PESTICIDE BIOCHEMISTRY AND PHYSIOLOGY 26, 365–374 (1986)

Effects of SC-0224 and Glyphosate on Inflated Duckweed (*Lemna gibba*) Growth and EPSP-Synthase Activity from *Klebsiella pneumoniae*[1]

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Received September 10, 1985; accepted April 1, 1986

The effects of the herbicides SC-0224 (trimethylsulfonium carboxymethylaminomethylphosphonate) and glyphosate [*N*-(phosphonomethyl)glycine] (PMG) on the inhibition of inflated duckweed (*Lemna gibba* L.) growth and on the conversion of shikimate to anthranilate in a cell-free extract of *Klebsiella pneumoniae* (ATCC 25306) were compared. SC-0224 is the trimethylsulfonium (TMS) salt of *N*-(phosphonomethyl)-glycine (PMG), whereas glyphosate is commercially formulated as the isopropylamine (IPA) salt of PMG. Both formulated and technical grade forms of SC-0224 were found to be much more phytotoxic to duckweed than either formulated or technical grade forms of glyphosate. The growth inhibition caused by glyphosate was partially prevented by different combinations of the aromatic amino acids Phe, Tyr, and Trp; whereas, the duckweed growth inhibition caused by SC-0224 could not be reduced by the same amino acid combinations. Trimethylsulfonium ion (TMS), the cationic constituent of the SC-0224 salt, and SC-0224 were found to be equally phytotoxic to duckweek indicating that the phytotoxic effects of TMS may be responsible for differences in the action of glyphosate and SC-0224 on duckweed. SC-0224 and glyphosate equally inhibited the production of anthranilate in the cell-free extract of *K. pneumoniae*, whereas TMS caused no inhibition. These results indicate that both constituents of the SC-0224 salt, TMS and PMG, are phytotoxic and may act independently.

INTRODUCTION

The herbicide SC-0224 is an experimental, nonselective, postemergence herbicide with potential for use on noncrop areas, in perennial crops, and on reduced tilalge cropping systems. Previous laboratory, greenhouse, and field studies have shown SC-0224 to have metabolic and phytotoxic effects very similar to those of glyphosate (3, 4, 7, 17, 24, 39) although differences have occurred in some cases. Bellinder (4) found that glyphosate and SC-0224 caused inhibition of both lipid and protein synthesis in isolated soybean [*Glycine max* (L.) Merr.] cells; however, SC-0224 caused a significantly greater inhibition in both cases. Bellinder (4) also found that SC-0224 caused greater cellular damage and necrosis in fall panicum (*Panicum dicotomiflorum* Michx.).

SC-0224 is the trimethylsulfonium (TMS) salt of *N*-(phosphonomethyl)-glycine (PMG), whereas the term glyphosate is often used to refer to both the acid and the isopropylamine (IPA) salt of PMG. Unless otherwise specified, the term glyphosate used in this paper refers to effects that have been proven to be true for both the IPA and acid forms of PMG (Fig. 1). Formulated SC-0224 and glyphosate both contain a surfactant as specified under Materials and Methods. TMS salts have previously been shown to have growth regulating and phytotoxic effects on a number of plant species including bermudagrass [*Cynodon dactylou* (L.) Pers.], carpetgrass [*Axonopus compressus* (Sw.) Beauv.], maize (*Zea*

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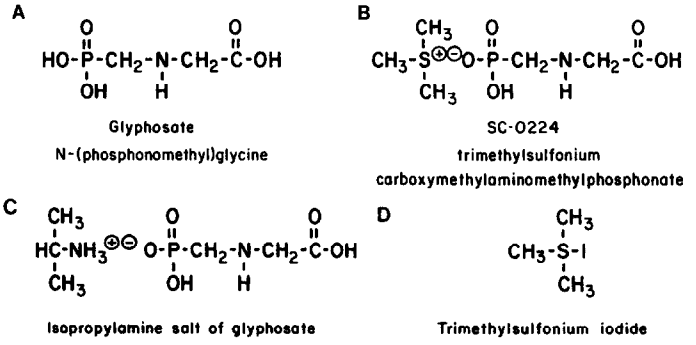

