsequent $^{14}CO_2$ evolved. Kaufman and Blake (52), working with ^{14}C-ethyl or "chain labeled" and ring labeled atrazine, found that $^{14}CO_2$ was evolved from the alkyl label, but found essentially no $^{14}CO_2$ from ring labeled atrazine in solution cultures. Labeling of the alkyl does not insure fast $^{14}CO_2$ evolution, since the ^{14}C evolved as $^{14}CO_2$ of the ethyl moiety of EPTC was very slow after four weeks exposure in the studies of MacRae and Alexander (65).

Parochetti and Warren (71) and Chisaka and Kearney (15) have used and evaluated autoclaving and KN_3 as controls for microbial degradation. The lack of residual activity with autoclaving adds emphasis to using azides as sterilizing agents. Parochetti and Warren (71) found that KN_3 was converted rapidly to HN_3 in acid soils and decomposed quite readily. Temperatures of 24 to 32 C also influenced volatilization of KN_3 and its subsequent loss. During the test (Table 19) from the second to the ninth week, there appeared to be more $^{14}CO_2$ evolved with KN_3 treatments than with autoclaving at the high temperature of 32 C under flooding. This difference between sterilization techniques was not observed under nonflooded conditions.

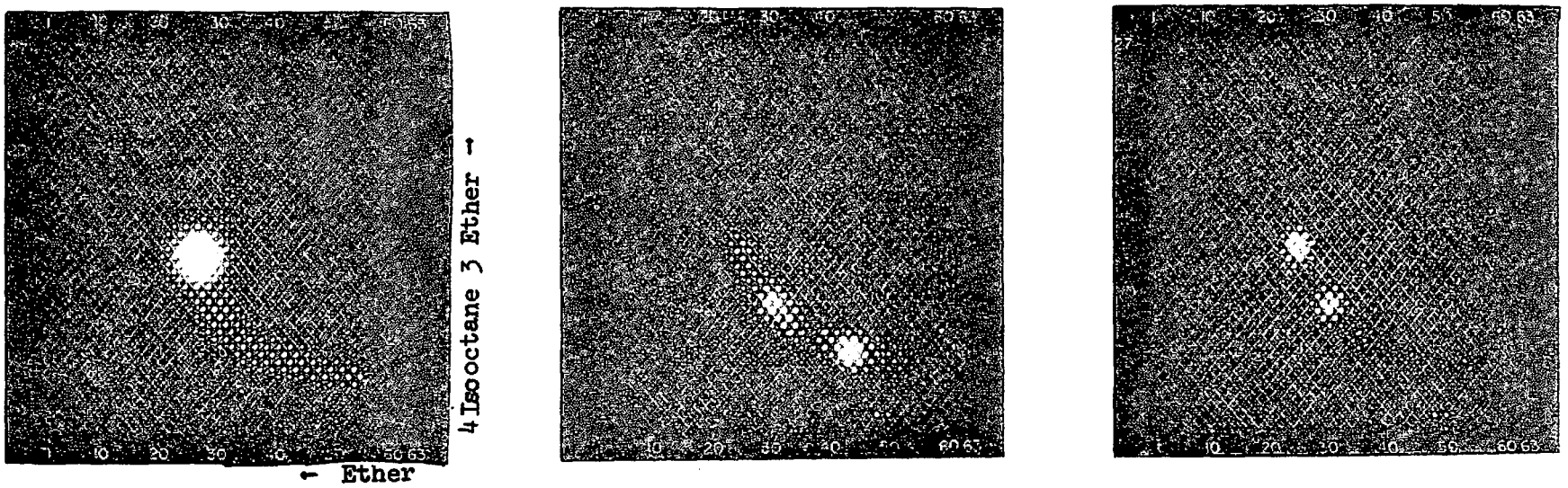
The role of specific environmental influences on the microbial degradation of butachlor to CO_2 has been shown to be indicative of conditions most suitable for microbial activity. With an increase in

temperature there is an increase in degradation; however, with flooding, there is an apparent inhibition in degradation as compared to moisture of the 15 percent level. It has been demonstrated that detoxication of butachlor is favored by flooding and high temperatures as revealed by plant bioassay. The data would indicate that the initial detoxified product of butachlor is not necessarily a decarbonylated product, but rather may be an N-dealkylated or an alpha hydroxylated product. The presence or absence of these products has not been proved with the methods employed; however, the data does indicate that detoxication of butachlor can proceed and occur under conditions not as relatively suitable for total degradation to CO_2.

Environmental Effects on Production in Soil of Extractable Organic and Aqueous Soluble Metabolites

Betagrams of the applied carbonyl labeled butachlor, laboratory standards of butachlor and the N-dealkylated and hydroxylated butachlor with respective R_f values as developed on two dimensional thin silica gel chromatograms are shown in Figure 5.

The extractable organic soluble products were chromatographed and resulting radioactive zones were measured as shown with the standards in the betagrams in Figure 5. The R_f value and percentage of amount of each aliquot was calculated for samples of eight and eleven weeks incubation (Table 27). Three organic soluble products other than butachlor were extracted. Two of these products were tentatively identified by R_f values as N-butoxymethyl-2-hydroxy-2',6'-diethylacetanilide ("B") and 2-Chloro-2',6'-diethylacetanilide ("A"), while the third was unknown origin material. The percentage of the aliquot that was butachlor tended to



	Applied Material			Lab Standard # 2			Lab Standard # 1		
	CP 2M*	%	R_f	CP 2M*	%	R_f	CF 2M*	%	R_f
Butachlor	86068	93.8	.66/.44	393	1.9	.66/.44	9660	56.8	.66/.44
NH "	3265	3.6	.53/.21	7745	38.4	.53/.21	7126	41.9	.53/.21
αCOH "	1332	1.4	.24/.05	11899	59.0	.24/.05	71	0.2	.24/.05
Origin	1094	1.2	.02/.00	144	0.7	.20/.00	102	0.6	.02/.00

Figure 5. Betagrams** of applied butachlor and standards of thin layer silica gel chromatograms.

