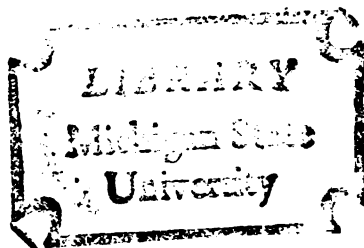


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Efficacy of CGA-43089
[α -(Cyanomethoximino)-Benzacetonitrile] As
A Herbicide Antidote for Sorghum
(Sorghum Vulgare Pers.)

presented by

Gary Lynn Leek

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EFFICACY OF CGA-43089
[α -(CYANOMETHOXIMINO)-BENZACETONITRILE] AS
A HERBICIDE ANTIDOTE FOR SORGHUM
(SORGHUM VULGARE PERS.)

By

Gary Lynn Leek

A DISSERTATION

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

DOCTOR OF PHILOSOPHY

Department of Crop and Soil Science

1981

ABSTRACT

EFFICACY OF CGA-43089 [α -(CYANOMETHOXIMINO)-BENZACETONITRILE] AS A HERBICIDE ANTIDOTE FOR SORGHUM (SORGHUM VULGARE PERS.)

By

Gary Lynn Leek

Seed treatment with CGA-43089 [α -(cyanomethoximino)-benzacetone nitrile] provided protection to sorghum (Sorghum vulgare Pers.) against the herbicides metolachlor [2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide], alachlor [2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide], diethatyl [N-(chloroacetyl)-N-(2,6-diethylphenyl)glycine], ethofumesate [($\pm$)-2-ethoxy-2,3-dihydro-3,3-dimethyl-5-benzofuranyl methanesulfonate], butylate + R-25788 [(S-ethyl diisobutylthiocarbamate) + (N,N-diallyl-2,2-dichloroacetamide)], and atrazine + metolachlor [2-chloro-4-ethylamino-6-isopropylamino-s-triazine + 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide] under greenhouse conditions.

CGA-43089 did not protect sorghum from the phytotoxic effects of diphenamid [N,N-dimethyl-2,2-diphenylacetamide], EPTC [S-ethyl dipropylthiocarbamate], EPTC + R-25788 [S-ethyl dipropylthiocarbamate + N,N-diallyl-2,2-dichloroacetamide], pronamide [3,5-dichloro(N-1,1-dimethyl-2-propynyl)benzamide], metribuzin [4-amino-6-tert-butyl-3-(methylthio)-as-triazin-5(4H)-one or buthidazole {3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-4-hydroxy-1-methyl-2-imidazolidinone}.

Under field conditions, CGA-43089 protected sorghum against high rates of metolachlor, alachlor, diethatyl, and atrazine + metolachlor

(4.5, 6.7, 6.7, and 3.4 + 4.2 kg/ha respectively), and low rates of ethofumesate and butylate + R-25788 (1.7 and 3.4 kg/ha respectively). Both forage and grain yields were significantly increased when sorghum was protected from herbicide damage by CGA-43089 compared with those plants not receiving the antidote.

The protective action of CGA-43089 on sorghum against metolachlor did not require light. The protective action of the antidote was not evident when sorghum was grown under flooded conditions. Untreated sorghum plants were not protected from metolachlor injury by the lateral displacement of CGA-43089 from treated seedlings growing in close proximity, unless the two types of seed were placed immediately adjacent to each other. Storage of antidote-treated sorghum seed for 11 months or more reduced germination.

CGA-43089 does not protect sorghum from metolachlor injury via reduced metolachlor absorption or retention. After germinating 24 h in 10^{-5}M ^{14}C -metolachlor, CGA-43089 treated sorghum seeds absorbed 36% more ^{14}C -metolachlor than untreated seeds. CGA-43089 treated seeds retained 78% of the radioactivity detected as parent metolachlor and 22% of the radioactivity as a polar metabolite, while untreated seeds converted 50% of the parent herbicide to a polar metabolite.

The protective action of CGA-43089 does not involve alterations in translocation of metolachlor to the site of action. Sorghum seedlings grown from seed treated with CGA-43089 translocated ^{14}C -metolachlor similarly to untreated seedlings.

The protective effects of CGA-43089 do not appear to involve increased rates of metolachlor metabolism. CGA-43089 treated sorghum

seedlings exposed to ^{14}C -metolachlor 24 h or 5 days following germination absorbed more ^{14}C -metolachlor and metabolized the ^{14}C -metolachlor at the same rate or less rapidly than unprotected seedlings.

These results suggest that the protective effects of CGA-43089 are not due to reduced herbicide absorption, modified herbicide translocation, or increased rates of metolachlor metabolism, but may involve other factors, such as interfering with the mechanism of herbicide action.

ACKNOWLEDGMENTS

The author wishes to express sincere appreciation to his major professor, Dr. Donald Penner, for his patience, guidance, technical expertise, and deep friendship that endured throughout the entire project. Special thanks are also expressed to Dr. William Meggitt, particularly for his assistance in supplying the mechanical equipment and technical help necessary to complete the field work associated with this project. The author would also like to express thanks to Dr. Alan Putnam, Dr. John Kaufmann, and Dr. Matthew Zabik for their help in planning this project and manuscript review. Thanks also to Ciba-Geigy and Dr. Homer LeBaron for supplying the untreated and treated sorghum seed, radiolabeled metolachlor, and financial assistance involved in this project. Special thanks are also expressed to fellow graduate students that helped make difficult days and nights infinitely more bearable, particularly my very close friends Dale Aaberg, Rich Voorman, Martin Mahoney, and Lynn Oakes. A final and special thanks is expressed to Claudia Haas for her deep friendship, caring, and help in the final preparation of this dissertation.

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Introduction

The use of chemicals to protect crops from herbicide injury is a relatively new approach to selective weed control. These chemicals, termed "protectants", "safeners", or "antidotes", selectively protect crop plants from herbicide injury without protecting weeds. Antidotes can be used to widen the selectivity or margin of safety of a herbicide so that higher herbicide doses can be applied, more potent herbicides can be used, longer periods of weed control can be obtained, and greater reliability under varying environmental conditions is possible. Antidotes may also permit the use of normally less selective herbicides, more economical herbicides, or more environmentally desirable herbicides.

A new plant protectant, [α -(cyanomethoximino)-benzacetone nitrile], known as CGA-43089 and trademarked Concep has recently been registered for use in sorghum against metolachlor injury. Existing herbicides used for sorghum have not provided sufficiently broad spectrum weed control. Broad spectrum herbicides, such as metolachlor, injured sorghum at rates required for effective weed control.

The objectives of this research were: a) to evaluate CGA-43089 as a potential antidote against selected herbicides for sorghum under greenhouse and field conditions, b) to determine the influence of light, flooding, leaching, and storage on the protective action of CGA-43089, and c) to determine if the protection provided to sorghum

by CGA-43089 was due to reduced herbicide absorption, modified translocation of the herbicide to the target site, or altered herbicide metabolism.

REVIEW OF LITERATURE

Introduction

A herbicide antidote is a compound that selectively protects crop plants from herbicide injury without protecting weeds. The site of antidote action can be external or internal. External protection could involve purely physical barriers to herbicide uptake, as in the case with activated carbon, or could involve competition with the herbicide for site of entry. Internal protection would involve more complex biochemical interaction, such as competition for a binding site of toxic action, or increased detoxication of the herbicide.

External Crop Protection - Activated Carbon

Activated carbon (activated charcoal) was one of the first protectants used, with varying degrees of success (1). Since it adsorbs most organic herbicides it can provide a physical barrier to herbicide uptake by the plant, thus reducing the risk of damage to the crop caused by herbicide treatment.

The use of activated carbon as a protectant has its limitations. Most obvious is that it can only be used with soil applied herbicides. Another drawback is that its range of action is severely localized. Seed coating with activated carbon generally does not protect the emerging shoot from herbicide injury since the seedlings rapidly grow out of the protected zone (8) (12). Placing the activated carbon in rows or as spot treatments is often uneconomical and also results in weed protection in the immediate vicinity of the crop (15) (27).

Furthermore, relatively large amounts of charcoal are required to obtain the desired protection (14) (23).

Internal Protection

The first observation that led ultimately to the concept of herbicide antidotes was made by Hoffmann in 1947 (28). He observed that tomato plants treated with both 2,4,6-T and 2,4-D did not show phenoxy-related injury symptoms. Later, in 1962, he described the use of chemical seed treatments that protected wheat from barban injury (16). These compounds were not developed for commercial use, but in 1969 Hoffmann reported the discovery of what ultimately became the first commercially developed herbicide protectant, 1,8-naphthalic anhydride (17) (Appendix A).

Despite a number of reports on various chemicals having protectant action (28), only two other compounds have been released for commercial use as herbicide protectants. These are N,N-diallyl-2,2-dichloroacetamide, known as R-25788 (Appendix A), and a newer compound [α -(cyanomethoximino)-benzacetone nitrile], known as CGA-43089 and trademarked Concep (Appendix A).

1,8-Naphthalic Anhydride

1,8-Naphthalic anhydride was introduced commercially in 1972 as a seed treatment that protected corn against EPTC (S-ethyl dipropylthiocarbamate) injury, and to a lesser extent butylate (S-ethyl diisobutylthiocarbamate) damage (28). Naphthalic anhydride has subsequently been shown to protect a number of crops against a variety of herbicides. Naphthalic anhydride has been reported to protect corn from various acetanilides (21), nearly all the thiocarbamates (3), and

limited protection from buthidazole {3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-4-hydroxy-1-methyl-2-imidazolidinone} injury (13). Naphthalic anhydride has also been reported to protect sorghum from alachlor [2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide] injury (29); sorghum, corn, and cotton from damage by the pyrrolidine urea herbicide 5328 [cis 2,5-dimethyl-1-pyrrolidine carboxanilide] (18); and corn (2) and oats (7) from foliar applications of barban [4-chloro-2-butynyl m-chlorocarbonilate].

Mechanism of Action of Naphthalic Anhydride

Little is known of the mode of action of naphthalic anhydride. However, it almost certainly does not act by preventing herbicide uptake into the plant (11) (18) (25).

Data concerning the effect of naphthalic anhydride on herbicide metabolism are fragmentary. There are conflicting reports on whether naphthalic anhydride alters EPTC metabolism in corn tissue (11) (25). Wilkinson and Smith (30) have demonstrated that 10^{-7} M naphthalic anhydride in combination with 10^{-5} M EPTC reversed EPTC-induced inhibition of acetate into lipids. They suggest that naphthalic anhydride may work by reversing the inhibition of fatty acid synthesis caused by thiocarbamate herbicides. Lay and Casida (19) were unable to demonstrate naphthalic anhydride induced stimulation of GSH levels or GSH-S-transferase activity in corn roots. Since this conflicts with results they obtained for R-25788, they concluded that naphthalic anhydride had a different mode of action from R-25788 (19).

R-25788

R-25788 was introduced commercially in 1973 as a protectant against EPTC and butylate injury in corn. R-25788 differs from naphthalic anhydride in that 1) R-25788 is equally effective in preventing EPTC injury to corn when applied as a soil spray as well as a seed treatment, 2) no weed species examined to date are protected (5), 3) R-25788 is more effective than naphthalic anhydride in protecting corn from injury by acetanilide herbicides and high rates of EPTC and butylate (3).

Mechanism of Action of R-25788

R-25788 could protect corn from EPTC injury by 1) inhibiting uptake and distribution of EPTC within the plant, 2) reversing EPTC induced inhibition of lipid synthesis, 3) enhancing EPTC detoxication, 4) any combination of the above.

