AN ABSTRACT OF THE THESIS OF

BARELD EGGE GROENWOLD (Name) for the DOCTOR OF PHILOSOPHY (Degree)

in Farm Crops (Major) presented on March 9, 1971 (Date)

Title: MECHANISMS OF UPTAKE AND SOME FACTORS INFLUENCING THE TOXICITY OF 2-chloro-2', 6'-diethyl-N-(methoxymethyl) acetanilide (alachlor) FOR SELECTED PLANT SPECIES

Redacted for Privacy

Abstract approved: William R. Furtick

A number of studies with ^{14}C-labeled 2-chloro-2', 6'-diethyl-N-(methoxymethyl) acetanilide (alachlor) were conducted to obtain information about the mechanisms of uptake of this herbicide.

In other studies, technical alachlor as well as the emulsifiable concentrate formulation of the chemical were used to investigate the effect of several factors on the toxicity of the herbicide.

Corn and oats absorbed alachor primarily in a passive way. The uptake of the herbicide in both species was slightly reduced in the presence of metabolic inhibitors. The reduction was somewhat stronger in corn than in oats.

A raise in temperature increased the uptake of alachlor only slightly. Q_{10} values of about 1.2 were found.

The part of the total uptake affected by metabolic inhibitors followed Michaelis-Menten kinetics over the alachlor concentration range of 0.1 mM to 0.5 mM.

Uptake of alachlor was measured in the presence of an analog, propachlor. Propachlor reduced the uptake of alachlor at a concentration of 0.3 mM for both chemicals. At a concentration of 0.03 mM for each herbicide alachlor uptake was less reduced.

Total uptake by corn and oats was nearly identical up to a concentration of 0.1 mM. At higher concentrations alachlor absorption by oats was about twice as high as by corn. This would suggest that selective uptake by the two species could at least partly account for the difference in tolerance towards alachlor.

A study was conducted to determine if P, K and Ca levels in oat plants were affected by alachlor treatment. P and Ca content was not affected by the presence of alachlor, but a gradual decrease in K level with increasing herbicide concentrations was observed.

Uptake of alachlor through the shoot region exceeded absorption by the roots in the two monocotyledonous species, corn and oats. Two dictos, soybean and cucumber, absorbed higher amounts of the herbicide through the roots. Shoot exposure of corn and cucumber was most damaging to the plant, while root exposure was more damaging to oats and soybean.

The effect of alachlor on the growth of oats and cucumber at various levels of sub-irrigation and carrier volume could be explained on the basis of differences in effective absorption regions in the plants.

Photoperiod had, in contrast with light intensity, no effect on the activity of the herbicide to corn, cucumber and oats. An increase in light intensity up to 1600 ft-c increased the toxicity of alachlor in corn and cucumber, but not in oats. The activity at 2000 ft-c was only slightly higher than at 60 ft-c, and less than at 660 ft-c. In the case of cucumber, an increase in alachlor concentration had very little effect at 2000 ft-c, in contrast with the other light intensities.

Mechanisms of Uptake and Some Factors Influencing the Toxicity of 2-chloro-2',6'-diethyl-N-(methoxymethyl) acetanilide (alachlor) for Selected Plant Species

by

Bareld Egge Groenwold

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

June 1971

APPROVED:

Redacted for Privacy

Professor of Agronomy
in charge of major

Redacted for Privacy

Head of Department of Farm Crops

Redacted for Privacy

Dean of Graduate School

Date thesis is presented March 9, 1971

Typed by Susie Kozlik for Bareld Egge Groenwold

ACKNOWLEDGEMENTS

The author wishes to express his gratitude to Dr. William R. Furtick for his guidance and encouragement throughout the graduate program.

Appreciation is further extended to the following individuals: Dr. Arnold P. Appleby and Dr. Thomas C. Moore for their excellent suggestions in the writing of this manuscript. Dr. Larry Boersma and Dr. John D. Lattin for serving on the graduate advisory committee. Kenneth Kirkland, Steven R. Radosevich and Charles E. Stanger for their assistance and cooperation.

The author further wishes to thank Monsanto Chemical Company for providing the technical and radioactive materials used in these studies.

Special acknowledgement must be extended to the author's wife Lien, whose patience and understanding have been instrumental in the completion of this step in his life.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDIX TABLES

MECHANISMS OF UPTAKE AND SOME FACTORS INFLUENCING THE TOXICITY OF 2-chloro-2', 6'-diethyl-N-(methoxymethyl) acetanilide (alachlor) FOR SELECTED PLANT SPECIES

INTRODUCTION

In spite of many years of intensive research by dedicated scientists a complete understanding of the processes by which plants absorb, accumulate and translocate organic and inorganic materials is still lacking.

Because of the early introduction of fertilizers far more is known about the absorption processes of inorganic nutrients than about the uptake of the more recently introduced organic growth regulators and pesticides.

Absorption of salts by plant roots can be either passive or active. Passive uptake mechanisms, including diffusion, mass flow, ion exchange across membrances and adsorption, are purely physical phenomena. On the contrary, active absorption, accounting for movement against a concentration gradient, requires energy. Most recent theories postulate the presence of a carrier system for transport across cellular membranes.

The introduction of many organic materials in recent years has directed interest to mechanisms by which absorption of these compounds occurs.

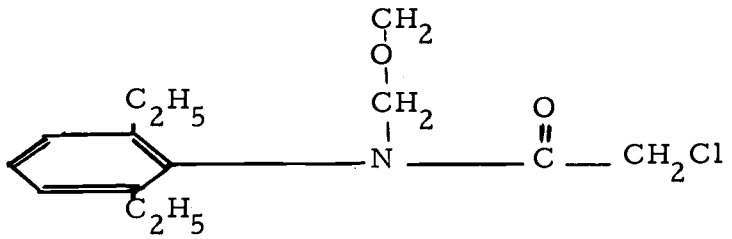
The objectives of the present study were:

1. to determine the relative importance of active and passive uptake of alachlor by corn and oats,
2. to determine if active uptake of alachlor follows Michaelis-Menten kinetics,
3. to determine the most effective site of uptake of alachlor by corn, oats, soybean and cucumber,
4. to determine the effect of light intensity, day length, carrier volume and amount of sub-irrigation on the toxicity of alachlor to selected plant species.

LITERATURE REVIEW

Physical and chemical properties of alachlor

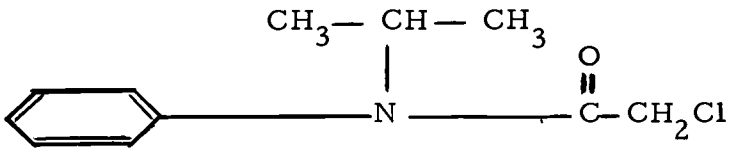
Alachlor is identified as: 2-chloro-2', 6'-diethyl-N-(methoxymethyl) acetanilide, with the following structural formula:



The technical material is odorless, cream colored, and solid at room temperature. The solubility is 148 ppm in water. The commercial formulation available is the emulsifiable concentrate. Alachlor has an estimated vapor pressure of 9.4×10^{-3} mm of Hg at 20 C.

Physical and chemical properties of propachlor

Propachlor is identified as: 2-chloro-N-isopropyl-acetanilide. The structural formula is as follows:



The technical material has a light tan color and is solid at room temperature. The solubility in water is 700 ppm. Commercial formulations available are the granular form and the wettable powder. The vapor pressure is 0.03 mm of Hg at 110 C.

Mechanism of action and metabolism

Duke (1967) suggested a role for 2-chloro-N-isopropylacetanilide at the level of protein or nucleic acid synthesis. Interference with the activity of GA could also be involved, though not directly since inhibition caused by propachlor was irreversible at very high levels of GA. Propachlor at a concentration of 10^{-3} M did not inhibit α amylase activity.

Edmondson (1969), following the same techniques as used by Duke (1967), was unsuccessful in his attempts to elucidate the mechanism of action of alachlor. Edmondson obtained similar effects on plant growth by using alachlor; however, the inhibition of ^{14}C-leucine incorporation into root protein as noticed by Duke was not obtained with alachlor. Also RNA metabolism and polyribosome formation were largely unaffected except for an increase in total RNA. Since treated tissue had also more DNA Edmondson concluded that alachlor exerts its primary effect on some more basic system than protein synthesis.

Based on the current knowledge of chloroacetamide and chloroacetanilide herbicides, it is apparent (Jaworski, 1969) that a generalization regarding their metabolic fates cannot be made.

The substitution of a phenyl group on the nitrogen might result in a completely different detoxification mechanism than when the nitrogens are substituted with aliphatic molecules, such as in CDAA.

Jaworski (1969) postulated that crop species resistant to these herbicides are capable of rapid detoxification by one of two major mechanisms. The mechanism active in alachlor and propachlor tolerant species would involve a reaction of the α-halogen with endogenous substances, and would lead to the formation of water soluble acidic metabolites.

Uptake of inorganic ions

A general description of ion absorption will be given in this section. Additional information can be found in a number of review articles, and in books that have been published in recent years: Briggs and Robertson (1957), Brouwer (1965), Crafts (1961), Dainty (1962), Epstein (1956), Jennings (1963), Laties (1957) and Steward and Sutcliffe (1959).

Absorption of inorganic ions, when studied over a period of time appears to be bi-phasic. A rapid initial uptake, not depending on metabolic energy, is followed by a slower steady rate uptake, depending on energy created in the plant (Maas, 1968; Moore, 1965). The initial uptake is a complex of physical phenomena such as diffusion, adsorption, mass flow, and exchange of ions.

The portion of the plant body accessible by physical processes is generally referred to as free space or apparent free space. The free space can be entered without transport across membranes. Since absorption appears to occur against a concentration gradient, the

term Donnan free space was introduced.

Accumulation in this space, being part of the apparent free space occurs according to the Donnan equilibrium theory (Hiatt, 1968). Ion exchange also accounts for accumulation against a concentration gradient.

Although some disagreement exists about the extent of the free space (Brouwer, 1965) most researchers now confine this space to the cell walls and the intercellular spaces, with the plasmalemma acting as the first barrier for inward movement.

Direct analyses of the vacuolar sap of plants immersed in solutions of known salt concentration have demonstrated that both anions and cations are accumulated by plants against concentration gradients. Furthermore, the extent of accumulation is such that known physical mechanisms, such as ion exchange and Donnan equilibria, cannot account for the extent of accumulation that occurs.

Since ion uptake is inhibited by low temperature, low oxygen tension and metabolic inhibitors, the assumption has to be made that ion accumulation as it occurs in plants requires metabolic energy. The slow steady rate uptake, following the rapid initial accumulation, requires metabolic energy, and has therefore been named active uptake.

A number of theories have been set forth to explain active transport. In general these theories suggest the existence of carrier

systems, which would accomplish the transport of ions across a differentially permeable membrane.

Accepting the plasmalemma as the inward boundary of the free space, this membrane will be the first obstacle to overcome. It is assumed (Epstein, 1960) that ions do not cross the plasmalemma in the form of free ion. Rather they combine with the carrier molecule at the outer surface of the membrane and then dissociate the ion being discharged into the "inner" space beyond.

Frequently it is found that a single ionic species is transported by two discrete mechanisms (Epstein, Rains and Elzam, 1963; Hiatt, 1968; Luttge and Laties, 1967; Osmond and Laties, 1968; Rains, 1967).

Mechanism I would be operative at concentrations up to 1 mM (Epstein, 1966; Osmond, 1968) and mechanism 2 would be operative at concentrations above 1 mM.

Although agreement exists concerning the two mechanisms controversial opinions are expressed as to the location of the mechanisms. Welch and Epstein (1968) consider the plasmalemma as the seat of both mechanisms, while Laties and his collaborators (Osmond and Laties, 1968) have put forward the contention that mechanism 1 is located in the plasmalemma, and mechanism 2 in the tonoplast.

The kinetics of ion absorption, expressed by both types of mechanism are similar to enzyme kinetics described by Michaelis and Menten (1913).

In analogy with the enzyme concept, the uptake theory states that ions complex with a carrier molecule and transverse membranes in this way. The two types of uptake mechanism originate from the fact that carrier sites differ considerably in their affinities for the ion. Studies conducted on the simultaneous uptake of ions support this theory (Epstein, 1956, 1962, 1966). Further support originates from studies revealing a maximum rate of uptake, indicating that all carrier sites might be occupied (Epstein and Hagen, 1952; Knauss and Porter, 1954).

Most workers favor the concept of active accumulation of ions, however others claim that anions only are absorbed actively, while the cations move passively to maintain the balance of electro-chemical potential.

Lundegardh (1950) proposed that transport of anions is mediated through cytochrome oxidase. According to his theory electrons produced by dehydrogenase reactions move outward via the cytochrome chain, while anions move in the opposite direction with the cations following passively. Robertson, Wilkins and Weeks (1951) found that 2, 4-dinitrophenol, an inhibitor of oxidative phosphorylation, increased respiration but decreased salt absorption. This implies that phosphorylation should be included in any theory of accumulation (Sutcliffe and Hackett, 1957; Laties, 1957).

Further evidence favoring phosphorylative mechanisms of ion transport has been presented by a number of authors (Budd and Laties,

1964; Higinbotham, 1959; Ordin and Jacobson, 1955; Weigl, 1963, 1964).

Attempts have been made to connect the transport of ions with protein synthesis (Sutcliffe, 1960; Jacoby and Sutcliffe, 1962). These workers showed that chloramphenicol, which inhibits protein synthesis, also inhibits ion accumulation in red beet and carrot tissue.

Stoner, Hodges and Hanson (1964) stated however that the effects observed by Sutcliffe may be ascribed to the action of the antibiotic as an inhibitor of the utilization of respiratory energy.

Radial transport of ions

It is generally thought that absorbed ions move rather freely into the root to the point where further penetration may be retarded by the Casparian strip. A theory to explain the radial movement of ions, as well as transport across the endodermis has been developed by Crafts and Broyer (1938). These workers maintain that there is a gradient of decreasing O_2 and increasing CO_2 from the cortex to the stele. Therefore ions accumulated in the cortical cells are moved into the stele through the symplast where they leak out into the xylem conduits. Crafts and Broyer proposed that the cells of the stele are "leaky" because of a deficiency of oxygen. However, Greenwood (1967) presents evidence for the diffusion of oxygen from shoots to roots in vegetable seedlings, and this raises questions about the

existence of oxygen deficiency in the stele. Laties and Budd (1964), supporting Crafts and Broyers theory, attributed the presumed leak iness of the stele cells to a volatile inhibitor of metabolism. Biddulph (1967), and Branton and Jacobson (1962) found no evidence of inhibition of accumulation in the stele, an observation confirmed by Yuh and Kramer (1968).

An alternative explanation, concerning leakage into xylem conduits, was offered by Hylmö (1953). This worker proposed that the protoplasts of the living xylem elements accumulate ions which are released into the xylem when the protoplasts disintegrate.

Yuh and Kramer (1969) doubted whether death of protoplasts in the maturing xylem elements would provide enough salt. They calculated from the rate of exudation and the volume of the xylem vessels in corn roots that the solution in the xylem vessels is being replaced at least three times per hour.

Arisz (1956) claimed that peristelar cells actively secrete salts into the xylem vessels.

Due to the presence of plasmodesmata connecting the protoplasts of the root cells, a pathway is provided for movement of ions from the root surface to the living cells adjoining the xylem vessels.

Composition and structure of cellular membranes

Electron micrographs show that most membranes, excluding those

of chloroplasts and mitchondria, have generally similar appearances. Cross sections indicate a thickness of 75 Å up to 100 Å. The membranes usually appear as two dark lines, each about 20 Å thick, separated by a lighter layer roughly 35 Å thick (Salisbury and Ross, 1969). The complete structure of three layers is referred to as a unit membrane. In many cells of both plants and animals the two outer layers are of equal thickness, and the membrane is then symmetrical. However, cases are known where the tonoplast and plasmalemma of plant cells are asymmetrical (Salisbury and Ross, 1969; Lehninger, 1970).

Models of membrane structure have been proposed by Frey-Wyssling (1955) and also by Danielli (1952). According to Danielli the greater part of the plasmalemma consists of a roughly bimolecular lipid layer covered on both sides by adsorbed protein molecules. Because of new fixation and staining techniques that are now employed, other models of membrane structure have been proposed (Lehninger, 1970). Two of these models are variants of the unit-membrane models. One of them proposes that the structural protein of the membrane is arranged within a lipid bi-layer, so that the hydrocarbon tails of the lipids intertwine with the polypeptide chain of the protein. Another proposes that structural protein molecules extend across the entire bi-layer at regular intervals. Membranes, consisting of conglomerations of globules have also been proposed. In these, the recurring

structural units are small globular lipo-proteins or alternating lipid micelles and globular proteins.

According to Lehninger (1970) membranes have a composition of about 60 percent protein and 40 percent lipid, although these proportions might vary somewhat. Collander (1959) emphasized the remarkable constancy in lipid: protein ratio. In the case of 17 different mammals studied the lipid/protein ratio varied only between 1/1.4 and 1/1.8 by weight. Ernster and Kuylenstierna (1969) gave phospholipid/protein ratios of 0.27 for the inner mitochondrial membrane, and 0.82 for the outer membrane.

Lauchli and Epstein (1970) stated that Ca is essential for maintenance of the functional integrity of root cell membranes and especially the plasmalemma. Omission of Ca causes membrane and transport phenomena to suffer various abnormalities and does so within minutes.

Carter and Lathwell (1967) concluded from their studies that absorption of orthophosphate, at high phosphorus concentrations, is dependent upon Ca. Grunwald (1968), studying the effects of sterols on the permeability of alcohol treated red beet tissue, noticed that $CaCl_2$, cholesterol, β-sitosterol and stigmasterol function as membrane stabilizers. Legget and Gilbert (1967) related Ca to the ion selectivity in soybean roots. Elzam and Hodges (1967) noticed an inhibition of energy dependent potassium transport during the initial phases of

transport caused by $CaSO_4$ or $CaCl_2$. After 30-45 min a stimulation of transport was observed. The authors concluded that the inhibition is of a non-competitive nature.

Uptake of organic substances

The absorption of many different organic compounds by plant tissues has been studied. These compounds include sugars, organic acids, inhibitors, synthetic auxins and other growth regulators, chelates, dyes, enzymes and other proteins, surfactants and pesticides (Foy and Yamaguchi, 1964).

A substantial number of studies have been conducted on absorption by leaves and isolated organelles. Total absorption by leaves might differ substantially from absorption by roots, due to presence of the cuticle on, and stomates in leaves (Leopold, 1964). However, investigations by Bowen (1969) give an indication of the similarities in mechanisms of membrane transport in leaves as compared to the membrane transport in roots.