* top to bottom, left to right

** Betagram is the term used by Baird-Atomic Manufacturers of the Model 6000 Beta Camera.

TABLE 27. Relative percentages of extractable organic soluble compounds from various butachlor soil treatments as determined on two dimensional thin layer chromatograms (silica gel)*

Incubation Temperature	Moisture Regime	Incubation Period	Percent of Soil Aliquote: butachlor	"A"	"B"	Unknown Origin
				$R\text{-}N(CH_2OC_4H_9)COCH_2Cl$	$R\text{-}N(H)COCHCl$	$R\text{-}N(CH_2OC_4H_9)COCH_2OH$	
16C	Nonflood	8 weeks	83.6	6.9	2.8	4.4
	Flood	"	87.8	4.6	2.4	3.2
24C	Nonflood	"	73.6	7.4	4.7	11.0
	Flood	"	72.9	7.6	4.5	13.0
32C	Nonflood	"	53.7	8.6	8.8	26.7
	Flood	"	59.9	20.4	6.2	10.8
16C	Nonflood	11 weeks	68.4	8.8	4.5	18.0
	Flood	"	89.4	5.0	2.1	3.5
24C	Nonflood	"	62.3	18.4	5.1	14.2
	Flood	"	82.0	7.2	4.1	6.6
32C	Nonflood	"	33.2	19.1	15.5	32.0
	Flood	"	17.7	19.6	14.9	47.8
Applied Material			93.0	4.6	1.4	1.0
Standard mixtures, No. 1			56.8	41.9	0.2	0.6
No. 2			1.9	38.4	59.0	0.7

* First development: 4 isooctane 3 ether. Second development: ether

R = 2',6'-diethylphenyl

decrease with temperature and incubation duration. The origin material, however, tended to increase with temperature and exposure duration. At eight weeks, the amount of the N-dealkylated product increased with temperature and was especially prominent under flooded conditions. There appeared to be less on a percentage of the aliquot basis of the alpha-hydroxy product than the N-dealkylated product initally at eight weeks and at the lower two temperatures at eleven weeks. A substantial increase in the alphahydroxyl product was observed at 32 C after eleven weeks under both moisture regimes.

The N-dealkylated, alphahydroxylated and origin materials appeared to be slightly more prominent under non-flooded than flooded conditions at 16 and 24 C after eleven weeks exposure. The origin material was most prominent at 32 C and flooded after eleven weeks while the N-dealkylated and hydroxylated products were relatively similar between both moisture regimes after eleven weeks at 32 C. This data tends to indicate that temperature, moisture and exposure duration influenced the development of the organic soluble degradation products of butachlor. High temperatures, ample moisture and long term exposure enhanced the relative production and concentration of these degradation products. Complete submersion of the substrate tended to initially enhance the relative increase of the N-dealkylated product and eventually of the origin material. The most significant increase of a degradation product with time, temperature and moisture appears to be with the origin material and this relative increase coincides with a similar decreasing pattern of butachlor itself. The increasing trend of the origin material also did not appear to be at the expense of the N-dealkylated or alphahydroxylated

product which would indicate that each degradation product could be produced simultaneously and possibly independently. The environmental conditions existing at a given time would appear to dictate the relative proportions and assumed rate of production of these organic soluble degradation products of butachlor.

The extractable aqueous soluble metabolites from various butachlor soil treatments for eleven weeks as developed electrophoretically with a buffer of pH 5.4 is shown in Figure 6. From three to five metabolites were evident when developed at buffer pH 5.4 electrophoretically. With an increase in incubation temperature, there appeared to be an increase in the percentage of more ionized metabolites. However, the same number of metabolitic products appeared at all three temperatures and both moisture regimes. The predominance of these aqueous soluble metabolites did not appear to be influenced by moisture levels during incubation.

A comparison of the incubation periods of eight and eleven weeks at 24 and 32 C (Figure 7) did not reveal a substantial shift or change in the type of aqueous soluble metabolite produced. One product did develop at 32 C under flooded conditions at eleven weeks (4-6 cm) that was not evident or as prominent at eight weeks. The lack of prominence of the relative amount of origin material was also observed at 32 C at eleven weeks.

When the aqueous soluble metabolites were electrophoretically developed at a buffer pH of 2.2 (Figure 8), there appeared to be less migration of the metabolites than witnessed at pH 5.4. This might indicate that the metabolites were acidic and in turn were not as highly ionized by increasing the acidity of the buffer solution. The same number of metabolites were apparent at pH 2.2 as at pH 5.4.