Chang et al. (6) examined the effects of R-25788 on the uptake and distribution of EPTC by corn seedlings, and found that R-25788 did not inhibit uptake of [^{14}C]-EPTC or affect the distribution of [^{14}C]-EPTC when measured over 1-7 days. In fact, greater rates of uptake of [^{14}C]-EPTC were reported upon treatment with R-25788.

Wilkinson and Smith (30) have demonstrated reversal of EPTC-induced inhibition of lipid synthesis in isolated spinach chloroplasts by R-25788. They suggest that R-25788 may act by reducing EPTC injury at a site of lipid synthesis.

Lay and Casida (19) have suggested that the mode of action of the protectant involves an increase in the rate of detoxication of the herbicide, via conjugation to glutathione. Specifically, they have

suggested that R-25788 acts by increasing the levels of GSH and GSH-S-transferase activity, and demonstrated these increases in corn following pretreatment with the protectant. They propose that plants sensitive to thiocarbamate herbicides are ones which lack initially higher GSH levels as well as the mechanism for synthesis of high GSH-S-transferase levels (20). The mechanisms by which R-25788 could increase GSH levels and GSH-S-transferase activity have not been elucidated.

Leavitt and Penner (22) have suggested that R-25788 protects corn primarily by stimulating sulfoxidation of EPTC, and as a result enhances its subsequent detoxication via conjugation to GSH. This hypothesis is supported by the work of Casida and co-workers (4) who have shown that corn treated with EPTC sulfoxide is not damaged, and that the EPTC sulfoxide is rapidly detoxified. This observation indicates that the levels of GSH or GSH-S-transferase activity cannot be the limiting factor, as even high levels of the sulfoxide can be detoxified. Thus, if EPTC is administered to corn, any sulfoxide formed can be detoxified, but sulfoxidation may not be fast enough to prevent EPTC from manifesting its toxicity. It follows then, that an R-25788 stimulated increase in levels of GSH or GSH-S-transferase activity in corn would not be critical in protecting corn.

CGA-43089

A new plant protectant, [α -(cyanomethoximino)-benzacetone nitrile], known as CGA-43089 and trademarked Concep has recently been registered for use as a seed treatment in sorghum against metolachlor herbicide injury. Existing herbicides used for sorghum have not provided

sufficiently broad spectrum weed control. Broad spectrum herbicides, such as metolachlor, injured sorghum at rates required for effective weed control. Seed treatment with CGA-43089 protected sorghum from metolachlor injury whether applied as a seed treatment or soil spray (9). However, since CGA-43089 is not specific for sorghum, and provides protection to a limited number of weed species (26), it is applied as a seed coating.

Presently, nothing is published explaining the mechanism of action of CGA-43089 in sorghum. The site of uptake of CGA-43089 has been shown to be the shoot zone (26). No protection to metolachlor is obtained when CGA-43089 is applied to the roots (26). Since metolachlor is also taken up in the shoot zone (10), the site of action of CGA-43089 may also be in the coleoptile of young seedlings.

MON-4606

In 1980, a safening agent [5-thiazolecarboxylic acid, benzyl ester, 2-chloro-4-(trifluoromethyl)] known as MON-4606 and trademarked Screen was discovered that will allow the use of alachlor on sorghum with a commercial level of selectivity (24). This safening agent can be applied as a seed treatment or in-furrow granule. Currently, there is no published data concerning the mode of action of MON-4606 in sorghum.

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CHAPTER 1

EVALUATION OF CGA-43089 AS A POTENTIAL ANTIDOTE AGAINST SELECTED HERBICIDES FOR SORGHUM (SORGHUM VULGARE PERS.)

Abstract

Seed treatment with CGA-43089 [α -(cyanomethoximino)-benzacetone nitrile] provided protection to sorghum (Sorghum vulgare Pers.) against the herbicides metolachlor [2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide], alachlor [2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide], diethatyl [N-(chloroacetyl-N-(2,6-diethylphenyl)glycine], ethofumesate [(+)-2-ethoxy-2,3-dihydro-3,3-dimethyl-5-benzofuranyl methanesulfonate], butylate + R-25788 [(S-ethyl diisobutylthiocarbamate) + (N,N-diallyl-2,2-dichloroacetamide)], and atrazine + metolachlor [2-chloro-4-ethylamino-6-isopropylamino-s-triazine + 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide] under greenhouse conditions. CGA-43089 did not protect sorghum from the phytotoxic effects of diphenamid [N,N-dimethyl-2,2-diphenylacetamide], EPTC [S-ethyl dipropylthiocarbamate], EPTC + R-25788 [S-ethyl dipropylthiocarbamate + N,N-diallyl-2,2-dichloroacetamide], pronamide [3,5-dichloro-(N-1,1-dimethyl-2-propynyl)benzamide], metribuzin [4-amino-6-tert-butyl-3-(methylthio)as-triazin-5(4H)-one, or buthidazole {3-[5-(1,1-dimethyl-ethyl)-1,3,4-thiadiazol-2-yl]-4-hydroxy-1-methyl-2-imidazolidinone}.

Under field conditions, CGA-43089 protected sorghum against high rates of metolachlor, alachlor, diethatyl, and atrazine + metolachlor (4.5, 6.7, 6.7, and 3.4 + 4.2 kg/ha respectively), and low rates of

ethofumesate and butylate + R-25788 (1.7 and 3.4 kg/ha respectively). Both forage and grain yields were significantly increased when sorghum was protected from herbicide damage by CGA-43089 compared with those plants not receiving the antidote.

Introduction

Numerous compounds have been examined for antidotal activity since the discovery by Hoffmann (2) that 4'-chloro-2-hydroxy-imino-acetanilide selectively protects wheat (Triticum aestivum L.) from injury caused by subsequent foliar applications of barban. Two compounds that have been evaluated as antidotes to protect sorghum against herbicide injury are NA (1,8-naphthalic anhydride) and R-25788 (N,N-diallyl-2,2-dichloroacetamide). Jordan and Jolliffe (3) and Rains and Fletchall (4) found that NA provided substantial protection to sorghum from high rates of alachlor. Spotanski and Burnside (5) confirmed in a number of field experiments that alachlor could be used for the selective control of annual grasses in sorghum provided the seeds were coated with NA. R-25788 as a tank mix did not protect sorghum from herbicide damage; however, seeds treated with a wettable powder formulation of R-25788 provided limited protection against both alachlor (5) and EPTC (1).

Sorghum seed treatment with CGA-43089 has been introduced as a protective measure against metolachlor injury. In addition to sorghum, rice, wheat, and proso millet have shown some increased tolerance to metolachlor following CGA-43089 seed treatment, but not to the extent obtained with sorghum. Some weeds, exemplified by Brachiaria plantaginea [(Link) Hitchc.] and Eleusine indica have also exhibited

increased tolerance to metolachlor following treatment of the seeds with the antidote.

Materials and Methods

Greenhouse Study

Plants were grown in greenhouse soil (1:1:1 soil, sand, peat) in 946 ml waxed cups. Formulated emulsifiable concentrates of the herbicides were sprayed on the surface of soil contained in 26 by 20 by 6 cm aluminum foil trays using a link belt sprayer at 2.1 kg/cm² pressure in 376 L/ha spray volume. The herbicide treated soil in these aluminum foil trays was placed in a rotary mixer and incorporated for 1 min. This herbicide incorporated soil was placed on top of untreated soil to a depth of 5.0 cm. The antidote-treated seed was provided by Ciba-Geigy and contained 1.25 g active ingredient of CGA-43089/kg seed prepared by spraying a concentrated formulation (2.09 F) of the antidote on seed rotating in a roller mill apparatus. The untreated seed was also provided by Ciba-Geigy and was similar in every respect to the treated seed except that it had not been treated with CGA-43089. Six antidote-treated or untreated Funk's G499 sorghum seeds were planted 25 cm deep into the soil of each cup. After planting, the cups were placed in a greenhouse with supplementary high pressure sodium lighting (240 $\mu\text{E m}^{-2}\text{s}^{-1}$) to give a 16 h day. Temperature ranged from 20 C at night to 33 C during the day. All plants were fertilized daily with a 150 ppm concentration of a commercial fertilizer testing 20:20:20 for NPK in a volume of water equal to that necessary to keep the plants fully turgid.

Thirty days after planting, the sorghum plants were photographed, harvested, and fresh weights determined. The data are expressed as g fresh weight per cup, and are the means of two experiments with four replications per experiment. A completely randomized design was used.

Field Study

In 1979, CGA-43089 treated and untreated sorghum seeds were planted the fourth week in June in 76 cm rows 6 m long at a rate of 2.3 kg/ha. Soil texture was a sandy clay loam with an organic matter content of 2.5%. The herbicides metolachlor, alachlor, diethatyl, ethofumesate, and butylate + R-25788 were applied both preplant incorporated and preemergence on the surface. Herbicides were incorporated twice to a depth of 5 cm using a spring tooth harrow. Plants were harvested 14 weeks after planting. Plant fresh weights and seed head dry weights were the parameters measured.

In 1980, CGA-43089 treated and untreated sorghum seeds were planted June 11 in 76 cm rows 7.6 m long at a rate of 2.3 kg/ha. The soil was a clay loam with an organic matter content of 2.8%. The herbicides metolachlor, alachlor, diethatyl, ethofumesate, butylate + R-25788, and atrazine + metolachlor were applied both preplant incorporated and preemergence. Herbicides were incorporated twice to a depth of 5 cm with a spring tooth harrow. Sorghum plants were harvested 18 weeks after planting. The parameters measured were plant fresh weights and seed head dry weights.

In all the treatments, herbicides were applied with a tractor mounted sprayer which traveled 6.4 km/h and delivered 234 L/ha at a pressure of 2.1 kg/cm². During both years of field tests, the study

was laid out in a split-split plot design to examine no antidote versus antidote treatment and preplant incorporated versus preemergence treatments. Each treatment was replicated three times.

Results and Discussion

In greenhouse tests CGA-43089 provided significant protection to sorghum from the phytotoxic effects of the herbicides metolachlor, alachlor, diethatyl, butylate + R-25788, and ethofumesate as measured by fresh weight (Table 1). CGA-43089 did not protect sorghum from injury caused by diphenamid, EPTC, pronamide, metribuzin, or buthidazole (Table 1). Propachlor did not injure sorghum even at relatively high use rates, and the addition of the antidote thus did not provide additional protection (Table 1).

In field studies CGA-43089 protected sorghum against high rates of metolachlor, alachlor, diethatyl, and atrazine + metolachlor, and low rates of ethofumesate and butylate + R-25788 (Tables 2-5). Sorghum receiving herbicide + CGA-43089 showed significantly less visible injury (data not presented) and had greater fresh weights (Table 2-5) and yield (Tables 2-5) than sorghum which did not receive the antidote. In all studies, the antidote alone did not significantly alter either the fresh weight or grain production of the sorghum (Tables 2-5).

In the 1979 field study, when the herbicides were applied preplant incorporated, use of the antidote resulted in significantly greater fresh weight production of sorghum exposed to the intermediate and high rates of the acetanilide herbicides metolachlor, alachlor, and diethatyl (Table 2). Sorghum was not damaged severely enough at

the low rates of these acetanilide herbicides that use of the antidote resulted in a significant increase in fresh weight production (Table 2). Against the non-acetanilide herbicides, CGA-43089 offered significant protection only against the low rate of butylate + R-25788 (Table 2). In terms of seed head yield, CGA-43089 offered significant protection to sorghum from all the acetanilide herbicides at all the rates tested (Table 2). Against the non-acetanilide herbicides, use of the antidote resulted in significantly higher seed head yield only against the low rate of butylate + R-25788 (Table 2).