Pennel and Weatherly (1958), studying the uptake of sucrose by leaf disks of Atropa belladonna, found two phases of uptake. The first phase was passive absorption, not requiring oxygen, while the second phase was dependent on metabolic energy. Grant and Beevers (1964) concluded that sugar uptake by carrot tissue and corn roots was an active process. Kriedemann and Beevers (1967) in studies with

castor bean seedling found a bi-phasic uptake by the cotyledon, and they suggested a lower capacity system at about 0.1 M sucrose. Glucose uptake by Nitella translucens cells was inhibited by 80 percent at a temperature of 4C, and by 90 percent in the presence of a metabolic inhibitor (Smith, 1967). Taylor (1959) obtained similar results in his studies with Scenedesmus quadricauda and stated that glucose transport across the plasmalemma is carrier-mediated and dependent on ATP production.

Shrift (1966) suggested an active uptake mechanism for ^{35}S-methionine in Chlorella. Birt and Hird (1958) studying the uptake of amino acids by carrot slices, found that uptake consisted of two phases. They further concluded that a common carrier system was involved in the active uptake of all amino acids tested. Berlin (1970), in his article on Specificities of Transport Systems and Enzymes cites work by Christensen. This worker, in his studies on intestines suggested the existence of a single membrane carrier, responsible for the transport of histidine, tyrosine, tryptophan, alanine and methionine.

The dual mechanism of ion uptake, extensively treated by Epstein, Rains and Elzam (1963) is also found for the uptake of the organic compound choline-sulfate (Nissen and Benson, 1964). These authors even proposed a model for the choline-sulfate carrier.

It has been postulated (Crafts, 1961) that herbicides are taken up

in the same manner as nutrient ions, as long as they are not highly toxic or otherwise physiologically active, Reinhold (1954), studying the uptake of indoleactic acid (IAA) by pea epicotyl segments and carrot disks, found that uptake occurred by two processes. One was purely physical and the other metabolically controlled. Poole and Thimann (1964) found a rapid initial uptake of ^{14}C-labeled IAA and indoleacetonitrile by Avena coleoptile sections. The rapid initial uptake was followed by a steady state uptake for four hours. The absorption of 2, 3, 5-triiodobenzoic acid (TIBA) by the roots and fronds of Lemna minor showed a rapid accumulation during the first half hour (Blackman and Sargent, 1959). At low concentrations these authors observed a continued progressive accumulation, while at concentrations greater than 2.5 mg/l. a loss from the plant occurred.

Johnson and Bonner (1956) followed the kinetics of uptake of 2, 4-D by oat coleoptile sections and found a rapid initial uptake followed by a slower steady uptake. The metabolic component of uptake has been identified with the second slower phase of uptake.

Davies and Seaman (1968), in studies with Elodea, found a rapid initial accumulation of diquat, followed by a slower accumulation. The initial phase was reversible in water and rapidly reached a saturation value while the slower uptake indicates an initial phase of passive absorption,

followed by active accumulation. Brian (1967) found a similar pattern of uptake for paraquat and diquat.

Saunders (1966) summarized some previous work done in conjunction with Saunders and Blackman. These workers found three different patterns of uptake, dependent on the nature of the chemical and the plant species. When segments excised from stem tissues of Pisum sativum, Avena sativa or Gossypium hirsutum were placed in solutions of phenoxyacetic acid or 2-chlorophenoxyacetic acid there was a progressive accumulation with time. Patterns of uptake of benzoic acid and 2-chlorobenzoic acid were also accumulative. On the other hand, the courses of uptake of 2, 4-D and 2, 4, 5- T by segments of Gossypium, and Pisum showed an initial phase of rapid uptake followed by a phase when there was a loss of radioactivity to the external solution. A similar course was observed for the uptake of 2, 4-dichlorobenzoic acid or 2, 5- dichlorobenzoic acid by segments of Pisum. A third pattern was found for the uptake of 2, 4-D or 2, 4, 5-T by tissues of Avena or Triticum. An initial period of rapid accumulation was followed by a phase when the rate slowed down and became negative so that there was a net loss of ^{14}C. However with time the rate changed to a positive value again.

Herbicide uptake and selectivity

Restricted herbicide uptake by tolerant plant species as compared

to susceptible species has for many years been recognized as a possible basis for selectivity. Blackman (1958) suggested that the differences in uptake and egress of 2, 4-D among species may be relevant to elucidation of the bases for herbicidal selectivity. Davies, Gramlich and Funderburk (1965), in studies with corn, cotton and soybeans, found that atrazine uptake by susceptible soybean exceeded the uptake by tolerant corn. Chow (1970) concluded that the difference in susceptibility to TCA between oats and barley is governed by the processes of absorption and dissipation. Sensitive lambsquarters accumulate higher amounts of pyrazon than tolerant sugar beets (Frank and Switzer, 1969). Neidermyer and Nalewaja (1969) found that resistant catchfly leaf sections absorbed more 2, 4-D than sensitive lambsquarters. However after 72 hours catchfly released 2, 4-D in solution, while lambsquarter continued to accumulate. Davies, Drennan, Fryer and Holly (1968) working with barley, pea, and mustard suggested that differential rates of entry into leaves contributed substantially to the selective toxicity of ioxynil.

At least as much evidence against discrimination in uptake has been presented, however. Colby and Warren (1965) found that a susceptible and a tolerant species absorbed solan at about the same rate. Majumdar and Muller (1969) found that initial uptake and transport of metobromuron occurred equally rapidly in sensitive Sinapis arvensis, and in tolerant Veronica persica Poir. Similar results were found for

tolerant and resistant potato varieties. The selectivity of propanil in rice, in contrast with barnyard-grass was attributed to the oxidative and hydrolytic metabolism of the herbicide in rice, and not to differences in foliage absorption (Yih, McRae and Wilson, 1968). Stoller and Wax (1968), in their work with amiben, did not find a correlation between differences in uptake and tolerance of species. Attempts by Nalewaja (1968) to link differential uptake and translocation to tolerance of di-allate were unsuccessful. Differences in amounts of prometryne absorbed by cotton and soybean were essentially identical, and this led Sikka and Davis (1968) to the conclusion that differential translocation appears to be a major factor contributing to the difference in susceptibility.

Although differential uptake as a basis for selectivity appears to exist, as indicated by various researchers, differences in translocation, dissipation and metabolism are often as important in the ultimate behavior of the plant.

Site of uptake

Many articles dealing with the effective site of uptake of herbicides have appeared in recent years. Appleby, Furtick and Fang (1965) reported severe injury in oats, caused by shoot exposure to EPTC, di-allate and propham. Similar results were obtained by Dawson (1963), working with EPTC, and by McKinley (1965) studying the uptake of DCPA. Parker (1966) noticed a higher degree of injury in

sorghum resulting from shoot exposure as compared to root exposure for the herbicides EPTC, CDEC, di-allate and CDAA. With dichlobenil, trifluralin and chlorpropham the effect was reversed. Oliver, Prendeville and Schreiber (1968) found that roots were the major site of EPTC uptake in barley, but that injury to wheat, oats, sorghum and giant foxtail from root exposure was equal to or slightly less than that from shoot exposure. Prendeville, Oliver and Schreiber (1968) observed severe injury in wheat, barley and oats when the coleoptilar internode was exposed to EPTC. This worker concluded that growth responses of species to EPTC applied to shoots is dependent on the stage of plant development at which treatment occurs.

Nishimoto (1968) tested several herbicides for their site of uptake. Avena sativa was found to absorb EPTC, dichlobenil, trifluralin, nitralin, propachlor, DCPA and CDEC primarily through the shoot region. The chemicals bromacil, pyrazon, diuron and atrazine were absorbed primarily by the roots. It was also found that atrazine was more effective on oats through root exposure, while shoot exposure to the same herbicide was more injurious to green foxtail and annual ryegrass. Gray and Weierich (1969) found that in the majority of grass species tested exposure of the roots to EPTC caused more injury than shoot exposure. Rahman and Ashford (1970) in their studies with trifluralin concluded that, due to morphological differences between green foxtail and wheat, selective control of foxtail is possible even though

both species absorb the herbicide through their coleoptilar node.

Little is known about the site of uptake of alachlor. Knake and Wax (1968) found that giant foxtail, although sensitive to alachlor, was not affected by placement of the herbicide in the root zone. Trivelli (1967) also found a higher degree of injury in barnyard-grass when shoots were exposed as compared to root exposure. Eshel (1969) noticed that exposure of the entire root system of cotton to alachlor caused severe growth inhibition. Placing the herbicide in both shoot and root zones increased only slightly the phytotoxic effect. Root uptake apparently is of more importance in cotton than shoot uptake.

Illumination

Studies to determine the effect of light on herbicidal toxicity have been conducted almost exclusively with chemicals which are known to interfere with the photosynthetic process. Ashton (1965) studied the effect of light intensity and quality on the toxicity of atrazine and monuron. He noticed an increase in injury from 30 to 4000 ft-c. The greatest injury occurred at wavelengths corresponding with peaks in the chlorophyll absorption spectrum. Allen and Palmer (1963) found a very limited activity of simazine on barley in the dark. Merkle, Leinweber and Bovey (1965) concluded that light is necessary for paraquat activity. They related the action of paraquat to the formation of a free radical, a reaction enhanced by higher light intensities.

Figuerola (1969) noticed an increase in activity of Igran under conditions of high temperature and high light intensity. It was found difficult to separate the individual action for either climatic factor. A similar conclusion was drawn by Jordan, Day and Clerx (1964) who studied the effects of ultraviolet light and temperature on the decomposition of triazines.

Brady (1969) found that absorption of the isooctyl ester of 2, 4, 5-trichlorophenoxy)-acetic acid varied more than 20 percent in four woody species when incident light intensity was increased from 40 to 4000 ft-c. Uptake by the two evergreens Pinus palustris Mill. and Ilex opaca Ait. increased linearly with an increase in light intensity. Two deciduous species showed maximum absorption at 2680 ft-c, while uptake was the same at 40 and 4000 ft-c.

EXPERIMENT I. THE EFFECT OF LIGHT INTENSITY ON THE TOXICITY OF ALACHLOR TO CORN, CUCUMBER AND OATS

Light intensity through its effect on biological processes in the plant may influence the uptake or metabolism of a herbicide and therefore affect the toxicity exerted. In this study the influence of light intensity on three plant species, treated with alachlor, was investigated.

Materials and Methods

Four experiments were conducted in which plants grown in alachlor-treated sand were exposed to four different light intensities: 2000, 1600, 660 and 60 ft-c. Quartz sand (El Monte El-20) was used as root medium for the three plant species. Plastic pots 7x7x7 cm were filled with alachlor- treated sand and five seeds were planted 2 cm deep in each pot. A hundred milliliters of water, containing the desired concentration of alachlor, were mixed uniformly with two kilograms of sand by shaking and stirring in a tin. The concentration of alachlor, expressed in ppm was based on the weight of dry sand. In all experiments the emulsifiable concentrate formulation of alachlor (lot HXA-49, 4 lbs/gal) was used. Corn was exposed to alachlor concentrations of 0, 10, 50 and 100 ppm; cucumber to concentrations of 0, 1, 5 and 10 ppm. Oats were exposed to alachlor concentrations of 0, 0.05, 0.1 and 0.3 ppm at two light intensities; and to 0, 0.1, 0.5,

1, 2 and 3 ppm at the other light intensities.

Immediately following planting the pots were placed on foam rubber in metal trays containing water. Nutrient solution with a composition given in table "A" was applied after emergence of the seedlings by sub-irrigation. The amount of solution was applied according to the needs of the check plants in each experiment. A total of 14 liters nutrient solution was added to 96 pots in the period between emergence and harvesting of the plants. Light intensity was varied by using different combinations of incandescent bulbs, fluorescent tubes and cheese cloth.

Foliage dry weight of all the plants per pot and the number of plants per pot were the parameters measured. The plants were harvested twelve days after planting. Foliage was dried in a forced air oven at 90 C for 18 hours. Eight replications per treatment were employed. During the experimental period the conditions maintained in the growth chamber were:

photoperiod	16 hr
day temperature	27 C
night temperature	16 C
R. H.	50 %

Table A. Recipe for half strength Hoagland's solution. Modified from Machlis and Torrey (1956).

Stock solution	Amount in grams	Final vol. (liters) in distilled H_2O	ml of stock solution to prepare 1 liter of final solution
1 M $Ca(NO_3) \cdot 4H_2O$	708.3	3	5
1 M KNO_3	303.3	3	5
1 M $MgSO_4$	120.4	1	2
1 M KH_2PO_4	136.1	1	1
* Fe chelate	42.5	1	1 per 50 l.
** Micronutrients		1	1

pH of final solution adjusted to 6.8 by adding 25 ml 4N KOH

* The iron chelate used in the solution was Geigy's Sequestrene 330. Fe chelate (sodium ferric diethylene triamine pentaacetate)

** The micro-nutrient stock solution contained 2.86 g of H_3BO_4; 1.81 g of $MnCl_2 \cdot 4H_2O$; 0.11 g of $ZnCl_2$; 0.07 g of $CuSO_4 \cdot 5H_2O$, and 0.025g of $Na_2MoO_4 \cdot 2H_2O$ per liter.

Results

Tables 1, 2, 3 and 4 show the results obtained in this study together with the analyses of variance. In figures 1, 2 and 3 the results are presented graphically. Appendix Tables A, B, C-1 and C-2 show the dry weight per pot and the number of plants per pot for each replication.

The dry weight per pot of the check plants appeared to depend on light intensity. Corn and oats were most vigorous at the highest

Table 1. The effect of light intensity on the toxicity of alachlor to corn.

Alachlor conc. (ppm)	Foliage dry wt. per pot (mg) Light intensity (ft-c) 2000	1600	660	60	Avg
0	608.3	473.8	427.3	366.9	469.1
10	548.4	366.4	348.7	353.8	404.3
50	365.5	200.5	204.0	261.0	257.8
100	148.8	38.7	75.7	90.0	88.3
	417.7	269.9	264.0	267.9	

Alachlor conc. (ppm)	Number of plants per pot Light intensity (ft-c) 2000	1600	660	60
0	4.6	5.0	5.0	4.9
10	4.8	5.0	4.9	4.8
50	4.8	4.9	5.0	5.0
100	4.1	4.4	4.3	4.9

Alachlor conc. (ppm)	Foliage dry wt. as percentage of check Light intensity (ft-c) 2000	1600	660	60
0	100	100	100	100
10	90	77	82	96
50	60	42	45	75
100	25	8	18	25

Table 2. The effect of light intensity on the effect of alachlor to cucumber.

Alachlor conc. (ppm)	Foliage dry wt. per pot (mg) Light intensity (ft-c) 2000	1600	660	60	Avg
0	140.3	167.1	98.9	92.1	124.6
1	92.3	110.2	87.3	88.7	94.6
5	73.7	64.3	53.5	67.5	64.7
10	75.1	57.8	40.3	50.8	56.0
AVG	95.4	99.8	70.0	74.8	

Alachlor conc. (ppm)	Number of plants per pot Light intensity (ft-c) 2000	1600	660	60
0	4.6	4.9	4.3	4.6
1	4.8	4.8	4.9	4.9
5	4.8	4.4	3.6	4.4
10	4.9	3.8	3.1	3.8

Alachlor conc. (ppm)	Foliage dry wt. as percentage of check Light intensity (ft-c) 2000	1600	660	60
0	100	100	100	100
1	66	66	88	95
5	53	38	54	73
10	54	35	41	63

Table 3. The effect of light intensity on the toxicity of alachlor to oats.

Alachlor conc. (ppm)	Foliage dry wt. per pot (mg) Light intensity (ft-c) 1600	60	Avg
0	52.6	40.7	46.7
0.05	53.3	33.6	45.9
0.1	39.8	31.4	19.6
0.3	20.8	17.6	19.2
AVG	41.6	32.1	

Alachlor conc. (ppm)	Number of plants per pot Light intensity (ft-c) 1600	60
0	5.0	4.8
0.05	5.0	4.9
0.1	4.3	5.0
0.3	3.0	4.8

Alachlor conc. (ppm)	Foliage dry wt. as percentage of check Light intensity (ft-c) 1600	60
0	101	100
0.05	101	95
0.1	76	77
0.3	40	43

Table 4. The effect of light intensity on the toxicity of alachlor to corn, cucumber and oats. Statistical analyses.

Corn		Analysis of Variance	
Source of variation	df	Mean square	F value
alachlor	3	917026	227.6**
light intensity	3	181345	45.0**
ala x light int.	9	13736	3.4**
error	112	4027	
$\bar{x}$ = 304.5	s = 63.4	CV = 20.8%	

Cucumber		Analysis of Variance	
Source of variation	df	Mean square	F value
alachlor	3	31092	128.2**
light intensity	3	7002	28.8**
ala x light int.	9	2076	8.5**
error	112	242	
$\bar{x}$ = 84.5	s = 15.5	CV = 18.4%	

Oats		Analysis of Variance	
Source of variance	df	Mean square	F value
alachlor	3	2622	37.6**
light intensity	1	1465	21.0**
ala x light int.	3	98	1.4
error	56	69	
$\bar{x}$ = 36.2	s = 8.3	CV = 23.0%	

** Significant at 1% level.

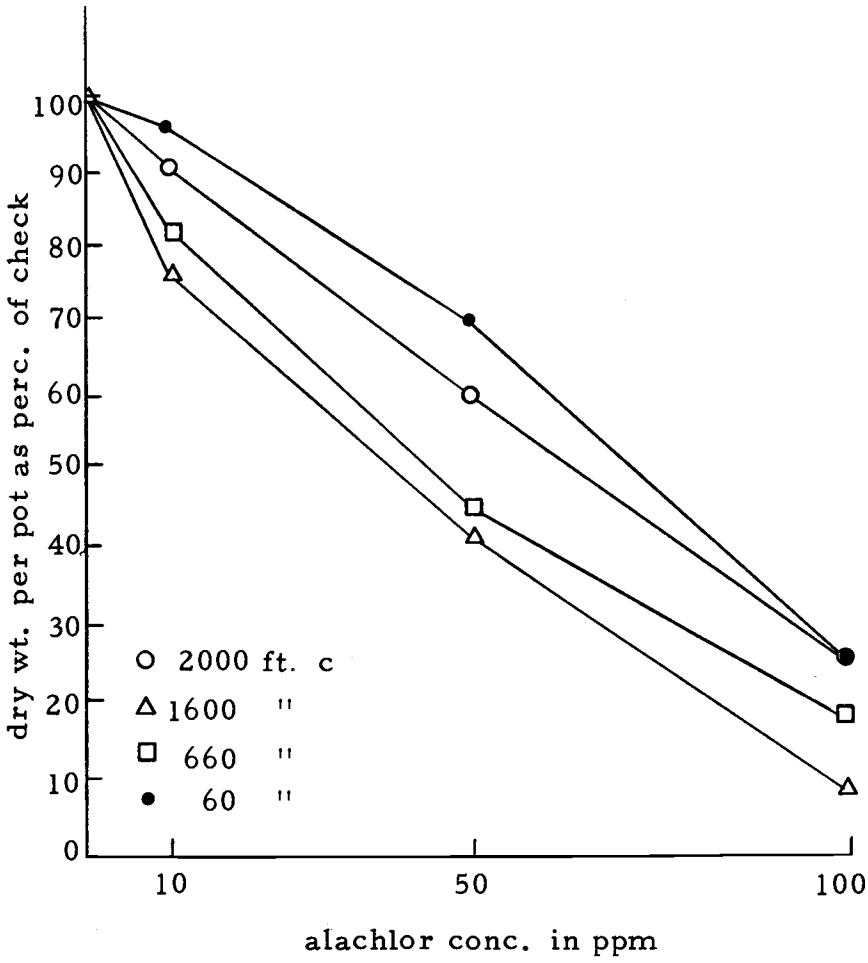


Figure 1. The effect of light intensity on the toxicity of alachlor to corn.