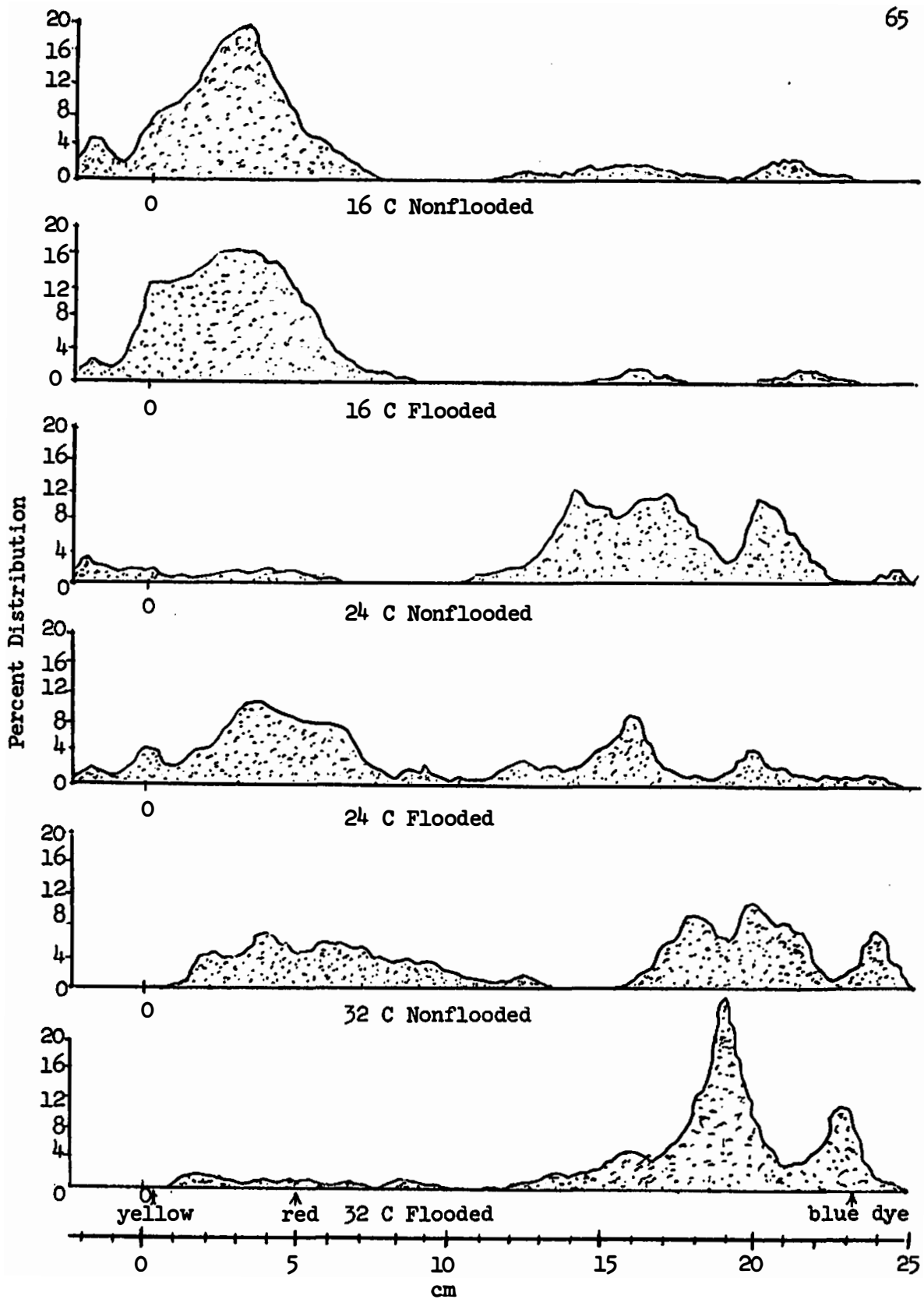


Figure 6. Electrophoretic distribution patterns of aqueous soluble ^{14}C metabolites (developed at buffer pH5.4) of butachlor treated soils at three temperatures and two moisture levels after eleven weeks incubation.

