Basis for Differential Chemical Selectivity of MG-191 Safener against Acetochlor and EPTC Injury to Maize*

Istvan Jablonkai

Central Research Institute for Chemistry, Hungarian Academy of Sciences, H-1525 Budapest, Hungary

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Chemical Selectivity, Safener, MG-191, Acetochlor, EPTC, Uptake, Translocation, Metabolism

The influence of MG-191 safener on the uptake, translocation and metabolism of [^{14}C]acetochlor and [^{14}C]EPTC was studied. The amounts of absorbed radioactivity by maize seedlings at 3, 6, 24, and 72 h after applications of [^{14}C]labeled herbicides and [^{14}C]MG-191 were different. Plants treated with [^{14}C]acetochlor took up 30- to 50-fold more radiolabel within 72 h than [^{14}C]EPTC- or [^{14}C]MG-191-treated plants. Addition of MG-191 caused only minor changes in the rate of herbicide absorption. EPTC and MG-191 and/or their metabolites moved quickly acropetally and partitioned equally between root and shoot tissues up to 72 h. The amount of acetochlor and/or its labeled metabolites translocated to shoot tissues was less than 10%. MG-191 practically had no influence on herbicide translocation rates. With all chemicals the amounts of water-soluble and unextractable fractions increased while the ratio of hexane-extractable metabolites decreased with time. TLC analyses of both water- and hexane-soluble metabolites confirmed the fast metabolism of acetochlor. The acetochlor metabolism took place *via* GSH conjugation and more polar, non-conjugated metabolites compared to parent molecule were detected in hexane-soluble fraction. MG-191 enhanced acetochlor metabolism by decreasing the portion of non-metabolized acetochlor. EPTC metabolism resulted in water-soluble metabolites having similar chromatographic properties to those of acetochlor. However, there was no safener effect on non-metabolized EPTC content of plants. It appears that MG-191 protects maize against EPTC by enhancing the early rate of conjugation with GSH after initial oxidative metabolism.

Introduction

The presently available safeners exhibit a high degree of botanical and chemical selectivity and protect only certain crops against injury from selected herbicides [1]. Herbicides antagonized by chemical safeners include primarily the soil-applied thiocarbamates and chloroacetanilides. The chemical specificity of dichloroacetamide safeners has been partially attributed to their structural similarities to thiocarbamates and chloroacetanilides [2, 3]. On the other hand, both types of herbicides cause similar injury symptoms and both are detoxified by glutathione (GSH) conjugation [4]. GSH conjugation of herbicides in plants can occur either directly as in chloroacetanilide metabolism or after an activation reaction as in the case of thiocarbamates. Furthermore, GSH conjugation of herbicides in plants can proceed non-enzymatically and/or enzymatically catalyzed by glutathione S-transferase enzymes (GST's) [5]. The contribution of non-enzymatic GSH conjugation may be significant depending on the reactivity of the electrophilic substrate [6]. The two major mechanisms by which a safener may confer its protective action are a) a safener induced enhancement of herbicide detoxication by elevating GSH content and GST activity and b) a competitive antagonism between the safener and the herbicide at a common site of action [1].

The dichloromethyl-dioxolane safener MG-191 protects maize against thiocarbamate and chlo-

Abbreviations: Acetochlor, 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(ethoxymethyl) acetamide; BAS 145138, 1-dichloroacetyl-hexahydro-3,3-8α-trimethyl-pyrrolo-[1,2-α]-pyrimidin-6-(2H)-one; EPTC, S-ethyl-N,N-dipropylthiocarbamate; Deethoxymethyl acetochlor, 2-chloro-N-(2-ethyl-6-methylphenyl)acetamide; Dichlormid, 2,2-dichloro-N,N-di-2-propenylacetamide; GSH, reduced glutathione; GST, glutathione S-transferase; MFO, mixed function oxidase; Metolachlor, 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide; MG-191, 2-dichloromethyl-2-methyl-1,3-dioxolane; NA, naphthalene-1,8-dicarboxylic acid anhydride; Propachlor, 2-chloro-N-(1-methylethyl)-N-phenylacetamide.

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roacetanilide herbicide injury [7]. MG-191, similar to the commercialized safener dichlormid, is more effective against thiocarbamates such as EPTC than chloroacetanilides such as acetochlor [8, 9]. The differential safening efficacy of MG-191 may be a consequence of its differential rate of absorption and translocation compared to those of EPTC and acetochlor or the existence of the dissimilar sensitive site(s) for safener and herbicide uptake as well as its differential action on the metabolism of EPTC and acetochlor.