FIG. 1. *Chemical structures of glyphosate, SC-0224, and trimethylsulfonium iodide.*

mays L.), cotton (*Gossypium hirsutum* L.), pigweed (*Amaranthus retroflexus* L.), and common duckweed (*Lemna minor* L.) (32). These species demonstrated varying degrees of sensitivity to like concentrations of TMS salts, whereas other species including radish (*Raphanus sativus* L.), mustard (*Brassica nigra* L.), barley (*Hordeum vulgaris* L.), and soybean were tolerant (32).

The structural similarities of SC-0224 and glyphosate and the observed similarities and differences in the phytotoxic effects of the two compounds has led to an interest in comparing the mechanism(s) of action of these herbicides. Previous studies which have been reviewed by Hoagland and Duke (18) have shown that glyphosate causes a number of biochemical responses in plants including alteration of photosynthesis (30, 33), respiration (28, 33, 36), and reduction of protein (9, 36, 37) and chlorophyll biosynthesis (22, 23). Although some presently believe that glyphosate has other sites of action (14), several recent studies give strong support to the hypothesis that the primary mechanism of action for glyphosate is the inhibition of the shikimic acid pathway (1, 3, 5, 10, 13, 26, 27, 34, 35). Hollander and Amrhein (19) found that glyphosate prevented the incorporation of [^{14}C]shikimate into the aromatic amino acids in buckwheat (*Fagopyrum esculentum* Moench). Amrhein *et al.* (1) found that glyphosate caused the accumulation of shikimate in buckwheat hypocotyls and cultured cells of *Galium mollugo* L. indicating a blockage of the shikimic acid pathway. Using a cell-free extract of *Klebsiella pneumoniae*, Steinrucken and Amrhein (34) identified 5-enolpyruvylshikimic acid-3-phosphate synthase (EPSP-synthase) as the enzyme of this pathway which is inhibited by glyphosate. Subsequent studies have shown glyphosate to competitively inhibit highly purified EPSP-synthase from *K. pneumoniae* (2, 35), as well as *Neurospora crassa* (5) and pea (*Pisum sativum* L.) (26). The shikimic acid pathway is responsible for the synthesis of the aromatic amino acids Tyr, Phe, and Trp, as well as many other secondary plant metabolites. Blockage of this pathway may thus lead to decreases in aromatic amino acid pool levels resulting in decreased protein synthesis and subsequent development of biochemical responses such as those listed above.

Partial or complete prevention of glyphosate-induced growth inhibition by supplemental aromatic amino acids has been shown in a number of microorganisms and plant species including inflated duckweed (*Lemna gibba* L.) (21), *Rhizobium japonicum* (21), *Eschericia coli* (15, 29), *Chlamydomonas reinhardi* (15); and in tissue cultures of carrot (*Daucus carota* L.) (15, 16, 38) and soybean (15). Supplemental aromatic amino acids have also prevented glyphosate-induced inhibition of transpiration in bean (*Phaseolus vulgaris* L.) shoots (31), anthocyanin synthesis in buckwheat (19),

and protein synthesis in isolated soybean leaf cells (36, 37). However, the feeding of aromatic amino acids was ineffective in preventing the glyphosate-induced inhibition of growth in some intact higher plants including maize (11), soybean (12), and wheat (*Triticum aestivum* L.) (9) and in tissue cultures of tobacco (*Nicotiana tabacum* L.) and soybean (25). Different compartmentalization of root-fed versus endogenous amino acids may be responsible for the failure of aromatic amino acids to prevent growth retardation in these cases (9, 12).