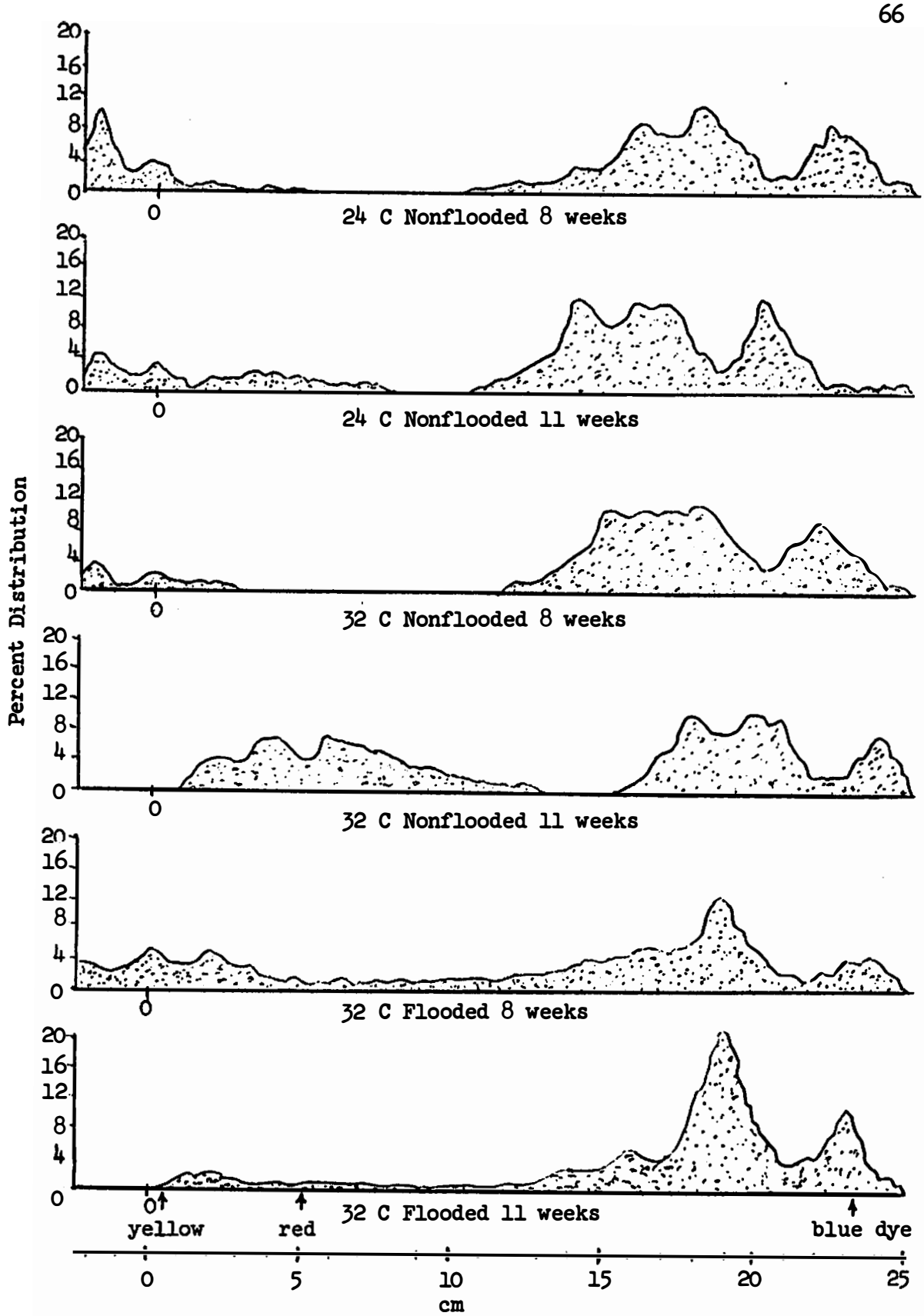


Figure 7. Comparative electrophoretic distribution of aqueous soluble metabolites of butachlor from two incubation durations at two temperatures.

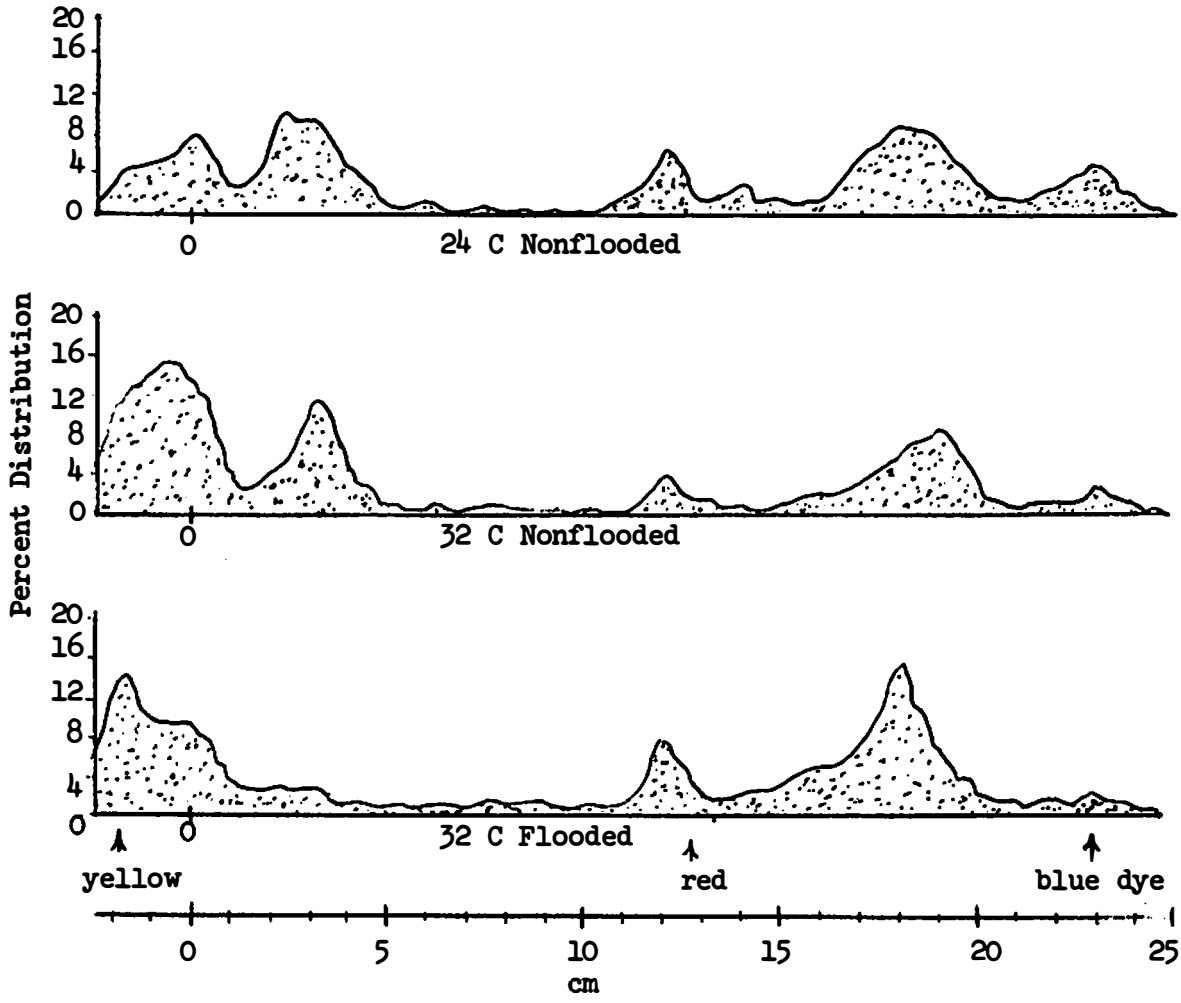


Figure 8. Electrophoretic distribution of aqueous soluble metabolites of butachlor (developed with buffer pH 2.2) from soils treated for eleven weeks at two temperatures.