Searching for a possible explanation of the differential safening efficacy of MG-191 against these two herbicides the objective of this study was to compare the uptake, translocation and metabolism of [^{14}C]EPTC and [^{14}C]acetochlor in unsafened and MG-191-safened maize seedlings as well as the absorption and translocation of [^{14}C]MG-191.

Materials and Methods

Chemicals

EPTC and acetochlor, provided by Nitrokemia (Fuzfogyartelep, Hungary), were purified by distillation and by silica gel column chromatography with benzene-hexane (30:70, v/v) as eluent. MG-191 was synthesized by the Department of Pesticide Research, Central Research Institute for Chemistry (Budapest, Hungary). [Carbonyl-^{14}C]acetochlor (sp. act. 37 MBq/mmol) and [2-^{14}C]MG-191 (sp. act. 111 MBq/mmol) were synthesized as described previously [10, 11]. [Carbonyl-^{14}C]EPTC (sp. act. 1.32 GBq/mmol) was provided by Dr. G. R. Stephenson (University of Guelph, Canada). Glutathione and cysteine conjugates of [^{14}C]acetochlor were obtained by non-enzymatic reactions [12]. Non-radioactive, postulated acetochlor plant metabolites and GSH and cysteine conjugates of acetochlor and its metabolites were prepared as described previously [13]. EPTC-sulfoxide and EPTC-sulfone were synthesized by the method of Lay and Casida [14]. All other chemicals and solvents were from Reanal (Budapest, Hungary).

Plant material

Seeds of hybrid maize (*Zea mays* L., Pioneer 3737) were soaked in tap water for 5 h. In experiments to determine the protective efficacy of safener at different application methods, plants were grown in plastic pots (11 × 11 × 12 cm) containing 1700 g of air dry quartz sand well mixed with 250 ml of water. The pots were watered every two days with half strength Hoagland's solution up to the original weights of pots. In uptake, translocation and metabolism studies four seedlings of 5 days old maize growing on a stainless steel screen were immersed into 100 ml of nutrient solution in a glass beaker. Experiments were carried out in a controlled plant growth room. The experimental conditions were: 23 ± 1 °C temperature, 60 ± 5% relative humidity, 16 h photoperiod with light intensity of 14 W m^{-2}.

Effect of application method on safener efficacy

In preplant treatments, 0.5 ml of acetone solutions of the herbicide and/or safener were solved in 250 ml of water to give a concentration of 10 and 50 μM and solutions were well mixed with 1700 g of sand. Eight seeds of maize hybrid were planted to a depth of 2.5 cm. In preemergence experiments, 1700 g sand moistured with 250 ml water was placed in plastic pots. Eight seeds of maize were planted 2.5 cm deep and equal amounts of herbicide and/or safener as in preplant use in 6 ml of 10% acetone-water were sprayed onto the sand surface then watered with 10 ml of nutrient solution. After 14 days plants were harvested and shoot heights and weights were measured.

Uptake, translocation of acetochlor, EPTC and MG-191

Seeds of maize hybrid were pre-germinated on moist filter paper for 2 days in an incubator at 24 °C and four uniformly sized seedlings were transferred to a glass beaker containing tap water. After three days the solutions in beakers were changed with 100 ml of half strength Hoagland's solutions contained 5 μmol of [^{14}C]labeled chemicals and nonlabeled safener. In the case of [^{14}C]EPTC, the amount of the herbicide was adjusted to 5 μmol by adding nonlabeled EPTC. The treated plants were harvested after 3, 6, 24 and 72 h. After weighing, the harvested plants were dissected separating roots, coleoptile, mesocotyl and leaves. Distribution of radioactivity in these tissues was determined by liquid scintillation counting (Packard TriCarb Scintillation System)

after combustion of samples. The distribution of radioactivity absorbed was visualized after autoradiography.

Metabolism of herbicides

5 Days old maize seedlings (4 per baker) absorbed equal amounts of labeled chemicals as in uptake studies by their roots. At 3, 6, 24 and 72 h after exposure the treated plants were removed from nutrient solution and their roots were thoroughly rinsed with water and wiped with paper towels. Then the plants were sectioned to root and shoot tissues. Separated root and shoot samples were placed in a mortar and were frozen with liquid nitrogen then were ground with a pestle. The homogenates were extracted with 10 ml of 80% methanol. The insoluble residues were filtered off and radioassayed by combustion and liquid scintillation counting. Methanol was removed from filtrates in vacuum below 30 °C by rotary evaporation. The methanol free concentrated filtrates were diluted with 1 ml of distilled water and partitioned three times with 5 ml of *n*-hexane. Then the hexane fractions were evaporated to 1 ml of final volume in 1 ml/min of stream of nitrogen at ambient temperature. Subsamples of water- and hexane-soluble extracts were radioassayed by liquid scintillation counting.

