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EFFICACY OF HERBICIDE SAFENERS

IN CORN [*Zea mays* L.] AND

GRAIN SORGHUM [*Sorghum bicolor* (L.) Moench]

By

Marulak Simarmata

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ABSTRACT

EFFICACY OF HERBICIDE SAFENERS IN CORN [*Zea mays* L.] AND GRAIN SORGHUM [*Sorghum bicolor* (L.) Moench]

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The efficacy of several commercial herbicide safeners was evaluated in greenhouse and laboratory studies for protecting corn and sorghum against injury from primisulfuron and metolachlor. Protection by the safeners against corn injury from primisulfuron plus terbufos insecticide and primisulfuron plus antioxidants, piperonyl butoxide and metyrapone, was also evaluated.

Significant protection against corn injury from metolachlor and from primisulfuron interaction with terbufos was observed with oxabetrinil, flurazole, naphthalic anhydride, dichlormid, and R-29148. Seed treatment with CGA-133205 killed the corn. None of the safeners provided protection against primisulfuron injury to sorghum, whereas all provided protection against metolachlor injury to sorghum.

Antioxidants, piperonyl butoxide (PPO) and metyrapone, increased primisulfuron injury to corn. Protection against the increased corn injury from primisulfuron plus PPO was most evident with naphthalic anhydride, flurazole, and R-29148. No protection was observed to the increased corn injury from

primisulfuron plus metyrapone. Naphthalic anhydride was also an effective safener for PPO enhanced primisulfuron injury when the PPO was applied foliarly 3 days prior to primisulfuron application.

Absorption of ^{14}C-primisulfuron by corn was not influenced by PPO or naphthalic anhydride. Metabolism of ^{14}C-primisulfuron was inhibited by PPO and this inhibition was not totally reversed by the safener, naphthalic anhydride.

These results suggest that safeners oxabetrinil, flurazole, naphthalic anhydrid, dichlormid, and R-29148 were effective in protecting corn against the injury from metolachlor and the injury from primisulfuron interaction with terbufos, and grain sorghum from metolachlor injury. Naphthalic anhydride, flurazole, and R-29148 were effective against the increased synergist primisulfuron injury induced by PPO. The increased corn injury from primisulfuron plus PPO was due to the inhibition of the primisulfuron metabolism. The safening action of naphthalic anhydride did not totally reverse this inhibition. Nomenclature: corn, [*Zea mays* L.]; sorghum, [*Sorghum bicolor* (L.) Moench]; primisulfuron, 2-[3-[4,6-bis(difluoromethoxy)-pyrimidin-2-yl]-ureidosulfonyl]-benzoic acid methylester; metolachlor, 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide; terbufos, S-[[(1,1-dimethylethyl)thio]methyl] O,O-diethylphosphorodithioate; piperonyl butoxide, α-[2-(2-butoxyethoxy) ethoxy] - 4, 5 - methylenedioxy-2-propyltoluene;

metyrapone, 2-methyl-1,2-di-3-pyridyl-1-propanone; oxabetrinil, α-[(1,3-dioxolan-2-yl-methoxy)imino]benzeneacetonitrile; flurazole, phenylmethyl 2-chloro-4-(trifluoromethyl)-5-thiazolecarboxylate; naphthalic anhydride, naphthalene-1,8-dicarboxylic acid anhydride; dichlormid, 2,2-dichloro-N,N-di-2-propenylacetamide; R-29148, 3-(dichloroacetyl)-2,2,5-trimethyl-1,3-oxazolidine; CGA-133205, O-(1,3-dioxolan-2-yl-methyl)-2,2,2-trifluoro-4′-chloroacetophenone-oxime.

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TABLE OF CONTENTS

Page

APPENDIX

LIST OF TABLES

INTRODUCTION

Application of herbicides in combination with other pesticides is one of the management practices in crop production that allows for control of a broad spectrum of pests with a single application. Herbicides are commonly applied before, simultaneously with, or after the use of other pesticides. Unwanted interaction among pesticides, such as an interaction between herbicides and insecticides can occur. Certain interactions between herbicides and insecticides may increase injury to crops and reduce herbicide selectivity. One way to alleviate this problem is to use chemicals, known as 'herbicide antidotes', 'herbicide safeners', 'herbicide protectants', or 'herbicide antagonists'. These chemicals can selectively protect crops from herbicide injury without protecting weeds.

The use of herbicide safeners has developed rapidly since Hoffmann (21) introduced the concept of herbicide safeners about three decades ago. There are numerous currently marketed crop safeners for herbicides, such as naphthalic anhydride (naphthalene-1,8-dicarboxylic acid anhydride), dichlormid (2,2-dichloro-N,N-di-2-propenylacetamide), R-29148 (3-[dichloroacetyl]-2,2,5-trimetyl-1,3-oxazolidine), cyometrinil ([Z]-

α[(cyanomethoxy)imino] benzeneacetonitrile), oxabetrinil (α-[(1,3-dioxolan-2-yl-methoxy)imino]benzeneacetonitrile), CGA-133205 (O-[1,3-dioxolan-2-yl-methyl]-2,2,2-trifluoro-4'-chloroacetophenone-oxime), CGA-154281 (4-[dichloroacetyl]-3,4-dihydro-3-methyl-2H-1,4-benzoxazine), flurazole (phenylmethyl 2-chloro-4-[trifluoromethyl]-5-thiazolecarboxylate), fenclorim (4,6-dichloro-2-phenyl-pyrimidine), MG-191 (2-dichloromethyl-2-methyl-1,3-dioxolane), and others. The mechanism of action of the herbicide safeners against herbicidal injury on crops is not clearly understood.

The objectives of this research were: (a) to evaluate and compare the potency of herbicide safeners in protecting corn [*Zea mays* L.] and grain sorghum [*Sorghum bicolor* (L.) Moench] from injuries caused by metolachlor (2-chloro-N-[2-ethyl-6-methylphenyl]-N-[2-methoxy-1-methylethyl]acetamide), primisulfuron (2-[3-[4,6-bis(difluoromethoxy)-pyrimidin-2-yl]-ureidosulfonyl]-benzoic acid methylester), and the interaction with terbufos (S-[[(1,1-dimethylethyl)thio]methyl]O,O-diethyl phosphorodithioate) insecticide; (b) to determine whether the safening action of herbicide safeners on corn was related to mixed function oxidase (MFO) activity by using the MFO inhibitors or antioxidants, piperonyl butoxide (α-[2-(2-butoxyethoxy)ethoxy]-4,5-methylenedioxy-2-propyltoluene) and metyrapone (2-methyl-1,2-di-3-pyridyl-1-propanone); (c) to study absorption and metabolism of ^{14}C- primisulfuron herbicide in the presence of the safener, naphthalic anhydride, and the antioxidant, piperonyl butoxide (PPO), an MFO inhibitor.