Environmental factors that generally enhance microbial activity also tend to enhance the degradation of butachlor to at least three organic soluble and three to five aqueous soluble metabolites. Temperature and exposure duration were prime factors, while moisture levels especially flooding did not appear to have any substantial effect on production of aqueous metabolites, but did effect production of specific organic soluble metabolites.

There was no attempt made in this study to identify the aqueous soluble metabolites. Recent work by Lamoureux, Stafford and Tanaka (58) with propachlor, N-isopropylacetanilide, indicated that in plants such as corn and sorghum, the metabolism of propachlor and subsequent detoxication was by conjugation with the amino acid, glutathione. The mechanism involved is the displacement of the 2-chloro group by a peptide sulfhydryl group (58). Two products have been identified from this displacement, glutathione and γ-glutamylcysteine conjugates of propachlor. The conjugates were water soluble and of an acidic nature. Glutathione conjugation of atrazine has been identified as the major mechanism of detoxication in corn (84) and is catalyzed by an enzyme identified as glutathione-S-transferase (25). This soluble enzyme was found to be present in species tolerant to atrazine, but not in susceptible species (31).

The data presented in the present investigation do not support identification of certain aqueous metabolites as glutathione conjugates nor is the author aware of any data presenting acetanilide soil evolved conjugations. However, conjugations with various soil amino acids and/or carbohydrates would not appear impossible and it is expected that the prevailing environmental conditions would significantly influence the rate and kind of metabolites evolved.

CHAPTER V

SUMMARY

This investigation of environmental factors influencing the detoxication and subsequent degradation of butachlor was initiated in September, 1970, and involved two phases. One phase dealt with factors effecting detoxication as bioassayed by barnyardgrass under greenhouse conditions. The second phase involved tracer studies determining the influence of environmental factors on $^{14}CO_2$ evolution from two labeling samples of butachlor and the presence and transitory patterns of organic and aqueous soluble metabolites.

Detoxication of butachlor was significantly influenced by the exposure duration to different moisture levels, temperatures, soil pH and organic matter content. The conditions conducive to microbial development, and more specifically to bacteria and actinomycetes, significantly enhanced detoxication and the primary detoxication mechanism of butachlor appeared to be microbial in nature. However, chemical detoxication was not eliminated as a probable, but definitely secondary, means of detoxication.

$^{14}CO_2$ evolution was influenced by environmental factors with the most significant amounts being evolved at high temperatures for the initial five weeks of incubation. After this time there was a decrease for the next six weeks with a total amount of 13 percent of the original applied material of 24 ppm (equivalent to 4 lb/A, 1/2", a.i. or 4.4 kg/ha cm) being evolved as $^{14}CO_2$ from carbonyl labeled butachlor and only 4.5 percent of ring labeled butachlor. Although flooding enhanced detoxication of butachlor after eight weeks exposure, the evolution of $^{14}CO_2$

from carbonyl or ring labels was inhibited by flooding in comparison to nonflooded moisture levels of 15 percent.

Electrophoretic and thin layer chromatograms of soil produced aqueous and organic soluble metabolites further emphasized the role of the environment and the intrinsic capacity of microbes to contribute to the degradation of a pesticide. Three organic soluble and five aqueous soluble metabolites of butachlor were separated. Two of the organic soluble compounds were tentatively identified as the N-dealkylated and the alpha hydroxy derivatives. Flooding only at high temperatures influenced the relative concentration but not the existence of the organic soluble metabolites while the presence of flooding or of non-flooding did not appear to effect the existence or concentration of the aqueous soluble metabolites.

LIST OF REFERENCES

LIST OF REFERENCES

1. Alexander, M. and M. J. H. Alem. 1961. Effect of Chemical Structure on Microbial Decomposition of Aromatic Herbicides. Journal of Agricultural and Food Chemistry. 9:44-47.

2. Armstrong, D. C., G. Chester and R. F. Harris. 1967. Atrazine Hydrolysis in Soil. Soil Sci. Soc. Amer. Proc. 31:61-66.

3. Audus, L. J. 1964. The Physiology and Biochemistry of Herbicides. Academic Press. London and New York.

4. Bailey, G. W., J. L. White and T. Rothberg. 1968. Adsorption of Organic Herbicides by Montmorillonite: Role of pH and Chemical Character of Adsorbate. Soil Sci. Soc. Amer. Proc. 32:222-234.

5. Baird, D. D., R. F. Husted and C. L. Wilson. 1965. Pre- and Post-emergence Herbicide Activity of N-isopropyl-2-Chloroacetanilide on Echinochloa crusgalli, Zea mays and Glycine max in the Greenhouse. Proceedings 18th Southern Weed Conference. p. 653-659.

6. Baird, D. D. and R. P. Upchurch. 1967. The Influence of Temperature on the Response of Six Grass Species to an Acetanilide Herbicide. Proceedings 20th Southern Weed Conference. p. 368.

7. Baird, D. D. and R. P. Upchurch. 1970. Characteristics of CP-53619 as a Herbicide for Rice. Proceedings 23rd Southern Weed Conference. p. 101-104.

8. Bartha, Richard, R. P. Lanzilotta and David Pramer. 1967. Stability and Effects of Some Pesticides in Soil. Applied Microbiology. 15:67-75.

9. Bartha, Richard and David Pramer. 1967. Pesticide Transformation to Aniline and Azo Compounds in Soil. Science. 156:1617-18.

10. Bartha, Richard. 1968. Biochemical Transformations of Anilide Herbicides in Soil. Journal of Agricultural and Food Chemistry. 16(4): 602-604.

11. Blair, A. M. and J. Holroyd. 1970. Herbicides for the Control of Agropyron Repens and Agrostic Gigantea. Proc. 10th Brit. Weed Control Conf. p. 326-330.