The water- and hexane-soluble metabolites were analyzed by thin-layer chromatography (TLC) on silica gel plates (Kieselgel 60, 0.25 mm thickness, Merck, Darmstadt, Germany). Subsamples (100 µl) were applied to the plates by Linomat III automatic sample applicator (Camag, Muttenz, Switzerland). The water-soluble metabolites were separated in *n*-butanol-acetic acid-water (12:3:5, v/v/v) solvent. The plates with hexane-soluble metabolites were developed in benzene-methanol (95:5, v/v). After development plates were subjected to autoradiography and the detected radioactive zones were scrapped from plates. The radioactivities in silica were quantified by liquid scintillation counting. Previously identified synthetic standards were cochromatographed with the plant extracts for the identification of detected metabolites.

Results and Discussion

Effect of application method on MG-191 protective efficacy against EPTC and acetochlor

The experiments on MG-191 protection were carried out with a sensitive maize hybrid grown in sand. The injuries caused by acetochlor and EPTC in sand were much more severe than in heavy soils [15]. Shoot length data in Table I demonstrate that under these extremely severe conditions the MG-191 safener provides good protection to maize against EPTC when it is incorporated together with the herbicide into the sand (preplant

Table I. The influence of application method on acetochlor and EPTC injury and the protective efficacy of MG-191.

Treatment	Rate [µM]	Application	Shoot length % of control	Protection[a]
MG-191	10	ppi[b]	100[d]	–
MG-191	50	ppi	96	
EPTC	50	ppi	62	–
EPTC + MG-191	50+10	ppi	88	68
EPTC + MG-191	50+50	ppi	95	86
MG-191	10	pre[c]	102	–
Acetochlor	50	pre	50	–
Acetochlor + MG-191	50+10	pre	57	15
Acetochlor + MG-191	50+50	pre	62	24
Acetochlor	50	ppi	61	–
Acetochlor + MG-191	50+10	ppi	75	37
Acetochlor + MG-191	50+50	ppi	80	49

[a] Based on shoot length protection (%) = 100 × [(herbicide + safener) − (herbicide)]/[(control) − (herbicide)];
[b] ppi = preplant incorporated treatment;
[c] pre = preemergent treatment;
[d] data represent the means of 2 experiments with 3 replicates.

application). All injury symptoms caused by EPTC were antagonized by both rates of the safener. When MG-191 was applied preemergence and combined with acetochlor the protection of maize against this herbicide was poor. However, when both safener and acetochlor were preplant incorporated as in the EPTC + MG-191 combination, a satisfactory protection was provided to maize particularly by the higher rate of MG-191. It should be noted however that a less severe shoot length inhibition of acetochlor was observed with this application. These data confirm earlier results which suggested that the root-applied MG-191 was more effective against acetochlor injury indicating the importance of root uptake in the safener efficacy [9].

Influence of MG-191 on acetochlor absorption and translocation

In time-course studies, 5 days old maize seedlings readily absorbed [^{14}C]acetochlor (Fig. 1) from nutrient solution. The amount of root-absorbed [^{14}C]acetochlor was continuously increased up to 72 h. As a result of increasing uptake the first detectable shoot length inhibition of acetochlor occurred at 24 h (Fig. 1). At the end of longest period studied 28% of shoot growth and 52% of root growth (data not shown) inhibition of acetochlor occurred. During this period the amount of [^{14}C]MG-191 increased from initial 2.94 to 7.26 µg/g fresh weight. Up to 72 h the ratio of herbicide/safener changed from initial 10 to 50. These data clearly demonstrate that small amounts of MG-191 can provide complete protection to maize against acetochlor in nutrient solution. Addition of MG-191 did not affect the acetochlor absorption into maize seedlings (Fig. 1).