CHAPTER 1

REVIEW OF LITERATURE

Introduction

The discovery and development of organic herbicides has revolutionized weed management in crop production. Selectivity obtained with organic herbicides has resulted in their widespread use in modern agriculture. But, the limited selectivity of certain herbicides has been a problem in controlling weeds that are botanically related to the crops.

Several approaches to overcome this problem have been reviewed by Hatzios (15). They involve development of new herbicides that are more selective than those currently available and enhancement of crop tolerance mechanically, genetically, or chemically. The chemical approach is based on the use of chemicals known as safeners, antidotes, protectants, or herbicide antagonists. Hoffmann (21) introduced this idea in 1962 and proposed that the use of safeners could permit: the use of higher rates of herbicides; the use of nonselective herbicides for selective weed control; the use of herbicides under conditions where crop damage is liable to occur; the protection of crops accidentally treated with nonselective herbicides. In addition, safeners could be a potential tool for elucidating sites and mechanism of herbicidal action (15).

History of herbicide safeners

Activated carbon (activated charcoal) was the first safener used directly on crop plants to protect them from herbicide injury (2). It has great adsorptive capacity and acts as a physical barrier to herbicide uptake. However, activated charcoal has some limitations. Coating seed with charcoal does not protect the emerging shoot from herbicide injury (14), placing charcoal in rows results in weed protection (19), and large amounts of charcoal are required to obtain the desired protection (1).

The use of chemicals to protect crops from herbicide injury is a relatively new approach to selective weed control. It started in 1947 when tomatoes [*Lycopersicon esculentum* Mill.] tested for their response to 2,4-D analogs were exposed accidentally to the fumes of 2,4-D (2,4-dichlorophenoxyacetic acid) and the epinastic responses were absent (20). Observed antagonistic responses that led to commercial products were 2,4,6-T (2,4,6-trichlorophenoxyacetic acid) antagonism of 2,4-D on tomato and the total inactivation of barban (4-chloro-2- butynyl-3-chlorophenylcarbamate) by 2,4-D on wheat [*Tritium aestivum* L.] (15).

Based on this antagonistic herbicide interaction, Hoffmann (21) evaluated several chemicals and introduced S-449 (4-chloro-2-hydroxyiminoacetanilide) in 1962 as an effective safener of wheat against injury from the herbicide barban. Hoffmann (22) was also successful in the discovery and development of naphthalic anhydride as an effective safener in corn against injury from thiocarbamate herbicides.

The efforts to develop new safeners have continued and resulted in several commercial safeners. Among them, chloroacetamide safeners of corn against thiocarbamate and chloroacetanilide herbicides have been most successful (31). Other safener groups that are also important are oxime ether derivatives (8, 10, 37) and disubstituted thiazole carboxylates (38). Both of them protect sorghum from chloroacetanilide herbicide injury. More recently, fenclorim was introduced as an effective safener of rice [*Oriza sativa* L.] against injury from metolachlor and pretilachlor (2-chloro-2′,6′-diethyl-N-[2-propoxyethyl]acetanilide) herbicide (9). In 1988, CGA-154281 was also introduced as a new safener to protect herbicidal injury of metolachlor to corn (35). The following review will describe the uses, proposed mode of action, and chemical structures of safeners that were used in this research.

Naphthalic anhydride

Naphthalic anhydride was patented in 1971 as a herbicide safener to protect corn against EPTC (S-ethyl dipropyl carbamothioate) injury (23). Naphthalic anhydride is moderately effective as a seed treatment safener for a number of chemically unrelated herbicides, such as phenylcarbamates, dithiocarbamates, chloroacetanilides, sulfonylureas, imidazolinones, cyclohexenones, and arylophenoxyalkanoic acids (15, 33) in primarily monocotyledoneous crops such as corn, grain sorghum, oats [*Avena sativa* L.], wheat, barley [*Hordeum vulgare* L.], and rice (40).

The mechanism of action of naphthalic anhydride has not been clearly defined. Naphthalic anhydride was found to enhance the rate of EPTC metabolism and it was postulated that naphthalic anhydride conferred protection by activating enzymes involved in EPTC detoxication (13).

In both corn and sorghum, chloroacetanilide herbicides, such as metolachlor, were detoxified by naphthalic anhydride by conjugation with the tripeptide glutathione (GSH) (13). Another report showed that the safener, naphthalic anhydride, conferred protection against metolachlor injury by inducing the *de novo* synthesis of GST-isozymes which catalyzed the detoxication of the herbicides (7).

Seed treatment of corn with naphthalic anhydride did not affect the activity of sulfonylurea and imazaquin (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3-quinolinecarboxylic acid) herbicides which inhibit acetolactate synthase (ALS) enzymes (3, 28). For imazethapyr

(2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid), naphthalic anhydride stimulated mixed function oxidase (MFO) activity that can rapidly metabolize imazethapyr to hydroxy-imazethapyr. The naphthalic anhydride safening effect can be antagonized by the MFO inhibitor aminobenzotriazole (ABT) (39).

Diclormid

The safener dichlormid was introduced commercially in 1973 as an antidote for EPTC injury to corn (32). Several researchers reported that dichlormid also protected corn seedlings from injury caused by acetanilides (26, 43), chlorsulfuron (2-chloro-N-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl] benzenesulfonamide) (34), and imazaquin herbicides (4). In addition to corn, grain sorghum has also been protected by dichlormid from thiocarbamate and acetanilide (12, 43) and from imazaquin herbicides (4).

Unlike naphthalic anhydride, which was ineffective at low rates or nonselective at high rates as a soil applied safener, dichlormid could be added to the EPTC formulations and applied preplant incorporated. Dichlormid was physiologically selective for preventing EPTC injury to corn without reducing EPTC effectiveness for weed control (40). Leavitt and Penner (26) reported that dichlormid was an effective safener whether applied preemergence, preplant incorporated, or as a tank mixture.