The objectives of this study were to determine and compare the effects of: (a) aromatic amino acids on the growth inhibition of inflated duckweed induced by SC-0224; (b) SC-0224 and the TMS ion on inflated duckweed growth; and (c) SC-0224 on EPSP-synthase activity. Glyphosate was included in these studies for the purpose of comparison.

MATERIALS AND METHODS

Duckweed Studies

Plant material. The aquatic flowering plant inflated duckweed (*Lemna gibba* L. strain G3) was obtained from the collection of Dr. C. F. Cleland of the Smithsonian Radiation Laboratory, Rockville, Maryland.

Growth medium. The E medium previously described by Cleland and Gibbs (8) was used in this study. This medium contained per liter: KH_2PO_4, 690 mg; KNO_3, 515 mg; $Ca(NO_3)_2 \cdot 4H_2O$, 1180 mg; $MgSO_4 \cdot 7H_2O$, 500 mg; H_3BO_3, 2.86 mg; $ZnSO_4 \cdot 7H_2O$, 0.22 mg; $Na_2MoO_4 \cdot 2H_2O$, 0.12 mg; $CuSO_4 \cdot 5H_2O$, 0.08 mg; $MnCl_2 \cdot 4H_2O$, 3.62 mg; $FeCl_3 \cdot 6H_2O$, 5.40 mg; tartaric acid, 3.00 mg; EDTA, 9.00 mg; sucrose, 10 g. The medium was adjusted to pH 4.6 with KOH and autoclaved 15 to 20 min at 15 psi.

Chemical treatments. The chemicals for each of the various treatments were added prior to pH adjustment and autoclaving. Preliminary studies, as well as previous reports (16), determined that autoclaving does not affect the biological activity of the herbicides used in this study. The formulated form of the herbicides were Roundup[3] in the case of glyphosate and SC-0224 4LC Lot No. WDC-1501 in the case of SC-0224. Roundup is a commercial formulation containing 1 part of the monoisopropylamine salt of glyphosate (4 lb/gal ai) to 0.5 part by weight of MON-0818 surfactant. Technical grades of glyphosate (79.0% pure solid) and SC-0224 (56.0% pure liquid) were used in this study. Trimethylsulfonium iodide (98% pure solid) was obtained from the Aldrich Chemical Company. The isopropylamine salt of glyphosate was prepared by mixing 1 part technical grade glyphosate (2.5 m*M*) with 1 part isopropylamine hydrochloride (2.5 m*M*). Isopropylamine hydrochloride was obtained from the Phaltz & Bauer Chemical Company. Shikimic acid and the amino acids, L-Phe, L-Tyr, and L-Trp, were obtained from the Sigma Chemical Company.

Growth assays. Growth studies were initiated by inoculating 50 ml of E medium in a 250-ml Erlenmeyer flask with one four-frond cluster of *Lemna gibba*. The flasks were stoppered with cotton plugs to maintain the axenic condition within the flasks, incubated at 29°C, and illuminated continuously under a mixture of fluorescent and incandescent lamps (80 μEinsteins/m^2/sec). These growth conditions were adequate for growth of inflated duckweed to cover the surface of the medium in approximately 10 days. Growth studies ran for 7-day periods after which the plants in each flask were harvested on a fishnet, rinsed with tap water, placed in preweighed aluminum trays, and dried in a drying oven at 55°C

[3] Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the authors or their employers, and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

for 48 hr. The aluminum trays were then weighed on an analytical balance to determine the dry weight of the tissue.

Enzyme Assay

Organism. A culture of the bacterium *K. pneumoniae* (ATCC 25306) was obtained from the American Type Culture Collection, Rockville, Maryland.