In the 1979 preemergence study, use of the antidote resulted in significantly greater sorghum fresh weight production and seed head yield against all of the acetanilide herbicides at all the rates tested, except for the high rate of diethatyl (Table 3). Concerning the non-acetanilide herbicides, use of CGA-43089 resulted in a significant increase in sorghum fresh weight production and seed head yield against the low rate of butylate + R-25788 (Table 3). Use of CGA-43089 also resulted in a significant increase in seed head yield against the low rate of ethofumesate (Table 3).

In the 1980 preplant incorporated study, use of CGA-43089 resulted in significantly greater sorghum fresh weight production against the high rates of metolachlor and diethatyl and all rates of alachlor (Table 4). Against the other three herbicides tested, use of CGA-43089 resulted in significant increases in fresh weight against the high rate of ethofumesate and atrazine + metolachlor (Table 4). In terms of seed head yield, the antidote offered significant protection to all the acetanilide herbicides at all the rates tested except the low rates of metolachlor and diethatyl (Table 4). Use of CGA-43089

also resulted in significant increases in seed head yield against the high rates of ethofumesate and atrazine + metolachlor (Table 4).

In the 1980 preemergence study, use of CGA-43089 resulted in significant increases in fresh weight production and seed head yield against all rates of all herbicides tested (Table 5).

During both years' field studies, the antidote provided greater protection when the herbicides were applied preemergence rather than preplant incorporated (Table 2-5). This is probably related to the heavy rains which occurred shortly after spraying both years, causing the incorporated herbicides to leach through the soil. Since those plants in the preplant incorporated trial were not exposed to high enough levels of herbicide to cause severe injury, the protecting effect of the antidote was not as evident.

CGA-43089 offered significant protection to sorghum from three classes of herbicides, namely the acetanilides metolachlor, alachlor, and diethatyl, the thiocarbamate herbicide butylate + R-25788 and the herbicide ethofumesate. However, there were differences in the relative efficacy of CGA-43089 to reduce phytotoxic damage dependent upon the class of herbicide. CGA-43089 was most effective in protecting sorghum from injury against the three acetanilide herbicides metolachlor, alachlor, and diethatyl, and less effective against butylate + R-25788 and ethofumesate. The herbicides metolachlor, alachlor, diethatyl, butylate + R-25788, and ethofumesate all inflict similar injury symptoms in sorghum. The characteristic symptoms often include abnormal leaf emergence from the coleoptile, leaves that don't unroll normally, or leaves that emerge twisted and stunted. Since the three classes of herbicides all cause similar injury symptoms at

approximately the same developmental stage in sorghum seedlings, it may be that the antidote acts at a similar site of action, and differences in the relative efficacy of CGA-43089 protection may be due to specific differences in the nature and structure of the herbicides in each class.

TABLE 1. Antidote potential of CGA-43089 - Greenhouse data^a.

Herbicide	Rate	Fresh weight	
		-antidote	+antidote
	(kg/ha)	(gm/pot)	
Control		39.2a	39.8a
Metolachlor	2.8	7.6e	28.3abc
Alachlor	5.6	1.2e	25.0bc
Diethatyl	5.6	0.9e	33.2ab
Butylate + R-25788	5.6	1.1e	19.4cd
Ethofumesate	2.8	1.3e	33.2ab
Diphenamid	4.5	9.4de	10.2de
EPTC	2.8	0 e	1.4e
Pronamide	2.8	29.9abc	22.9bc
Propachlor	6.7	39.5a	33.9ab
Metribuzin	2.8	0 e	0 e
Buthidazole	1.7	0 e	0 e

^aMeans followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

TABLE 2. Antidote potential of CGA-43089 - Field data for 1979 herbicides applied preplant incorporated.

Herbicide	Rate (kg/ha)	Fresh weight (kg/6.1 m row)		Seed head yield (gm/6.1 m row)	
		-antidote	+antidote	-antidote	+antidote
Control		15.3a	14.7ab	1576ab	1660a
Metolachlor	2.2	7.7d-i	13.2a-d	472g-h	1242bc
	3.4	5.7e-j	13.6abc	227h-k	972cde
	4.5	4.6g-j	11.6a-d	150jk	921c-f
Alachlor	2.2	9.1b-h	13.3a-d	606e-h	1129cd
	4.5	4.2g-j	11.6a-d	181i-k	862c-g
	6.7	1.6j	10.6a-f	32k	661e-g
Diethaty1	2.2	8.4c-h	10.8a-f	556f-i	1121c
	4.5	3.7h-j	11.0a-e	327h-k	735d-g
	6.7	4.8g-j	11.5a-d	188i-k	806d-g
Ethofumesate	1.7	11.5a-d	13.2a-d	832d-g	1106cd
	3.4	8.3c-h	8.7c-h	479g-j	852c-g
Butylate + R-25788	3.4	3.8h-j	9.4b-g	252h-k	811d-g
	6.7	1.5j	5.3f-j	84jk	337h-k

^aMeans followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

TABLE 3. Antidote potential of CGA-43089 - Field data for 1979 herbicides applied preemergence

Herbicide	Rate (kg/ha)	Fresh weight (kg/6.1 m row)		Seed head yield (gm/6.1 m row)	
		-antidote	+antidote	-antidote	+antidote
Control		13.4ab	12.7ab	1601a	1598a
Metolachlor	2.2	2.2h	12.3ab	105h	1261ab
	3.4	1.4i	12.3ab	70h	1008bc
	4.5	0.2i	7.9b-g	2h	532d-g
Alachlor	2.2	2.7g-i	14.7g	113h	1611a
	4.5	1.2i	13.0ab	47h	1293ab
	6.7	1.3i	9.6a-d	17h	658c-f
Diethatyl	2.2	3.6fg	10.1a-c	265gh	1010bc
	4.5	1.7i	10.2a-c	73h	717c-e
	6.7	0.7i	5.0c-i	8h	258gh
Ethofumesate	1.7	4.0e-g	8.7b-f	335e-h	814cd
	3.4	0.0i	2.3hi	0h	166gh
Butylate + R-25788	3.4	3.5fg	10.7ab	238gh	873cd
	6.7	1.6i	4.4d-i	99h	289f-h

^aMeans followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

TABLE 4. Antidote potential of CGA-43089 - Field data for 1980 herbicides applied preplant incorporated

Herbicide	Rate (kg/ha)	Fresh weight (kg/7.6 m row)		Seed head yield (g/15.2 m row)	
		-antidote	+antidote	-antidote	+antidote
Control		15.7a-g	15.9a-h	7467a-c	7149a-d
Metolachlor	2.2	17.0a-g	17.4a-g	6984a-e	7798a-c
	3.4	13.6d-i	17.8a-f	5131d-g	7425a-c
	4.5	12.3h-j	20.6a	3977gh	8113ab
Alachlor	2.2	11.8g-h	19.6ab	3895	8288ab
	4.5	7.6jk	20.7a	2815hi	8028ab
	6.7	6.4k	20.2a	1724i	8708ab
Diethatyl	2.2	15.5a-h	17.0a-g	6383b-e	7224a-d
	4.5	13.2e-i	18.2a-f	4927e-h	7450a-c
	6.7	12.4g-h	18.5a-f	4232f-h	7195a-d
Ethofumesate	1.7	13.1f-i	16.8a-h	5578c-g	7163a-d
	3.4	10.3i-k	18.8a-d	4103f-h	7727a-c
Butylate + R-25788	2.2	16.9a-h	15.5a-h	6825a-e	6166b-f
	4.5	14.7b-i	17.8a-f	5109d-g	6832a-e
Atrazine + Metolachlor	2.2 + 2.8	14.0c-i	17.8a-f	5186d-g	7140a-d
	3.4 + 4.2	7.6jk	18.8a-d	2838hi	7688a-c

^aMeans followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

TABLE 5. Antidote potential of CGA-43089 - Field data 1980 herbicides applied preemergence

Herbicide	Rate (kg/ha)	Fresh weight (kg/7.6 m row)		Seed head yield (gm/15.2 m row)	
		-antidote	+antidote	-antidote	+antidote
Control		18.1ab	15.9bc	6327a-c	6873a-c
Metolachlor	2.2	8.2c-f	19.6ab	1306d-f	8448ab
	3.4	6.6c-f	19.3ab	1758d-f	7586a-c
	4.5	7.1c-f	19.5ab	1573d-f	7852ab
Alachlor	2.2	4.5d-g	19.8ab	1283d-f	8763a
	4.5	2.3e-g	20.0ab	583f	8336ab
	6.7	1.7fg	22.7a	207f	8109ab
Diethatyl	2.2	9.4c-f	20.0ab	3089de	7790ab
	4.5	8.2c-f	20.5ab	2090d-f	7598a-c
	6.7	5.0e-g	20.8ab	1514d-f	7724ab
Ethofumesate	1.7	8.7c-f	18.0ab	3207d	7440a-c
	3.4	2.0fg	18.1ab	575f	6619a-c
Butylate + R-25788	2.2	7.5c-f	19.2ab	1936d-f	5421c
	4.5	1.0g	11.5c-e	106f	2824de
Atrazine + Metolachlor	2.2 + 2.8	6.5c-f	18.9ab	1521d-f	7788ab
	3.4 + 4.2	2.5e-g	19.0ab	995ef	7612a-c

^aMeans followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

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CHAPTER 2

FACTORS INFLUENCING EFFICACY OF CGA-43089

Abstract

The protective action of the herbicide antidote CGA-43089 [α -(cyanomethoximino)-benzacetone nitrile] on sorghum (Sorghum vulgare Pers.) against metolachlor [2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide] did not require light. The protective action of the antidote was not evident when sorghum was grown under flooded conditions. Untreated sorghum plants were not protected from metolachlor injury by the lateral displacement of CGA-43089 from treated seedlings growing in close proximity, unless the two types of seed were placed immediately adjacent to each other. Storage of antidote-treated sorghum seed for 11 months or more reduced germination.

Introduction

Environmental factors that influence herbicide efficacy also have the potential to affect antidote action. In an unpublished report, Ciba-Geigy indicated that the protective action of CGA-43089 involved light and the phytochrome system of sorghum. Although Nyffeler et al. (3) reported that the protective activity of CGA-43089 on sorghum against metolachlor was not significantly influenced by soil moisture, Ketchersid and Merkle (2) have reported that under extremely wet environmental conditions, the effectiveness of CGA-43089 in protecting sorghum may be reduced. Nyffeler et al. (3) have also reported that some weeds like alexandergrass [Brachiaria plantaginea, (Link)

A. Hitch], Eleusine spp., and itchgrass (Rottboellia exaltata L.) exhibit increased tolerance to metolachlor following exposure of seeds to CGA-43089. Therefore, possible displacement of CGA-43089 from treated sorghum seeds could potentially impart undesirable protection to weed seedlings against metolachlor.

Treatment of seeds with the herbicide antidote 1,8-naphthalic anhydride has resulted in decreased germination with some species (1). Since CGA-43089 is applied as a seed treatment, the antidote potentially could affect sorghum seed germination.

The purpose of this study was to 1) determine whether light was a prerequisite for antidote action, 2) determine if flooded conditions influence antidote activity, 3) evaluate potential displacement of CGA-43089 from treated sorghum seed which could impart undesirable weed seedling tolerance to metolachlor, 4) determine the effect of CGA-43089 on sorghum seed germination.