There has been, and still is, much controversy surrounding the mode of action of dichlormid as to whether it acts at a site of herbicide action or protection is the result of enhanced herbicide metabolism (11). Early studies indicate that it did not act by inhibiting the uptake or translocation of EPTC in plants (6). Lay and Casida (25) suggested that the mechanism of action of dichlormid involved an increase in the rate of detoxication of the herbicide, via conjugation to glutathione. They demonstrated that corn pretreated with dichlormid had higher GSH levels, GST activity, and metabolized EPTC sulfoxide faster than nontreated plants. They proposed that dichlormid protection was the result of enhanced EPTC metabolism through elevation in GSH levels and GST activity. Leavitt and Penner (27) confirmed the EPTC sulfoxide conjugation to GSH, and proposed that dichlormid acted by stimulating the formation of the EPTC sulfoxide in corn.

R-29148

The safener R-29148 was first discovered in 1971 as a safener for protecting corn from thiocarbamate injury (42). It is normally incorporated into the soil applied as a tank mixture with EPTC, butylate (*S*-ethyl bis[2-methylpropyl]carbamothioate) or vernolate (*S*-propyl dipropylcarbamothioate). It is also effective as a seed treatment. The safener R-29148 provided partial protection to corn from the phytotoxic effects of chlorimuron (2-[[[[(4-chloro-6-methoxy-2-pyrimidinyl) amino] carbonyl] amino] sulfonyl] benzoic acid) and sulfometuron (2-[[[[(4,6-dimethyl-2-pyrimidinyl)amino]carbonyl] amino]sulfonyl]benzoic acid) (46). Currently, there is no published data concerning the mode of action of R-29148.

Cyometrinil, Oxabetrinil, CGA-133205

Cyometrinil was marketed as CONCEP I in 1978, oxabetrinil was marketed as CONCEP II in 1982, and CGA-133205 was marketed as CONCEP III in 1986. All of them are used as herbicide safeners against injury from metolachlor and other acetanilide herbicides in grain sorghum (17). They are effective when applied as a seed treatment. When applied as a tank mixture, they may also provide some protection to several weeds of the genus sorghum. Turner *et al.* (41) reported the adverse effects caused by cyometrinil on the seed germination of grain sorghum. As a result, CIBA-GEIGY developed a chemical analog, oxabetrinil. But, there was an undesirable interaction of oxabetrinil with downy mildew. To solve this problem CIBA-GEIGY introduced CGA-133205 which is expected to replace oxabetrinil in grain sorghum markets (8). Yenne and Hatzios (44) reported that metolachlor with seed-applied CGA-133205 had no effect on sorghum germination, while metolachlor with seed applied oxabetrinil significantly reduced germination of grain sorghum.

The mechanism by which these safeners confer protection are not clearly known, but several hypothesis have been advanced. In an early study, Winkle *et al.* (43) reported that cyometrinil did not affect the metabolism of metolachlor in sorghum seedlings. But, recent studies showed that cyometrinil and oxabetrinil seed treatment caused accelerated metolachlor metabolism in sorghum shoots, decreased metolachlor content, and increased formation of the glutathione conjugate (12). Zama and Hatzios (45) reported that oxabetrinil enhanced metolachlor metabolism in grain sorghum seedlings. It has also been proposed that oxabetrinil promoted nonenzymatic GSH conjugation of chloroacetanilides by a direct activation of the reaction. However, Gronwald *et al.* (13) were unable to confirm the activation of nonenzymatic conjugation by oxabetrinil.

Unlike cyometrinil and oxabetrinil, the studies about CGA-133205 are still limited. Moreland and Corbin (29) proposed that grain sorghum tissue from CGA-133205 treated seed metabolized metolachlor more extensively than untreated seed. But, they have not yet separated the contribution to the enhanced metabolism of metolachlor by the glutathione system from those of the monooxygenase system in the safener enhanced seedlings.

Flurazole

Flurazole was discovered by MONSANTO in 1980 and marketed under the trade name SCREEN. Flurazole is a seed treatment protectant which safens grain sorghum and corn seedlings from injury due to alachlor (2-chloro-N-[2,6-diethylphenyl]-N-[methoxymethyl]acetamide), metolachlor, and other chloroacetanilide herbicides (5, 38). Schafer (38) reported that flurazole could be effective when applied in furrow, but because it affords protection to weed species of the genus sorghum, flurazole is marketed as a seed treatment for sorghum seeds. Hatzios (16) applied flurazole as a preplant incorporated treatment, but it was not very effective in protecting corn against thiocarbamate and chloroacetanilide herbicides.

The mechanism of the safening effect of flurazole is poorly understood (5). Mozer *et al.* (30) showed that flurazole caused an increased in GST activity in corn seedlings. Flurazole has also been reported to enhance the level of GSH in grain sorghum (36). Breaux and his coworkers (5) confirmed these two reports. They reported that flurazole did not detectably change the metabolic pathway that was responsible for detoxication of acetochlor (2-chloro-N-[ethoxymethyl]-N-[2-ethyl-6-methyl-phenyl]acetamide), but it did alter the rate of metabolism. They also stated that acetochlor was converted to GSH conjugates in both safened and unsafened corn and grain sorghum seedlings, but the use of flurazole caused an increase in both GSH levels and GST activity which catalyzed the conjugation of acetachlor with GSH.

Summary

Currently, there are numerous herbicide safeners available in the market. Research on the action of these safeners can assist the process of discovering and developing new herbicides. The herbicide safeners may allow cheaper herbicides to replace the more expensive one for the control of similar weed problem.

In a previous review (15), several reports have proposed mechanism of actions for herbicide safeners on various crops and herbicides. But, their protective actions are not totally understood. Further investigations to understand how the herbicide safeners provide protection to crops have been suggested by Hatzios and Hoagland in 1989 (18).

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CHAPTER 2

EVALUATION AND COMPARISON OF HERBICIDE SAFENERS IN CORN [*Zea mays* L.] AND GRAIN SORGHUM [*Sorghum bicolor* (L.) Moench]

Abstract

Combinations of certain insecticides and herbicides can produce severe crop injury. Greenhouse studies were conducted to evaluate and compare herbicide safeners for protection of corn and grain sorghum from herbicidal injury of metolachlor, primisulfuron, and the interaction with terbufos insecticide.

Primisulfuron applied at 40 g ai/ha to corn not treated with terbufos showed only slight injury, but corn tretated with terbufos at 750 g/1000 m of row showed severe injury. A high rate of metolachlor produced severe injury to corn seedlings. The level of metolachlor injury was not affected by terbufos. With the exception of CGA-133205 that killed corn, the safeners oxabetrinil, flurazole, naphthalic anhydride, dichlormid, and R-29148 provided significant protection against injury to corn from the interaction between primisulfuron and terbufos, and from metolachlor.