Growth medium. The organism was grown in the following citric acid–mineral salts medium (34). The following chemicals were dissolved in distilled water to make a final concentration of 1 liter: $MgSO_4 \cdot 7H_2O$, 200 mg; citric acid $\cdot H_2O$, 2 g; KH_2PO_4-anhydrous, 10 g; $NaNH_4HPO_4 \cdot H_2O$, 3.5 g; glucose, 1.6 g; acid-hydrolyzed casein, 50 mg; indole, 2 mg. The indole, glucose, and acid-hydrolyzed casein were aseptically added to the medium after the other ingredients had been autoclaved for 15 to 20 min at 15 psi. This medium has a pH of about 7.0.

Preparation of cell-free extract. Five hundred milliliter plastic Erlenmeyer flasks containing 85 ml of the medium described above were inoculated with 10^9 cells from a broth culture shaken for 7 hr (100 rpm, 30°C). The flasks were then incubated with shaking (100 rpm, 30°C) in a reciprocal shaking bath (Fisher shaking water bath Model 127) for 12 to 13 hr and the cell suspension was then centrifuged at 4000*g* for 10 min at 4°C. The pellet was resuspended in 250 ml of 0.9% NaCl and then recentrifuged. The cells of the washed pellet were resuspended in 4 ml of 0.1 *M* Tris/HCl buffer (pH 7.8) per gram fresh weight of cells. The cells of this suspension were disrupted in a French pressure-cell at 16,000 psi and then centrifuged for 30 min at 24,000*g* at 4°C. The supernatant was then frozen until used.

Protein determination. The protein concentration of the cell-free extract was determined by the Coomasie method (6). A stock dye solution was prepared by bringing 100 mg of Coomasie G-250 dye, 50 ml of 95% ethanol, and 100 ml of 85% H_3PO_4 to a final volume of 200 ml with distilled H_2O. This stock solution was diluted 1:5 with distilled water and filtered through Whatman No. 1 filter paper. Five milliliters of this diluted dye solution were added to a 0.1-ml aliquot of the extract. The absorbance at 595 nm was determined between 2 and 60 min after addition of the dye solution to the sample. Maximum absorbance readings are obtained during this time period. The absorbance reading was then compared to a standard protein curve which was prepared using bovine serum albumin.

Assay procedure. Shikimic acid was converted to anthranilic acid by the enzymes in the cell-free extract (34). A reaction mixture containing Tris/HCl buffer (pH 8.2) 30 m*M*, $MgCl_2$ 5 m*M*, ribose-5-phosphate 1 m*M*, NAD 1 m*M*, glutamine 5 m*M*, shikimic acid 1 m*M*, protein 2.5 mg, and the appropriate inhibitor treatment was brought to a final volume of 1 ml and then incubated in test tubes at 37°C for the appropriate time period. All inhibitor solutions were titrated to pH 8.2 prior to addition to the reaction mixture. Anthranilic acid was extracted from the reaction mixture by addition of 0.1 ml of 1 *N* HCl followed by 4 ml of ethyl acetate. The tubes were mixed with a test tube mixer and then centrifuged at 216*g* for 15 min. The absorbance at 336 nm of the ethyl acetate layer was then measured against an ethyl acetate blank. This absorbance reading was then compared to those of a standard anthranilic acid curve to determine the amount of anthranilate produced during the reaction.

RESULTS AND DISCUSSION

The effects of various concentrations of SC-0224 and glyphosate, in both their technical grades and formulations, on the growth of inflated duckweed, are presented in Table 1. This comparison shows SC-0224 to be a stronger inhibitor of duckweed growth than glyphosate. Table 1 also shows that there are no significant differences between the growth inhibition induced by

TABLE 1
The Effect of Various Concentrations of Formulated (Form.) and Technical Grade (Tech.) Glyphosate and SC-0224 on the Dry Weight of Lemna gibba Grown in E Medium (8) for 7 Days

Concn (μ*M*)	Chemical (mg)[a]			
	SC-0224 (Tech.)	SC-0224 (Form.)	Glyphosate (Tech.)	Glyphosate (Form.)
0	30.12 z	30.12 z	30.12 y	30.12 y
10	10.07 a y	10.96 a y	30.08 b y	29.92 b y
50	3.97 a x	4.47 a x	18.90 b x	18.22 b x
100	1.66 a w	1.33 a w	9.93 b w	10.33 b w