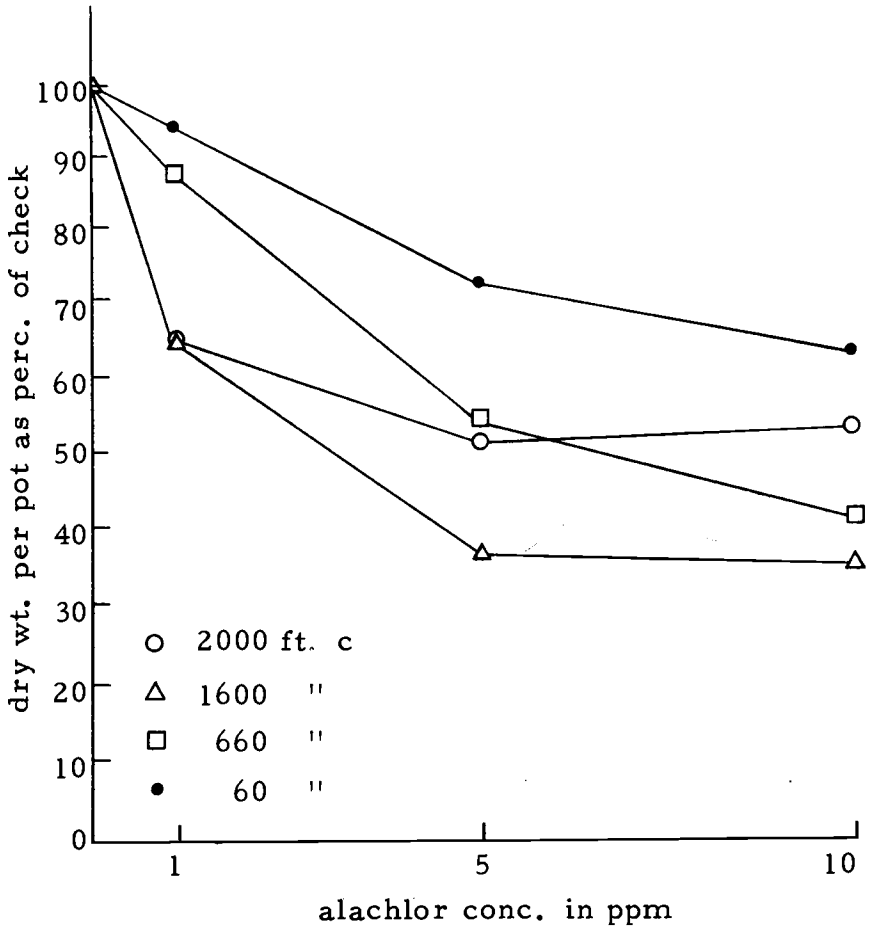


Figure 2. The effect of light intensity on the toxicity of alachlor to cucumber.

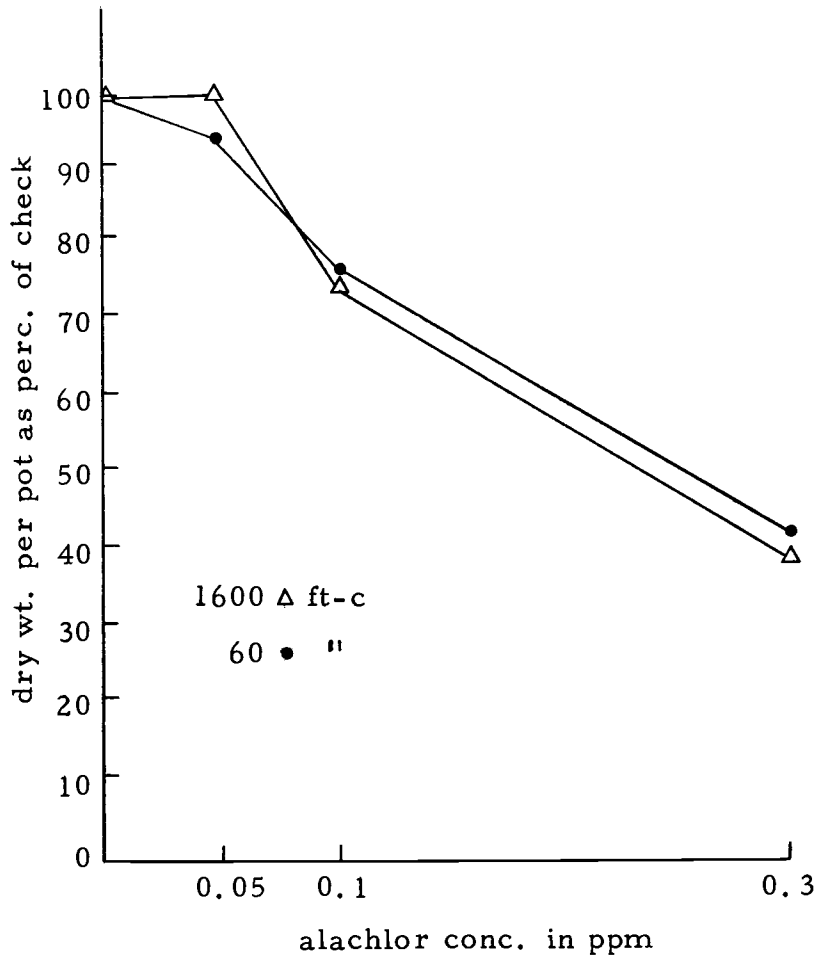


Figure 3. The effect of light intensity on the toxicity of alachlor to oats.

light intensity while cucumber gave higher yields at 1600 ft-c than at 2000 ft-c.

Oats were far more susceptible to alachlor than the other two species. Corn was extremely tolerant to the herbicide under the conditions of this experiment. Alachlor injury was apparent in all species at the higher doses. Symptoms of herbicide injury were growth reduction and dark green coloration of the leaves. Twisting and curling of the first leaf in oats and corn occurred frequently.

Increase in light intensity from 60 ft-c to 1600 ft-c increased alachlor toxicity for corn and cucumber. However, the light intensity of 2000 ft-c appeared to be less effective in the stimulation of alachlor activity than 1600 ft-c for cucumber or 1600 ft-c and 660 ft-c for corn. The interaction between alachlor and light intensity was significant for corn and cucumber but not for oats.

EXPERIMENT II. THE EFFECT OF DAY LENGTH ON THE TOXICITY OF ALACHLOR TO CORN, CUCUMBER AND OATS

Plants differ markedly in their response to photoperiod. The objective of this study was to investigate if day length might have an effect on the action of alachlor.

Materials and Methods

Two experiments were conducted in which plants grown in alachlor-treated sand were exposed to photoperiods of 16 hours and 8 hours.

Materials and methods were the same as described in Experiment 1, except that 16 liters of nutrient solution were administered between emergence and harvesting.

During the experimental period the conditions maintained in the growth-chamber were:

light intensity	1800 ft-c
temperature	27 C
R. H.	50 %

Results

Tables 5, 6, 7 and 8 give a summary of the results and the statistical analyses. The information concerning each replication is presented in Appendix tables D, E and F.

Table 5. The effect of day length on the toxicity of alachlor to corn.

Alachlor conc. (ppm)	Foliage dry wt. per pot (mg) Day length (hr) 8	16	Avg
0	694.2	779.9	737.0
10	589.0	603.6	596.3
50	474.2	472.0	473.1
100	153.3	176.8	165.0
AVG	477.6	508.1	

Alachlor conc. (ppm)	Number of plants per pot Day length (hr) 8	16
0	4.9	4.9
10	4.9	4.6
50	4.9	4.9
100	4.3	4.4

Alachlor conc. (ppm)	Foliage dry wt. as percentage of check Day length (hr) 8	16
0	100	100
10	85	77
50	68	60
100	22	22

Table 6. The effect of day length on the toxicity of alachlor to cucumber.

Alachlor conc. (ppm)	Foliage dry wt. per pot (mg) Day length (hr) 8	16	Avg
0	118.6	143.6	131.1
1	84.5	91.4	88.0
5	61.9	78.0	69.9
10	59.5	62.3	60.9
AVG	81.1	93.8	

Alachlor conc. (ppm)	Number of plants per pot Day length (hr) 8	16
0	4.9	5.0
1	4.8	4.8
5	4.0	4.8
10	4.1	4.5

Alachlor conc. (ppm)	Foliage dry wt. as percentage of check Day length (hr) 8	16
0	100	100
1	71	64
5	52	54
10	50	43

Table 7. The effect of day length on the toxicity of alachlor to oats.

Alachlor conc. (ppm)	Foliage dry wt. per pot (mg) Day length (hr) 8	16	Avg
0	66.3	72.8	69.5
0.05	54.0	58.6	56.3
0.1	41.7	38.8	40.3
0.3	23.0	23.4	23.2
AVG	46.2	48.4	

Alachlor conc. (ppm)	Number of plants per pot Day length (hr) 8	16
0	5.0	5.0
0.05	5.0	5.0
0.1	5.0	3.9
0.3	4.4	3.4

Alachlor conc. (ppm)	Foliage dry wt. as percentage of check Day length (hr) 8	16
0	100	100
0.05	81	81
0.1	62	53
0.3	35	32

Table 8. The effect of day length on the toxicity of alachlor to corn, cucumber and oats. Statistical analyses.

Corn		Analysis of Variance	
Source of variance	df	Mean square	F Value
alachlor	3	950230	94.2**
day length	1	14813	1.4
ala x day length	3	5900	0.5
error	56	10082	
$\bar{x}$ = 492.7	s = 100.4	CV = 20.3%	

Cucumber		Analysis of Variance	
Source of variation	df	Mean square	F Value
alachlor	3	15552	79.3**
day length	1	2586	13.2**
ala x day length	3	394	2.0
error	56	195	
$\bar{x}$ = 87.0	s = 13.9	CV = 16.0%	

Oats		Analysis of Variance	
Source of variance	df	Mean square	F Value
alachlor	3	6418	43.7**
day length	1	75	0.5
ala x day length	3	71	0.5
error	56	146	
$\bar{x}$ = 47.0	s = 12.0	CV = 25.7%	

** Significant at 1 % level.

Untreated test species yielded higher dry weights at the longer day length.

Cucumber responded significantly to the change in photoperiod. However, no species showed a significant interaction between alachlor and day length.

EXPERIMENT III. THE EFFECT OF CALCIUM NITRATE ON THE TOXICITY OF ALACHLOR TO OATS

Both nitrogen and calcium are essential elements in the nutrition of plants, and calcium nitrate supplies both elements in the ionic form in which they are preferentially absorbed. Changes in the level of calcium nitrate may very well affect the uptake and consequently the activity of a herbicide.

Materials and Methods

The effect of calcium nitrate on alachlor-treated oat plants was studied by growing pre-germinated oat plants in 30-ml test tubes. The oat seeds were allowed to imbibe water for 24 hours. After this period the seeds were placed in the dark for three days and allowed to germinate. The seedlings, held in place by cork gaskets, were then cultured for two days in test tubes, containing tap water. Thereafter, the plants were transferred to test tubes containing water with the desired concentrations of calcium nitrate and alachlor. The solution was replaced every two days to prevent the occurrence of anaerobic conditions.

Alachlor concentrations of 0, 0.05 and 0.1 ppm and calcium nitrate levels of 10^{-5}, 10^{-4} and 10^{-3} M were studied. Water uptake was measured three times over 48 hour periods. The first measurement was made 96 hours before harvesting. The plants were

harvested, after being in contact with the experimental solution for 15 days. Dry weights of shoots and roots were determined. Each treatment was replicated three times. During the experimental period the conditions maintained in the growth chamber were:

photoperiod	14 hr
light intensity	2000 ft-c
temperature	27 C
R. H.	50 %

Results

Dry weights of foliage and roots are presented in tables 9 and 10 together with the statistical analyses. Water uptake is given in table 11.

Growth reduction caused by alachlor was more severe for roots than for shoots. Stimulation of growth by calcium nitrate was less for roots than for shoots.

Water uptake appeared to be closely related to the dry weights of the $Ca(NO_3)_2$ + 0 alachlor treatment for shoots, and to the dry weight of the alachlor + 0 $Ca(NO_3)_2$ treatment for the roots. This phenomenon is illustrated in table 11.

Calcium nitrate at a concentration of 10^{-3} M acted antagonistically with regard to alachlor. Growth reduction at 0.05 ppm alachlor was more severe than at 0.1 ppm.

Table 9. The effect of calcium nitrate on the toxicity of alachlor to the foliage of oats.

Alachlor conc. (ppm)	Foliage dry wt. per plant (mg) $Ca(NO_3)_2$ conc. (M) 0	10^{-5}	10^{-4}	10^{-3}	Avg
0	30.8	46.4	60.0	49.1	46.6
0.05	34.7	45.4	45.1	47.8	43.2
0.1	30.6	38.4	47.9	55.6	43.1
AVG	32.0	43.4	51.0	50.8	

Analysis of Variance

Source of Variation	df	Mean square	F Value
alachlor	2	61	0.4
calcium nitrate	3	953	6.3**
ala x ca-nitrate	6	119	0.8
error	36	150	

$\bar{x}$ = 44.0 s = 12.2 CV = 27.7%

**Significant at 1% level

Alachlor conc. (ppm)	Foliage dry wt. as percentage of check $Ca(NO_3)_2$ conc. (M) 0	10^{-5}	10^{-4}	10^{-3}
0	100	151	194	159
0.05	112	147	146	155
0.1	99	124	155	180

Table 10. The effect of calcium nitrate on the toxicity of alachlor to the roots of oats.

	Root dry wt. per plant (mg) $Ca(NO_3)_2$ conc. (M)				
Alachlor conc. (ppm)	0	10^{-5}	10^{-4}	10^{-3}	Avg
0	18.8	22.3	23.3	21.0	21.3
0.05	12.5	18.1	23.3	16.7	17.7
0.1	12.4	15.1	17.4	16.3	15.3
AVG	14.6	18.5	21.3	18.0	

Analysis of Variance

Source of variation	df	Mean square	F Value
alachlor	2	147	8.8**
calcium nitrate	3	92	5.6**
ala x ca-nitrate	6	10	0.6
error	36	16	

$\bar{x}$ = 17.6 s = 4.1 CV = 23.1%

** Significant at 1% level

Alachlor	Root dry wt. as percentage of check $Ca(NO_3)_2$ conc. (M)			
conc. (ppm)	0	10^{-5}	10^{-4}	10^{-3}
0	100	119	124	112
0.05	67	97	125	89
0.1	67	81	93	87

Table 11. The effect of calcium nitrate and alachlor on the uptake of water by oats.

Alachlor conc. (ppm)	$Ca(NO_3)_2$ conc. (M)	Water uptake by 4 plants in 48 hrs (ml) t_1	t_2	t_3	AVG
0	0	28	24	15	22
0.05	0	17	14	8	13
0.1	0	18	13	7	13
0	10^{-5}	42	38	31	37
0	10^{-4}	52	43	33	33
0	10^{-3}	38	27	17	27
0.05	10^{-5}	27	24	14	22
0.01	10^{-5}	23	17	10	17
0.05	10^{-4}	44	36	23	34
0.1	10^{-4}	34	26	17	26
0.05	10^{-3}	36	23	15	25
0.01	10^{-3}	30	24	16	23

Alachlor conc. (ppm)	Dry wt. as percentage of check Roots	Shoots	Water uptake as percentage of check
0	100	100	100
0.05	67	112	79
0.1	67	99	66
$Ca(NO_3)_2$ conc. (M)			
0	100	100	100
10^{-5}	119	151	158
10^{-4}	124	194	194
10^{-3}	112	159	157

The dry weight of each treatment replication is presented in Appendix Table G.

EXPERIMENT IV. THE EFFECT OF CARRIER VOLUME ON THE TOXICITY OF ALACHLOR TO OATS AND CUCUMBER

Carrier volume is an important factor in the determination of the activity of a herbicide, especially when the water solubility of the material is low. Three rates of carrier volume were tested for their influence on the activity of alachlor.

Materials and Methods

Oat and cucumber plants were grown as described for experiment I. The herbicide was applied with a pot-sprayer, equipped with a Tee jet 8001-E nozzle, and was calibrated so that one pass would deliver a quantity of water corresponding to 20 gal/ac. The desired levels of carrier volume were obtained by making 1, 2, or 4 passes. The alachlor concentrations tested were 0, 1, 2 and 4 lb/ac. Following emergence, 2 liters of half strength nutrient solution were added on six consecutive days, by sub-irrigation. Dry weights of the above ground parts were determined ten days after treatment. Every treatment was replicated four times. Growing conditions during the experimental period were:

photoperiod	14 hr
light intensity	2150 ft-c
day temperature	27 C

night temperature	16 C
R. H.	50 %

Results

The results and the statistical analyses are presented in tables 12 and 13, and in Appendix tables H and I. Oats, although very sensitive to alachlor as shown in earlier experiments, reacted differently in this study. For both species alachlor activity increased only slightly when the concentration was raised.

Carrier volume was a significant factor for oats, but not for cucumber. This difference in response suggests a difference in site of uptake. In experiment VI, when corresponding plant parts were exposed, different responses of oats and cucumber to alachlor were indeed observed.

Table 12. The effect of carrier volume on the toxicity of alachlor to oats.

Alachlor conc. (lb/ac)	carrier volume (gal/ac) 20	40	80	AVG
0	41.5	42.1	33.4	39.0
1	46.5	26.7	40.1	34.4
2	39.6	29.5	17.2	28.7
4	34.5	30.8	28.9	31.4
AVG	40.5	32.2	27.3	

Analysis of Variance

Source of variation	df	Mean square	F Value
alachlor	3	233	1.8
carrier volume	2	705	5.5**
ala x carrier volume	6	124	0.9
error	36	128	

$\bar{x}$ = 33.0 s = 11.3 CV = 34.3%

**Significant at 1% level.

Table 13. The effect of carrier volume on the toxicity of alachlor to cucumber.