Primisulfuron applied at 10 g ai/ha and metolachlor applied at 4.5 kg ai/ha caused severe injury to sorghum

seedlings with or without terbufos. Partial protection was provided by the safeners, oxabetrinil, CGA-133205, flurazole, naphthalic anhydride, dichlormid, and R-29148, against metolachlor injury only. Nomenclature: corn, [*Zea mays* L.]; sorghum, [*Sorghum bicolor* (L.) Moench]; metolachlor, 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide; primisulfuron, 2-[3-[4,6-bis(difluoromethoxy)-pyrimidin-2-yl]-ureidosulfonyl]benzoic acid methylester; terbufos, S-[[(1,1-dimethylethyl)thio]methyl]O,O-diethyl phosphorodithioate; CGA-133205,O-(1,3,dioxolan-2-yl-methyl)-2,2,2-trifluoro-4′-chloroacetophenone-oxime; oxabetrinil, α-[(1,3-dioxolan-2-yl-methoxy)imino]benzeneacetonitrile); flurazole, phenylmethyl 2-chloro-4-(trifluoromethyl)-5-thiazolecarboxylate; naphthalic anhydride, naphthalene-1,8-dicarboxylic acid anhydride; dichlormid, 2,2-dichloro-N,N-di-2-propenylacetamide; R-29148, 3-(dichloroacetyl)-2,2,5-trimethyl-1,3-oxazolidine

Introduction

Many herbicides are available that will selectively control grass weeds in corn and grain sorghum, but certain herbicides may cause injury problems at the seedling stage. Metolachlor is a preemergence herbicide for corn and grain sorghum. Under certain condition, metolachlor may injure corn seedlings (8) and grain sorghum seedlings (6). Primisulfuron is a sulfonylurea herbicide that provides postemergence control of several annual and perennial grasses plus many broadleaf weeds in corn (7), but some varieties of corn may be injured from the application of primisulfuron (1).

The use of multiple pesticides has become the usual practice in crop production. Preemergence or postemergence herbicides may follow the application of soil insecticides. Although these two chemicals act independently, certain soil insecticides may increase herbicidal injury (4). Of the 121 herbicide-insecticide interactions listed by Hatzios and Penner (3), more than 93 percent were synergistic. Severe corn injury from interactions between primisulfuron and organophosphate insecticides have been reported (1, 2, 4, 5).

One way to reduce the injury problems is by using chemicals known as herbicide safeners. The main purpose for using safeners is to broaden the margin of herbicide selectivity. Herbicide safeners may be applied as a seed treatment, preplant incorporated, or as a tank mixture with the herbicide. Commercial safeners evaluated in this study include oxabetrinil, CGA-133205, naphthalic anhydride, flurazole, dichlormid, and R-29148.

The objectives of this research were: a) to evaluate the interaction between metolachlor and primisulfuron with terbufos insecticide, and b) to evaluate and compare the effectiveness of herbicide safeners against injury from metolachlor, primisulfuron, and the interaction with terbufos insecticide in corn and grain sorghum.

Materials and Methods

Three safener-treated or untreated corn seeds 'Northrup King 9283' and six safener-treated or untreated sorghum seeds 'DK 46' were planted in 945-ml plastic pots containing an air dried Spinks loamy sand soil with 1.0 percent organic matter and pH 7.2. The safeners oxabetrinil (1.5 g ai/kg seed), CGA-133205 (1.5 g ai/kg corn seed only), naphthalic anhydride (10 g ai/kg seed), and flurazole (2.5 g ai/kg seed) were applied as seed treatment, dichlormid and R-29148 were applied as a preplant incorporated treatment or tank mixture with herbicide application at 0.6 kg ai/ha. Sorghum seed treated or untreated with safener CGA-133205 (0.4 g ai/kg seed) were provided by CIBA-GEIGY Corp.

Terbufos insecticide was preplant incorporated at a rate equivalent to 750 g/1000 m of row in the top 3 cm of soil in the pots.

Metolachlor was applied preemergence at the rates of 6.7 and 4.5 kg ai/ha for corn and grain sorghum, respectively. Primisulfuron plus nonionic surfactant X-77 (0.1% v/v) was applied postemergence at the three- to four-leaf stage visible or with collars of corn and grain sorghum at the rates 40 and 10 g ai/ha, respectively. The rates were lower for sorghum because preliminary studies indicated sorghum was more sensitive to metolachlor and primisulfuron injury than corn. The herbicides were sprayed with a continuous link belt sprayer at 193 kPa pressure and 230 L/ha volume.

Plants were grown in the greenhouse at 24 ± 2 C with supplemental lighting to provide a total mid-day light intensity of 1200 $uE\ m^{-2}s^{-1}$ from both supplemental and natural light. Sorghum seedlings for the primisulfuron experiment were thinned to two plants per pot. The plants were watered as needed and fertilized weekly with water-soluble fertilizer (20% N, 20% P_2O_5, 20% K_2O) at the rate of 1500 ppm.

All experiments were factorial combinations of herbicides, terbufos and safeners in a completely randomized design. Each treatment had four replications and all experiments were repeated. Plant heights were measured 14 and 28 days after treatment (DAT) for primisulfuron and metolachlor, respectively. The mean of the plants from each pot was considered as one observation. Following the analysis of variance the means were separated using Fisher's Protected LSD at the 5 percent level of significance.

Result and Discussion

Primisulfuron by itself reduced corn height slightly (Table 1). The combination of primisulfuron and terbufos resulted in severe injury to corn seedlings (Table 1). Metolachlor at high rate of 6.7 kg ai/ha caused severe injury to corn seedlings, but no interactions with terbufos insecticide was evident (Table 2). In a previous study, corn hybrid 'NK 9283' has been shown premierly sensitive to metolachlor at a high rate (9).

Significant protection against primisulfuron-terbufos interaction injury to corn was provided by oxabetrinil, flurazole, naphthalic anhydride, dichlormid, and R-29148 (Table 1).

Corn was protected from metolachlor injury by the safeners oxabetrinil, flurazole, naphthalic anhydride, dichlormid, and R-29148 whether or not it was treated with terbufos (Table 2).