[a] Values represent the average of two experiments with five replications per treatment. The letters a and b correspond to comparisons within rows and the letters w, x, y, and z correspond to comparisons within columns. Means which are followed by the same letter are not significantly different at the 0.05 level according to Duncan's multiple range test.

technical grade versus the formulated forms of either herbicide; this finding has two implications. One is that the added constituents of the formulated versus the technical grade forms of the herbicides have no significant growth effects in these cases. The second implication of this comparison is that the use of surfactants as an aid to penetration into the plant is unnecessary in the case of duckweed. This is reasonable in light of the fact that in this study duckweed was floating in continous contact with the treatment solutions and the chemicals may therefore be taken into the plant either by the roots or by the surface of the fronds.

The effects of supplemental aromatic amino acids on the growth inhibition of duckweed induced by formulated and technical grade glyphosate are presented in Tables 2 through 6.

Addition of Phe (0.20 m*M*) to the growth medium was found to be effective in preventing growth inhibition induced by both the formulated and technical grade forms of glyphosate (0.10 m*M*) (Table 2). The addition of both Phe (0.10 m*M*) and Tyr (0.10 m*M*) was more effective than Phe alone in preventing growth inhibition by the two forms of glyphosate (Table 3). The combination of Phe, Tyr, and Trp was no more effective than the combination of Phe and Try only in preventing growth inhibition of duckweed induced by glyphosate, but was more effective than Phe alone. The data presented in Table 5 demonstrate that these amino acids were equally effective in preventing growth inhibition induced by the isopropylamine salt of glyphosate without surfactant. Because it was found to be a stronger inhibitor of duckweed growth, SC-0224 was used at 0.05 m*M* compared with 0.10 m*M* for glyphosate in these

TABLE 2
The Effect of Phe on the Growth Inhibition of Lemna gibba Induced by Formulated (Form.) and Technical Grade (Tech.) Glyphosate and SC-0224

Treatment[a]	Chemical concn (m*M*)	Dry wt[b] (mg)
Control	—	27.90 a
Phe	0.20	29.39 a
Glyphosate (Tech.)	0.10	10.14 c
Glyphosate (Form.)	0.10	11.04 c
SC-0224 (Tech.)	0.05	3.15 d
SC-0224 (Form.)	0.05	2.53 d
Glyphosate (Tech.) + Phe	0.10 0.20	16.75 b
Glyphosate (Form.) + Phe	0.10 0.20	19.46 b
SC-0224 (Tech.) + Phe	0.05 0.20	3.80 d
SC-0224 (Form.) + Phe	0.05 0.20	2.13 d

[a] Plants were grown in E medium (8) for 7 days.

[b] Values represent the average of two experiments with five replications per treatment. Means followed by the same letter are not significantly different at the 0.05 level according to Duncan's multiple range test.

TABLE 3
The Effect of Phe and Tyr on the Growth Inhibition of Lemna gibba Induced by Formulated (Form.) and Technical Grade (Tech.) Glyphosate and SC-0224

Treatment[a]	Chemical concn (m*M*)	Dry wt[b] (mg)
Control	—	31.18 a
Phe + Tyr	0.10 + 0.10	33.19 a
Glyphosate (Tech.)	0.10	10.79 c
Glyphosate (Form.)	0.10	10.73 c
SC-0224 (Tech.)	0.05	2.59 d
SC-0224 (Form.)	0.05	3.37 d
Glyphosate (Tech.) + Phe + Tyr	0.10 + 0.10 + 0.10	24.21 b
Glyphosate (Form.) + Phe + Tyr	0.10 + 0.10 + 0.10	22.31 b
SC-0224 (Tech.) + Phe + Tyr	0.05 + 0.10 + 0.10	3.16 d
SC-0224 (Form.) + Phe + Tyr	0.05 + 0.10 + 0.10	2.77 d

[a] Plants were grown in E medium (8) for 7 days.
[b] Values represent the average of two experiments with five replications per treatment. Means followed by the same letter are not significantly different at the 0.05 level according to Duncan's multiple range test.