Alachlor conc. (lb/ac)	carrier volume (gal/ac) 20	40	80	AVG
0	105.7	95.1	105.3	102.0
1	91.6	91.0	76.5	86.3
2	97.0	66.4	91.5	84.9
4	104.0	72.5	81.6	86.0
AVG	99.5	81.2	88.7	

Analysis of Variance

Source of variation	df	Mean square	F Value
alachlor	3	797	1.8
carrier volume	2	1356	3.1
ala x carrier volume	6	398	0.9
error	36	431	0.9

$\bar{x}$ = 89.5 s = 20.8 CV = 23.2%

EXPERIMENT V. THE EFFECT OF SUB-IRRIGATION AND CARRIER VOLUME ON THE TOXICITY OF ALACHLOR TO OATS

Movement of a herbicide can be drastically affected by sub-irrigation carrier volume. In greenhouse or growth chamber studies, where thin layers of soil ordinarily are employed the effects of these two factors, as well as their interaction, may completely mask other factors studied.

Materials and Methods

Oat plants were cultured as described in Experiment I. The 2 lb/ac rate of alachlor at carrier volumes of 20 and 100 gal/ac were applied as indicated in Experiment IV.

Eight pots, comprising four duplicated sets of alachlor carrier volume treatments were placed in a glass tray and treated as one level of sub-irrigation. Three levels of sub-irrigation: 100, 200 and 300 ml per glass tray per day, were used. Each sub-irrigation level was replicated twice.

The experimental lay-out of this study was a split-plot design, with sub-irrigation as the largest unit. Since the interaction between carrier volume and sub-irrigation was of special interest, the foliage dry weights were statistically treated as a factor analysis.

Growing conditions were the same as described in Experiment IV, with day length being 16 hours, instead of 14 hours.

Results

Table 14 and Appendix Table J show the results, and the statistical analysis. The means of the main factors are also given in Appendix Table J.

Only the factors carrier volume, and alachlor appeared to give significant effects. Although the sub-irrigation effect was not significant, increasing volume of sub-irrigation tended to reduce herbicide toxicity. This corresponds with the results obtained for oats in the previous experiment. A high level of sub-irrigation will cause an upward movement of the herbicide, and thus will decrease the possibilities for root uptake. This effect will be opposed by a high carrier volume. The results in the following table illustrate this phenomenon.

carrier volume	Foliage dry wt. as perc. of check sub-irrigation (ml/day)		
(gal/ac)	100	200	300
20	91	89	95
100	87	81	80

Variation between replications obscured any significant effect of both the main factors and their interaction.

Table 14. The effect of sub-irrigation and carrier volume on the toxicity of alachlor to oats.

Sub-irrigation per day (ml)	Carrier volume (gal/ac)	Alachlor conc. (lbs/ac)	Foliage dry weight per pot (mg)
100	20	0	55.0
100	100	0	53.1
100	20	2	50.1
100	100	2	46.3
200	20	0	60.1
200	100	0	58.5
200	20	2	53.6
200	100	2	47.5
300	20	0	60.4
300	100	0	59.5
300	20	2	57.3
300	100	2	47.4

Analysis of Variance

source of variation	df	Mean square	F Value
replication	1	29.3	0.3
subirrigation	2	56.6	0.5
error a	2	106.7	
carrier volume	1	97.2	17.1**
carrier vol x sub-irr	2	4.2	0.7
error b			
alachlor	1	339.7	12.1*
ala x sub-irr	2	5.4	0.2
ala x carrier vol	2	36.7	1.3
ala x sub-irr x carrier vol	2	4.6	0.2
error c	7	28.1	

** Significant at 5% level.

EXPERIMENT VI. THE SITE OF UPTAKE OF ALACHLOR BY CORN, OATS, SOYBEAN AND CUCUMBER

Site of uptake of a herbicide frequently differs between plant species. Shoot or root uptake of the herbicide might be of fundamental importance for the selectivity of the chemical. Four plant species were studied in their specific response to alachlor treatment by exposing the roots and both roots and shoots.

Materials and Methods

The emulsifiable concentrate formulation of alachlor was mixed through sand as already described. Four seeds per pot were planted at a depth of 2.5 cm. In the case of root exposure the shoot region was protected by means of a plastic cylinder (length 2.5 cm, diameter 2.0 cm). The seed was placed in the center at the base of the cylinder. A 0.5 cm layer of activated charcoal was placed in the cylinder directly above the seed.

Alachlor concentrations that would give approximately 50 percent growth reduction with both shoots and roots exposed, were employed in this study. For the tolerant corn and soybean the alachlor concentration was 50 ppm, for the sensitive oats 0.3 ppm and for cucumber 5 ppm. Two liters of 5×10^{-4} M $Ca(NO_3)_2$ were added daily by sub-irrigation. Foliage dry weights were determined 15 days after planting. Each treatment was replicated eight times.

^{14}C-Alachlor was used to investigate whether the site of uptake most injurious to the plant was correlated with the uptake of herbicide by that plant part. The test species were grown for two weeks with vermiculite as root medium. Calcium nitrate at a concentration of 5×10^{-4} M was the only nutrient applied throughout this period. Before exposure to the experimental solution the roots and shoots were excised and placed in 5×10^{-4} M calcium nitrate for one hour. Two excised roots of each species were exposed to the experimental solution for three hours. Five to six excised shoot regions per species were treated in a similar way. Each treatment was replicated twice. The composition of the experimental solution was: 5 ppm unlabeled alachlor, 5×10^{-4} M $Ca(NO_3)_2$, and 0.08 μc ^{14}C-alachlor. Growing conditions were the same as described in Experiment IV.

Results

Results and statistical analyses are presented in tables 15, 16 and in Appendix table K.

Table 16 shows that for all species there was a significant difference in dry weight obtained by exposing different plant parts to alachlor.

Shoot exposure was more damaging to corn and cucumber, while the reverse was true for oats and soybean. The studies with ^{14}C-alachlor indicated that shoot uptake for the monocotyledonous species

Table 15. The site of uptake of alachlor by corn, oats, soybean and cucumber.

Exposed plant part	Growth reduction (%) Plant species			
	Corn	Oats	Soybean	Cucumber
none	0	0	0	0
root	10	36	36	12
shoot	46	20	15	52
root & shoot	56	56	51	64

Exposed plant part	Uptake of ^{14}C-alachlor (ppbw per mg dry wt.) Plant species			
	Corn	Oats	Soybean	Cucumber
root	17.4	29.5	24.9	33.1
shoot	20.5	55.7	18.1	22.6

Summary of results

Species	Plant part accounting for most of the uptake and growth reduction	
	Uptake	Reduction
corn	shoot	shoot
oats	shoot	root
soybean	root	root
cucumber	root	shoot

Table 16. The site of uptake of alachlor by corn, oats, soybean and cucumber.

Statistical analyses.

Corn		Analysis of Variance	
Source of variation	df	Mean square	F Value
plant part	2	1127161	18.9**
error	21	59570	

Oats		Analysis of Variance	
Source of variation	df	Mean square	F Value
plant part	2	8108	22.6**
error	21	358	

Soybean		Analysis of Variance	
Source of variation	df	Mean square	F Value
plant part	2	83973	7.8**
error	21	10691	

Cucumber		Analysis of Variance	
Source of variation	df	Mean square	F Value
plant part	2	23309	18.7**
error	21	1178	

** Significant at 1% level.

exceeded root uptake on a dry-weight basis. However, the dicotyledonous species show a higher uptake by the roots.

The two tolerant species, corn and soybean, reacted in a similar manner towards alachlor treatment. Corn, which absorbed more herbicide by the shoot region, was also more reduced in growth by shoot exposure. Soybean showed the same effect for root exposure.

The reverse was true for oats and cucumber. Oats, although taking up more herbicide through the shoots, were affected more by root exposure. Cucumber was most seriously reduced in weight by shoot exposure although root uptake was more important.

EXPERIMENT VII. THE EFFECT OF ALACHLOR ON THE UPTAKE OF POTASSIUM, PHOSPHORUS AND CALCIUM BY OATS

Herbicides, through their effect on cellular membranes, may influence the uptake of nutrients. Large amounts of proteins are present in these membranes. Chemicals that influence protein synthesis could eventually affect the uptake of other materials.

Materials and Methods

The effect of alachlor on the uptake of potassium, phosphorus and calcium by oat plants was investigated in a study in which alachlor-treated oat plants were exposed to half strength nutrient solution for four days. Based on the results obtained in the concentration study for oats (Experiment X) alachlor rates of 0, 0.04, 0.05, 0.1 and 0.2 mM were employed. According to the concentration study, plants were exposed to the various alachlor concentrations for one hour and then transferred to test tubes containing half strength nutrient solution.

The plants were cultured as described in Experiment III, with the solution in the 10-ml test tubes replaced daily. Four days after a one-hour exposure period to alachlor the plants were harvested, and dry weights of shoots and roots were determined. Water uptake was measured daily over the same period. P, K and Ca analyses were performed on the combined plant parts of all ten replications of

each treatment. P and Ca were determined by the atomic absorption technique, and K by colorimetric absorption. Growing conditions during the experimental period were as described in Experiment IV, except that continuous light was employed.

Results

Tables 17, 18 and Appendix table L present the information obtained in this study.

At the lower alachlor concentrations the chemical appeared to have a more pronounced effect on shoot growth than on root growth. At 0.2 mM alachlor, root growth was more reduced than shoot growth.

Plants treated with 0.2 mM alachlor did not show any increase in water uptake over the experimental period (Table 18). This is in contrast to the reaction of plants exposed to the lower concentrations of herbicide.

The uptake of nutrients was not affected by alachlor, with the exception of K. The decrease in K content, with increasing alachlor concentrations appeared to be gradual.

Table 17. The effect of alachlor on the uptake of potassium, phosphorus and calcium by oats. Dry weight of shoots and roots.

Alachlor conc. (mM)	Dry wt. of shoots and roots per plant (mg) Plant part Shoot	Root
0	51.1	20.7
0.04	40.1	17.1
0.05	42.6	18.6
0.1	43.2	18.7
0.2	40.3	14.2

Shoots	Analysis of Variance		
Source of variation	df	Mean square	F Value
alachlor	4	201	2.7*
error	45	73	
$\bar{x}$ = 9.5	s = 43.5	CV = 19.6%	

Roots	Analysis of Variance		
Source of variation	df	Mean square	F Value
alachlor	4	56	6.1**
error	45	9	
$\bar{x}$ = 17.8	s = 3.0	CV = 16.8%	

** Significant at 1% level.
* Significant at 5% level.

Table 18. The effect of alachlor on the uptake of potassium, phosphorus, and calcium by oats. Nutrient level and water uptake.

	Level of P, K and Ca (%)		
Alachlor conc. (mM)	P	K	Ca
0	0.68	5.30	0.90
0.04	0.68	4.60	0.85
0.05	0.63	4.35	0.85
0.1	0.68	4.40	0.90
0.2	0.68	4.20	0.85

	Water uptake by ten plants in 24 hr. (ml)				
Alachlor conc. (mM)	t_1	t_2	t_3	t_4	AVG
0	44	37	56	72	52
0.04	44	37	49	52	46
0.05	44	33	46	52	44
0.1	46	35	47	54	46
0.2	36	26	38	37	35

EXPERIMENT VIII. THE UPTAKE OF ALACHLOR BY CORN PLANTS AS A FUNCTION OF TIME

Uptake of a herbicide can be dependent on energy generated by metabolic processes within the plant. In this case uptake has been designated as being active. When uptake is not dependent upon energy created in the plant the process is of a physical nature. Uptake of a herbicide might also be a combination of the active and passive absorption processes.

Materials and Methods

The uptake of alachlor by corn and oat plants was studied by using ring-labeled ^{14}C-alachlor with a specific activity of 1.02 mc/mM. To determine whether uptake of alachlor is a metabolically controlled or a physical process two metabolic inhibitors, dinitrophenol (DNP) at 10^{-5} M, and sodium azide (NaN_3) at 10^{-4} M, were employed.

Corn and oat seeds were placed in petri dishes and allowed to soak up water for 24 hours. The petri dishes were then transferred to a dark growth chamber at a temperature of 27 C. Three days later the seedlings were placed in test tubes containing 10 ml of a 5×10^{-4} M calcium nitrate solution. The seedlings were grown continuously in light. The calcium nitrate solution was replaced daily. After three days for corn, and five days for oats, the plants were transferred to the experimental solution.

Exposure time ranged from 15 minutes to 2.5 hours. Longer periods of exposure were avoided to prevent undesirable side effects caused by the metabolic inhibitors. The experimental solution contained 5 ppm alachlor, 5×10^{-4} M calcium nitrate, a pre-determined amount of ^{14}C-alachlor and dinitrophenol or sodium azide as required. The plants were rinsed for one minute, following exposure to alachlor. The composition of the rinsing solution was identical with the experimental solution, except that it did not contain ^{14}C-alachlor. The purpose of this rinse was to replace ^{14}C-alachlor adsorbed on the root surface, by unlabeled alachlor. Following the one-minute rinse, the plants were exposed for one hour to a solution of the same composition as used in the rinsing.

Alachlor taken up in the presence of metabolic inhibitors and leached out during the one hour period is referred to as "exchangeable", while the remainder has been termed "non-exchangeable". Exchangeable plus non-exchangeable uptake is referred to as "passive uptake" in this experiment and also in the remaining part of the thesis, while that part of the total uptake affected by the presence of metabolic inhibitors has been termed "active uptake".

The amount of alachlor absorbed, adsorbed or replaced was determined with the liquid scintillation counter by measuring the activity of ^{14}C-alachlor left in the experimental solution, or obtained in the rinsing and leaching solution. One liter of scintillation fluid contained

666 ml Toluene, 333 ml of Triton X-100, 5.5 g of 2, 5-diphenyloxazole and 0.1 g of 1, 4-bis-2-(5-phenyloxazolyl)-benzene. One-ml samples were taken and added to 10 ml of scintillation fluid. To this mixture 2 ml of toluene were added.

Immediately following the experimental period the dry root weights of the plants were determined. Each treatment was replicated twice.

Results

Results are presented in table 19, and in Appendix tables M and N. Total uptake, in the presence and absence of metabolic inhibitor is illustrated graphically in figure 4.

The effect of a metabolic inhibitor appeared to be very small during the initial accumulation period. After 15 minutes the inhibitors reduced total uptake by approximately 25 percent. Dinitrophenol expressed a slightly less inhibitory effect as compared with sodium azide. Alachlor uptake appeared to be mainly a physical process.

Table 19. The uptake of alachlor by corn plants as a function of time.

Total uptake in ppb(w) per mg dry root weight			
Exposure time (min)	Check H_2O	Metabolic inhibitor DNP	Metabolic inhibitor NaN_3
15	77	62	55
30	95	69	76
60	101	71	58
90	102	108	95
150	120	92	60

Exchangeable uptake in ppb(w) per mg dry root weight			
Exposure time (min)	Check H_2O	Metabolic inhibitor DNP	Metabolic inhibitor NaN_3
15	9	6	7
30	9	9	8
60	10	8	7
90	11	12	11
150	12	8	8

Active and Non-exchangeable uptake in ppb(w) per mg dry root weight			
Exposure time (min)	Check H_2O	Metabolic inhibitor DNP	Metabolic inhibitor NaN_3
15	69	56	48
30	86	60	68
60	91	63	51
90	91	96	84
150	108	84	52

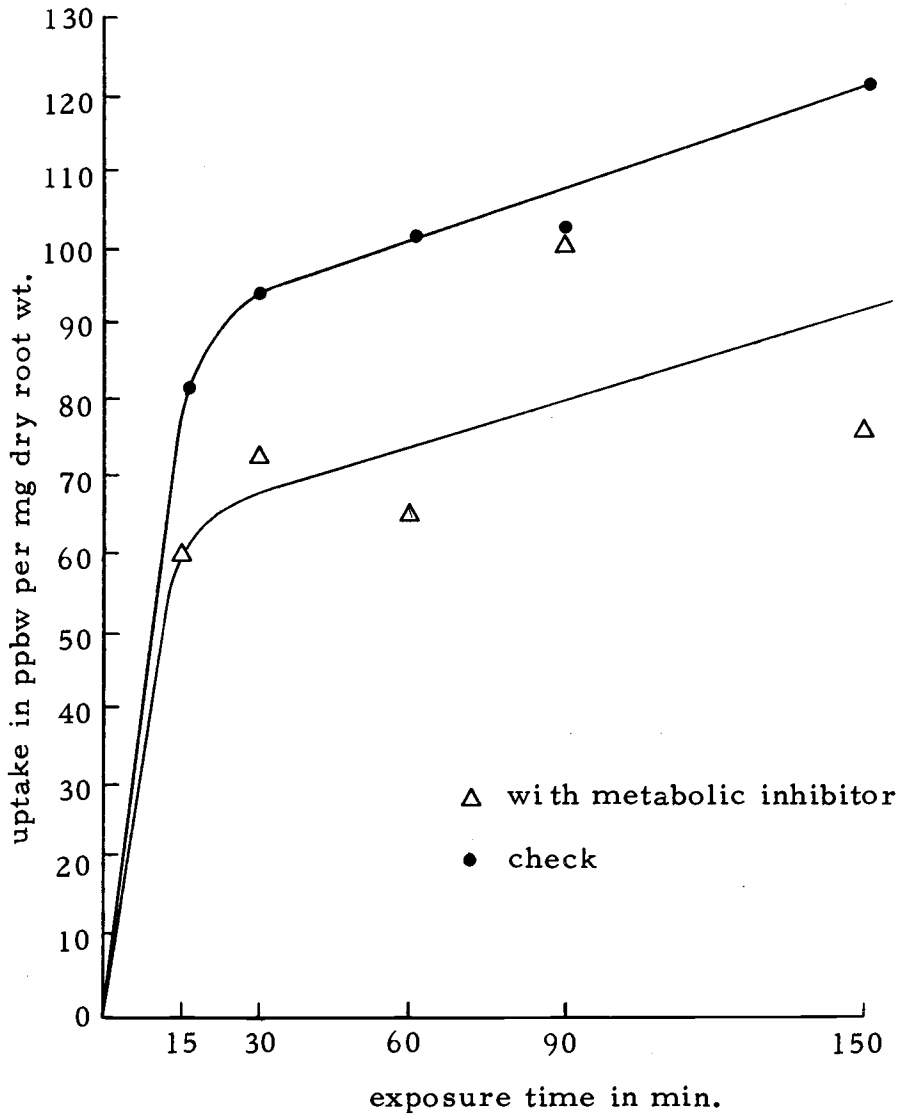


Figure 4. Total uptake of alachlor by corn as a function of time.

EXPERIMENT IX. THE UPTAKE OF ALACHLOR BY OAT PLANTS AS A FUNCTION OF TIME

As indicated in the introduction for the previous experiment, uptake of a herbicide can be a metabolically controlled or a physical process.

It is of interest to investigate the absorption of a tolerant and a sensitive species to determine if the two species discriminate in total uptake, or if active and passive absorption might take place in different proportions.

Materials and Methods

Materials and methods were the same as described in Experiment VIII, except that three replications were employed.

Results

Experimental data are presented in table 20 and in Appendix tables O, P and Q. In figure 5 a graphical presentation of total uptake has been made.