Materials and Methods

The soil used in the light requirement and displacement experiments was a greenhouse soil mix (1:1:1 soil, sand, peat). A formulated emulsifiable concentrate of metolachlor was sprayed on the surface of soil placed in 27 by 20 by 6 cm aluminum foil trays with a link belt sprayer at 2.1 kg/cm² pressure with 376 L/ha spray volume. The herbicide-treated soil in the trays was placed in a mechanical mixer and incorporated for 1 min. This herbicide-treated soil was placed in 454 ml double-waxed cups and sorghum seeds were planted 2.0 cm deep. The antidote-treated seed was provided by Ciba-Geigy and contained 1.25 g active ingredient of CGA-43089/kg seed prepared

by spraying a concentrated formulation (2.09 F) of the antidote on seed rotating in a roller mill apparatus. Ciba-Geigy also provided the untreated sorghum seed, and it was the same in every respect to the treated seed except that it had not been treated with the antidote. In the light requirement study, ten untreated or antidote-treated sorghum seeds were planted in each cup. In the displacement study, three treated sorghum seeds were planted, and then four untreated sorghum seeds were planted 1 cm, 0.5 cm, or adjacent to each of the three treated sorghum seeds. Plants in the light requirement study were placed in a dark chamber with temperatures ranging from 25 C at night to 30 C during the day. Plants in the displacement study were placed in a greenhouse supplemented with high-pressure sodium lighting ($240 \mu\text{E m}^{-2}\text{sec}^{-1}$) to give a 16 h day with temperatures ranging from 23 C at night to 33 C during the day. Plants in the light requirement study were not fertilized while those in the leaching study were fertilized daily with a commercial fertilizer testing 20:20:20 for NPK at 150 ppm in that volume of water necessary to keep the plants turgid. Seven days after planting, photographed seedlings were harvested, and fresh weights recorded in the light requirement study. The data presented are the means of two experiments with four replications per experiment. The displacement study was repeated three times, and photographs taken 10 days after planting. Both the light and displacement studies were conducted with a completely randomized design.

For the experiment designed to determine the effect of flooding on CGA-43089 activity, the antidote-treated and untreated sorghum seeds were grown in vermiculite until the seedlings were approximately 2 cm

tall. These plants were then transferred to 12 ml test tubes holding 8 ml half-strength Hoagland's No. 1 solution containing either 0, $1 \times 10^{-3}M$, $1 \times 10^{-4}M$, $1 \times 10^{-5}M$, or $1 \times 10^{-6}M$ formulated emulsifiable metolachlor. In one study, the incubation solution was changed daily, in another the tubes were supplemented with the appropriate solution only to replace losses due to evaporation or transpiration. In follow-up studies, 2-fold and 3-fold supplements of technical grade CGA-43089 were also added to culture tubes. The experiment was repeated three times, and photographs were taken 7 days after growing in the culture solution.

Seeds tested in the germination study were stored at ambient temperature for eleven months following treatment with CGA-43089. The seeds were placed in an incubation chamber in the dark at 27 C. After 6 days, the percentage of germinated seeds was determined. The data presented are the means of three experiments with ten replications per treatment.

Results and Discussion

The protective action of CGA-43089 did not require light (Table 1). Antidote-treated sorghum seedlings grown in a dark chamber in the presence of metolachlor had significantly greater fresh weight than sorghum not receiving the antidote (Table 1). Sorghum seedlings were not damaged by metolachlor even at relatively high use rates (e.g. up to 4.5 kg/ha) when grown in the dark (data not presented). Only at very high rates of metolachlor, 6.7 kg/ha, 9.0 kg/ha, 11.0 kg/ha, and 13.1 kg/ha was there sufficient injury so that the protective effect of the antidote was evident (Table 1).

The protective action of the antidote was not evident when sorghum was grown under flooded conditions (Figure 1). Antidote-treated sorghum seedlings growing in half-strength Hoaglands No. 1 solution supplemented with $1 \times 10^{-3}M$, $1 \times 10^{-4}M$, $1 \times 10^{-5}M$, or $1 \times 10^{-6}M$ metolachlor were compared photographically with those growing in a similar solution culture, but not treated with CGA-43089 (Figure 1). In both antidote-treated and untreated seedlings, plant growth was stunted and abnormal in direct relation to the concentration of metolachlor included in the nutrient solution. To insure that the loss of protection to sorghum was not the result of diluting out the antidote in the nutrient solution, additional 2-fold or 3-fold supplements of the antidote were added to each culture tube. Even with these additional 2-fold or 3-fold supplements of the antidote, sorghum was not protected from metolachlor injury.

Untreated sorghum plants were not protected from metolachlor injury by the lateral displacement of CGA-43089 from treated seedlings when seeds were separated by 1 cm and 0.5 cm distances (Figure 2). However, there was sufficient displacement from treated sorghum seeds to provide protection to untreated sorghum when these two types of seeds were planted adjacent to each other (Figure 2). Since only a very limited number of weeds species have been shown to be protected by CGA-43089 (3), and only those seeds in direct physical contact with antidote-treated seed are protected, it is unlikely that CGA-43089 would offer undesirable protection to weed seedlings.

In petri plate germination tests, sorghum seeds treated with antidote and stored for more than 11 months at ambient temperature had only 62.5% germination compared with untreated seeds that had 90%

germination. This represents a 28% loss in germination due to the storage of antidote-treated seed.

TABLE 1. Effect of light on efficacy of CGA-43089 in protecting sorghum against metolachlor injury^a

Metolachlor rate (kg/ha)	Light	Antidote	Fresh weight (g/10 plants)
0	+	-	1.58 a
0	+	+	1.47 a
0	-	-	1.53 a
0	-	+	1.88 a
6.7	+	-	.84 b
6.7	+	+	1.55 a
6.7	-	-	.77 b
6.7	-	+	1.67 a
9.0	+	-	.73 b
9.0	+	+	1.48 a
9.0	-	-	.74 b
9.0	-	+	1.51 a
11.0	+	-	.64 b
11.0	+	+	1.47 a
11.0	-	-	.59 b
11.0	-	+	1.22 a
13.1	+	-	.60 b
13.1	+	+	1.31 a
13.1	-	-	.64 b
13.1	-	+	1.32 a

^aMeans followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

FIGURE 1. Top Photo: Sorghum growing in half-strength Hoagland's No. 1 nutrient solution. The two tubes on the left contain untreated plants, and the two tubes on the right contain antidote-treated seedlings.

Bottom Photo: Sorghum growing in half-strength Hoagland's No. 1 nutrient solution supplemented with 1×10^{-4} M metolachlor. The two sets of plants on the left are untreated, and the two sets on the right have CGA-43089 treatment.

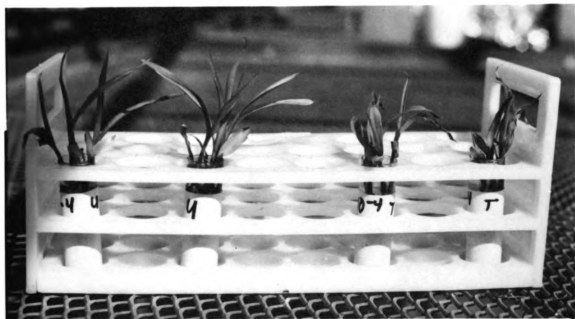
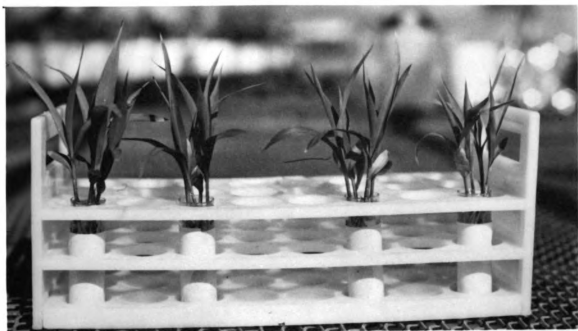


FIGURE 1.

FIGURE 2. Top Photo:

Container on the left holds three treated sorghum seeds, each surrounded by four untreated sorghum seeds planted 0.5 cm away. Soil was not sprayed with herbicide.

Container on the right holds sorghum planted in similar fashion. Soil was sprayed with 1×10^{-5} M metolachlor.

Bottom Photo:

Container on the left holds three treated sorghum seeds, each surrounded by four untreated sorghum seeds planted adjacent. Soil was not sprayed with herbicide.

Container on the right holds sorghum planted in similar fashion. Soil was sprayed with 1×10^{-5} M metolachlor.



FIGURE 2.

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CHAPTER 3

INFLUENCE OF CGA-43089 ON METOLACHLOR ABSORPTION, TRANSLOCATION, AND METABOLISM IN SORGHUM (SORGHUM VULGARE PERS.)

Abstract

CGA-43089 [α -(cyanomethoximino)-benzacetone nitrile] did not protect sorghum from metolachlor injury via reduced metolachlor absorption or retention. After germinating 24 h in $10^{-5}M$ ^{14}C -metolachlor, CGA-43089 treated sorghum seeds absorbed 36% more ^{14}C -metolachlor than untreated seeds. CGA-43089 treated seeds retained 78% of the radioactivity detected as parent metolachlor and 22% of the radioactivity as a polar metabolite, while untreated seeds converted 50% of the parent herbicide to a polar metabolite.

The protective action of CGA-43089 did not involve alterations in translocation of metolachlor to the site of action. Sorghum seedlings grown from seed treated with CGA-43089 translocated ^{14}C -metolachlor similarly to untreated seedlings.

The protective effects of CGA-43089 did not appear to involve increased rates of metolachlor metabolism. CGA-43089 treated sorghum seedlings exposed to ^{14}C -metolachlor 24 h or 5 days following germination absorbed more ^{14}C -metolachlor and metabolized the ^{14}C -metolachlor at the same rate or less rapidly than unprotected seedlings.

These results suggest that the protective effects of CGA-43089 may be due to factors other than reduced herbicide absorption, modified herbicide translocation, or increased rates of metolachlor, but may

involve other factors, such as interfering with the mechanism of herbicide action.

Introduction

CGA-43089 may protect sorghum against herbicide injury by reducing herbicide absorption, modifying translocation of herbicide to the site of action, altering herbicide metabolism or interacting with the mechanism of herbicide action.

Ketchersid and Merkle (2) suggested that less metolachlor was absorbed by antidote-protected sorghum coleoptiles than by non-protected coleoptiles grown under identical soil conditions. They found that disappearance of metolachlor from an incubation medium was slower in the presence of CGA-43089 indicating that absorption of the herbicide was decreased. They further found that metolachlor was rapidly metabolized in sorghum, and suggested that CGA-43089 might protect by decreasing the rate of absorption sufficiently to prevent phytotoxic accumulation at the site of action.

Currently, there are no published reports available which explain the protective effects of CGA-43089 in sorghum in terms of altered herbicide translocation or its effects on herbicide metabolism.

The purpose of this study was to determine whether the protective effects of CGA-43089 is due to reduced herbicide absorption, modified translocation of herbicide to the site of action, or increased herbicide metabolism.

Materials and Methods

The absorption study was conducted to determine whether metolachlor absorption was affected by treating sorghum seeds (Funk's

G623) with CGA-43089. The antidote-treated seed was provided by Ciba-Geigy and contained 1.25 g/active ingredient of CGA-43089/kg seed prepared by spraying a concentrated formulation (2.09 F) of the antidote on seed rotating in a roller mill apparatus. The untreated seed was also provided by Ciba-Geigy and was the same in every respect to the untreated seed except that it had not been treated with CGA-43089. Approximately 50 antidote-treated or untreated seeds were germinated at 27 C in 9 cm plastic petri plates lined with two sheets of Whatman No. 4 filter paper containing 10 ml of $1 \times 10^{-5}M$ uniformly ring-labeled ^{14}C -metolachlor (6.36 m Ci/m mole). After 24 h, 30 seeds were selected for uniformity of size and development from each germination plate. These seeds were frozen in liquid nitrogen and ground to a fine powder using mortar and pestle. These samples were extracted in 15 ml methanol:water (9:1) on a water bath shaker oscillating 60 times per minute at 25 C for 1 h in 30 ml test tubes positioned horizontally. After extraction, the residue was filtered with Whatman No. 1 filter paper, and the remaining supernatant fluid was evaporated to dryness under a nitrogen gas stream. The dried samples were redissolved in 0.5 ml methanol:water (9:1) and 15 μ l subsamples were radioassayed by liquid scintillation spectrometry. Ten μ l subsamples were spotted on 250 μ silica gel GF thin layer chromatography plates developed in hexane:chloroform:ethanol (70:20:10) and scanned for radioactivity. Results presented are the means of two experiments with four replications per experiment.