TABLE 4
The Effect of Phe, Tyr, and Trp on the Growth Inhibition of Lemna gibba Induced by Formulated (Form.) and Technical Grade (Tech.) Glyphosate and SC-0224

Treatment[a]	Chemical concn (m*M*)	Dry wt[b] (mg)
Control	—	28.42 a
Phe + Tyr + Trp	0.01 + 0.01 + 0.01	30.89 a
Glyphosate (Tech.)	0.10	9.61 c
Glyphosate (Form.)	0.10	9.99 c
SC-0224 (Tech.)	0.05	3.13 d
SC-0224 (Form.)	0.05	2.06 d
Glyphosate (Tech.) + Phe + Tyr + Trp	0.10 + 0.10 + 0.10 + 0.10	22.76 b
Glyphosate (Form.) + Phe + Tyr + Trp	0.10 + 0.10 + 0.10 + 0.10	22.76 b
SC-0224 (Tech.) + Phe + Tyr + Trp	0.05 + 0.10 + 0.10 + 0.10	3.37 d
SC-0224 (Form.) + Phe + Tyr + Trp	0.05 + 0.10 + 0.10 + 0.10	1.79 d

[a] Plants were grown in E medium (8) for 7 days.
[b] Values represent the average of two experiments with five replications per treatment. Means followed by the same letter are not significantly different at the 0.05 level according to Duncan's multiple range test.

studies. None of the aromatic amino acid treatments was effective in preventing inhibition of duckweed growth by either the technical grade or the formulated form of SC-0224 (Tables 2–4). When used at 5 μ*M*, SC-0224 caused less growth inhibition than at higher concentrations; however, the amino acid treatments were not effective in reducing growth inhibition at this concentration either (Table 6).

These supplemental aromatic amino acid studies are in general agreement with the work of Jaworski with glyphosate (21). The failure of the amino acid treatments to prevent growth inhibition caused by SC-0224 indicates that the effect of this herbicide on duckweed is different from that of glyphosate. The fact that SC-0224 is structurally different from glyphosate only in the cationic constituent of its salt leaves two possible explanations for the different effects of the two herbicides. One explanation would be that the TMS ion acts in an additive or synergistic manner when in combination with PMG to cause the observed differences. A second explanation would be that the TMS ion acts independently of PMG causing the different effects of these herbicides.

The effects of both the TMS and the isopropylamine (IPA) ions on the growth of duckweed are presented in Table 7. These data show that IPA-hydrochloride causes no significant reduction in duckweed growth, whereas the TMS salts inhibited

TABLE 5
The Effect of Phe, Tyr, and Trp on the Growth Inhibition of Lemna gibba Induced by the Isopropylamine Salt of Glyphosate

Treatment[a]	Chemical concn (m*M*)	Dry wt[b] (mg)
Control	—	29.30 a
Phe + Tyr + Trp	0.10 + 0.10 + 0.10	29.89 a
IPA-glyphosate[c]	0.10	9.70 c
IPA-glyphosate + Phe + Tyr + Trp	0.10 + 0.10 + 0.10 + 0.10	25.68 b

[a] Plants were grown in E medium (8) for 7 days.
[b] Values represent the average of two experiments with five replications per treatment. Means followed by the same letter are not significantly different at the 0.05 level according to Duncan's multiple range test.
[c] IPA-glyphosate = isopropylamine salt of glyphosate.