The effect of a metabolic inhibitor on the uptake was very small throughout the experimental period. Also in this study DNP was less effective in preventing uptake than NaN_3.

Total uptake per mg dry root weight by oats exceeded uptake by corn and appeared again to be mainly a physical process.

Table 20. The uptake of alachlor by oat plants as a function of time.

Total uptake in ppb(w) per mg dry root weight			
Exposure time (min)	Check H_2O	Metabolic inhibitor DNP	Metabolic inhibitor NaN_3
15	69	61	66
30	121	96	103
60	103	106	123
90	146	128	131
150	151	153	113

Exchangeable uptake in ppb(w) per mg dry root weight			
Exposure time (min)	Check H_2O	Metabolic inhibitor DNP	Metabolic inhibitor NaN_3
15	8	11	11
30	16	17	17
60	13	14	15
90	17	17	33
150	18	20	27

Active and Non-exchangeable uptake in ppb(w) per mg dry root weight			
Exposure time (min)	Check H_2O	Metabolic inhibitor DNP	Metabolic inhibitor NaN_3
15	61	50	55
30	105	79	86
60	90	92	108
90	129	111	98
150	133	133	86

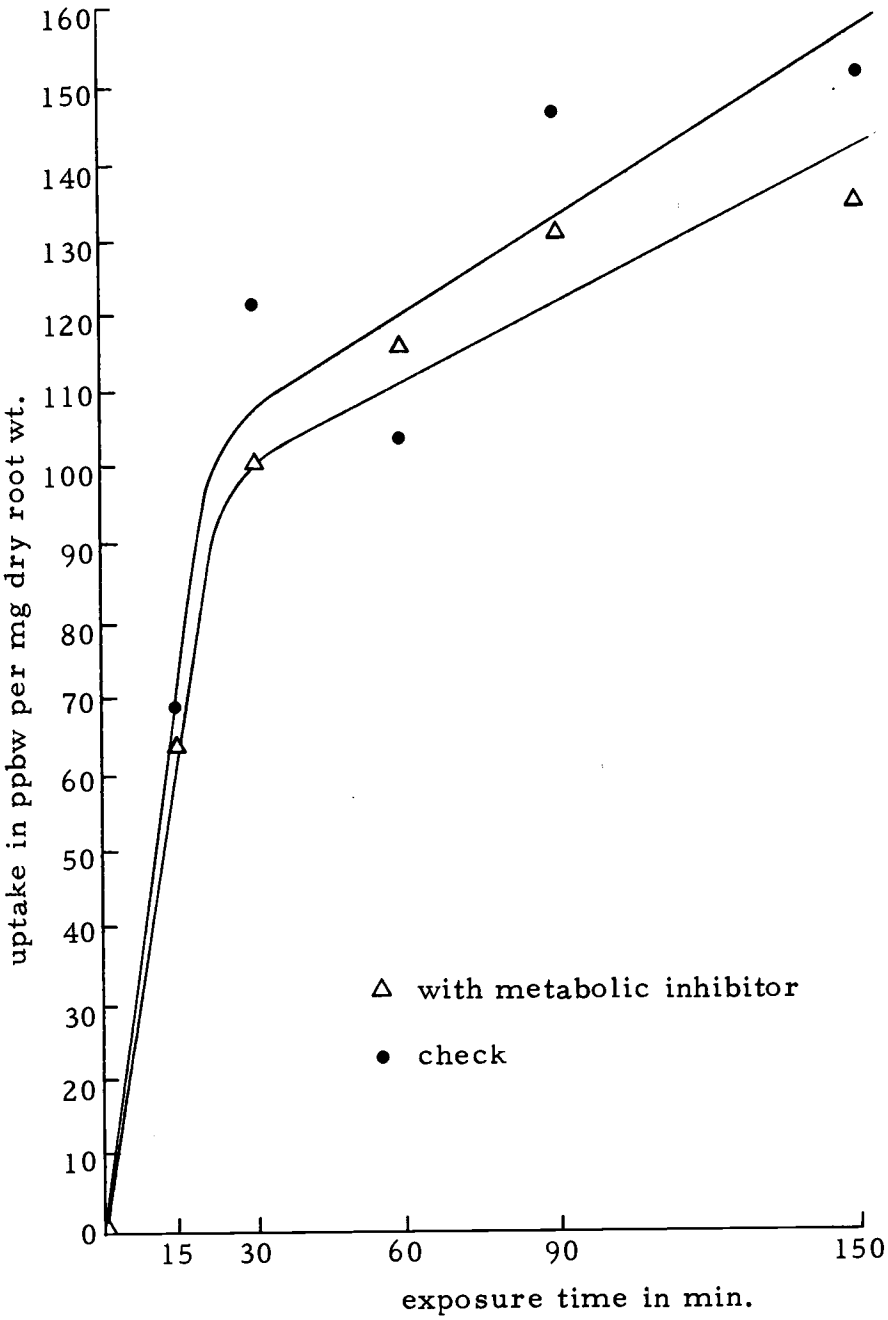


Figure 5. Total uptake of alachlor by oats as a function of time.

EXPERIMENT X. THE UPTAKE OF ALACHLOR BY CORN AND OAT PLANTS AS A FUNCTION OF CONCENTRATION

Absorption of several ions, sugars and growth regulators by plants, when studied over a concentration range, follows patterns similar to those described by Michaelis and Menten (1913) for enzyme catalyzed reactions.

In this experiment an attempt was made to determine if uptake of the phytotoxic organic chemical alachlor, at a range of concentrations, responds in a similar manner. Corn, a tolerant species, and oats, a sensitive species, were compared in their reaction to alachlor exposure.

Materials and Methods

The techniques employed in this study have been described in Experiment VIII.

For each species, the concentrations used were prepared by dilution from a stock solution containing 1 mM alachlor and 14 μc of ^{14}C-alachlor. In this way a constant ratio between labeled and unlabeled alachlor was obtained. Alachlor concentrations studied were; 0.5, 0.4, 0.3, 0.2, 0.1, 0.05, 0.04, 0.03, 0.02 and 0.01 mM. The 0.5 mM strength corresponds with a concentration of 135 ppm and 0.0185 mM consequently with the 5 ppm concentration used in the previous studies.

Plant roots were left in contact with the experimental solution for one hour and thereafter rinsed for one minute and exposed to the leaching solution for one hour, as described before. Each treatment was replicated four times.

Results

The data in table 21 and in figures 6 and 7 show that in the 0.01 to 0.05 mM alachlor concentration range total uptake for both species was almost identical. At the higher concentrations, uptake by oats was approximately twice as high as uptake by corn. The data obtained were based on the disappearance of the ^{14}C-alachlor activity from the experimental solution.

In previous experiments active uptake of alachlor by corn and oats was 25 percent and five percent of the total uptake respectively. These figures were used to calculate the amount of alachlor accumulated actively at each concentration.

It was assumed that during the one-hour exposure period the ratio between total uptake and active uptake remained the same over the range of concentrations studied.

The reciprocal values of the concentrations, as well as those of the amount of alachlor absorbed actively, are given in table 21 and

Table 21. The total uptake of alachlor by corn and oat plants as a function of concentration.

Total uptake in μ M per mg dry root weight		
Alachlor	Plant species	
conc. (mM)	Corn	Oats
0.5	6.03	12.50
0.4	5.85	10.43
0.3	4.40	10.05
0.2	3.33	7.38
0.1	2.28	7.38
0.05	1.08	1.15
0.04	0.76	0.89
0.03	0.38	0.60
0.02	0.33	0.35
0.01	0.13	0.19

Reciprocal values of alachlor concentration and active uptake per hour		
	Plant species	
1/conc.	Corn	Oats
2.0	0.66	1.59
2.5	0.69	1.92
3.3	0.91	2.00
5.0	1.20	2.70
10.0	1.75	2.70
20.0	3.70	16.60
25.0	5.27	25.00
33.0	10.00	33.33
50.0	12.50	50.00
100.0	33.34	100.00

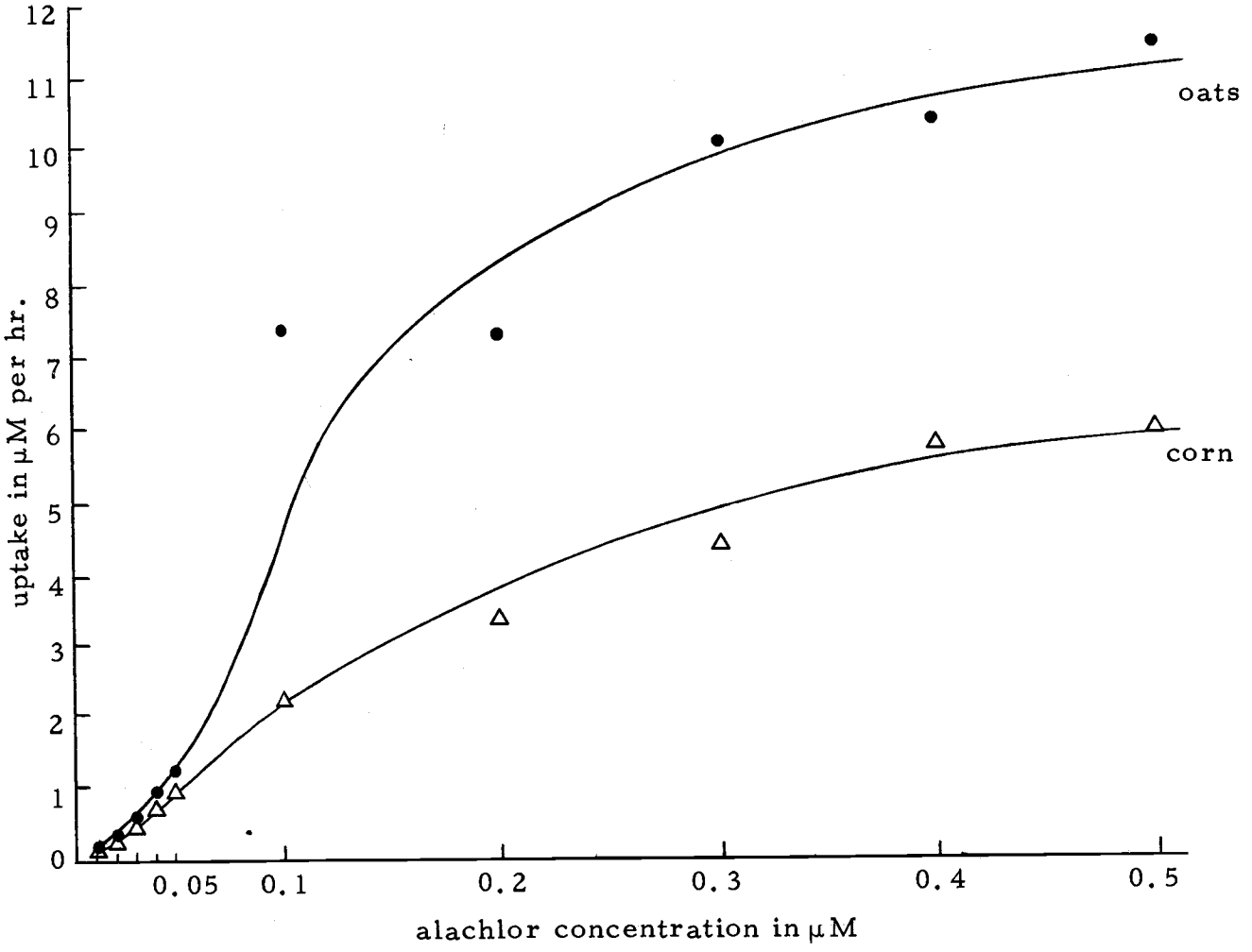


Figure 6. Total uptake of alachlor by corn and oats as a function of concentration

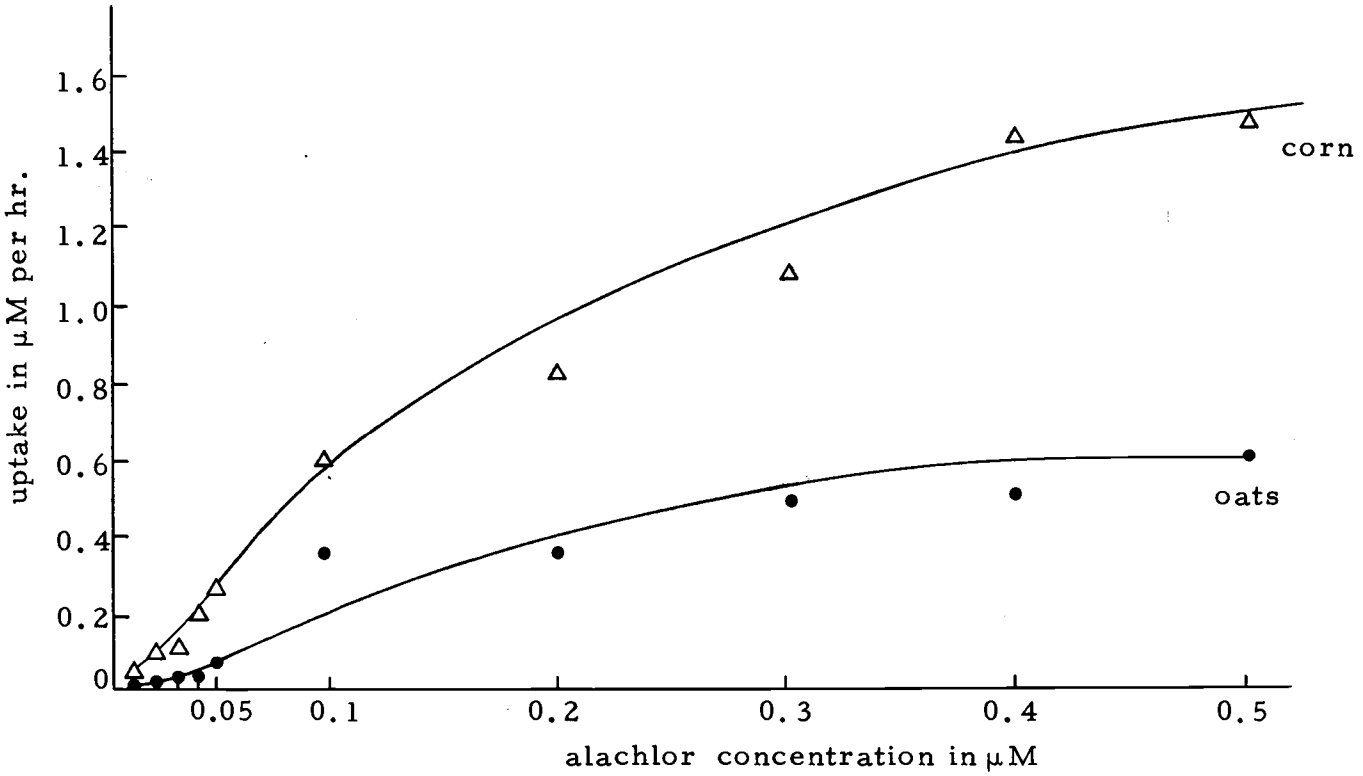


Figure 7. Active uptake of alachlor by corn and oats as a function of concentration.

plotted in figure 8 (Lineweaver and Burk, 1936). Concentration is referred to as S and the rate of uptake as V in figure 8, and also in the remaining part of the text. The equations for the regression lines in figure 8 are presented in table 22 together with the correlation coefficients.

From figure 8 the Michaelis constant (Km), and the maximum rate of uptake (V max) values were determined for both species. For these calculations the experimental values obtained at the higher concentrations only were used. The V max and Km values are given in table 22.

Uptake at the lower concentrations studied was somewhat lower than would be expected on the basis of the relationship established for the high concentration range.

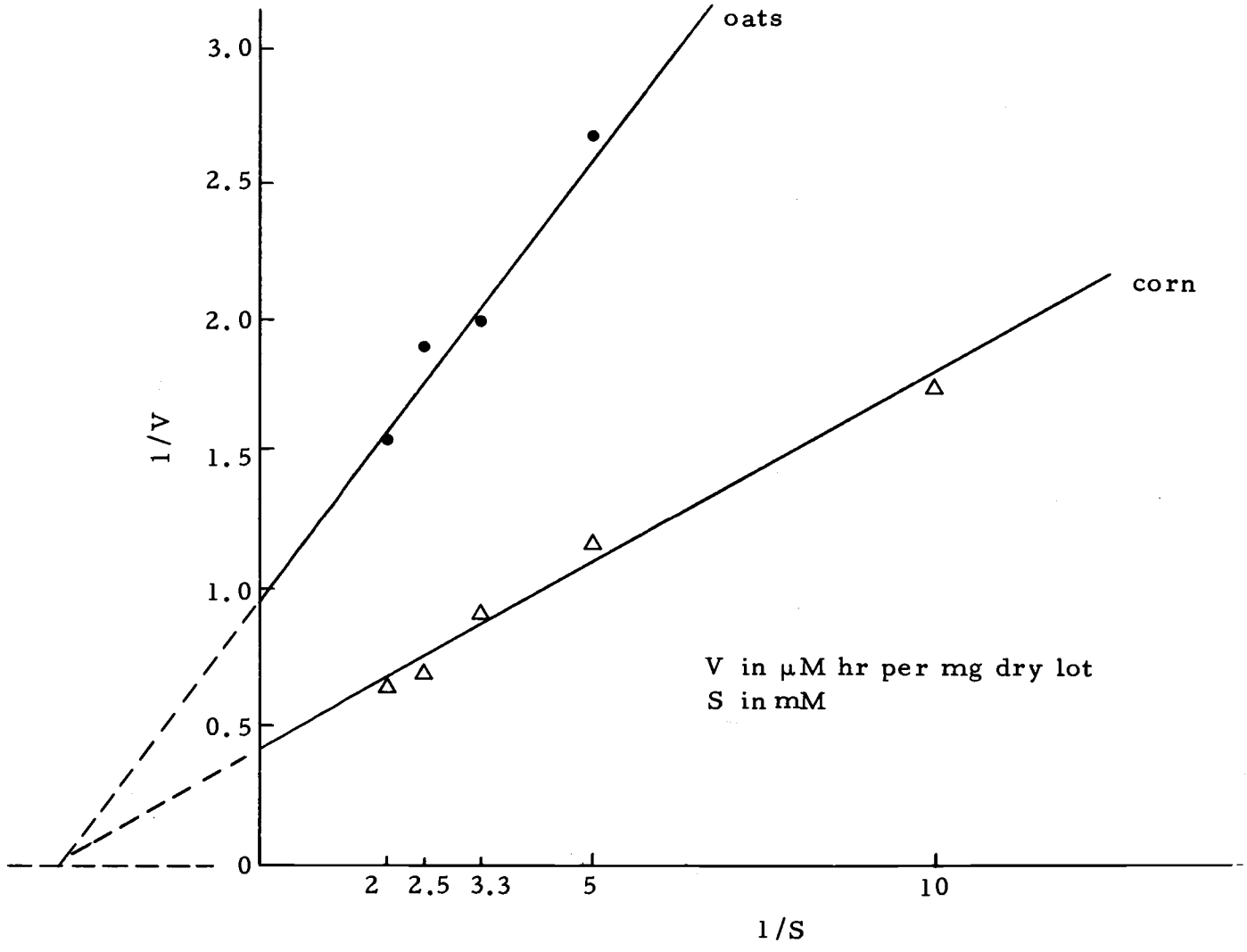


Figure 8. Lineweaver-Burk plot for the uptake of alachlor by corn and oats.