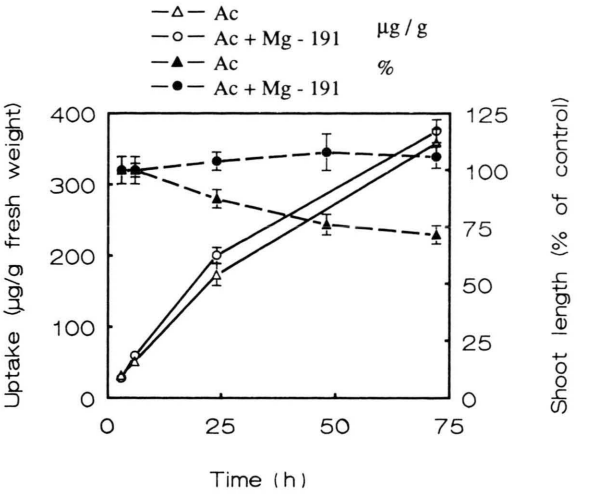

Fig. 1. Influence of MG-191 safener on uptake and shoot length inhibition of root-applied [^{14}C]acetochlor. The values represent the means ± SD of 2 experiments with 3 replicates. The SD is not shown when smaller than the data point.

Regarding the acetochlor distribution within the plants almost all root-applied radioactivity remained in roots shortly after treatment (Table II). Following 72 h exposure less than 10% of absorbed radioactivity moved acropetally towards the shoots accumulating in tips of older leaf tissues (autoradiogram not shown). MG-191 did not have any effect on the mobility of radioactivity from [^{14}C]acetochlor in safened plants. These results indicate that the antagonism of the acetochlor-

Table II. Distribution of radioactivity in maize seedlings following root application of [^{14}C]acetochlor as influenced by the safener MG-191.

Plant tissue	Percent (%) of absorbed radioactivity[a] Time [h]					
	3		24		72	
	–	+ MG-191	–	+ MG-191	–	+ MG-191
Primary roots	35.1	32.6	21.4	42.7	19.1	22.9
Adventitious roots	64.5	66.8	75.5	55.4	71.4	68.5
Mesocotyl + coleoptile	0.3	0.3	1.8	0.4	1.4	0.9
1st leaf	0.2	0.3	1.0	0.9	5.9	4.5
2nd leaf	–	–	0.2	0.6	2.2	3.2

[a] Data represent the means of 2 experiments with 3 replicates in terms of percentage of radioactivity absorbed by whole plants.

induced effects on maize roots and shoots by the safener MG-191 do not result from alterations in acetochlor uptake and translocation.

Influence of MG-191 on EPTC absorption and translocation

Time dependent shoot growth inhibition and uptake of EPTC and influence of MG-191 are shown in Fig. 2. The uptake of rootapplied [^{14}C]EPTC reached a maximum in the first 6 h and decreased up to 72 h. Despite of the early measurable absorption peak of EPTC, a perceptible shoot length inhibition was detected only at 48 h and 38% of shoot growth inhibition was observed after 72 h exposition (Fig. 2). The low recovery (30%) of [^{14}C] obtained at 72 h may be due to the high volatility of EPTC [15, 20]. In general, when [^{14}C]EPTC was applied together with the safener MG-191 the herbicide uptake was not significantly altered by safener (Fig. 2). An exception was observed at 24 h when the safener enhanced EPTC uptake by 1.5-fold compared to that of unsafened plants. Nevertheless, MG-191 conferred a perfect protection to maize against EPTC throughout the study. At 3 h the uptake of EPTC in both safened and unsafened plants was 18- and 23-fold of MG-191 safener uptake. At 72 h safener uptake was hardly below that of EPTC and the ratio of herbicide/safener was 1.7. The influence of MG-191 on distribution of radioactivity from root-applied [^{14}C]EPTC within the plants is represented by Table III. The majority of radioactivity absorbed in [^{14}C]EPTC treated maize plants is primarily located in the roots shortly after treatment. After 72 h 55% of radioactivity was detected in shoots and no accumulation into leaf tips occurred (autoradiogram not shown). In the safened plants similar distribution patterns and no safener effect was detected up to 24 h. At 72 h a slight retardation of absorbed radioactivity was observed throughout the plant more significantly in adventitious roots, mesocotyl and coleoptile. Apparently, the minor effects of MG-191 on EPTC uptake and translocation are adequately explained by its protection efficacy. Similar results have been reported previously with the safener dichlormid [16].