Seed treatment application of the safener CGA-133205 killed the corn (Tables 1 and 2). With the exception of CGA-133205, there were no statistically significant effects of safeners on the height of corn seedlings when applied by themselves or in combination with terbufos (Tables 1 and 2). There was no significant differences between tank mixtures and preplant incorporated application of the safeners dichlormid and R-29148 (Tables 1 and 2). Terbufos by itself also did not reduced the height of corn seedlings (Tables 1 and 2).

Both primisulfuron and metolachlor caused severe injury to sorghum seedlings (Tables 3 and 4). No interaction between terbufos and primisulfuron or terbufos and metolachlor was evident on sorghum (Tables 3 and 4).

None of the tested safeners protected sorghum from primisulfuron injury (Table 3). Protection from metolachlor injury was provided by oxabetrinil, CGA-133205, flurazole, naphthalic anhydride, dichlormid, and R-29148 (Table 4). Preliminary studies showed that terbufos by itself and terbufos plus safeners did not reduce the height of sorghum seedlings. Safeners by themselves did not influence the growth of sorghum seedlings (Tables 3 and 4).

Summary

A Synergistic interaction between primisulfuron and terbufos was observed in corn hybrid 'NK 9283'. The safeners oxabetrinil, flurazole, naphthalic anhydride, dichlormid, and R-29148 provided protection against corn injury from primisulfuron interaction with terbufos. Protection was also provided by these safener against corn injury from metolachlor.

Primisulfuron and metolachlor caused severe injury to grain sorghum. Protection by the safeners, oxabetrinil, CGA-133205, flurazole, naphthalic anhydride, dichlormid, and R-29148 was only evident against metolachlor injury.

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TABLE 1. Protection of corn seedlings with herbicide safeners from primisulfuron-terbufos interaction injury.

Primi-sulfuron	Safeners[a]	Shoot height(14DAT) -Terbufos	+Terbufos
(g/ha)		-----(cm/plant)------	
0	None	51	50
	Oxabetrinil (st)	49	51
	CGA-133205 (st)	0	0
	Flurazole (st)	51	50
	NA (st)	48	47
	Dichlormid (pre)	52	51
	Dichlormid (ppi)	50	46
	R-29148 (pre)	49	47
	R-29148 (ppi)	47	45
40	None	42	33
	Oxabetrinil (st)	45	43
	CGA-133205 (st)	0	0
	Flurazole (st)	46	39
	NA (st)	43	40
	Dichlormid (tm)	45	38
	Dichlormid (ppi)	45	39
	R-29148 (tm)	47	43
	R-29148 (ppi)	41	39
LSD (0.05)		------- 5 ----------	

[a]st=seed treatment; pre=preemergence; ppi= preplant incorporated; tm=tank mixture.

TABLE 2. Protection of corn seedlings with herbicide safeners from metolachlor-terbufos injury.

Metolachlor	Safener	Shoot height (28 DAT) -Terbufos	Shoot height (28 DAT) +Terbufos
(kg ai/ha)		--- (cm/plant)	-----
0.0	None	51	50
	Oxabetrinil (st)	49	51
	CGA-133205 (st)	0	0
	Flurazole (st)	51	50
	NA (st)	48	47
	Dichlormid (pre)	52	51
	Dichlormid (ppi)	50	46
	R-29148 (pre)	49	47
	R-29148 (ppi)	47	45
6.7	None	23	23
	Oxabetrinil (st)	42	42
	CGA-133205 (st)	0	0
	Flurazole (st)	48	50
	NA (st)	40	45
	Dichlormid (tm)	45	47
	Dichlormid (ppi)	46	46
	R-29148 (tm)	45	48
	R-29148 (ppi)	44	46
LSD (0.05)		------ 5	-----------

TABLE 3. Protection of sorghum seedlings with herbicide safeners from primisulfuron-terbufos injury.

Primi-sulfuron	Terbufos	Safener	Shoot height (14 DAT)
(g ai/ha)	(g/1000 m row)		(cm/plant)
0	0	None	58
		Oxabetrinil (st)	57
		CGA-133205 (st)	55
		Flurazole (st)	55
		NA (st)	54
		Dichlormid (post)	55
		R-29148 (post)	51
10	0	None	36
		Oxabetrinil (st)	33
		CGA-133205 (st)	33
		Flurazole (st)	29
		NA (st)	27
		Dichlormid (tm)	32
		R-29148 (tm)	31
10	750	None	31
		Oxabetrinil (st)	31
		CGA-133205 (st)	32
		Flurazole (st)	29
		NA (st)	28
		Dichlormid (tm)	30
		R-29148 (tm)	32
LSD (0.05)			7

TABLE 4. Protection of sorghum seedlings with herbicide safeners from metolachlor-terbufos injury.

Meto-lachlor	Terbufos	Safener	Shoot height (28 DAT)
(kg ai/ha)	(g/1000 m row)		(cm/plant)
0.0	0	None	55
		Oxabetrinil (st)	59
		CGA-133205 (st)	60
		Flurazole (st)	54
		NA (st)	53
		Dichlormid (pre)	54
		R-29148 (pre)	50
4.5	0	None	19
		Oxabetrinil (st)	47
		CGA-133205 (st)	44
		Flurazole (st)	46
		NA (st)	46
		Dichlormid (tm)	33
		R-29148 (tm)	50
4.5	750	None	17
		Oxabetrinil (st)	45
		CGA-133205 (st)	39
		Flurazole (st)	45
		NA (st)	46
		Dichlormid (tm)	37
		R-29148 (tm)	47
LSD (0.05)			8

CHAPTER 3

INCREASED PRIMISULFURON INJURY TO CORN [*Zea mays* L.] WITH ANTIOXIDANTS AND PROTECTION WITH HERBICIDE SAFENERS

Abstract

Greenhouse and laboratory studies were conducted to evaluate the efficacy of herbicide safeners against corn injury from primisulfuron and primisulfuron plus antioxidants.

Antioxidants, piperonyl butoxide and metyrapone, applied preemergence at 5000 ppm were observed to increase primisulfuron injury to corn. Piperonyl butoxide (PPO) by itself did not influence shoot height of corn seedlings, but metyrapone reduced shoot height significantly. Naphthalic anhydride, oxabetrinil, flurazole, dichlormid, and R-29148 provided protection against primisulfuron injury to corn. The increased corn injury from primisulfuron plus PPO was also protected by those safeners. Protection was most evident with naphthalic anhydride, flurazole, and R-29148. None of the safeners provided protection against increased injury from metyrapone. Naphthalic anhydride was also an effective safener against increased corn injury when 3000 ppm PPO was applied foliarly 3 days prior to primisulfuron application.