In the translocation study, six sorghum seeds were planted in 55 g of No. 7 silica sand in 40 ml centrifuge tubes. The plants were grown in a greenhouse supplemented with artificial lighting (240 μ

$\text{m}^{-2} \text{ s}^{-1}$) to give a 16 h day with temperature ranging from 23 C at night to 33 C during the day. The plants were watered daily with one-half strength Hoagland's solution. Ten days after planting, the sorghum seedlings were watered with one-half strength Hoagland's solution containing $1 \times 10^{-5} \text{ M}$ uniformly ring-labeled ^{14}C -metolachlor (6.23 m Ci/m mole). Twenty-four hours after the ^{14}C -metolachlor treatment, sorghum plants were removed from the centrifuge tubes, freeze-dried, and radioautographed. The experiment was conducted twice, with three replications per experiment.

The in vivo ^{14}C -metolachlor metabolism study was conducted with CGA-43089-treated and untreated sorghum seedlings to determine what influence the antidote may have in altering herbicide metabolism. Approximately 40 sorghum seeds were placed in 9 cm plastic petri plates lined with two sheets of Whatman No. 4 filter paper and filled with 10 ml of distilled water. Twenty-four hours after germination, twenty antidote-treated or untreated seedlings were selected from each plate based on uniformity of size and stage of germination. Three microliters of uniformly ring-labeled ^{14}C -metolachlor (6.36 m Ci/m mole) were placed on each seedling, including the radicle and emerging coleoptile. These seedlings were then placed on paper towels saturated with $1 \times 10^{-5}\text{M}$, $5 \times 10^{-5}\text{M}$, $1 \times 10^{-4}\text{M}$, $4 \times 10^{-4}\text{M}$, or $1 \times 10^{-3}\text{M}$ solutions of metolachlor. The seeds were rolled up in the towels and placed on edge in 200 ml beakers containing 50 ml of metolachlor of the same molarity as the herbicide saturated towel. Seeds were positioned approximately 5 cm above the level of herbicide solution in the beaker. Twelve hours, 24 h, or 72 h later the seedlings were frozen in liquid nitrogen and ground to a fine powder with mortar and

pestle. The samples were then extracted, filtered, dried, redissolved, and radioassayed by liquid scintillation spectrometry and scanning as described previously.

CGA-43089-treated and untreated sorghum seedlings growing in vermiculite under greenhouse conditions were used to study how the antidote influenced in vivo ^{14}C -metolachlor metabolism in older, more developed plants. The plants were supplemented with artificial lighting ($240 \mu \text{m}^{-2} \text{s}^{-1}$) to give a 16 h day with temperature ranging from 23 C at night to 33 C during the day. Plants were fertilized daily with a 150 ppm concentration of commercial fertilizer testing 20:20:20 for NPK. Immediately following emergence of the sorghum coleoptile above the vermiculite surface, 3 μl of uniformly ring-labeled ^{14}C -metolachlor (6.36 m Ci/m mole) was placed on the emerging coleoptile. Twenty-four or 72 h later, the sorghum plants were harvested, extracted, filtered, dried, redissolved, and radioassayed as described earlier. All experiments were conducted twice with three replications per experiment.

In studies utilizing in vitro mixed function oxidase preparations, antidote-treated and untreated sorghum plants were grown under the same conditions described in the metabolism study using more mature plants. Five or eight days after planting, sorghum seedlings were harvested, separated into roots and shoots, frozen with liquid nitrogen, and ground to a fine powder using mortar and pestle. Cold 0.1M phosphate buffer (pH 7.4)(2 ml/g plant material) was added to the frozen powder. This slurry was filtered through four layers of cheesecloth, and the liquid filtrate centrifuged for 30 minutes at 20,000 x g. The supernatant fluid was centrifuged a second time at

40,000 x g for 75 min. Seven ml aliquots of the supernatant fluid were placed into 15 ml test tubes containing 1 ml 40 μ M NADPH and 2 ml 1×10^{-5} M metolachlor. These samples were incubated on a water bath shaker oscillating 40 times per minute at 25 C in 15 ml test tubes positioned horizontally. One-half hour, 1 h, or 3 h later, the reaction was terminated by adding 2 ml toluene to each test tube and mixing on a water bath shaker for 15 minutes. Samples from both antidote-treated and untreated plants were then analyzed and compared for remaining parent metolachlor by gas chromatography employing an OV-1 column at 170 C. The mixed function oxidase study was repeated four times, with three replications per experiment.

Results and Discussion

After germinating 24 h in 10^{-5} M 14 C-metolachlor, CGA-43089 treated sorghum seeds absorbed 36% more radiolabeled herbicide than untreated seeds. CGA-43089 treated seeds retained 78% of the radioactivity detected as a parent metolachlor (R_f 0.71), while untreated seed converted 50% of the parent herbicide to a polar metabolite (R_f 0.12) (Figure 1). These data indicate that CGA-43089 did not protect sorghum from metolachlor injury via reduced metolachlor absorption or retention. These results are in contrast to the conclusion of Ketchersid and Merkle (2) that CGA-43089 might protect sorghum by decreasing the rate of metolachlor uptake sufficiently to prevent a phytotoxic accumulation at the site of action. However, their conclusion was based on data measuring metolachlor absorption by sorghum coleoptiles rather than seed. Our data suggests that treatment of sorghum seed with CGA-43089 does not act as a physical

barrier reducing metolachlor absorption. This is consistent with the observation by Nyffeler et al. (3) that treatment of the shoot zone with CGA-43089 protected sorghum completely, even greater than offered by the standard seed dressing.

Sorghum seedlings grown from seed treated with CGA-43089 translocated ^{14}C -metolachlor no differently than untreated seedlings (Figure 2). This result indicates that the protective action of CGA-43089 did not involve alterations in translocation of metolachlor to the site of action. Non-involvement of CGA-43089 in metolachlor translocation is consistent with results obtained by Nyffeler et al. (3) and Gerber et al. (1) indicating that both metolachlor and CGA-43089 are most effective early in the development of the sorghum plant, between seed germination and emergence, and before translocation of herbicide over relatively long distances would become an important factor.

CGA-43089 treated sorghum seedlings exposed to ^{14}C -metolachlor 24 h following germination absorbed more ^{14}C -metolachlor and metabolized the radiolabeled herbicide no more rapidly than unprotected seedlings (Figures 3-6)(Table 1). Antidote-treated seedlings harvested 12 h, 24 h, or 72 h following ^{14}C -metolachlor treatment absorbed 37%, 31%, and 14% more ^{14}C -metolachlor, respectively, than unprotected sorghum seedlings (Table 1). Twelve hours following application of ^{14}C -metolachlor, both antidote-treated and untreated sorghum seedlings retained 74% of the radioactivity detected as ^{14}C -metolachlor, and metabolized 26% to a polar metabolite (Figure 3)(Table 1). As a result of combined absorption and metabolism, antidote-treated plants contained 59% more ^{14}C -metolachlor and radiolabeled polar metabolite than untreated sorghum (Table 1). Twenty-four hours following

application of ^{14}C -metolachlor, CGA-43089 treated seedlings retained 64% of the radioactivity as parent metolachlor and 36% as polar metabolite, but unprotected seedlings retained only 34% of the radioactivity as ^{14}C -metolachlor and metabolized 66% of the herbicide to a polar metabolite (Figure 4)(Table 1). As a result of both absorption and metabolism, CGA-43089 treated sorghum contained 2.7 fold more ^{14}C -metolachlor and 21% less radiolabeled polar metabolite than untreated sorghum (Table 1). Seventy-two hours following ^{14}C -metolachlor treatment, both antidote treated and untreated seedlings had metabolized approximately 83% of the ^{14}C -metolachlor to the polar metabolite, and retained approximately 17% of the radioactivity as parent metolachlor (Figure 5)(Table 1). In terms of combined absorption and metabolism, antidote-treated sorghum contained 16% more ^{14}C -metolachlor and radiolabeled polar metabolite than untreated sorghum (Table 1). A relatively consistent pattern of metolachlor metabolism occurred at all concentrations of herbicide tested, namely $1 \times 10^{-5}\text{M}$, $5 \times 10^{-5}\text{M}$, $1 \times 10^{-4}\text{M}$, $5 \times 10^{-4}\text{M}$, and $1 \times 10^{-3}\text{M}$, such that CGA-43089 treated sorghum seedlings did not show increased metabolism of ^{14}C -metolachlor compared with unprotected plants (Figure 6).

To further study the role of CGA-43089 in ^{14}C -metabolism, older more mature plants were used. Twenty-four and 72 h following ^{14}C -metolachlor application to emerging shoots of five-day old plants, there was little difference in ^{14}C -metolachlor metabolism between CGA-43089 treated and untreated sorghum plants (Figures 7-9). Twenty-four hours after application, both antidote-treated and untreated plants retained approximately 28% of the radioactivity detected as ^{14}C -metolachlor, approximately 11% as a metabolite with R_f 0.47, and

61% as a polar metabolite (Figure 7). In the 72 h study, both antidote-treated and untreated plants metabolized over 90% of radioactivity detected to a polar metabolite (Figures 8-9).

These results indicate that under these experimental conditions, CGA-43089 did not protect sorghum by increasing the rate of metolachlor metabolism. Further studies using in vitro mixed function oxidase preparations support the results of our metabolism studies. The rate of metolachlor disappearance from mixed function oxidase preparations isolated from CGA-43089 treated sorghum roots, shoots, or coleoptiles differed little from preparations isolated from untreated plants. This suggests that CGA-43089 did not alter metolachlor metabolism via an induction of sorghum mixed function oxidases.

In conclusion, the observed results indicate that treatment of sorghum seeds with CGA-43089 did not reduce herbicide absorption, modify translocation of herbicide to the target site, or increase metolachlor metabolism compared with untreated plants. These results suggest that the protective effect of CGA-43089 may be due to some other factor or set of factors, perhaps by interfering with the mechanism of herbicide action.

The suggestion that CGA-43089 may protect by interfering with the mechanism of herbicide action is supported by several observations. The herbicides for which the antidote offered protection were ones which affect, or have been reported to affect lipid synthesis and surface wax deposition, and except for ethofumesate, are alkyl amide herbicides. Excluding EPTC and propachlor, those compounds for which the antidote did not offer protection are herbicides not considered

to affect lipid synthesis or surface wax deposition, and are conjugated or ring amides.

TABLE 1. Radioactivity detected in twenty sorghum seedlings due to absorption, metabolism, and combined absorption and metabolism. Sorghum seedlings were treated with ^{14}C -metolachlor 24 h after germination, and extracted for radioassay 12 h, 24 h, or 72 h later.^{ab}

	Extraction Period	Absorption		Absorption % of Treated Seed	Metabolism		Combined Absorption and Metabolism	
		DPM Detected			% Polar Metabolite	% Parent Herbicide	DPM for Polar Metabolite	DPM for Parent Herbicide
Untreated Seed	12 h	2,386e		63*	26de	74ab	620ij	1765fg
	24 h	4,213d		69*	66bc	34d	278lde	1432gh
	72 h	9,307ab		86	83a	17ef	7725ab	1582fgh
Treated Seed	12 h	3,788d		100	26de	74ab	985i	2803de
	24 h	6,106c		100	36d	64bc	2198ef	3908c
	72 h	10,823a		100	83a	17ef	8983a	1840efg

^aMeans followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

^bAsterisks(*) within rows indicate significant differences at the 5% level between the means of measurements from untreated seedlings after extractions at 12, 24, and 72 h and those from treated seedlings determined by student's t-test.