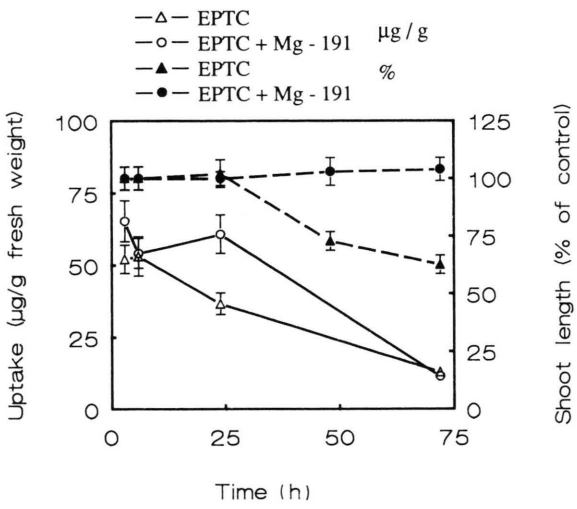

Fig. 2. Influence of MG-191 safener on uptake and shoot length inhibition of root-applied [^{14}C]EPTC. The values represent the means ± SD of 2 experiments with 3 replicates. The SD is not shown when smaller than the data point.

Table III. Distribution of radioactivity in maize seedlings following root application of [^{14}C]EPTC and as influenced by the safener MG-191.

Plant tissue	Percent (%) of absorbed radioactivity[a] Time [h]					
	3		24		72	
	–	+ MG-191	–	+ MG-191	–	+ MG-191
Primary roots	25.5	27.2	31.9	26.3	15.5	17.3
Adventitious roots	50.8	48.8	38.2	42.7	29.5	41.2
Mesocotyl + coleoptile	12.8	13.8	16.0	14.2	27.3	17.9
1st leaf	10.9	10.2	8.1	10.3	18.8	16.3
2nd leaf	–	–	5.8	6.5	8.9	7.3

[a] Data represent the means of 2 experiments with 3 replicates in terms of percentage of radioactivity absorbed by whole plants.

Influence of MG-191 on acetochlor metabolism

The metabolism of acetochlor was characterized by analyses of soluble polar and apolar as well as insoluble metabolite fractions in both roots and shoots of maize seedlings. A major part (87%) of the total absorbed radioactivity was found in water-soluble form at 3 h after [^{14}C]acetochlor application and by the end of experiment (72 h) increased to 96%. The amount of initial portion of hexane-extractable radioactivity (11%) decreased to 2% at 72 h. Data in Table IV indicate the higher detoxication capacity of roots since the initial ratio of water-soluble/hexane-soluble radioactivity in the roots was 9.2 while 0.9 in shoot. Further metabolism appeared to be time dependent. The ratios of polar to apolar metabolite fraction continuously increased. The amount of bound residue was much higher in roots in comparison to that of shoots. When MG-191 safener was applied together with acetochlor differences could be detected in the ratios of hexane-soluble metabolites to water-soluble ones. At 3 h in both root and shoot tissues of safened plants the amount of hexane-soluble fraction containing the parent acetochlor molecule and its apolar metabolites fell down more definitely in shoots. At 6 h following MG-191 treatment the amount of this fraction decreased only in the shoots. At this time more hexane-extractable radioactivity was recovered from roots of safened plants. Later no significant differences were detected in the amount of this fraction. Safener treatment increased the portions of nonextractable residue after 24 and 72 h only in the roots. Bound [^{14}C] residues in maize following propachlor and metolachlor application also tended to be slightly higher as a result of treatment with the safener BAS 145138 [17].

TLC analyses of water-soluble metabolites (Table V) confirmed that the main metabolic pathway of acetochlor metabolism in maize was conjugation with glutathione. The main detoxication product at 3 h was the acetochlor GSH conjugate ($R_f = 0.33$). The increasing amount of acetochlor cysteine conjugate ($R_f = 0.47$) with time indicates that this conjugate is a product of catabolism of acetochor GSH conjugate. No qualitative changes were shown on acetochlor metabolism after MG-191 treatment in roots. The only detectable influence of safener is that in the roots of protected plants higher levels of herbicide-GSH conjugate were maintained in each period than in nonsafened plants. At 72 h in shoots of safened plants the cysteine conjugate of deethoxymethyl acetochlor ($R_f = 0.43$) appeared which may derive from deethoxymethylation of acetochlor cysteine conjugate. This cannot be the product formed by catabolism of deethoxymethyl acetochlor GSH conjugate ($R_f = 0.28$) because the level of this conjugate is constant in roots and not detectable in shoots. However, further investigations are necessary to elucidate the exact biological significance of these conjugates.

Table IV. Effect of MG-191 safener on time-dependent acetochlor metabolism.