^{14}C-Primisulfuron absorption by corn was not influenced by the antioxidant, PPO, or the safener, naphthalic anhydride. However, metabolism of ^{14}C-primisulfuron was inhibited by PPO. This inhibition was not totally reversed by the safener, naphthalic anhydride. Nomenclature: corn, [*Zea mays* L.]; primisulfuron, 2-[3-[4,6-bis(difluoromethoxy)-pyrimidine-2-yl-] ureidosulfonyl]-benzoic acid methylester; piperonyl butoxide, α-[2-(2-butoxyethoxy)ethoxy]-4,5-methylenedioxy-2-propyltoluene; metyrapone, 2-methyl-1,2-di-3-pyridyl-1-propanone; naphthalic anhydride, naphthalene-1,8-dicarboxylic acid anhydride; oxabetrinil, α-[(1,3-dioxolan-2-yl-methoxy)imino]benzeneacetonitrile; flurazole, phenylmethyl 2-chloro-4-(trifluoromethyl)-5-thiazolecarboxylate; dichlormid, 2,2-dichloro-N,N-di-2-propenylacetamide; R-29148, 3-(dichloroacetyl)-2,2,5-trimethyl-1,3-oxazolidine.

Introduction

Primisulfuron is a new sulfonylurea herbicide for postemergence weed control in corn. Ahrens (1) reported that some varieties of corn may be injured by postemergence application of primisulfuron. As with other sulfonylurea herbicides, inhibition of acetolactate synthase (ALS) is the site of action of primisulfuron (2). Selectivity of this class of herbicide in cereal crops, corn, and soybean has been correlated to the rapid metabolism of the sulfonylureas to herbicidally inactive products (2).

Metabolism of primisulfuron in corn occurs by hydroxylation and subsequent sugar conjugation of the phenyl and pyrimidine rings, whereas the sulfonylurea bridge cleavage constitutes a minor pathway (3). Hydroxylation of primisulfuron was inhibited *in vitro* by tetcyclasis as well as CO in the presence of oxygen. Fonne-Pfister *et al.* (3) concluded that hydroxylation of primisulfuron is catalyzed by an inducible cytochrome P450 monooxygenase system.

Molecular oxygen is an obligatory substrate required for the function of a group of hemoproteins known as mixed function oxidase (MFO) enzymes (9). These enzymes are actively involved in the metabolism of a number of herbicides by plants (5). The activity of MFO is known to be inhibited by antioxidant compounds or MFO inhibitors, such as piperonyl butoxide (PPO), sesamex (a component of sesame oil), EDU (N-[2-2-oxo-1-imidazolidinyl]ethyl-N-phenylurea), and n-propyl gallate (8). Furthermore Rubin *et al.* (8) observed that PPO acted as an herbicide and insecticide synergists.

The use of chemicals to protect crops from herbicidal injury has been an accepted practice in modern weed management programs (7). Hoffmann (6) introduced this concept about three decades ago. Today, there are numerous commercial crop safeners, such as oxabetrinil, flurazole, naphthalic anhydride, dichlormid and R-29148. The mechanism of action of these safeners is not clearly defined and several theories have been proposed. According to Hatzios (4), a safener-induced enhancement of herbicide detoxication in protected plants seems to be the major mechanism involved in the protective action of currently developed safeners. The enhancement of oxidative reactions is currently viewed as possibly involved in the protective action of herbicide safeners.

The objectives of these research were: a) to determine the effect of the antioxidants, PPO and metyrapone, on primisulfuron activity, b) to study the efficacy of herbicide safeners against primisulfuron injury to corn in the presence of the antioxidants, PPO and metyrapone, and c) to study the absorption and metabolism of ^{14}C-primisulfuron in the presence of the safener, naphthalic anhydride, and the antioxidant, PPO.

Materials and Methods

General greenhouse study: Corn 'NK 9283' treated or untreated with herbicide safeners was planted three seeds per pot in 945-ml plastic pots containing air-dried Spinks loamy sand with 1.0 percent organic matter and pH 7.2. The safeners naphthalic anhydride, oxabetrinil and flurazole were applied as seed treatment, dichlormid and R-29148 were applied as tank mixtures with the herbicide. The rates for the safeners were 10 g ai/kg seed, 1.5 g ai/kg seed, 2.5 g ai/kg seed, 0.6 kg ai/ha, and 0.6 kg ai/ha for naphthalic anhydride, oxabetrinil, flurazole, dichlormid and R-29148, respectively.

The antioxidants, PPO and metyrapone, were applied as a 5000 ppm solution containing ethanol 0.5 % v/v 1.5 ml per pot immediately after planting. Primisulfuron plus nonionic surfactant X-77 (0.1 % v/v) was applied postemergence at the rates 40 or 80 g ai/ha to corn at the three to four-leaf stage.

The efficacy of the safener, naphthalic anhydride, against the increased injury from primisulfuron at 40, 80 and 120 g ai/ha plus PPO to corn was evaluated in a greenhouse study. In this study the corn was planted in a BACCTO potting soil mix. Naphthalic anhydride (10 g ai/kg seed) was applied as a seed treatment, but PPO at 3000 ppm plus ethanol (0.5 % v/v) and nonionic surfactant X-77 (0.1 % v/v) were applied postemergence 3 days prior to primisulfuron application. Primisulfuron and PPO were applied postemergence using a continuous link belt sprayer at 193 kPa pressure and 230 L/ha volume.

The plants were grown in the greenhouse at 24 ± 2 C with supplemental lighting to provide a total mid-day light intensity of 1200 uE $m^{-2}s^{-1}$ from both supplemental and natural light. The plants were watered as needed and fertilized weekly with water-soluble fertilizer (20 % N, 20 % P_2O_5, 20 % K_2O) at the rate of 1500 ppm.

These studies used a factorial treatment arrangement of primisulfuron, antioxidants and safeners in a completely randomized design. Each treatment combination had four replications and all experiments were repeated. Shoot heights were measured 14 days after treatment (DAT) and calculated as a percent of control. The mean of three plants from each pot was considered as one observation. Following the analysis of variance the means were separated with Fisher's Protected LSD at the 5 percent level of significance.

^{14}C-Primisulfuron absorption and metabolism: Corn 'NK 9283' treated or untreated with naphthalic anhydride was planted 1 seed per pot in 945-ml plastic pots containing BACCTO potting soil mix. Plants were grown in the greenhouse for 1 week and then moved to the growth chamber. Temperature in the growth chamber was maintained at 24 C during the day and at 20 C at night, and lighting was provided for 16 h period by high pressure sodium lamps.