FIGURE 1. Radioscans of thin-layer chromatograms of extracts from sorghum seed germinated in ^{14}C -metolachlor solution for 24 h. Scan of unprotected seeds on top, scan of CGA-43089 treated seeds on bottom. The polar metabolite has an R_f value of 0.12 and the parent metabolite an R_f value of 0.71. The developing system was Hexane:Chloroform:Ethanol (70:20:10).

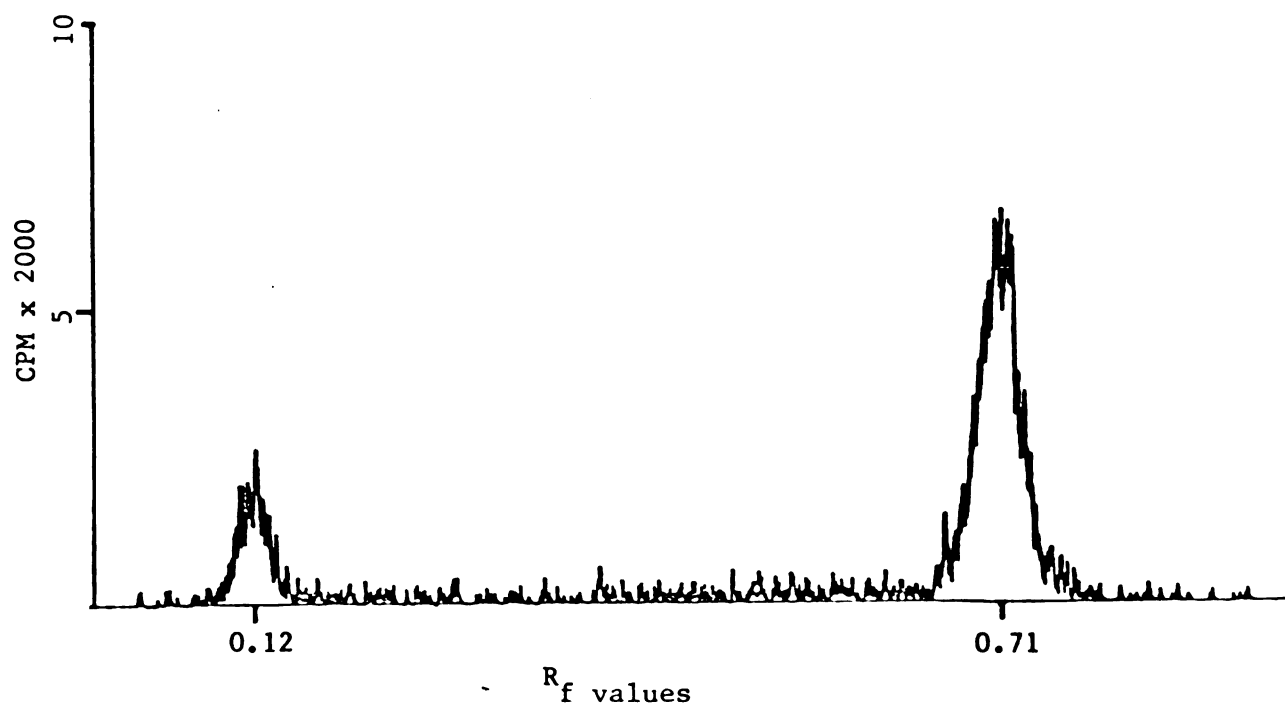
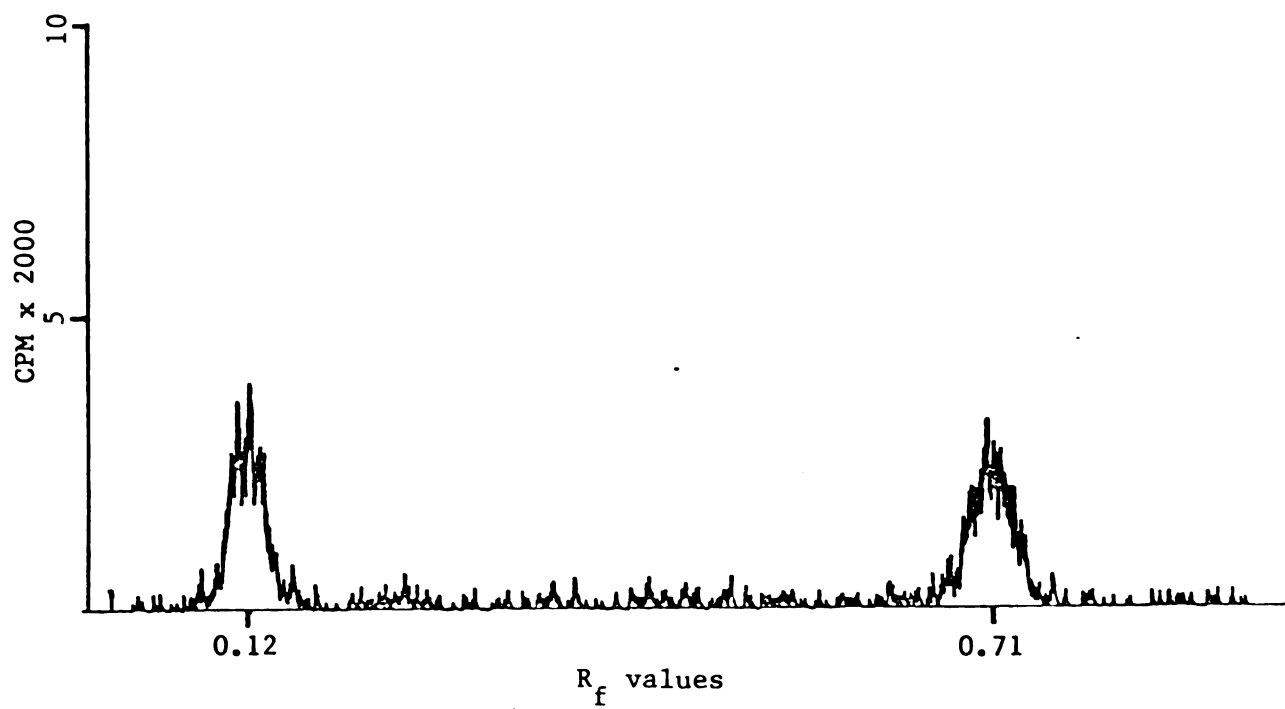


FIGURE 1.

FIGURE 2. Translocation of ^{14}C -metolachlor in sorghum seedling.
Unprotected plants are on the upper tier, CGA-43089 treated
plants on the lower tier.

FIGURE 3. Radioscans of thin-layer chromatograms of extracts from sorghum seedlings. Seedlings were treated with ^{14}C -metolachlor 24 h after initiation of germination, and extracts taken 12 h later. Scan of unprotected seedling on top, scan of CGA-43089 treated seedling on bottom. The polar metabolite has an R_f value of 0.12 and the parent metolachlor an R_f value of 0.71. The developing system was Hexane:Chloroform:Ethanol (70:20:10).

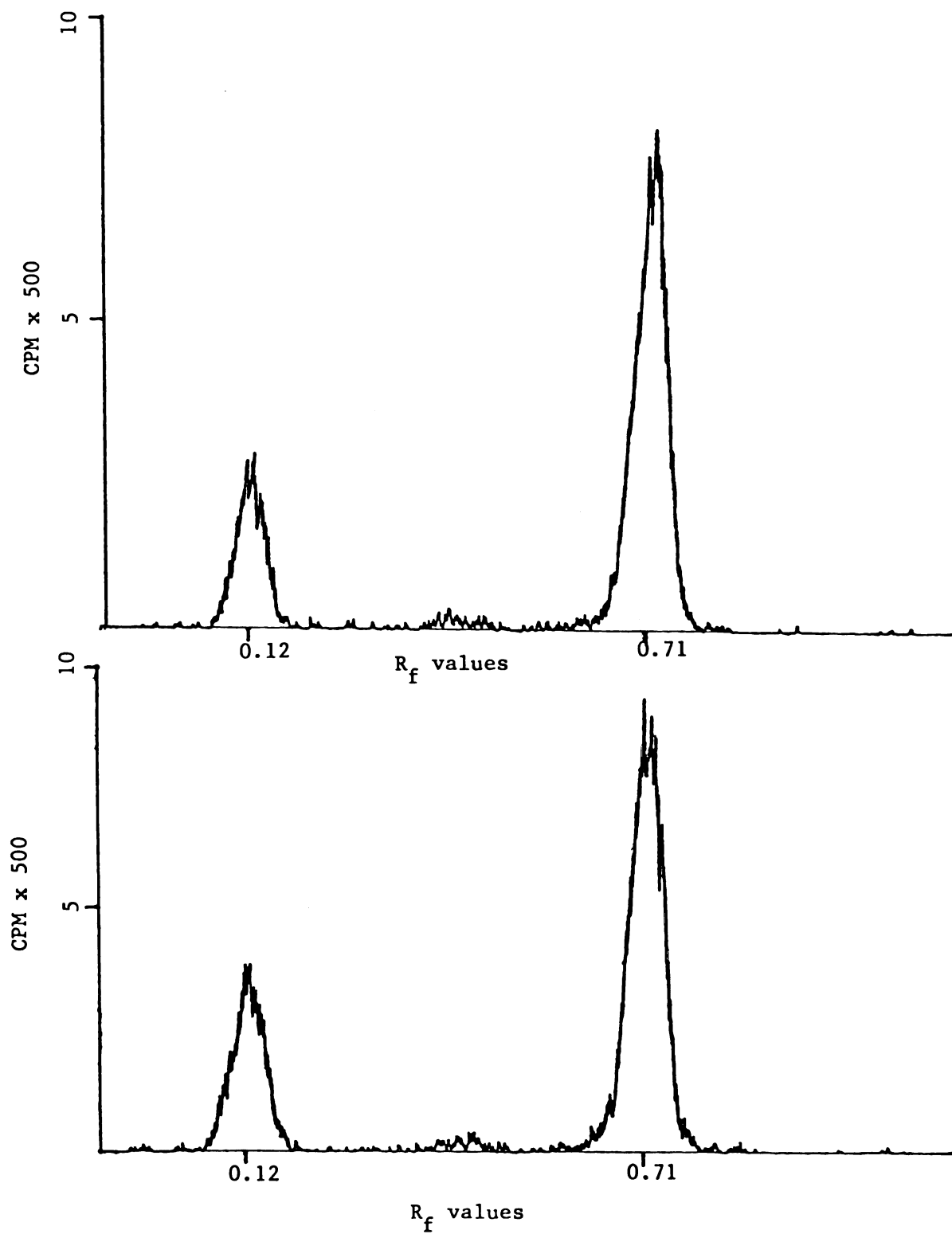


FIGURE 3.

FIGURE 4. Radioscans of thin-layer chromatograms of extracts from sorghum seedlings. Seedlings were treated with ^{14}C -metolachlor 24 h after initiation of germination, and extracts taken 24 h later. Scan of unprotected seedlings on top, scan of CGA-43089 treated seedlings on bottom. The polar metabolite had an R_f value of 0.12 and the parent metolachlor an R_f value of 0.71. The developing system was Hexane:Chloroform:Ethanol (70:20:10).