MG-191 treatment [μM]	Time [h]	Percent (%) of absorbed radioactivity[a] Metabolite fraction					
		Water-soluble		Hexane-soluble		Unextractable	
		Root	Shoot	Root	Shoot	Root	Shoot
0	3	86.1	1.5	9.4	1.7	1.1	0.2
	6	94.0	1.2	2.9	0.5	1.2	0.2
	24	92.4	2.4	4.3	0.3	0.6	0.1
	72	87.6	8.7	0.9	1.1	1.3	0.4
50	3	89.3	2.1	6.6	0.5	1.1	0.4
	6	92.5	1.5	4.3	0.2	1.5	0.1
	24	93.5	1.3	2.9	0.1	2.0	0.1
	72	88.6	6.4	1.0	0.8	2.9	0.3

[a] Data represent the means of 2 experiments with 3 replicates in terms of percentage of absorbed radioactivity by whole plants.

Table V. Influence of MG-191 safener on distribution of water-soluble acetochlor metabolites in roots and shoots of maize seedlings.

MG-191 treatment [μM]	Time [h]	Percent (%) of recovered radioactivity[a] R_f 0.26	0.28	0.33	0.38	0.43	0.47
				Roots			
0	3	19.4	5.3	56.5	2.3	11.0	5.5
	6	28.7	9.7	41.7	5.9	6.3	5.2
	24	22.8	8.1	35.5	8.7	7.3	15.7
	72	17.7	3.4	40.6	7.6	4.2	23.7
50	3	25.4	–	55.2	5.8	6.1	7.5
	6	22.5	4.3	51.0	3.2	6.1	11.4
	24	16.1	4.5	50.7	4.6	5.7	17.2
	72	11.0	3.0	48.2	4.8	7.2	22.2
				Shoots			
0	3	55.5	–	45.5	–	–	–
	6	34.4	–	56.5	–	–	9.1
	24	19.4	–	26.3	19.2	–	35.2
	72	7.7	–	13.4	30.0	–	48.9
50	3	47.3	–	52.7	–	–	–
	6	37.6	–	57.4	–	–	5.0
	24	17.6	–	20.1	23.5	–	38.9
	72	6.4	–	18.6	15.1	25.3	34.6

[a] Data represent the means of 2 experiments with 3 replicates in terms of percentage of radioactivity in either roots or shoots.

After TLC separation of hexane-soluble acetochlor metabolites 6 radioactive zones were detected (data not shown). The time dependent distribution of hexane-soluble metabolites showed that acetochlor metabolism yielded more polar compounds as compared to parent molecule ($R_f = 0.50$). At 3 h about 70% of hexane-extractable radioactivity from roots was present as acetochlor. The decrease in amount of acetochlor is quite detectable. At 72 h, the percentage of unmetabolized acetochlor fell down to 17% in roots and 2% in shoots.

The safener definitely reduced the levels of acetochlor content of plants up to 24 h (Fig. 3). At this time only 0.3% of the total absorbed radioactivity was present as parent acetochlor in safened plants compared to 1.9% acetochlor content of unsafened plants. Recent evidence supports the previous findings that safeners protect maize from chloroacetanilide herbicide injury by enhancement of herbicide degradation [18–20]. These reduced amounts of acetochlor already appeared to be below the phytotoxic level.

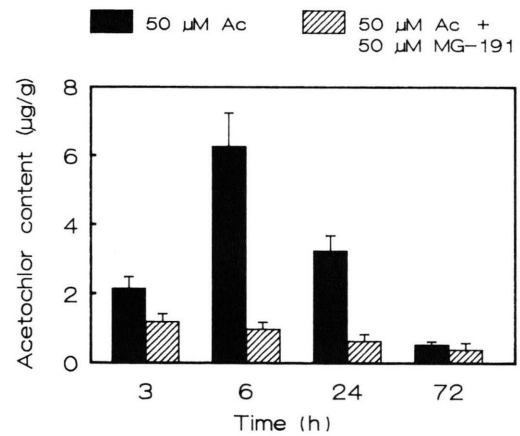

Fig. 3. Content of non-metabolized [^{14}C]acetochlor in protected and unprotected maize seedlings. Vertical bars represent the means and the standard deviations of 2 experiments with 3 replicates.

Influence of MG-191 on EPTC metabolism

Analysis of metabolite distribution after [^{14}C]EPTC treatment (Table VI) revealed that the

Table VI. Effect of MG-191 safener on time-dependent EPTC metabolism.