Table 22. The uptake of exchangeable alachlor by corn and oat plants as a function of concentration.

Exchangeable uptake expressed as percentage of total uptake		
Alachlor conc. (mM)	Plant species Corn	Oats
0.5	12	14
0.4	11	12
0.3	11	9
0.2	6	6
0.1	3	3
0.05	13	9
0.04	14	9
0.03	12	12
0.02	19	9
0.01	13	16

Plant species	Concentration range (mM)	Equation regression line	Correlation coefficient	Level of sign. of regression
oats	0.01 - 0.1	$y = 3.85 + 1.05 X$	0.997**	1 %
	0.2 - 0.5	$y = 0.94 + 0.35 X$	0.983*	5 %
corn	0.01 - 0.05	$y = 3.68 + 0.36 X$	0.994**	1 %
	0.1 - 0.5	$y = 0.42 + 0.14 X$	0.989**	1 %

** Significant at 0.5% level.
* Significant at 1% level.

Plant species	Concentration range (mM)	Km (M)	V max μM/hr per mg dry wt.
oats	0.5 - 0.2	0.37×10^{-3}	1.06
corn	0.5 - 0.1	0.33×10^{-3}	2.38

EXPERIMENT XI. THE EFFECT OF TEMPERATURE ON THE UPTAKE OF ALACHLOR BY CORN AND OATS

Temperature has a pronounced effect on physical and metabolic processes in the plant. A temperature increase of ten degrees centigrade accelerates most chemical reactions by a factor of about 2.5 and physical processes such as diffusion only slightly.

Hence determination of the temperature coefficient (Q_{10}) for the absorption of a chemical by plant roots can provide clues as to whether the uptake of the chemical is a passive or an active process.

Materials and Methods

This experiment was conducted as described before (Experiment VIII).

The temperatures employed to study herbicide uptake by corn and oats were 27 C and 17 C. In addition the uptake by oats was determined at 13 C and 8 C. The alachlor concentrations used were 0.5, 0.3, 0.05 and 0.03 mM. Each treatment was replicated four times.

Results

The results are presented in table 23, and in Appendix tables T

Table 23. The effect of temperature on the uptake of alachlor by corn and oats.

Total uptake by corn in μ M per mg dry root weight

Alachlor conc. (mM)	Temperature (C) 27	17	Q_{10}
0.3	5.59	4.38	1.28
0.03	0.50	0.41	1.22

Total uptake by oats in μ M per mg dry root weight

Alachlor conc. (mM)	Temperature (C) 27	17	13	8	Q_{10}
0.5	12.50		7.9		1.36
0.3	8.42	7.84			1.07
0.05	1.15			1.20	0.98
0.03	0.63	0.74			0.85

and U. Q_{10} values calculated from the uptake data indicate that uptake of alachlor for both species was primarily a physical process.

EXPERIMENT XII. THE EFFECT OF PROPACHLOR ON THE UPTAKE OF ALACHLOR BY CORN AND OATS

If active absorption of a herbicide follows enzyme kinetics, then this absorption could be reduced in the presence of an analog. If the same carrier system is utilized for uptake of the two materials the analog will inhibit uptake by competing with the herbicide for sites on the carrier molecule. When uptake is a physical process the presence of an analog has no effect on uptake.

The use of propachlor, which has a molecular structure very similar to alachlor, should give further information about the nature of the absorption process.

Materials and Methods

The techniques used in this study have been described before (Experiment VIII).

Alachlor concentrations of 0.3 and 0.03 mM were employed. Uptake was determined in the presence and absence of propachlor. The analog and alachlor were used at the same concentration, expressed in mM.

Results

Results are presented in table 24 and Appendix tables V and W. Active uptake as given in table 24, has been calculated from the

Table 24. The effect of propachlor on the uptake of alachlor by corn and oats.

Total uptake in μ M per mg dry root weight			
Alachlor conc. (mM)	Propachlor conc. (mM)	Plant species Corn	Oats
0.3	0	5.59	8.42
0.3	0.3	4.03	4.26
0.03	0	0.50	0.63
0.03	0.03	0.43	0.54

Active uptake in μ M per mg dry root weight			
Alachlor conc. (mM)	Propachlor conc. (mM)	Plant species Corn	Oats
0.3	0	1.40	0.42
0.3	0.3	1.01	0.21
0.03	0	0.13	0.03
0.03	0.03	0.10	0.03

total uptake data. Active uptake has been considered as being 25 percent for corn and five percent for oats.

The data indicated that alachlor uptake was affected by the presence of propachlor in all cases, except for uptake by oats at the low concentration. A 50 percent reduction in alachlor uptake, caused by propachlor, was observed at the 0.3 mM concentration for oats. At the same concentration the inhibitory effect on corn was approximately 30 percent, while this was about 20 percent at the low concentration.

DISCUSSION AND CONCLUSIONS

Studies conducted to examine the effects of light intensity on the toxicity of alachlor to three plant species revealed that the activity of the herbicide increases with an increase in light intensity up to 1600 ft-c. The toxicity of alachlor to corn, at each concentration studied, was highest at 1600 ft-c, followed by 660 ft-c, 2000 ft-c, and 60 ft-c respectively. For cucumber a similar effect was noticed. However, in this case the toxicity at 2000 ft-c was less dependent on the concentration of the herbicide. The activity of alachlor at 1 ppm was identical at 2000 ft-c and 1600 ft-c. At 5 ppm the activity at 2000 ft-c and 660 ft-c was nearly identical, and at 10 ppm the level of 2000 ft-c was less effective than the 660 ft-c level.

While the lowest and the highest activities of alachlor for corn and cucumber were found at 60 ft-c and 1600 ft-c respectively, this was not so for oats. The effect of light intensity on the toxicity of the herbicide was very small for oats.

The results indicate that a detoxification mechanism, if present, becomes more effective above a light intensity of 1600 ft-c. The increase in activity from 60 ft-c to 1600 ft-c probably was due to an increase in uptake of the herbicide.

Photoperiod appeared to have very little effect on alachlor activity, as demonstrated by the results of experiment II.

To determine the most effective site of uptake of alachlor, two different experimental methods were used. The first technique involved plants grown in sand, treated with unlabeled alachlor, while in the second approach excised root and shoot regions were exposed to a mixture of unlabeled and ^{14}C-labeled alachlor.

To obtain a maximum amount of information four test species were used. Corn, as the tolerant monocotyledonous species, had a shoot uptake that was slightly higher than root uptake, expressed on a weight basis, while damage exerted by shoot exposure was much more severe than by root exposure.

In oats, the susceptible monocot tested, uptake by the shoot region was almost twice as high as that by the root system. However, damage caused by exposure of both shoot and root region exceeded only slightly that caused by root exposure.

In the two dicotyledonous species tested, root uptake was more important than shoot uptake. Damage in soybean was more severe through root exposure, but this was not the case in cucumber.

It appears that in monocots the shoot region is the main site of uptake while in dicots the root region is more important for uptake.

The comparison between the alachlor tolerant and susceptible species indicates that corn and soybean showed more damage when the plant part with the highest uptake was exposed. In the two susceptible species the reverse held true.

Absolute amounts of alachlor taken up by the four species appear to be related to their relative tolerance to alachlor. Uptake increases from corn, to soybean, to cucumber, to oats and this reflects the respective sensitivity to the herbicide. This phenomenon would indicate that discrimination in uptake might be a factor in the selective action of alachlor.

The way in which this experiment was conducted did not make it possible to draw conclusions about the role of a detoxification mechanism in the selective action of alachlor towards corn and soybean.

The effects of carrier volume and amount of sub-irrigation on the activity of alachlor were studied in two experiments. The results apparently reflect movement of the herbicide through the sand. Downward movement of the herbicide, enhanced by an increase in carrier volume is reversibly affected by an increase in amount of sub-irrigation.

The growth of oats was markedly reduced with increasing amounts of sub-irrigation at 20 gal/ac, but only slightly at 100 gal/ac. At one level of sub-irrigation the toxicity of alachlor to cucumber was highest at a carrier volume of 40 gal/ac as compared to carrier volumes of 20 and 80 gal/ac. In oats 80 gal/ac caused more reduction in growth than 20 and 40 gal/ac. Although contact with the herbicide

probably is enchanced by a higher carrier volume, the less effective uptake by the roots of cucumber might have caused the reduced activity at 80 gal/ac.

Since calcium nitrate was to be used in all experiments involving ^{14}C-labeled alachlor, an experiment was conducted to determine if calcium nitrate would interact with the herbicide.

The results indicated that calcium nitrate markedly enhanced shoot growth of oats with a maximum increase at a concentration of 10^{-4} M. Root growth was also stimulated, though not to the same degree.

No evidence of an interaction between calcium nitrate and alachlor was noticed for roots. An antagonistic effect for shoots was observed however. The most severe reduction in shoot weight at the 10^{-5} M concentration of calcium nitrate was observed at the highest alachlor concentration. This was in contrast with the results obtained at the 10^{-3} M calcium nitrate concentration.

Changes in membrane permeability, that could have resulted from the calcium treatments, cannot account for this reaction towards alachlor.

The difference in nitrogen level might enhance the detoxification of alachlor through a stimulatory effect on some part of the biological system. This however cannot account for the observed reaction toward alachlor.

Since the coefficient of variation was 32 percent in this study, the observed effect might not have been realistic.

A number of studies were conducted to obtain information about the mechanisms of uptake of alachlor by corn and oats.

It appeared that the total uptake of alachlor was slightly reduced in the presence of dinitrophenol or sodium oxide. The reduction was somewhat stronger in corn than in oats. These results seem to indicate that passive uptake is of more importance in the sensitive oat plants than in the tolerant corn plants.

The part of the total uptake affected by the presence of metabolic inhibitors followed Michaelis-Menten kinetics at an alachlor concentration range of 0.1 mM to 0.5 mM. At the lower concentrations these kinetics were not followed.

Absorption of alachlor studied at different temperatures gave Q_{10} values in the order of 1, 2, and this seems to confirm the previous observation that alachlor uptake is primarily a passive process.

Propachlor, at 0.3 mM, reduced active uptake of alachlor by 50 percent in oats at an alachlor concentration of 0.3 mM. This effect was less pronounced with concentrations of 0.03 mM for both chemicals. In corn, less competition by propachlor was noticed at both concentrations.

Total uptake by corn and oats was nearly identical up to an alachlor concentration of 0.1 mM. Above this concentration,

absorption by oats was about twice as high as compared to corn. The effect of alachlor on the absorption of nutrients by oat plants was investigated by studying the uptake of K, P and Ca. It appeared that while the P and Ca content of the oat plants was not changed at the different alachlor concentrations studied this was not so for K. A gradual decrease in K content with increasing alachlor concentrations was noticed.

BIBLIOGRAPHY

Allen, W. S. and R. D. Palmer. 1963. The mode of action of simazine in barley. Weeds 11:27-31.

Appleby, A. P., W. R. Furtick., and S. C. Fang. 1965. Soil placement studies with EPTC and other carbamate herbicides on Avena sativa. Weed Res. 5:115-122.

Arisz, W. H. 1956. Significance of the symplasm theory for transport across the root. Protoplasma 46:1-62.

Ashton, F. M. 1965. Relationship between light and toxicity symtoms caused by atrazine and monuron. Weeds 13:164-168.

Berlin, R. D. 1970. Specificities of transport systems and enzymes. Science 168:1539-1545.

Biddulph, S. F. 1967. A microautoradiographic study of ^{45}Ca and ^{35}S distribution in the intact bean root. Planta (Berlin) 74: 350-367.

Birt, L. M., and F. J. R. Hird. 1958. Kinetic aspects of the uptake of amino acids by carrot tissue. Biochem. J. 70:286-292.

Blackman, G. E. 1958. Selective toxicity in relation to specific differences in retention, penetration and uptake. Proc. IV thInt. Conf. Plant. Protection, 4th Meeting (Hamburg, Germany, Sept. 8-15, 1957).

Blackman, G. E., and J. A. Sargent. 1959. The uptake of growth substances II. The absorption and accumulation of 2,3,5-triiodobenzoic acid by the root and frond of Lemna minor. J. Exp. Bot. 10:480-503.

Bowen, J. E. 1969. Absorption of copper, zinc, and manganese by sugarcane leaf tissue. Plant Physiol. 44:255-261.

Brady, H. A. 1969. Light intensity and the absorption of 2,4,5-T by woody plants. Weed Sci 17:320-323.

Branton, D., and L. Jacobson. 1962. Ion transport in pea plants. Plant Physiol. 37:539-545.

Brian, R. C. 1967. The uptake and adsorption of diquat and paraquat by tomato, sugar beet, and cocksfoot. Ann. Appl. Biol. 59:91-99.

Briggs, G. E. and R. N. Robertson. 1957. Apparent free space. Ann. Rev. Plant Physiol. 8:11-30.

Brouwer, R. 1965. Ion absorption and transport in plants. Ann Rev. Plant Physiol. 16:241-266.

Budd, K. and G. G. Laties. 1964. Ferricyanide-mediated transport of chloride by anaerobic corn roots. Plant Physiol. 39:648-654.

Carter, O. G. and D. J. Lathwell. 1967. Effects of temperature on orthophosphate absorption by excised corn roots. Plant Physiol. 43:484-488.

Chow, P. N. P. 1970. Absorption and dissipation of TCA by wheat and oats. Weed Sci 18:492-496.

Colby, S. R. and G. F. Warren. 1965. Selective action of solan on tomato and eggplant. Weeds: 13:257-263.

Collander, R. 1959. Cell membranes: their resistance to penetration and their capacity for transport. In Plant Physiol. (ed. Steward.) Academic Press, New York.

Crafts, A. S. and T. C. Broyer. 1938. Migration of salts and water into xylem of the roots of higher plants. Am. J. Bot. 25:529-535.

Crafts, A. S. 1961. Translocation in plants. Holt, Rinehart and Winston. New York. 182 pp.

Dainty, J. 1962. Ion transport and electrical potentials in plant cells. Ann. Rev. Plant Physiol. 13:379-402.

Danielli, J. F. 1952. In "The permeability of natural membranes" by Davson, H. and J. F. Danielli 2nd ed. Cambridge Univ. Press, London and New York.

Davies, P. J., D. S. H. Drennan, J. D. Fryer and K. Holly. 1968. The basis of the differential phytotoxicity of 4-hydroxy-3,5-diiodobenzonitrile: II Uptake and translocation. Weed Res. 8:233-240.

Davies, P. J. and D. E. Seaman. 1968. Uptake and translocation of diquat in Elodea. Weed Sci 16:293-296.

Davis, D. E., J. V. Gramlich, H. H. Funderburk. 1965. Atrazine absorption and degradation by corn, cotton and soybeans. Weeds 13:252-255.

Dawson, J. H. 1963. Development of barnyardgrass seedlings and their response to EPTC. Weeds 11:60-67.

Duke, W. B., F. W. Slife, and J. B. Hanson. 1967. Studies on the mode of action of 2-chloro-N-isopropylacetanilide. Weed Soc. Am., Abst., 50.

Edmonson, J. B. 1969. Effects of 2-chloro-2', 6'-diethyl-N-(methoxymethyl) acetanilide on cucumber growth. Ph.D. thesis, Urbana, Univ. of Illinois, 46 pp.

Elzam, O. E. and T. K. Hodges. 1967. Calcium inhibition of potassium absorption in corn root. Plant Physiol. 42:1483-1488.

Epstein, E. and C. E. Hagen. 1952. A kinetic study of the absorption of alkali cations by barley roots. Plant Physiol. 27:457-474.

Epstein, E. 1956. Mineral nutrition of plants: mechanisms of uptake and transport. Ann. Rev. Plant Physiol. 7:1-24.

Epstein, E. 1960. Spaces, barriers, and ion carriers: Ion absorption by plants. Am. J. Bot. 47:393-399.

Epstein, E., D. W. Rains, and W. E. Schmid. 1962. Course of cation absorption by plant tissue. Science 136:1051-1052.

Epstein, E., D. W. Rains, and O. E. Elzam. 1963. Resolution of dual mechanisms of potassium absorption by barley roots. Proc. Natl. Acad. Sci. 49:684-692.

Epstein, E. 1966. Dual pattern of ion absorption by plant cells and by plants. Nature 212:1324-1327.

Ernster, L. and B. Kuylenstierna. 1969. Structure, composition, and function of mitochondrial membranes. In "Mitochondria structure and function" (ed. Ernster, L. and Z. Drahota), Acad. Press, New York.

Eshel, Y. 1969. Phytotoxicity, leachability, and site of uptake of 2-chloro-2', 6'-diethyl-N-(methoxymethyl) acetanilide. Weed Sci 17: 441-444.

Figuerola, L. F. 1969. The influence of various climatic and edaphic factors on the toxicity of 2-tert. butylamino-4-ethylamino-6-methylthio-s-triazine (Igran) to winter wheat (Triticum aestivum) Ph. D. thesis, Corvallis, Oregon State Univ. 97 pp.

Foy, C. L. and S. Yamaguchi. 1964. Mechanisms of root absorption of organic molecules. In "Absorption and translocation of organic substances in plants." (ed. J. Hacskaylo) 7th Ann. Symposium, Southern section, Am. Soc. Plant. Physiol. Emory University, Texas.

Frank, R., and C. M. Switzer. 1969. Absorption and translocation of pyrazon by plants. Weed Sci 17: 365-370.

Frey-Wyssling, A. 1955. Die submikroskopische Struktur des Cytoplasmas In Protoplasmatologia, Vol. II, A-2: 1-244, Springer, Vienna.

Grant, B. R., and H. Beevers. 1964. Absorption of sugars by plant tissues. Plant Physiol. 39: 78-85.

Gray, R. H. and A. J. Weierich. 1969. Importance of root, shoot and seed exposure on the herbicidal activity of EPTC. Weed Sci 17: 223-229.

Greenwood, D. J. 1967. Studies on the transport of oxygen through the stems and roots of vegetable seedlings. New Phytologist 66: 337-347.

Grunwald, C. 1968. Effects of sterols on the permeability of alcohol treated red beet tissue. Plant Physiol. 43: 484-488.

Hiatt, A. J. 1968. Electrostatic association and Donnan phenomena as mechanisms of ion accumulation. Plant Physiol. 43: 893-901.

Higinbotham, H. 1959. The possible role of adenosine triphosphate in rubidium absorption as revealed by the influence of external phosphate, dinitrophenol and arsenate. Plant Physiol. 34: 645-650.