12. Burnside, O. C., C. L. Schmidt and R. Behrens. 1961. Dissipation of Simazine from the soil. Weeds. 9:477-484.

13. Burschel, Peter and V. H. Freed. 1959. The Decomposition of Herbisides in Soils. Weeds. 7:157-161.

14. Chandio, Purna, W. R. Furdick and W. B. Bollen. 1960. The Effects of Four Herbicides on Microorganisms in Nine Oregon Soils. Weeds. 8:589-598.

15. Chisaka, Hideo and Philip C. Kearney. 1970. Metabolism of Propanil in Soils. J. Agr. Food Chem. 18:854-858.

16. Clark, C. G. and S. J. L. Wright. 1970. Degradation of the Herbicide Isopropyl N-Phenylcarbamate by Arthrobacter and Achromobacter spp. from Soil. Soil Biol. Biochem. 2:217-226.

17. Corbin, F. T. and R. P. Upchurch. 1967. Influence of pH on Detoxication of Herbicides in Soil. Weeds. 150:370-376.

18. Dalton, R. L., A. W. Evans, and R. C. Rhodes. 1966. Disappearance of Diuron from Cotton Field Soils. Weeds. 14:31-33.

19. Danielson, L. L., W. A. Gentner, and L. L. Jansen. 1961. Persistence of Soil-incorporated EPTC and other Carbamates. Weeds. 9:463-475.

20. Davies, F. L. and S. T. Williams. 1970. Studies on the Ecology of Actinomycetes in Soil I. The Occurrence and Distribution of Actinomycetes in a Pine Forest Soil. Soil Biol. Biochem. 2:227-238.

21. Deming, J. M. 1963. Determination of Volatility Losses of C^{14}-CDAA from Soil Surfaces. Weeds. 11:91-96.

22. Donaldson, T. W. and Chester L. Foy. 1964. The Phytotoxicity and Persistence in Soils of Benzoic Acid Herbicides. Weeds. 12:195-201.

23. Dubrey, H. D. and J. F. Freeman. 1963. Influence of Soil Properties and Microbial Activity on the Phytotoxicity of Linuron and Diphenamid. Soil Sci. 97:334-340.

24. Engvild, K. G. and H. L. Jensen. 1969. Microbiological Decomposition of the Herbicide Pyrazon. Soil Biology and Biochemistry. 1:295-300.

25. Enzymes Detoxify Herbicides. 1970. Agr. Res. 19, No. 4, 8-9.

26. Fields, M. L. and D. D. Hemphil. 1966. Effect of Zytron and Its Degradation Products on Soil Microorganisms. Applied Microbiology. 14:724-731.

27. Fink, R. J. 1970. Herbicide Carryover. Crops and Soils. 22:18-19.

28. Flinchum. W. T. 1970. Evaluation of CP-53619 and CP-57177 for Weed Control in Drill and Broadcast Seeded Rice. Proc. 24rd Southern Weed Conf. p. 105.

29. Frank, Peter A. 1966. Persistence and Distribution of Monuron and Neburon in an Aquatic Environment. Weeds. 14:219-222.

30. Frear, D. S., H. R. Swanson, and F. S. Tanaka. 1969. N-Dimethylation of Substituted 3-(Phenyl)-1-Methylureas: Isolation and Characterization of a Microsomal Mixed Function Oxidase from Cotton. Phytochemistry. 8:2157-2169.

31. Frear, D. S. and H. R. Swanson. 1970. Biosynthesis of S-(4-Ethylamino-6-Isopropylamino-2-S-triazino)Glutathione: Partial Purification and Properties of a Glutathione S-Transferase from Corn. Phytochemistry. 9:1-10.

32. Geissbuhler, H., C. Haselback, H. Aibi and L. Ebner. The Fate of Tenoran in Soils and Plants. Weed Research. 3:277-297.

33. Geissbuhler, H. and J. A. Buth. 1970. Behavior of Urea Herbicides in Soil with Special Reference to Environmental Contamination Problems. Proc. 10th Brit. Weed Control Conf. p. 307-313.

34. Guenzi, W. D. and W. E. Beard. 1968. Anaerobic Conversion of DDT to DDD and Aerobic Stability of DDT in Soil. Soil Sci. Soc. Amer. Proc. 32:522-524.

35. Hamdi, Y. A. and M. S. Tewfik. 1970. Degradation of 3,5-Dinitro-o-Cresol by Rhizobium and Azotobacter spp. Soil Biol. Biochem. 2:163-166.

36. Hance, R. J. and G. Chesters. 1969. The Fate of Hydroxyatrazine in a Soil and a Lake Sediment. Soil Biol. and Biochem. 1:309-315.

37. Harris, C. I. 1965. Hydroxysimazine in Soil. Weed Res. 5:275-276.

38. Harris, C. I. 1967. Fate of 2-Chloro-s-triazine Herbicides in Soil. J. Agr. Food Chem. 15:157-161.

39. Hebblethwaite, J. F. 1970. The Performance of Alachlor on Some Annual Summer Crops and Weeds in South Africa. Proc. 10th Brit. Weed Control Conf. p. 452-459.

40. Hill, G. D., et al. 1955. The Fate of Substituted Urea Herbicides in Agricultural Soils. Agron. Jour. 47:93-104.

41. Hirsch, P. and M. Alexander. 1960. Microbial Decomposition of Halogenated Propionic and Acetic Acids. Canadian Journal of Microbiol. 6:241-249.

42. Halstun, J. T., Jr. and W. E. Loomis. 1956. Leaching and Decomposition of 2,2-Dichloropropionic Acid in Several Iowa Soils. Weeds. 4:205-217.