PPO at 3000 ppm plus ethanol (0.5 % v/v) and nonionic surfactant X-77 (0.1 % v/v) was applied postemergence 3 days prior to primisulfuron application. Prior to treatment with the ^{14}C-primisulfuron, corn at the three- to four-leaf stage was sprayed with primisulfuron plus X-77 (0.1 % v/v). While spraying, leaves used for the ^{14}C-primisulfuron absorption and metabolism studies were covered with plastic wrap.

A 2 *u*l drop of the ^{14}C-primisulfuron (activity 2.22×10^2 Bq, uniform ring labelled) and 18 1-*u*l drops (activity 4.44×10^3 Bq) for absorption and metabolism studies, respectively, were applied to the second leaf of seedlings. After the spots were dry, the seedlings were placed in growth chamber until harvest. After 8 h the plants were harvested for absorption study and after 10 h the plants were harvested for the metabolism study. The leaves were rinsed for 45 seconds with 5 ml of 50 % MeOH (v/v). The leaves for the metabolism study were placed in dry ice at -30 C until extracted.

Absorption of ^{14}C-primisulfuron was determined by radioassaying the MeOH rinsate by liquid scintillation spectrometry. Data are presented as a percent of total applied.

For the metabolism study, the leaves were ground with a Virtis grinder in 40 ml of 80 % acetone (v/v) for 6 minutes.

The extract was filtered under vacuum with a Whatman no 1 filter paper. The filtered solution was transferred to a 250-ml round bottom flask and concentrated to 3 ml *in vacuo* at 35 C using a rotary evaporator. The aqueous plant extract was placed in a 15-ml vial and the round bottom flask was rinsed with 5 ml acetonitrile and then combined with the aqueous plant extract.

The volume of the aqueous extract was reduced under a stream of nitrogen to 2 ml. The aqueous plant extract was then filtered under vacuum with a millipore-filter paper (0.45 *m*M) and the vial was rinsed with 2 to 3 ml acetonitrile. The volume was then reduced under a stream of nitrogen to approximately 0.6 ml volume. Following the addition of 0.4 ml of 80 % acetone (v/v) the extract was placed on silica gel 60 F_{254} TLC plates that were developed with the solvent system of methanol : benzene (2:1 v/v).

The location of the ^{14}C-primisulfuron and its metabolites on the TLC plates were determined with a scanner. The ^{14}C-labelled spots were removed and radioassayed by liquid scintillation spectrometry. Metabolism data are presented as percent of ^{14}C absorbed. Both absorption and metabolism studies were analyzed using a completely randomized design with eight replications. Means were separated by Fisher's Protected LSD at the 5 percent level of significance.

Results and Discussions

Effect of MFO inhibitors and herbicide safeners: Primisulfuron applied at 40 and 80 g ai/ha injured the hybrid 'NK 9238' corn seedlings (Table 1). The level of injury increased in the presence of the antioxidants, PPO and metyrapone. PPO by itself did not injure corn seedlings, but metyrapone caused a significant decrease in corn shoot height (Table 1). Corn injury from primisulfuron applied at 40 and 80 g ai/ha was reversed by the safeners naphthalic anhydride, oxabetrinil, flurazole, dichlormid, and R-29148 (Table 1). The increased corn injury from PPO plus primisulfuron at 40 and 80 g ai/ha was also protected by those safeners. Protection against the incresed corn injury from primisulfuron plus PPO was most evident from naphthalic anhydride, flurazole, and R-29148 (Table 1). None of the safeners provided protection against increased injury from metyrapone (Table 1).

Further study to evaluate primisulfuron activity in the presence of PPO and naphthalic anhydride is presented in Table 2. Postemergence application of PPO also increased corn injury from primisulfuron applied at 40, 80 and 120 g ai/ha (Table 2). The safener, naphthalic anhydride, reversed the increased injury from the antioxidant, PPO, applied 3 days prior to primisulfuron application (Table 2).

^{14}C-Primisulfuron absorption and metabolism: Absorption of ^{14}C-primisulfuron was not influenced by the safener, naphthalic anhydride, the antioxidant, PPO, or both the PPO and the naphthalic anhydride (Table 3). The increased corn injury from primisulfuron plus PPO appeared due to the inhibition of the ^{14}C-primisulfuron metabolism by the PPO (Table 3). The safener, naphthalic anhydride, partially reversed this inhibition (Table 3). Naphthalic anhydride itself did not influence the rates of ^{14}C-primisulfuron metabolism (Table 3).

Summary

The antioxidants, piperonyl butoxide (PPO) and metyrapone, increased corn injury from primisulfuron. The increased injury from PPO was reversed by the safeners, flurazole and R-29148. However, protection by the safeners against increased injury from metyrapone was not evident. The increased injury from PPO was due to inhibition of primisulfuron metabolism. The safener, naphthalic anhydride, did not totally reverse this inhibition.

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TABLE 1. Efficacy of herbicide safeners against injury from the interaction of primisulfuron and antioxidants.

Primi-sulfuron	Safener[a]	Shoot height (14 DAT)		
		None	PPO	Metyrapone
(g ai/ha)		(percent of untreated)		
0	None	100	98	92
40	None	85	64	81
	NA (st)	94	89	80
	Oxabetrinil (st)	94	81	84
	Flurazole (st)	97	97	85
	Dichlormid (tm)	93	81	87
	R-29148 (tm)	98	96	86
80	None	83	70	74
	NA (st)	89	84	81
	Oxabetrinil (st)	91	85	82
	Flurazole (st)	93	88	79
	Dichlormid (tm)	91	78	76
	R-29148 (tm)	94	92	83
LSD (0.05)		--------- 8 ---------------		

[a]st=seed treatment; tm=tank mixture.

TABLE 2. Efficacy of nahpthalic anhydride against increased primisulfuron injury to corn with the PPO.

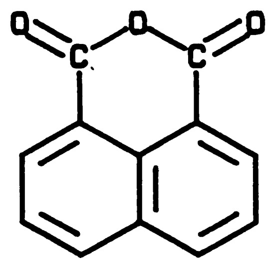
Primisulfuron	PPO	Shooth height (14 DAT) -NA	+NA
(g ai/ha)	(ppm)	(percent of control)	
40	0	90	99
	3,000	86	99
80	0	88	94
	3,000	81	95
120	0	85	94
	3,000	77	94
LSD (0.05)		-------- 7 --------	

TABLE 3. Absorption and metabolism of ^{14}C-primisulfuron in corn seedlings in the presence of the safener, naphthalic anhydride and the antioxidant, PPO.