Hylmo, B. 1953. Transpiration and ion absorption. Physiol. Plantarum 6: 333-405.

Jacoby, B., and J. F. Sutcliffe. 1962. Connection between protein synthesis and salt absorption in plant cells. Nature 195: 1014.

Jaworski, E. G. 1969. Chloroacetamides. In "Degradation of herbicides" (ed. Kearny, P. C. and D. D. Kaufman) Marcel Dekker, Inc. New York.

Jennings, D. H. 1963. The absorption of solutes by plant cells. Iowa State University Press, Ames, Iowa. 204 pp.

Johnson, M. P. and J. Bonner. 1956. The uptake of auxin by plant tissue. Physiol. Plantarum 9: 102-118.

Jordan, L. S., B. E. Day and W. A. Clerx. 1964. Photodecomposition of triazines, Weeds 12: 5-6.

Knake, E. L. and L. M. Wax. 1968. The importance of the shoot of giant foxtail for uptake of pre-emergence herbicides. Weed Sci 16: 393-395.

Knauss, H. J. and J. W. Porter. 1954. The absorption of inorganic ions by Chlorella pyrenoidosa. Plant Physiol. 29: 229-234.

Kriedemann, P. and H. Beevers. 1967. Sugar uptake and translocation in the castor bean seedling. I Characteristics of transfer in intact and excised seedlings. Plant Physiol. 42: 161-173.

Laties, G. G. 1957. Respiration and cellular work and the regulation of the respiration rate in plants. Survey of Biol. Progress III: 216-287.

Laties, G. G. and K. Budd. 1964. The development of differential permeability in isolated steles of higher plants. Am. J. Bot. 25: 529-535.

Lauchli, A. and E. Epstein. 1970. Transport of potassium and rubidium in plant roots. Plant Physiol. 45: 639-641.

Legget, J. E. and W. A. Gilbert. 1967. Localization of the Ca-mediated apparent ion selectivity in the cross-sectional volume of soybean roots. Plant Physiol. 42: 1658-1664.

Lehninger, A. L. 1970. Biochemistry, Worth Publ. Inc., New York.

Leopold, A. C. 1964. Plant growth and development, McGraw-Hill Publ. New York.

Lineweaver, H. and D. Burk. 1936. The determination of enzyme dissociation constants. J. Am. Chem. Soc. 56: 658-666.

Lundegardh, H. 1950. The translocation of salts and water through wheat roots. Physiol. Plantarum 3: 103-151.

Luttge, U. and G. G. Laties. 1967. Selective inhibition of absorption and long distance transport in relation to the dual mechanisms of ion absorption in maize seedlings. Plant Physiol. 42: 181-184.

Maas, E. V., D. P. Moore and B. J. Mason. 1968. Manganese absorption by excised barley roots. Plant Physiol. 43: 527-530.

Machlis, L. and J. G. Torrey. 1956. Plants in action: A laboratory manual of plant physiology. Freeman. San Francisco.

Majumdar, J. C. and F. Muller. 1969. Untersuchungen über Aufnahme und Translokation von ^{14}C-Metobromuron in unterschiedlich empfindlichen Pflanzen. Weed Res. 9: 322-332.

McKinley, J. 1965. Factors influencing the herbicidal activity of dimethyl 2, 3, 5, 6-tetrachloroterephthalate. Master's thesis, Corvallis, Oregon State University. 66 pp.

Merkle, M. G., C. L. Leinweber, and R. W. Bovey. 1965. The influence of light, oxygen and temperature on the herbicidal properties of paraquat. Plant Physiol. 40: 832-835.

Michaelis, L. and M. L. Menten. 1913. Die Kinetik der Ivertinwirkung. Biochem. Zeitschrift 49: 333-369.

Moore, D. P. 1965. Accumulation of calcium in exudate of individual barley roots. Plant Physiol. 40: 641-644.

Nalawaja, J. D. 1968. Uptake and translocation of di-allate in wheat, barley, flax and wild oat. Weed Sci 16: 309-312.

Neidermyer, R. W. and J. D. Nalewaja. 1969. Uptake, translocation and fate of 2, 4-D in night flowering catchfly and common lambsquarters. Weed Sci 17: 528-533.

Nishimoto, R. K. 1968. The influence of herbicide placement in soil on toxicity to emerging seedlings. Master's thesis, Corvallis, Oregon State University. 68 pp.

Nissen, P. and A. A. Benson. 1964. Active transport of choline sulfate by barley roots. Plant Physiol. 39: 586-589.

Oliver, L. R., G. N. Prendeville, and M. M. Schreiber. 1968. Species differences in site of root uptake and tolerance to EPTC. Weed Sci 16: 534-537.

Ordin, L. and L. Jacobson. 1955. Inhibition of ion absorption and respiration in barley roots. Plant Physiol. 30: 31-37.

Osmond, C. B. and G. G. Laties. 1968. Interpretation of the dual mechanism for ion absorption in beet tissue. Plant Physiol. 43: 747-755.

Parker, C. 1966. The importance of shoot entry in the action of herbicides applied to the soil. Weeds 14: 117-121.

Pennel, G. A. and P. E. Weatherley. 1958. On the mechanism of sugar uptake by floating leaf disks. New Phytologist 57: 326-339.

Poole, R. J. and K. V. Thimann. 1964. Uptake of indole-3-acetic acid and indole-3-acetonitrile by Avena coleoptile sections. Plant Physiol. 39: 98-103.

Prendeville, G. N., L. R. Oliver and M. M. Schreiber. 1968. Species differences in site of root uptake and tolerance to EPTC. Weed Sci 16: 538-540.

Rahman, A. and R. Ashford. 1970. Selective action of trifluralin for control of green foxtail in wheat. Weed Sci 18: 754-759.

Rains, D. W. 1967. Sodium absorption by barley roots: role of the dual mechanisms of alkali cation transport. Plant Physiol. 42: 314-318.

Reinhold, L. 1954. The uptake of indole-3-acetic acid by pea epicotyl segments and carrot discs. New Phytologist 53: 217-239.

Robertson, R. N., M. J. Wilkins, and D. C. Weeks. 1951. Studies in the metabolism of plant cells. IX The effects of 2, 4-dinitrophenol on salt accumulation and salt respiration. Australian J. Biol. Res. 4:248-264.

Saunders, P. F., C. F. Jenner and G. E. Blackman. 1966. The uptake of growth substances VI. A comparative study of the factors determining the patterns of uptake of phenoxyacetic acid and 2, 4, 5-trichlorophenoxyacetic acid, weak and strong auxins by Gossypium tissues. J. Exp. Bot. 17:241-269.

Salisbury, F. B. and C. Ross. 1969. Plant Physiology (ed. Jensen, W. A. and L. G. Kavaljian.) Wadsworth Publ. Comp. Inc. Belmont, California.

Shrift, A. 1966. Methionine transport in Chlorella vulgaris. Plant Physiol. 41:405-410.

Sikka, H. C. and D. E. Davis. 1968. Absorption, translocation and metabolism of prometryne in cotton and soybean. Weed Sci 16:474-478.

Smith, F. A. 1967. Links between glucose uptake and metabolism in Nitella translucens. J. Exp. Bot. 18:348-358.

Steward, F. C. and J. F. Sutcliffe. 1959. Plants in relation to inorganic salts. In Plant Physiology (ed. Steward, F. C.) Acad. Press. New York.

Stoller, E. W. and L. M. Wax. 1968. Amiben metabolism and selectivity. Weed Sci 16:283-288.

Stoner, C. D., T. K. Hodges and J. B. Hanson. 1964. Chloramphenicol as an inhibitor of energy-linked processes in maize mitochondria. Nature 203:258-261.

Sutcliffe, J. F. and D. P. Hackett. 1957. Efficiency of ion transport in biological systems. Nature 180:95-96.

Sutcliffe, J. F. 1960. New evidence for a relationship between ion absorption and protein turnover in plant cells. Nature 188: 294-297.

Taylor, F. J. 1959. The absorption of glucose by Scenedesmus quadricauda. I. Some kinetic aspects. Proc. R. Soc. B. 151: 400-418.

Trivelli, O. 1967. Some herbicidal properties of 2-chloro-2' 6'-diethyl-N-(methoxymethyl) acetanilide. Master's thesis, Corvallis, Oregon State University, 59 pp.

Weigl, J. 1963. Die Ledeutung der Energiereichen Phosphate bei der Ionenaufnahme durch Wurzeln. Planta 60: 307-321.

Weigl, J. 1964. Zur Hemmung der aktiven Ionenaufnahme durch Arsenat. Z. Naturforschg. 19b: 646-648.

Welch, R. M. and E. Epstein. 1969. The plasmalemma: Seat of the type 2 mechanisms of ion absorption. Plant Physiol. 44: 301-304.

Yih, R. Y., D. H. McRae, and H. F. Wilson. 1968. Mechanism of selective action of 3', 4'-dichloropropionanilide. Plant Physiol. 43: 1291-1296.

Yuh, G. H. and P. J. Kramer. 1969. Radial transport of ions in roots. Plant Physiol. 44: 1095-1100.

APPENDIX

Table A. The effect of light intensity on the toxicity of alachlor to corn.

Foliage dry weight (mg) and number of plants per pot.

Alachlor conc. (ppm)	Replication	Light intensity (ft - c)			
		2000	1600	600	60
0	1	560.7-5	474.2-5	426.4-5	432.8-5
	2	793.9-5	508.8-5	485.9-5	403.6-5
	3	675.1-5	573.5-5	434.3-5	318.9-4
	4	616.0-5	451.1-5	371.9-5	335.4-5
	5	468.8-4	448.5-5	411.0-5	369.8-5
	6	441.4-4	421.6-5	384.7-5	323.5-5
	7	617.2-5	353.3-5	450.0-5	410.4-5
	8	693.5-4	559.6-5	454.7-5	341.5-5
10	1	732.3-5	340.9-5	329.7-5	364.2-5
	2	329.5-3	380.2-5	359.4-5	390.8-5
	3	663.0-5	423.0-5	234.8-4	308.5-4
	4	531.7-5	405.1-5	364.5-5	443.0-5
	5	533.2-5	406.3-5	413.0-5	300.6-4
	6	535.6-5	328.4-5	478.1-5	336.9-5
	7	550.2-5	294.0-5	332.2-5	343.9-5
	8	512.1-5	353.7-5	278.6-5	343.0-5
50	1	290.4-5	190.8-5	239.2-5	333.7-5
	2	410.1-5	208.7-5	259.5-5	314.0-5
	3	302.9-4	199.4-4	217.5-5	243.6-5
	4	373.0-5	220.4-5	175.8-5	182.7-5
	5	293.3-5	286.4-5	222.3-5	251.8-5
	6	466.2-5	280.2-5	246.7-5	263.2-5
	7	296.0-4	225.2-5	117.3-5	205.6-5
	8	492.1-5	93.6-5	154.4-5	293.8-5
100	1	147.1-5	13.8-4	110.9-3	69.8-4
	2	147.7-4	32.6-5	83.1-5	92.2-5
	3	160.0-4	35.2-5	63.0-3	133.0-5
	4	169.0-3	89.5-4	97.7-4	123.1-5
	5	102.2-3	53.7-5	73.5-5	53.7-5
	6	198.4-5	7.8-3	97.4-5	72.9-5
	7	175.6-4	35.9-5	61.9-5	57.3-5
	8	90.8-5	41.7-4	18.8-4	118.4-5

Table B. The effect of light intensity on the toxicity of alachlor to cucumber.

Foliage dry weight (mg) and number of plants per pot.

Alachlor conc. (ppm)	Replication	Light intensity (ft - c)			
		2000	1600	660	60
0	1	154.0-5	166.5-5	88.0-4	109.9-5
	2	173.9-5	155.8-5	97.3-4	67.3-4
	3	192.8-5	167.3-5	101.9-4	98.0-5
	4	149.3-5	152.2-4	69.7-3	97.2-5
	5	115.5-4	167.5-5	108.9-5	80.4-4
	6	117.2-4	171.6-5	95.0-4	91.2-5
	7	87.5-4	186.7-5	130.4-5	111.6-5
	8	132.9-5	169.6-5	100.1-5	81.7-4
1	1	109.7-5	110.2-5	85.7-5	88.5-5
	2	92.8-5	133.9-5	95.6-5	96.0-5
	3	71.1-4	110.2-5	83.2-5	92.8-5
	4	82.9-5	95.0-4	97.3-5	89.4-5
	5	83.0-4	118.9-5	75.6-4	97.8-5
	6	101.1-5	110.6-5	91.2-5	94.9-5
	7	96.4-5	90.9-4	86.8-5	97.1-5
	8	102.1-5	111.9-5	83.7-5	63.7-4
5	1	71.6-5	53.3-5	59.6-4	67.8-4
	2	77.0-5	72.3-5	46.9-3	81.7-5
	3	63.9-4	77.6-5	53.0-4	66.2-4
	4	87.5-5	61.0-4	70.7-5	74.2-5
	5	65.8-5	71.9-5	46.0-3	59.6-4
	6	73.7-5	34.9-2	33.2-2	68.6-5
	7	80.5-5	85.0-5	51.5-4	49.8-3
	8	69.7-4	58.4-4	67.6-4	72.3-5
10	1	68.9-5	78.2-5	20.1-2	45.6-3
	2	86.5-5	47.0-3	41.8-3	45.2-4
	3	70.6-5	49.3-3	40.0-3	40.1-3
	4	86.7-4	62.8-4	46.8-3	86.9-5
	5	63.2-5	76.0-5	43.8-4	72.6-4
	6	74.3-5	12.4-1	42.1-3	36.7-5
	7	85.0-5	63.3-4	37.9-3	50.1-3
	8	65.0-5	73.5-5	50.2-4	29.4-3

Table C-1. The effect of light intensity on the toxicity of alachlor to oats.

Foliage dry weight (mg) and number of plants per pot.

Alachlor conc. (ppm)	Replication	Light intensity (ft - c)	
		1600	60
0	1	49.7-5	46.8-5
	2	46.4-5	42.1-5
	3	35.2-5	37.9-5
	4	54.5-5	36.6-5
	5	55.6-5	43.6-5
	6	56.7-5	29.6-3
	7	52.3-5	43.6-5
	8	69.8-5	46.8-5
0.05	1	63.3-5	48.3-5
	2	47.0-5	38.4-5
	3	56.8-5	45.1-4
	4	59.9-5	39.0-5
	5	49.1-5	30.4-5
	6	54.8-5	38.4-5
	7	38.6-5	41.1-5
	8	57.3-5	27.0-5
0.1	1	44.2-4	30.7-5
	2	37.0-5	28.7-5
	3	53.9-4	44.8-5
	4	31.6-3	37.7-5
	5	30.7-4	25.2-5
	6	53.9-5	19.2-5
	7	29.5-5	40.6-5
	8	37.8-4	14.5-5
0.3	1	17.5-3	35.7-5
	2	23.9-3	15.9-4
	3	18.8-3	19.2-5
	4	9.6-1	12.5-5
	5	33.4-4	16.9-5
	6	19.6-4	16.2-5
	7	26.9-3	3.0-5
	8	17,3-3	21.6-4

Table C-2. The effect of light intensity on the toxicity of alachlor to oats.

Foliage dry weight (mg) and number of plants per pot.

Alachlor conc. (ppm)	Replication	2000 ft - c	Alachlor conc. (ppm)	Replication	660 ft - c
0	1	94.9-5	0	1	47.3-5
	2	64.1-5		2	63.5-5
	3	44.8-5		3	52.5-5
	4	71.5-5		4	48.2-5
	5	60.0-5		5	60.3-5
	6	89.2-5		6	65.7-5
	7	59.5-5		7	55.7-5
	8	85.8-5		8	54.0-5
1	1	4.5-1	0.1	1	36.8-4
	2	10.7-1		2	26.6-5
	3	9.6-1		3	15.0-4
	4	0		4	30.7-5
	5	5.2-3		5	34.2-5
	6	9.2-2		6	34.5-5
	7	15.0-3		7	30.0-3
	8	12.2-3		8	34.4-5
2	1	0	0.5	1	0
	2	1.8-1		2	0.4-1
	3	0		3	5.2-4
	4	0		4	17.6-3
	5	0		5	10.1-3
	6	0		6	8.1-4
	7	0		7	0.2-2
	8	0		8	2.3-5
3	1	0	1	1	1.1-2
	2	0		2	0.2-2
	3	2.1-1		3	0.1-1
	4	0		4	1.3-2
	5	0		5	0.8-3
	6	0		6	1.0-2
	7	0		7	0
	8	0		8	0

Table D. The effect of day length on the toxicity of alachlor to corn. Foliage dry weight (mg) and number of plants per pot.

Alachlor conc. (ppm)	Replication	Day length	
		8 hours	16 hours
0	1	614.7-5	605.6-5
	2	509.4-4	733.0-5
	3	681.2-5	726.1-5
	4	708.4-5	992.4-5
	5	739.0-5	673.3-5
	6	818.5-5	510.9-4
	7	813.8-5	1012.6-5
	8	668.6-5	985.9-5
10	1	692.8-5	521.7-5
	2	488.8-4	699.7-5
	3	703.5-5	679.6-5
	4	618.8-5	634.4-5
	5	603.9-5	595.3-5
	6	493.0-5	556.1-3
	7	535.1-5	605.4-4
	8	576.1-5	546.7-5
50	1	412.0-5	380.4-5
	2	425.0-5	505.3-5
	3	528.2-5	384.0-4
	4	434.8-4	448.5-5
	5	393.9-5	636.1-5
	6	447.1-5	565.7-5
	7	590.6-5	428.6-5
	8	562.2-5	427.7-5
100	1	153.7-5	109.5-4
	2	139.2-5	236.1-4
	3	249.7-5	254.0-5
	4	103.2-2	157.1-5
	5	126.1-3	44.1-5
	6	176.2-5	173.9-5
	7	172.9-4	272.4-5
	8	105.7-5	167.2-4

Table E. The effect of day length on the toxicity of alachlor to cucumber.

Foliage dry weight (mg) and number of plants per pot.

Alachlor conc. (ppm)	Replication	Day length	
		8 hours	16 hours
0	1	131.8-5	104.1-5
	2	124.5-5	140.1-5
	3	123.6-5	161.8-5
	4	97.1-4	134.8-5
	5	119.9-5	154.9-5
	6	116.5-5	151.8-5
	7	118.5-5	127.5-5
	8	117.1-5	174.3-5
1	1	76.3-5	91.6-5
	2	89.5-5	71.8-4
	3	76.2-5	106.8-5
	4	69.2-4	76.9-4
	5	101.0-5	94.6-5
	6	95.6-5	91.6-5
	7	93.9-5	115.5-5
	8	75.0-4	83.1-5
5	1	61.0-4	74.3-5
	2	77.5-5	53.7-3
	3	68.2-4	85.6-5
	4	59.8-4	80.3-5
	5	29.5-2	88.1-5
	6	61.9-4	82.0-5
	7	75.0-5	80.5-5
	8	62.5-4	80.0-5
10	1	58.0-4	68.0-5
	2	62.4-5	47.7-5
	3	71.0-5	67.9-5
	4	46.3-4	78.5-5
	5	42.4-3	53.7-4
	6	53.1-3	64.8-4
	7	70.9-4	42.7-3
	8	72.4-5	75.5-5

Table F. The effect of day length on the toxicity of alachlor to oats. Foliage dry weight (mg) and number of plants per pot.