MG-191 treatment [μM]	Time [h]	Percent (%) of absorbed radioactivity[a] Metabolite fraction Water-soluble Root	Water-soluble Shoot	Hexane-soluble Root	Hexane-soluble Shoot	Unextractable Root	Unextractable Shoot
0	3	50.7	14.3	21.0	12.7	0.8	0.6
	6	48.1	24.2	16.8	9.1	0.9	0.9
	24	50.8	30.1	11.5	5.2	1.2	1.1
	72	40.7	45.2	5.0	2.7	7.1	3.7
50	3	60.8	17.4	11.0	6.4	0.8	0.6
	6	53.5	25.1	11.9	8.6	0.5	0.5
	24	43.1	31.1	16.5	7.1	0.5	1.2
	72	44.3	38.6	5.4	3.8	5.0	3.0

[a] Data represent the means of 2 experiments with 3 replicates in terms of percentage of absorbed radioactivity by whole plants.

greater part (65%) of radioactivity taken up by maize seedlings was converted to polar, water-soluble metabolites at 3 h. The amount of this fraction from roots and shoots of maize seedlings increased to 86% at 72 h. The initial 34% of apolar, hexane-extractable radioactivity in whole plant fell to 8% at 72 h. At 3 h after [^{14}C]EPTC application maize roots contained a higher ratio of water-soluble to hexane-soluble metabolites as compared to that of shoots. These ratios increased with time to a greater extent in maize shoots. The amount of nonextractable residue continuously increased up to 72 h. Treatment with MG-191 resulted in an early enhancement of EPTC metabolism. Already after 3 h the amount of hexane-soluble radioactivity were decreased by 50% in both root and shoot tissues of safened plants compared to those of nonsafened plants. At 6 h, the safener-induced decrease of hexane-soluble fraction was only shown in roots of safened plants. Later the safener slightly elevated the proportion of this metabolite fraction to water-soluble fraction. However, safener treatment definitely decreased the amount of nonextractable residue.

TLC analysis of water-soluble fraction revealed (Table VII) that in general MG-191 influenced the metabolism of EPTC quantitatively but not qualitatively in either the shoot or the root tissues. In this fraction 4 radioactive bands were detected. None of these metabolites were identified in absence of standards. A band with the lowest retention value ($R_f = 0.29$) was detected only after 72 h in roots and shoots of nonsafened plants. Previous findings on EPTC metabolism suggest that the molecule is detoxified by plants *via* glutathione conjugation after oxidation to its sulfoxide [21]. R_f values described previously for EPTC metabolites [12, 22] and similar chromatographic properties of

Table VII. Influence of MG-191 safener on distribution of water-soluble EPTC metabolites in roots and shoots of maize seedlings.

MG-191 treatment [μM]	Time [h]	Percent (%) of recovered radioactivity[a] R_f 0.29	0.35	0.40	0.50
				Roots	
0	3	–	9.8	56.4	33.8
	6	–	36.6	46.0	17.4
	24	–	30.2	43.4	26.4
	72	16.5	11.9	51.4	20.2
50	3	–	38.0	34.3	27.7
	6	–	33.5	43.7	22.8
	24	–	51.1	29.6	19.3
	72	–	12.8	53.3	33.9
				Shoots	
0	3	–	41.1	32.0	26.9
	6	–	42.8	27.4	29.8
	24	–	31.9	28.0	40.1
	72	18.6	23.8	38.1	19.4
50	3	–	54.5	28.6	16.9
	6	–	18.0	22.4	59.6
	24	–	24.7	29.5	45.8
	72	–	22.4	25.4	52.2

[a] Data represent the means of 2 experiments with 3 replicates in terms of percentage of radioactivity in either roots or shoots.

water-soluble EPTC metabolites to those of acetochlor also suggest that the metabolism of EPTC by maize yields glutathione and cysteine conjugates after initial oxidation. Considering the R_f values and chromatographic characteristics of these metabolites, the metabolite with $R_f = 0.35$ is probably the S-(N,N-dipropyl-carbamoyl)glutathione while metabolite with $R_f = 0.50$ is probably the S-(N,N-dipropyl)carbamoyl cysteine. The trends in changes of relative amounts of metabolites also indicates that EPTC metabolism takes place *via* glutathione conjugation following catabolism of GSH conjugate yielding cysteine conjugate. A higher level of metabolite with $R_f = 0.35$ was found in roots of protected plants. Similarly, a faster formation of metabolite with $R_f = 0.50$ in shoots appeared to be promoted by safener.