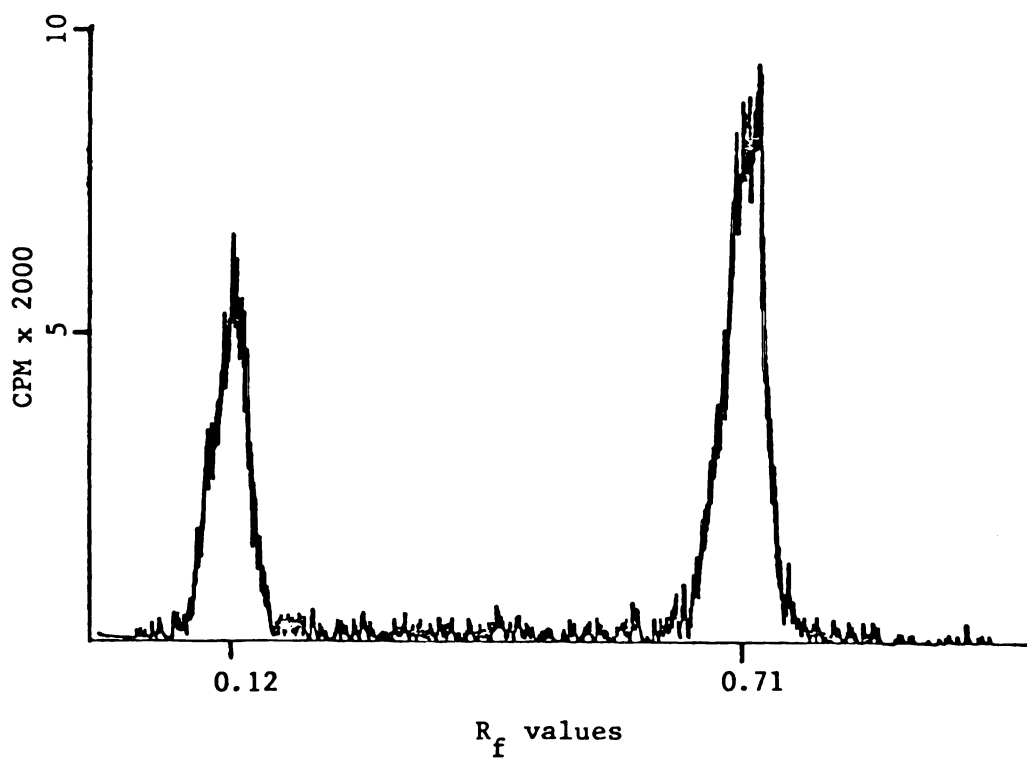
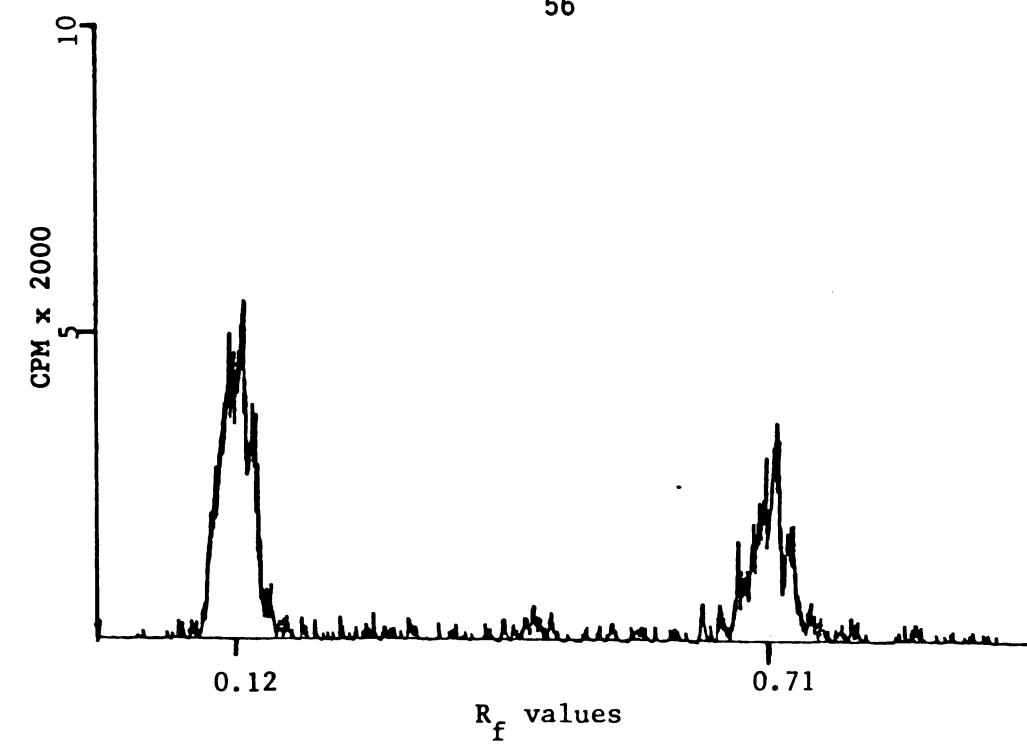


FIGURE 4.

FIGURE 5. Radioscans of thin-layer chromatogram of extracts from sorghum seedlings. Seedlings were treated with ^{14}C -metolachlor 24 h after initiation of germination, and extracts taken 3 days later. Scan from unprotected seedlings on top, scan from CGA-43089 treated seedlings on bottom. The polar metabolite had an R_f value of 0.12 and the parent metolachlor an R_f of 0.71. The developing system was Hexane:Chloroform:Ethanol (70:20:1).

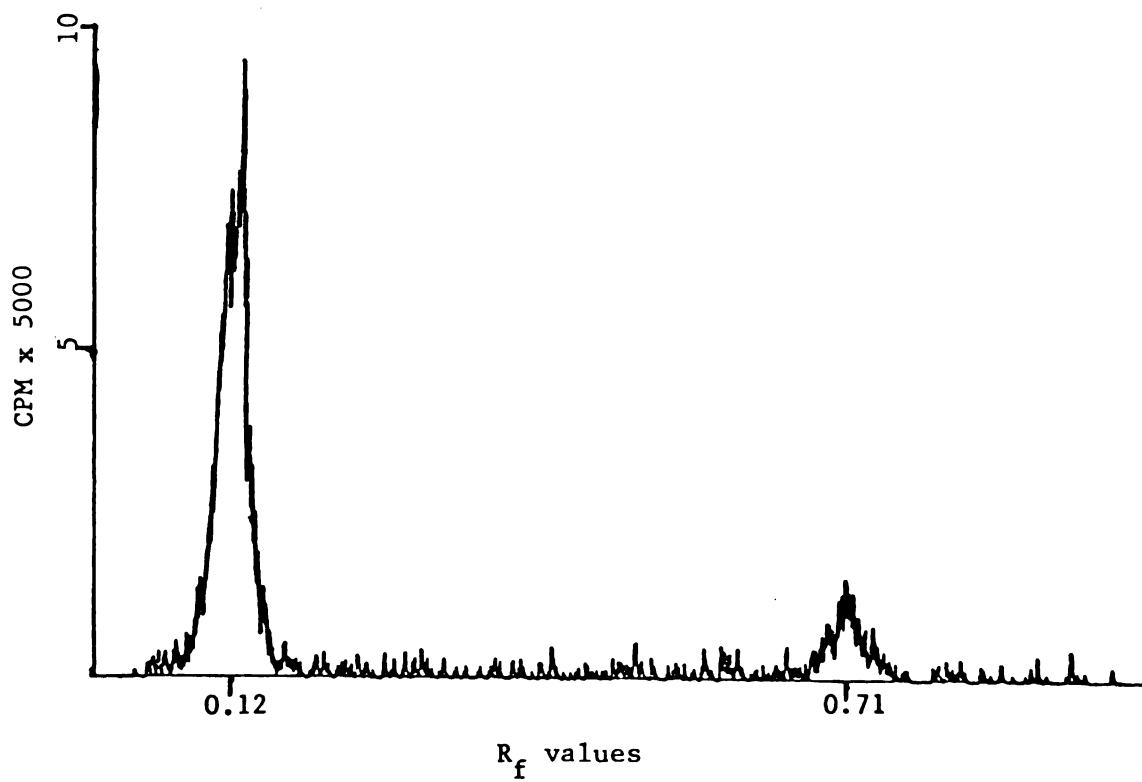
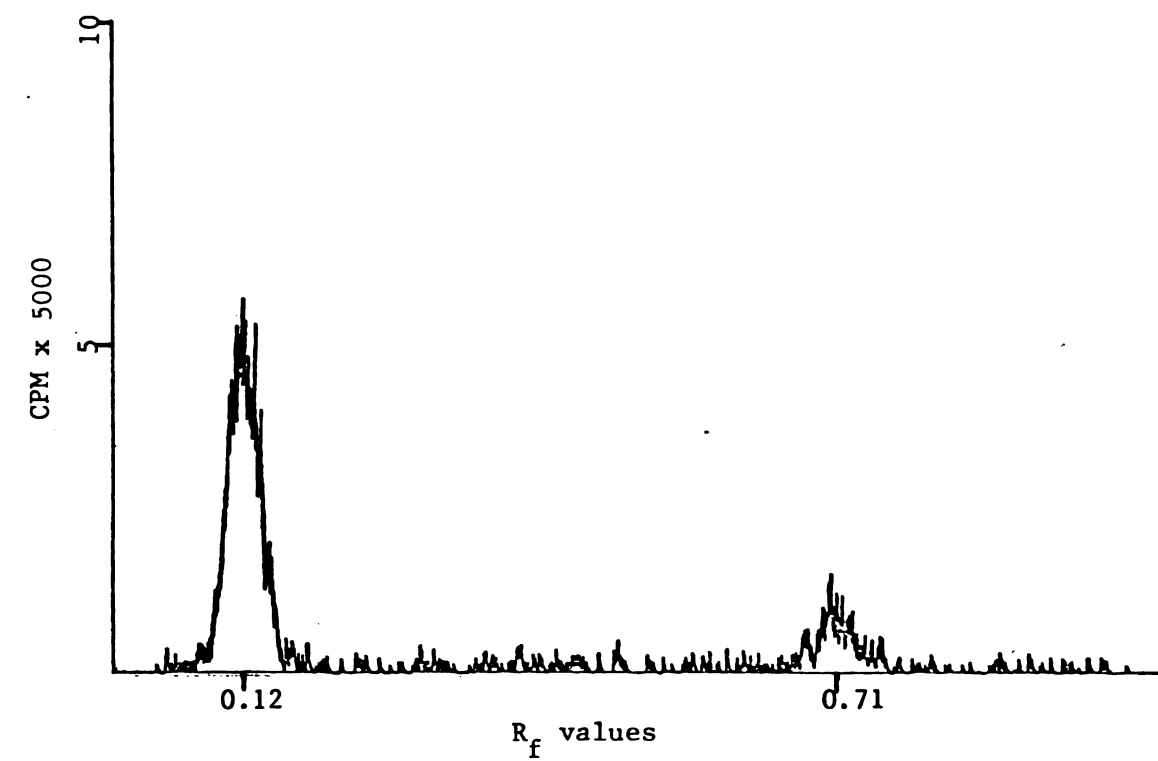


FIGURE 5.