Alachlor conc. (ppm)	Replication	Day length	
		8 hours	16 hours
0	1	60.8-5	80.0-5
	2	68.4-5	59.1-5
	3	65.3-5	66.4-5
	4	57.3-5	78.2-5
	5	76.8-5	89.5-5
	6	72.2-5	55.5-5
	7	57.8-5	86.3-5
	8	71.8-5	67.1-5
0.05	1	46.0-5	41.1-5
	2	45.9-5	66.0-5
	3	49.0-5	36.6-5
	4	50.5-5	43.5-5
	5	63.9-5	74.3-5
	6	47.8-5	86.4-5
	7	64.6-5	46.0-5
	8	64.5-5	75.6-5
0.1	1	55.1-5	12.9-2
	2	36.7-5	46.2-5
	3	41.2-5	38.6-4
	4	44.6-5	58.3-5
	5	26.4-5	48.9-4
	6	43.3-5	27.4-4
	7	40.4-5	41.4-3
	8	46.4-5	37.1-4
0.3	1	18.3-4	13.1-3
	2	17.1-4	27.8-3
	3	42.0-5	44.9-5
	4	21.7-5	16.2-2
	5	20.2-4	42.5-5
	6	17.6-5	17.4-4
	7	18.9-4	6.0-2
	8	28.3-4	20.0-3

Table G. The effect of calcium nitrate on the toxicity of alachlor to oats.

Dry weight of foliage (mg).

Alachlor conc. (ppm)	Replication	$Ca(NO_3)_2$ conc. (M)			
		0	10^{-5}	10^{-4}	10^{-3}
0	1	49.8	53.4	79.3	56.1
0	2	34.4	36.1	48.0	43.8
0	3	18.6	32.4	53.0	55.4
0.05	1	31.6	31.2	44.5	48.1
0.05	2	30.3	60.0	37.9	44.9
0.05	3	40.0	30.7	43.1	33.3
0.05	4	36.9	59.8	54.9	65.1
0.1	1	20.1	38.2	63.2	74.2
0.1	2	30.0	51.6	46.1	34.9
0.1	3	40.2	39.5	45.6	50.6
0.1	4	32.4	24.4	36.6	62.9
Dry weight of roots (mg)					
0	1	23.9	28.6	25.7	25.6
0	2	21.0	15.2	22.9	20.8
0	3	12.8	18.9	20.4	24.4
0	4	17.6	26.8	24.3	13.2
0.05	1	13.5	18.0	22.9	12.9
0.05	2	10.1	17.9	17.3	20.1
0.05	3	14.4	21.3	22.5	15.3
0.05	4	12.3	15.4	30.7	18.7
0.1	1	7.3	11.8	16.1	17.8
0.1	2	12.9	15.5	20.7	13.9
0.1	3	10.2	19.9	17.8	15.2
0.1	4	19.4	13.4	15.3	18.5

Table H. The effect of carrier volume on the toxicity of alachlor to oats.

Foliage dry weight (mg) and number of plants per pot.

Alachlor conc. (lbs/ac)	Replication	Carrier volume (gal/ac)		
		20	40	80
0	1	45.1-5	37.3-4	15.9-3
	2	37.4-5	53.1-5	33.1-4
	3	31.6-5	42.8-5	44.7-5
	4	52.0-5	35.2-5	40.0-4
1	1	53.5-5	23.9-3	10.0-2
	2	44.1-5	27.1-3	24.1-5
	3	38.1-5	29.2-5	60.5-5
	4	50.2-5	26.5-4	25.7-3
2	1	51.5-5	29.0-3	14.3-3
	2	42.7-5	20.7-4	17.3-4
	3	38.9-5	18.2-4	27.0-5
	4	25.2-5	50.0-5	10.0-2
4	1	45.5-5	30.7-5	26.3-4
	2	30.9-5	42.4-5	19.8-4
	3	23.1-3	17.0-4	22.9-4
	4	38.6-5	32.9-5	46.7-5

Table I. The effect of carrier volume on the toxicity of alachlor to cucumber.

Foliage dry weight (mg) and number of plants per pot.

Alachlor conc. (lbs/ac)	Replication	Carrier volume (gal/ac)		
		20	40	80
0	1	81.7-4	110.2-5	92.8-5
	2	114.7-5	63.8-4	97.0-5
	3	103.2-5	86.5-4	110.9-5
	4	123.3-5	120.0-5	111.8-5
1	1	93.0-5	80.8-4	97.0-5
	2	65.2-3	127.7-5	65.0-3
	3	111.4-5	80.9-4	62.8-4
	4	96.8-5	74.7-3	81.3-4
2	1	79.3-4	89.0-5	82.9-5
	2	110.615	46.9-2	113.5-5
	3	119.6-5	94.7-5	88.4-4
	4	78.7-5	35.0-2	81.3-5
4	1	83.9-4	37.1-2	67.1-4
	2	124.8-5	76.3-4	69.3-4
	3	113.7-5	85.3-5	83.7-5
	4	93.8-5	91.6-5	106.3-5

Table J. The effect of sub-irrigation and carrier volume on the toxicity of alachlor to oats.

Foliage dry weight per pot in mg.

Alachlor conc. (lbs/ac)	Carrier volume (gal/ac)	Replication	Duplicate	Sub-irrigation per day (ml)		
				100	200	300
0	20	1	1	56.2	51.7	68.8
0	20	1	2	53.5	69.3	64.5
0	20	2	1	54.4	62.0	43.8
0	20	2	2	54.2	57.4	64.3
0	100	1	1	54.5	66.7	56.5
0	100	1	2	51.7	54.4	64.9
0	100	2	1	56.2	62.6	51.8
0	100	2	2	49.8	50.2	64.6
2	20	1	1	48.5	59.2	64.3
2	20	1	2	40.6	56.6	47.7
2	20	2	1	53.1	47.1	60.1
2	20	2	2	57.9	51.5	55.0
2	100	1	1	48.0	56.8	53.8
2	100	1	2	30.2	53.9	47.6
2	100	2	1	64.0	50.5	47.7
2	100	2	2	43.0	28.6	39.4

Factor	Foliage dry weight per pot (mg) level		
	1	2	3
alachlor	57.7	50.2	
sub-irrigation	51.0	54.9	56.0
carrier volume	56.0	51.9	
replication	55.1	52.8	

Table K. The site of uptake of alachlor by corn, oats, soybean and cucumber.

Foliage dry weight (mg) and number of plants per pot.

Plant part exposed	Replication	Plant species			
		Corn	Oats	Soybean	Cucumber
None	1	1100.7-5	92.1-5	369.5-5	184.2-3
	2	1013.7-5	137.0-5	410.3-5	172.5-3
	3	1468.2-5	118.2-5	326.6-3	232.8-5
	4	1952.2-5	112.2-5	258.6-3	207.7-5
	5	858.5-5	129.0-5	498.4-5	111.6-5
	6	1261.5-5	85.2-5	410.9-3	132.9-5
	7	1488.2-3	90.8-5	328.5-3	122.5-5
	8	956.5-3	133.3-5	520.0-5	181.3-5
Root	1	1129.5-5	75.2-5	334.1-5	71.2-2
	2	1158.5-5	42.4-3	211.8-2	126.2-3
	3	1164.5-5	41.3-3	68.4-1	186.1-5
	4	1054.6-5	68.6-5	318.4-3	187.3-5
	5	1160.4-5	89.1-5	50.1-1	163.0-5
	6	1553.6-5	88.0-5	411.5-5	181.2-5
	7	883.0-5	92.4-5	239.7-2	137.5-3
	8	1012.4-5	76.8-5	353.1-5	129.4-5
Root and Shoot	1	524.1-5	57.0-5	97.2-1	77.6-5
	2	408.4-3	46.1-3	205.3-3	70.4-5
	3	766.2-5	43.6-5	282.2-3	64.8-5
	4	604.3-5	47.7-3	189.7-3	69.6-5
	5	524.2-5	71.2-5	305.6-5	80.5-5
	6	603.1-5	67.2-5	233.3-3	49.0-3
	7	509.5-5	40.8-5	131.0-1	51.5-3
	8	537.2-5	21.8-3	87.2-1	65.9-5

Table L. The effect of alachlor on the uptake of potassium, phosphorus and calcium by oats.

Replication	Dry root weight per plant (mg) Alachlor conc. (mM)				
	0.	0.04	0.05	0.1	0.2
1	24.9	17.8	19.9	19.4	13.6
2	22.5	16.6	17.5	19.6	8.4
3	22.7	15.9	21.6	19.0	18.9
4	18.6	16.6	17.3	14.5	14.5
5	14.5	15.1	20.8	21.4	12.6
6	21.6	19.8	21.9	19.1	17.9
7	20.0	22.8	18.0	17.7	11.5
8	17.2	11.7	20.0	21.8	15.2
9	27.0	17.9	14.4	19.5	13.5
10	16.7	17.5	14.7	15.1	15.9

Replication	Dry shoot weight per plant (mg) Alachlor conc. (mM)				
	0	0.04	0.05	0.1	0.2
1	62.6	43.6	49.7	56.8	46.7
2	55.8	35.2	45.1	45.8	27.5
3	60.7	29.3	57.3	40.9	43.8
4	42.9	50.8	45.6	30.4	44.7
5	40.6	36.0	36.8	54.3	38.3
6	54.1	36.8	41.5	49.6	40.0
7	47.0	52.0	43.3	40.2	36.1
8	39.4	33.2	49.9	40.1	43.6
9	62.2	43.7	25.1	44.7	29.6
10	46.1	40.6	32.4	29.5	53.0

Table M. The uptake of alachlor by corn plants as a function of time. (check, and in presence of DNP)

Total uptake, Exchangeable uptake and Active & Non-exchangeable uptake in the absence of metabolic inhibitor (dpm per mg dry root weight)

Exposure time (min)	Replication	Total uptake	Exchangeable uptake	Active & Non-exchangeable uptake
15	1	325	44	281
	2	393	39	354
30	1	457	51	406
	2	427	36	391
60	1	409	41	368
	2	528	57	471
90	1	461	53	408
	2	486	48	438
150	1	555	43	512
	2	567	66	501

Total uptake, Exchangeable uptake and Non-exchangeable uptake in the presence of DNP (dpm per mg dry root weight)

Exposure time (min)	Replication	Total uptake	Exchangeable uptake	Non-exchangeable uptake
15	1	268	28	240
	2	313	29	284
30	1	365	25	340
	2	278	61	217
60	1	450	40	410
	2	211	28	183
90	1	593	61	532
	2	411	45	366
150	1	308	29	279
	2	550	37	513

Table N. The uptake of alachlor by corn plants as a function of time. (in presence of NaN_3)

Total uptake, Exchangeable uptake and Non-exchangeable uptake in the presence of NaN_3 (dpm per mg dry root weight)				
Exposure time (min)	Replication	Total uptake	Exchangeable uptake	Non-exchangeable uptake
15	1	305	33	272
	2	206	28	178
30	1	483	35	448
	2	221	39	182
60	1	262	35	227
	2	278	25	253
90	1	438	56	382
	2	444	67	377
150	1	258	41	217
	2	300	32	268

Table O. The uptake of alachlor by oat plants as a function of time. (check)

Total uptake, Exchangeable uptake and Active and Non-exchangeable uptake in the absence of metabolic inhibitor (dpm per mg dry root weight)

Exposure time (min)	Replication	Total uptake	Exchangeable uptake	Active & Non-exchangeable uptake
15	1	216	20	196
	2	133	24	109
	3	344	41	303
30	1	400	58	342
	2	397	46	351
	3	418	60	358
60	1	342	44	298
	2	397	37	360
	3	301	47	254
90	1	538	70	468
	2	413	54	359
	3	524	44	480
150	1	419	57	362
	2	509	66	443
	3	591	60	531

Table P. The uptake of alachlor by oat plants as a function of time. (in presence of DNP)

Total uptake, Exchangeable uptake and Non-exchangeable uptake in the presence of DNP (dpm per mg dry root weight)				
Exposure time (min)	Replication	Total uptake	Exchangeable uptake	Non-exchangeable uptake
15	1	134	38	177
	2	289	48	241
	3	113	22	91
30	1	437	88	349
	2	366	54	312
	3	167	29	138
60	1	275	31	244
	2	343	46	297
	3	451	63	388
90	1	370	44	328
	2	499	54	445
	3	410	67	462
150	1	529	67	462
	2	607	80	527
	3	392	57	335

Table Q. The uptake of alachlor by oat plants as a function of time. (in presence of NaN_3)

Total uptake, Exchangeable uptake and Non-exchangeable uptake in the presence of NaN_3 (dpm per mg dry root weight)				
Exposure time (min)	Replication	Total uptake	Exchangeable uptake	Non-exchangeable uptake
15	1	134	16	118
	2	243	32	211
	3	293	61	232
30	1	291	37	254
	2	414	63	351
	3	328	71	257
60	1	348	17	331
	2	341	57	284
	3	554	78	476
90	1	451	110	341
	2	--	--	--
	3	426	114	312
150	1	330	97	233
	2	402	85	317
	3	407	87	320

Table R. The total uptake of alachlor by corn and oat plants as a function of concentration.

Alachlor conc. (mM)	Total uptake by corn plants in dpm per mg dry root weight Replication 1	2	3	4
0.5	1983	2582	929	1547
0.4	1296	2210	1244	2235
0.3	1209	1988	871	1410
0.2	1057	1158	965	1238
0.1	971	843	1074	784
0.05	305	307	111	476
0.04	145	237	163	296
0.03	56	142	110	113
0.02	76	63	139	85
0.01	25	78	21	20

Alachlor conc. (mM)	Total uptake by oat plants in dpm per mg dry root weight Replication 1	2	3	4
0.5	3268	2019	2429	2100
0.4	2246	2464	1657	2355
0.3	1813	2887	2536	1897
0.2	1686	1478	1939	1814
0.1	1548	3109	1964	2162
0.05	154	293	334	190
0.04	191	243	174	155
0.03	155	151	103	99
0.02	94	36	85	82
0.01	45	27	49	37

Table S. The uptake of exchangeable alachlor by corn and oat plants as a function of concentration.

Exchangeable uptake by corn plants in dpm per mg dry root weight				
Alachlor	Replication			
conc. (mM)	1	2	3	4
0.5	205	430	116	196
0.4	137	299	130	261
0.3	105	287	77	122
0.2	72	73	50	74
0.1	32	27	39	25
0.05	27	28	11	44
0.04	12	25	14	33
0.03	12	16	11	11
0.02	8	8	11	6
0.01	3	12	3	5

Exchangeable uptake by oat plants in dpm per mg dry root weight				
Alachlor	Replication			
conc. (mM)	1	2	3	4
0.5	368	247	235	373
0.4	190	308	176	298
0.3	200	282	250	229
0.2	114	90	98	136
0.1	45	99	71	73
0.05	25	42	39	25
0.04	21	37	21	19
0.03	16	15	14	16
0.02	12	15	23	6
0.01	6	4	5	5

Table T. The effect of temperature on the total uptake of alachlor by corn and oats.

Total uptake by corn
in μ M per mg dry root weight

Alachlor conc. (mM)	Temperature (C)	Replication 1	2	3	4
0.3	27	5.82	3.75	7.55	5.24
0.3	17	30.8	5.60	4.61	4.21
0.03	27	0.60	0.50	0.37	0.53
0.03	17	0.42	0.51	0.36	0.35

Total uptake by oats
in μ M per mg dry root weight

Alachlor conc. (mM)	Temperature (C)	Replication 1	2	3	4
0.5	27	16.10	10.00	13.50	10.40
0.5	13	8.40	7.00	7.50	8.80
0.3	27	11.33	9.86	7.25	5.24
0.3	17	7.01	10.33	7.38	6.65
0.05	27	0.73	1.38	1.58	0.90
0.05	8	1.00	1.70	1.20	0.90
0.03	27	0.67	0.57	0.78	0.49
0.03	17	0.68	0.69	0.68	0.89

Table U. The effect of temperature on the uptake of exchangeable alachlor by corn and oats.

Exchangeable uptake by corn
in μ M per mg dry root weight

Alachlor	Temperature	Replication			
conc. (mM)	(C)	1	2	3	4
0.3	27	0.83	0.66	1.21	0.72
0.3	17	0.43	0.73	0.52	0.61
0.03	27	0.06	0.06	0.04	0.06
0.03	17	0.05	0.06	0.05	0.03

Exchangeable uptake by oats
in μ M per mg dry root weight

Alachlor	Temperature	Replication			
conc. (mM)	(C)	1	2	3	4
0.5	27	1.81	1.22	1.16	1.84
0.5	13	1.26	0.69	1.17	1.86
0.3	27	1.12	1.03	0.89	0.65
0.3	17	0.90	1.11	1.03	0.84
0.05	27	0.12	0.20	0.18	0.12
0.05	8	0.10	0.15	0.09	0.15
0.03	27	0.10	0.09	0.09	0.06
0.03	17	0.08	0.10	0.08	0.12

Table V. The effect of propachlor on the total uptake of alachlor by corn and oats.

Total uptake in μ M per mg dry root weight				
Alachlor conc. (mM)	Propachlor conc. (mM)	Replication	Plant species	
			Corn	Oats
0.3	0	1	5.82	11.33
		2	3.75	9.86
		3	7.55	7.25
		4	5.24	5.24
0.3	0.3	1	3.52	3.81
		2	5.54	3.34
		3	3.67	3.83
		4	3.39	6.05
0.03	0	1	0.60	0.67
		2	0.50	0.57
		3	0.37	0.78
		4	0.53	0.49
0.03	0.03	1	0.38	0.69
		2	0.40	0.53
		3	0.40	0.49
		4	0.53	0.45

Table W. The effect of propachlor on the uptake of exchangeable alachlor by corn and oats.

Alachlor conc. (mM)	Propachlor conc. (mM)	Replication	Exchangeable uptake in μ M per mg dry root weight: Plant species Corn	Oats
0.3	0	1	0.83	1.12
		2	0.66	1.03
		3	1.21	0.89
		4	0.72	0.65
0.3	0.3	1	0.72	0.60
		2	0.86	0.64
		3	0.79	0.78
		4	0.74	0.99
0.03	0	1	0.06	0.10
		2	0.06	0.09
		3	0.04	0.09
		4	0.06	0.06
0.03	0.03	1	0.04	0.08
		2	0.06	0.07
		3	0.06	0.07
		4	0.08	0.05