TLC of hexane-soluble radioactive materials revealed the detection of three metabolites (data not shown). Compound with $R_f = 0.67$ cochromatographed with parent EPTC, compound with $R_f = 0.63$ cochromatographed with EPTC-sulfone and an unknown metabolite with $R_f = 0.48$ were detected. The absence of EPTC-sulfoxide (R_f = 0.17) among metabolites detected is due to the its reactive nature. Shortly after [^{14}C]EPTC treatment 34% of parent molecule was detectable in roots of safened plants. The great part of EPTC was transformed to its respective sulfone (46%) and unknown metabolite ($R_f = 0.48$) at 3 h. Addition of the MG-191 safener resulted in the detection of only EPTC indicating that the safener enhances the rate of conjugation of oxidized products with GSH. The EPTC-sulfoxide and sulfone can react immediately with GSH both non-enzymatically and enzymatically to form of S-(N,N-dipropylcarbamoyl)glutathione [22, 23]. Fig. 4 demonstrates that MG-191 treatment did not decrease the EPTC content of maize plants. At 24 h the EPTC level in safened plants was even higher than that of nonsafened plants. These findings are consistent with the results of Barta and Dutka [24] that derivatives of the monooxygenase inhibitor amino-benzotriazole act as EPTC antagonists and also suggest that EPTC-sulfoxide may be more phytotoxic than the parent molecule.

In summary, the data suggest that there are great differences in the absorption of root-applied acetochlor and EPTC (Fig. 1 and 2). The maize plants initially took up less amounts of acetochlor

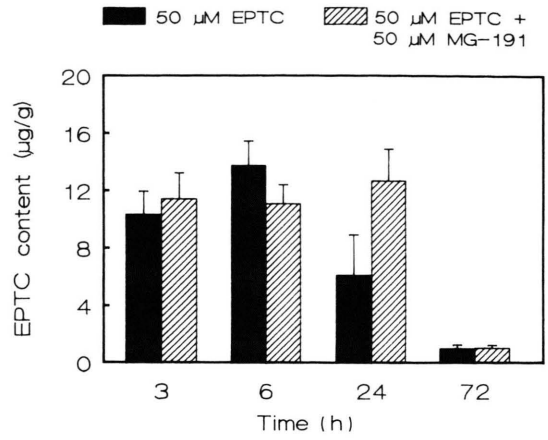

Fig. 4. Content of non-metabolized [^{14}C]EPTC in protected and unprotected maize seedlings. Vertical bars represent the means and the standard deviations of 2 experiments with 3 replicates.

than EPTC treated plants and there were no differences in the absorbed radioactivities up to 6 h. Later, however, the acetochlor treated plants contained 5- to 30-fold amount of radiolabel than EPTC treated plants. The rate of EPTC translocation was greater than that of acetochlor and the translocation pattern was similar to that of safener (data not shown). Conversely, a slow translocation of acetochlor was observed as compared to those of EPTC and MG-191. The relatively fast translocation of MG-191 and/or its metabolites show a similar distribution to that of EPTC. These findings suggest that the safener should move similarly or more rapidly than the herbicide to the site(s) of action to provide satisfactory protection. The contribution of competition for target site(s) seems to be of principal importance in the protective mechanism of action of MG-191 against EPTC injury to maize. These sites in case of chloroacetanilides and thiocarbamates are considered to be located in meristematic tissues of maize [25]. This may be one of the reasons why the much more absorbed and less mobile acetochlor was perfectly antagonized by root-applied MG-191. Because of its greater translocation rate the safener and/or its bioactive metabolite(s) reach the target site(s) sooner than acetochlor and/or its metabolites. Moreover, a safener-induced enhancement on acetochlor metabolism by GSH conjugation may be due to the ability of MG-191 to increase the levels of enzymes

(GST, monooxygenase) and the cofactor GSH involved in herbicide metabolism [26]. Against EPTC, the safener MG-191 appeared to have no influence on early oxidative metabolism mediated by a monooxygenase system. It should be noted, however, that neither dichlormid nor NA changed the levels of cytochrome P-450 in protected maize [27]. From analyses of water-soluble EPTC metabolites it can be concluded that MG-191 confers its protection by enhancing early GSH conjugation rate of either EPTC-sulfoxide or sulfone. The surprisingly low internal levels of MG-191 as compared to those of the herbicides also indicate that either these small amounts could provide complete protection or bioactive metabolites formed during its metabolism may have a key role in protection of maize against herbicide injury.

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