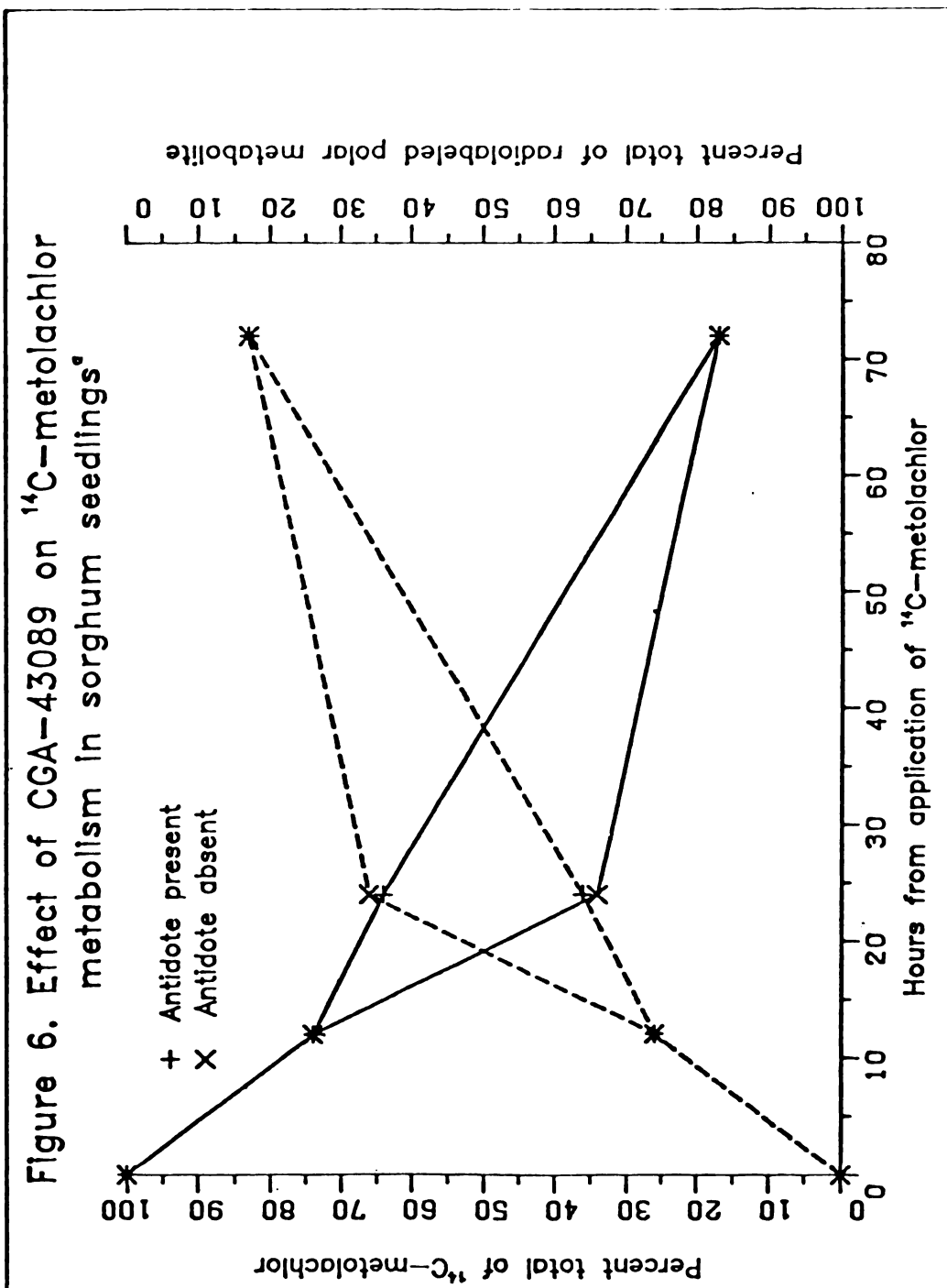


FIGURE 7. Radioscans of thin-layer chromatograms of extracts from sorghum seedlings. Seedlings were treated with ^{14}C -metolachlor 5 days after initiation of germination and at the time of shoot emergence, and extracts were taken 24 h later. Scan from unprotected seedlings on top, scan from CGA-43089 protected seedlings on bottom. The polar metabolite had an R_f value of 0.12, an intermediate metabolite had an R_f of 0.47, and the parent metolachlor had an R_f of 0.71.

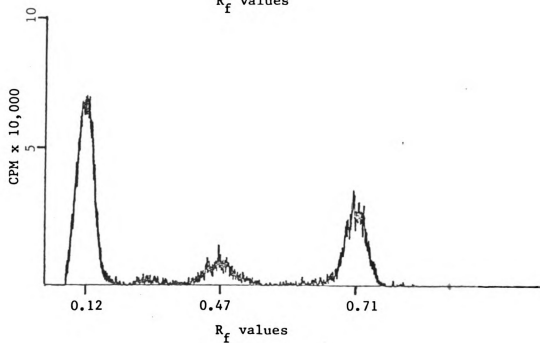
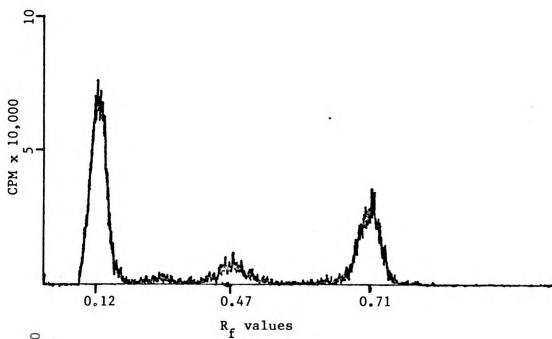


FIGURE 7.

FIGURE 8. Radioscan of thin-layer chromatogram of extracts from sorghum seedlings. Seedlings were treated with ^{14}C -metolachlor 5 days after initiation of germination and at the time of shoot emergence, and extracts were taken 3 days later. Scan for unprotected seedlings on the top, scan for CGA-43089 treated seedlings on bottom. The polar metabolite had an R_f value of 0.12 and the parent metolachlor had an R_f of 0.71. The developing system was Hexane:Chloroform:Ethanol (70:20:10).

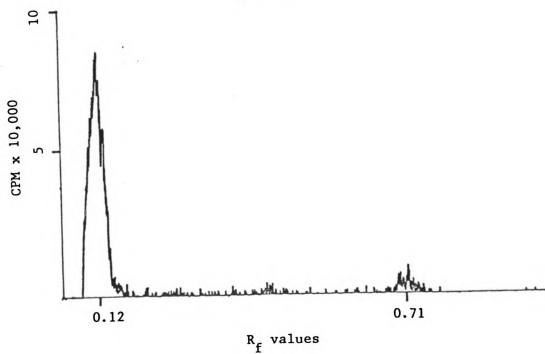
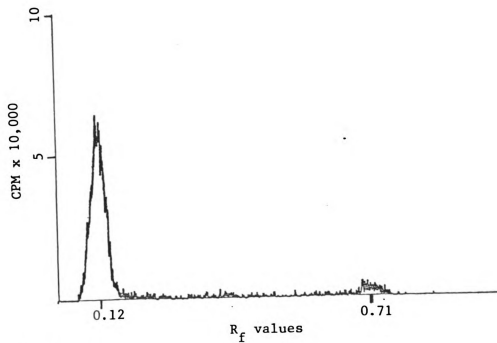
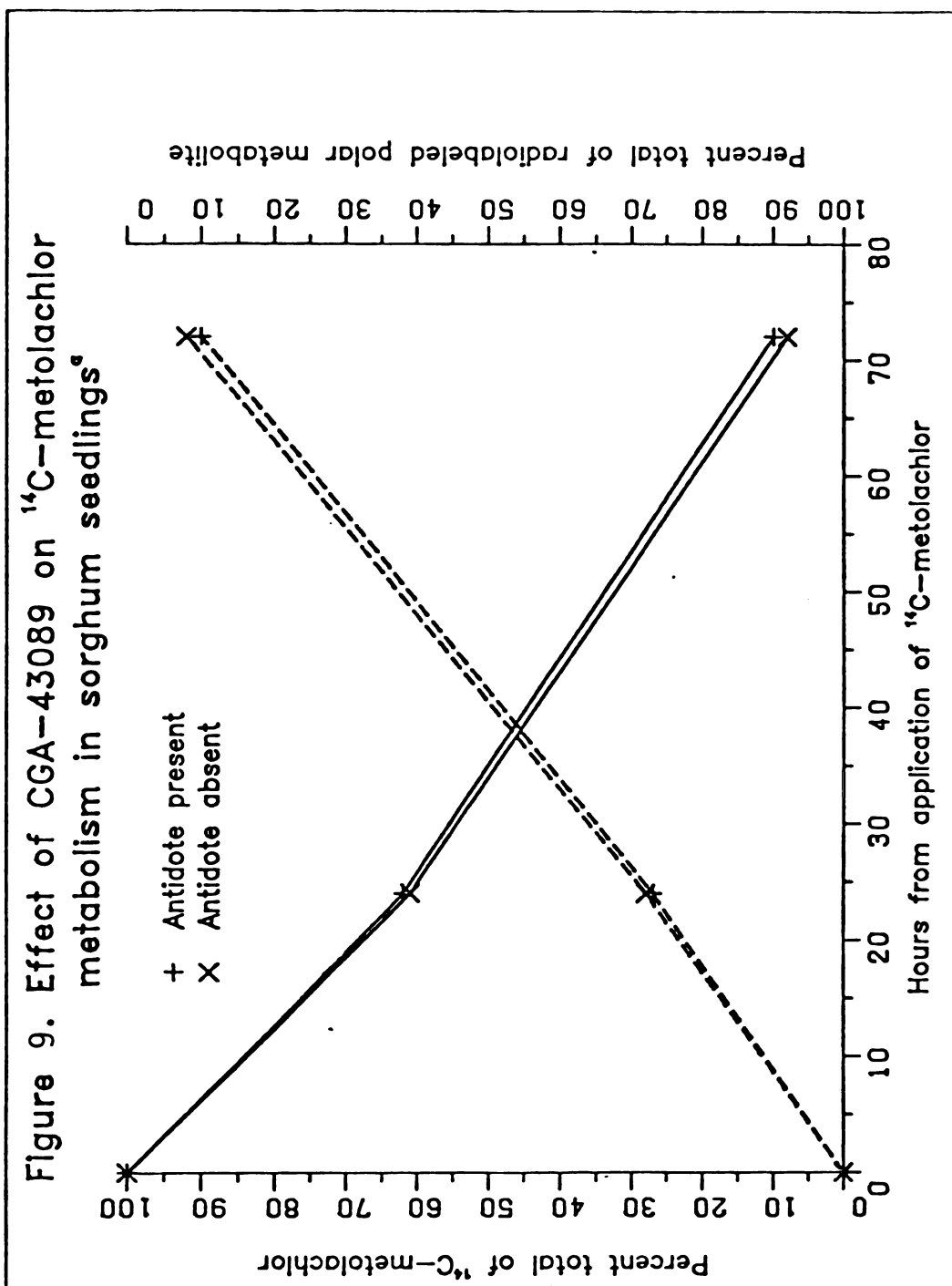


FIGURE 8.

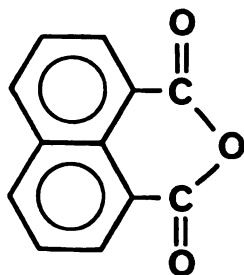


^a ^{14}C -Metolachlor applied five days after onset of germination.

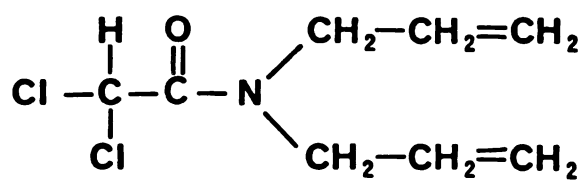
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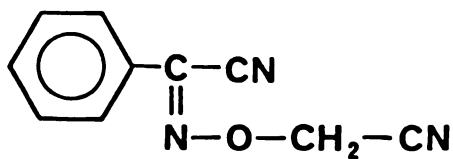
APPENDIX A - Structures of 1,8-naphthalic anhydride, R-25788, and
CGA-43089



1,8 - NAPHTHALIC ANHYDRIDE



R-25788



CGA-43089

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