43. Husted, R. F., D. D. Baird and R. P. Upchurch. 1967. Characteristics of CP-52223 as a Preemergence Herbicide for Sugar Beets. (Beta vulgaris) and other Row Crops. Proc. North Central Weed Control Conf. p. 31-33.

44. IRRI Annual Report. 1964. p. 253.

45. Jordan, L. S., J. M. Lyons, W. H. Isom and B. C. Daz. 1968. Factors Affecting Performance of Preemergence Herbicides. Weed Science. 16:457-462.

46. Kaufman, Donald D. 1963. Simazine: Degradation by Soil Microorganisms. Science. 142-405.

47. Kaufman, Donald D. 1964. Microbial Degradation of 2,2-Dichloropropionic Acid in Five Soils. Canadian Jour. of Microbiology. 10:843-852.

48. Kaufman, D. D. 1964. Pesticide Breakdown. Agricultural Research. 1212:11.

49. Kaufman, D. D. 1966. An Inexpensive Positive Pressure Soil Perfusion System. Weeds. 14:90-91.

50. Kaufman, D. D. 1967. Degradation of Carbamate Herbicides in Soil. J. Agr. Food Chem. 15:582-590.

51. Kaufman, D. D., J. R. Plimmer, P. C. Kearney, J. Blake, and F. S. Guardia. 1968. Chemical Versus Microbial Decomposition of Amitrole in Soil. Weed Science. 16:266-272.

52. Kaufman, D. D. and Joan Blake. 1970. Degradation of Atrazine by Soil Fungi. Soil Biol. Biochem. 2:73-80.

53. Kearney, P. C. and D. D. Kaufman. 1965. Enzyme from Soil Bacterium Hydrolyses Phenylcarbamate Herbicides. Science. 147:740.

54. Kearney, P. C. and D. D. Kaufman. 1969. Degradation of Herbicides. Mercel Dekker, Inc. New York.

55. Kearney, P. C. and J. R. Plimmer. 1969. Prometryne Degradation in Soils and Light. Abstr. Papers, Am. Chem. Soc. No. 158 PEST 30.

56. Koren, Ephraim, C. L. Foy and F. M. Ashton. 1969. Absorption Volatility and Migration of Thiocarbamate Herbicides in Soils. Weed Science. 17:148-153.

57. Kowalenke, C. G. and R. B. McKercher. 1970. An Examination of Methods for Extraction of Soil Phospholipids. Soil Biol. Biochem. 2:269-273.

58. Lamoureux, G. L., L. C. Stafford and F. S. Tanaka. 1970. The Metabolism of 2-Chloro-N-Isopropylacetanilide (Propachlor) in the Leaves of Corn, Sorghum, Sugarcane, and Barley. Jour. Agr. Food Chem. 18:81-93.

59. Lanzilotta, R. P. and David Palmer. 1970. Herbicide Transformation I. Studies with Whole Cells of Fusarium solanie. Applied Microbiology. 19:301-306.

60. Lanzilotta, R. P. and David Palmer. 1970. Herbicide Transformation II. Studies with an Acylamidase of Fusarium solanie. Applied Microbiology. 19:307-313.

61. Lembeck, William J. and A. R. Colmer. 1967. Effect of Herbicides on Cellulose Decomposition by Sporocytophaga myxococcoides. Applied Microbiology. 15:300-303.

62. Linscott, J. J., O. C. Burnside and T. L. Lavy. 1969. Phytotoxicity and Movement of Amiben Derivatives in Soil. Weed Science. 17: 170-174.

63. Loeppky, Carol and B. G. Tweedy. 1969. Effects of Selected Herbicides upon Growth of Soil Algae. Weed Science. 17:110-113.

64. Loos, M. A., J. M. Ballagand and M. Alexander. 1967. Phenoxyacetate Herbicide Detoxification by Bacterial Enzymes. Jour. of Agric. and Food Chemistry. 15:858-863.

65. MacRae, I. C. and Martin Alexander. 1969. Microbial Degradation of Selected Herbicides in Soil. Jour. of Agric. and Food Chem. 13: 72-76.

66. McClure, George W. 1970. Accelerated Degradation of Herbicides in Soil by the Application of Microbial Nutrient Broths. Contributions from Boyce Thompson Institute. 24:235-240.

67. McCormick, L. L. and A. E. Hiltbold. 1966. Microbiological Decomposition of Atrazine and Diuron in Soil. Weeds. 14:77-82.

68. Meikle, R. W., A. E. Williams and C. T. Redemann. 1966. Metabolism of Tordon Herbicide (4-Amino-3,5,6-trichloropicolinic Acid) in Cotton and Decomposition in Soil. Jour. of Agric. and Food Chem. 14:384-387.

69. Murray, D. S., W. L. Rieck and J. Q. Lyad. 1969. Microbial Degradation of Five Substituted Urea Herbicides. Weed Science. 17:52-55.

70. Ogle, R. E. and G. F. Warren. 1954. Fate and Activity of Herbicides in Soils. Weeds. 3:257-271.

71. Parochetti, J. V., G. F. Warren. 1970. Behavior of Potassium Azide in the Soil. Weed Science. 18:555-560.

72. Plimmer, J. R., P. C. Kearney, H. Chisaka, J. B. Yount and U. I. Klingebiel. 1970. Jour. of Agric. and Food Chem. 18:859-61.

73. Plimmer, J. R., P. C. Kearney, D. D. Kaufman and F. S. Guardia. 1967. Amitrole Decomposition by Free Radical-Generating Systems and by Soils. Jour. of Agric. and Food Chem. 15:996-999.