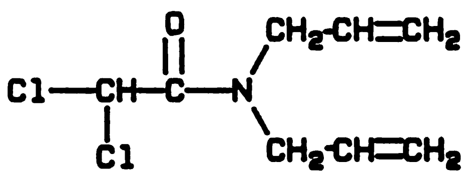
Herbicide interaction	Absorption	Metabolism
	(% of treated)	(% of absorbed)
Primisulfuron	20.4	10.2
Primisulfuron + NA	21.4	11.1
Primisulfuron + PPO	21.4	5.7
Primisulfuron + NA + PPO	18.9	7.8
LSD (0.05)[a]	4.7	1.7

[a]Comparisons valid within columns.

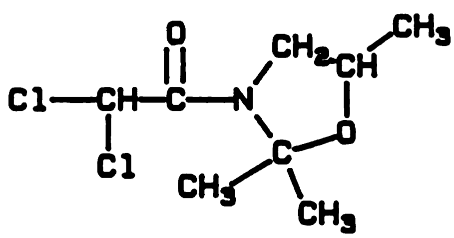
APPENDIX A. Chemical structure of naphthalic anhydride, dichlormid, R-29148, oxabetrinil, CGA-133205, and flurazole.



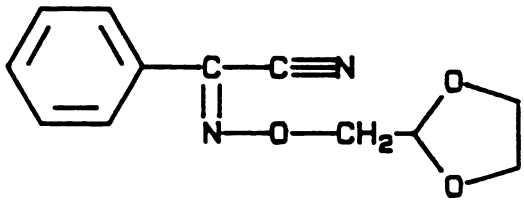
Naphthalic Anhydride



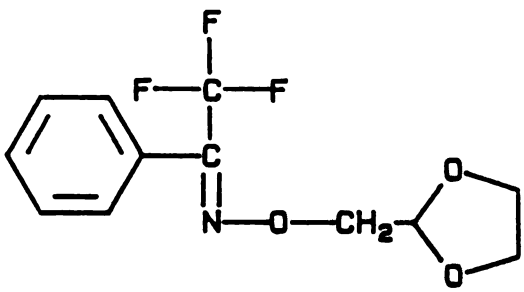
Dichlormid



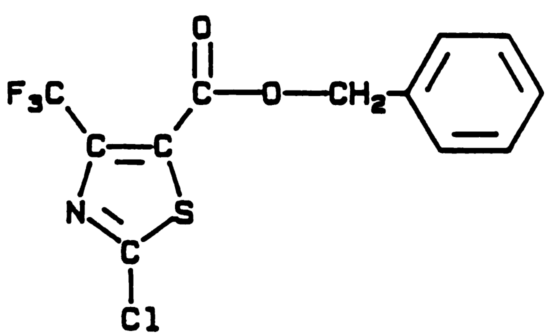
R-29148



Oxabetrinil

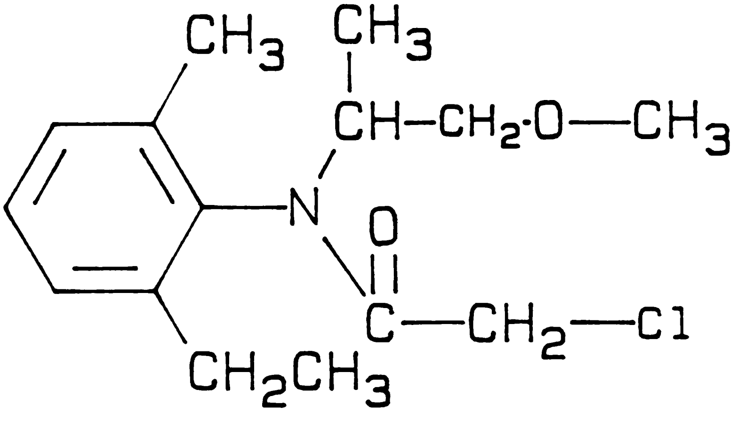


CGA-133205

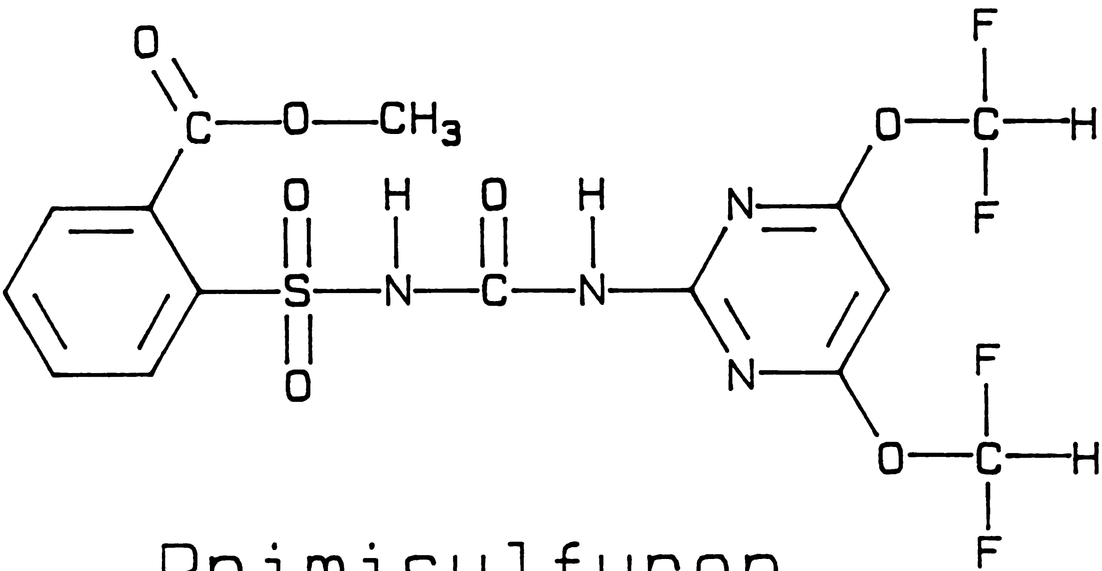


Flurazole

APPENDIX B. Chemical structure of metolachlor and primisulfuron.



Metolachlor



Primisulfuron