TABLE 6
The Effect of Phe, Tyr, and Trp on the Growth Inhibition of Lemna gibba Induced by SC-0224 (Technical Grade)

Treatment[a]	Chemical concn (m*M*)	Dry wt[b] (mg)
Control	—	29.43 a
Phe + Tyr + Trp	0.10 + 0.10 + 0.10	29.96 a
SC-0224	0.005	9.39 b
SC-0224 + Phe + Tyr + Trp	0.005 + 0.10 + 0.10 + 0.10	9.14 b

[a] Plants were grown in E medium (8) for 7 days.
[b] Values represent the average of two experiments with five replications per treatment. Means followed by the same letter are not significantly different at the 0.05 level according to Duncan's multiple range test.

duckweed growth. Two different TMS salts were used in this study to account for any effect that the halide anion may have on growth. No significant difference was found between the iodide and the chloride salts of TMS. An additional study (data not shown) showed that potassium iodide (0.10 m*M*) did not cause growth inhibition of duckweed, indicating that the iodide anion did not inhibit duckweed growth at the concentrations used in this study. These data (Table 7) indicate that the TMS ion alone has strong phytotoxic effects on duckweed growth. A comparison of the effects of SC-0224 and TMS iodide on duckweed growth is presented in Table 8. These data show that there is no significant difference in the effects of the TMS ion and SC-0224 on duckweed growth. The data of Tables 7 and 8 indicate that the differences found between the effects of glyphosate and SC-0224 on duckweed may be accounted for by the effects of the TMS ion acting alone.

TABLE 7
The Effect of Isopropylamine Hydrochloride (IPA-HCl), Trimethylsulfonium Iodide (TMS-I), and Trimethylsulfonium Chloride (TMS-Cl) on the Growth of Lemna gibba

Treatment[a]	Chemical concn (m*M*)	Dry wt[b] (mg)
Control	—	28.59 a
IPA-HCl	0.10	27.30 a
TMS-I	0.10	1.24 b
TMS-Cl	0.10	1.43 b

[a] Plants were grown in E medium (8) for 7 days.
[b] Values represent the average of two experiments with five replications per treatment. Means within a column which are followed by the same letter are not significantly different at the 0.05 level according to Duncan's multiple range test.

TABLE 8
The Effects of Various Concentrations of SC-0224 (Technical Grade) and Trimethylsulfonium Iodide (TMS-I) on the Dry Weight of Lemna gibba[a]

Concn (μ*M*)	Dry wt[b] (mg)	
	SC-0224	TMS-I
0	29.26 a	29.26 a
1	27.49 a	29.99 a
10	10.24 b	9.45 b
50	4.33 c	4.50 c

[a] Plants were grown in E medium (8) for 7 days.
[b] Values represent the average of two experiments with five replications per treatment. Means within a column which are followed by the same letter are not significantly different at the 0.05 level according to Duncan's multiple range test. An analysis of variance test showed no significant difference between chemical treatments.

The finding that TMS has a stronger and more rapid effect than PMG on inflated duckweed does not preclude the possibility that PMG may also act independently of TMS on the shikimic acid pathway. The effects of TMS, PMG, and SC-0224 on the conversion of shikimate to anthranilate in a cell-free extract of *K. pneumoniae* were assayed. The reactions of this assay involved four enzymes including shikimate kinase, EPSP-synthase, chorismate synthase, and anthranilate synthase. The results presented in Tables 9 and 10 are in agreement with the previous findings of Steinrucken and Amrhein (34) and indicate that SC-0224, like glyphosate, is effective in inhibiting these reactions, whereas TMS-iodide caused no inhibition. Although the enzymes were not assayed individually in the present study, previous studies have shown that of the four enzymes glyphosate inhibits EPSP-synthase exclusively (34). Because of the structural similarities of gly-

TABLE 9

The Effects of Glyphosate (PMG), SC-0224, and Trimethylsulfonium Iodide (TMS-I) on the Conversion of Shikimate to Anthranilate in a Cell-Free Extract of Klebsiella pneumoniae after Various Time Intervals

Time (min)	Treatment[a] (μmol anthranilate)[b]			
	Control	PMG	SC-0224	TMS-I
0	45 b u	48 b u	49 b u	43 b u
15	65 a v	42 b u	43 b u	58 a v
30	75 a w	48 b u	38 b u	88 a w
40	120 a x	40 b u	45 b u	115 a x
60	131 a y	50 b u	49 b u	120 a y
120	183 a z	52 b u	60 b u	190 a z

[a] Technical grade glyphosate, SC-0224, and TMS-I (1 m*M*) were used in this experiment.