74. Probst, G. W., Tomasz Tolab, R. J. Herberg, F. J. Holzer, S. J. Parka, Cornelius Van Der Schans and J. B. Tepe. 1967. Fate of Trifluralin in Soils and Plants. Jour. of Agric. and Food Chem. 15:592-599.

75. Razab, Mohamed Tawfik H. and John P. McCollum. 1961. Degradation of C^{14}-Labeled Simazine by Plants and Soil Microorganisms. Weeds. 9:72-84.

76. Roeth, F. W., T. L. Lavey and O. C. Burnside. 1969. Atrazine Degradation in Two Soil Profiles. Weed Science. 17:202-205.

77. Rogoff, Martin H. and James J. Reid. 1956. Bacterial Decomposition of 2,4-Dichlorophenoxyacetic Acid. Journal of Bacteriology. 71:303-307.

78. Rosen, J. D. and R. F. Strusz. 1968. Photolysis of 3-(p-Bromophenyl)-1-methoxy-1-methylurea. Jour. of Agric. and Food Chem. 16:568-570.

79. Sharabi, N. C. and L. M. Bordeleau. 1969. Biochemical Decomposition of the Herbicide N-(3,4-Dichlorophenyl)-2-methylpentanamide and Related Compounds. Applied Microbiology. 18:369-375.

80. Sheets, T. J. and A. S. Crafts. 1957. The Phytotoxicity of Four Phenylurea Herbicides in Soil. Weeds. 5:93-101.

81. Sheets, T. J. 1963. Photochemical Alteration and Inactivation of Amiben. Weeds. 11:186-190.

82. Sheets, T. J. 1964. Review of Disappearance of Substituted Urea Herbicides from Soil. Jour. of Agric. and Food. Chem. 12:30-33.

83. Sheets, T. J. 1970. Degradation and Effects of Herbicides in Soils. First FAO International Conference on Weed Control.

84. Shimabukuro, R. H., H. R. Swanson, W. C. Walsh. 1970. Glutathione Conjugation Atrazine Detoxication Mechanism in Corn. Plant Physiol. 46:103-107.

85. Smith, A. E., and A. Fitzpatrick. 1970. The loss of Five Thiolcarbamate Herbicides in Nonsterile Soils and their Stability in Acidic and Basic Solutions. Jour. of Agric. and Food Chem. 18: 720-722.

86. Stickler, R. L., E. L. Knake and T. D. Hinesly. 1969. Soil Moisture and Effectiveness of Preemergence Herbicides. Weed Sci. 17:257-259.

87. Suneson, C. A. and L. G. Jones. 1960. Herbicides May Produce Instability. Agronomy Journal. 52:120-121.

88. Taylorson, R. B. 1966. Influence of Temperature and other Factors on Loss of CDEC from Soil Surfaces. Weeds. 14:294-296.

89. Tweedy, B. G., Kikki Turner and M. Actitun. 1968. The Interactions of Soil Borne Microorganisms and D.C.P.A. Weed Sci. 16:470-473.

90. Tweedy, B. G., C. Loeppky, and J. A. Ross. 1970. Metobromuron: Acetylation of the Analine Moiety as a Detoxification Mechanism. Science. 168-482-483.

91. Upchurch, R. P., F. L. Selman, D. D. Mason and E. J. Kamprath. 1966. The Correlation of Herbicidal Activity with Soil and Climatic Factors. Weeds. 14:42-46.

92. Wain, R. L. and F. Wightman. 1956. The Chemistry and Mode of Action of Plant Growth Substances. Academic Press, Inc. New York. p. 187-194.

93. Walker, R. L. and R. S. Newman. 1956. Microbial Decomposition of 2,4-Dichlorophenoxyacetic acid. Applied Microbiology. 4:201-206.

94. Walter, J. P., C. F. Eastin and M. G. Merkle. 1970. The Persistence and Movement of Fluorodifen in Soils and Plants. Weed Research. 10:165-171.

95. Weber, J. B., P. W. Perry and K. Ioaraki. 1968. Effect of pH on the Phytotoxicity of Prometryne Applied to Synthetic Soil Media. Weed Science. 16:134-136.

96. Weed Control. 1968. National Academy of Sciences, Washington, D. C. Vol. 2, pp. 197-209. Publication 1597.

97. Youngson, C. R. 1967. Factors Influencing the Decomposition of Tordon Herbicide in Soils. Down to Earth. 23:3-11.

VITA

The author, Douglas D. Baird, of Bridgeton, Missouri, was born in Montclair, New Jersey, on January 21, 1934. After graduation from Summit High School, Summit, New Jersey, in 1952 he entered the Army of the United States and served as a Dental Technician Instructor at Brooks Army Medical Center, Fort Sam Houston, Texas. He entered The University of Tennessee in September, 1956, and received the Bachelor of Science degree in 1960 with a major in Horticulture.

Upon graduation from the University, he was employed by The University of Tennessee Agriculture Experimental Station as a Research Assistant in Horticulture. During this period, he entered graduate school at The University of Tennessee and received the Master of Science degree with a major in Horticulture in 1963. After graduation, he was employed as an Instructor of Horticulture with The Virginia Polytechnic Institute at Blacksburg, Virginia. In 1964, he joined the Agricultural Research Department of Monsanto Company, St. Louis, Missouri, as a Research Biologist. In 1969, as a Research Specialist, he was granted an academic leave and in 1971, was granted the Doctor of Philosophy degree from The University of Tennessee with a major in Agriculture Plant and Soil Science.

He is married to the former Nancy Ruth Hollingsworth of Stillwater, Oklahoma. They have four children; Carolyn Susan, Joanna Elizabeth, Douglas Delmar, Jr., and Stephanie Ruth.