[b] Values represent the average of two experiments with five replications per treatment. The letters a and b correspond to comparisons within rows and the letters u through z correspond to comparisons within columns. Means which are followed by the same letter are not significantly different at the 0.05 level according to Duncan's multiple range test.

phosate and SC-0224 and the failure of TMS iodide to inhibit these reactions, it is reasonable to assume that SC-0224 was inhibiting EPSP-synthase in this study. Although the enzymes used in this study were from a bacterium, previous studies have shown that glyphosate inhibits EPSP-synthase from other organisms also (5, 10, 13, 26). EPSP-synthase isolated from a higher plant (pea) was found to be more sensitive to glyphosate than EPSP-synthase isolated from a microorganism (*Neurospora crassa*) (5, 26). Based on these previously reported findings and the data presented here, it can be assumed that SC-0224 inhibits EPSP-synthase in higher plants.

TABLE 10

The Effects of Various Concentrations of Glyphosate (PMG), SC-0224, and Trimethylsulfonium Iodide (TMS-I) on the Conversion of Shikimate to Anthranilate in a Cell-Free Extract of Klebsiella pneumoniae after 40 min

Concn	Treatment[a] (μmol anthranilate)[b]		
	PMG	SC-0224	TMS-I
0	120 z	120 z	120 x
1	83 a y	90 a y	125 b x
10	50 a x	53 a x	135 b x
100	40 a x	45 a x	115 b x

[a] Technical grade herbicides were used in this experiment.

[b] Values represent the average of two experiments with three replications per treatment. The letters a and b correspond to comparisons within rows and the letters x, y and z correspond to comparisons within columns. Means which are followed by the same letter are not significantly different at the 0.05 level according to Duncan's multiple range test.

The data presented here have shown that the two ions of the SC-0224 structure, TMS and PMG, may act independently of each other with each exhibiting phytotoxic effects. These data do not indicate that TMS and PMG act synergistically when used in combination; however, the data do not eliminate possible synergistic action in other plant species and/or on other physiological processes. SC-0224, like glyphosate, was found to be a strong inhibitor of the shikimic acid pathway. Inhibition of this pathway is believed to be the primary mechanism of action for glyphosate, with PMG being the active consitituent of the herbicide. This study has indicated that use of PMG as its TMS salt (SC-0224) does not alter its ability to inhibit this pathway. The present study has also indicated that the phytotoxic effect of the TMS ion is more important than that of PMG in the case of duckweed, an aquatic species. However, this relationship may not hold in the case of

terrestrial species which may be less sensitive to TMS.

Glyphosate and SC-0224 have been shown to have similar efficacy in many field studies (3, 4, 17, 24, 39), which suggests that PMG is the most active of the two SC-0224 ions in these terrestrial species. However, Hutchinson and Banks (20) have reported that, in some cases, bermudagrass is more sensitive to SC-0224 than to glyphosate. These similarities and differences in the action of glyphosate and SC-0224 may be due to differences in the sensitivity of various species to TMS. Previous studies (32) have found TMS to be much more phytotoxic to bermudagrass than to many other plant species. These findings suggest that in some plant species PMG may be the most phytotoxic ion of the SC-0224 structure, whereas in other species TMS may be the most active ion.

In conclusion, SC-0224 contains two constituent parts which apparently act independently in the systems studied here. TMS is apparently the more important of the two ions in the case of duckweed, exerting an acute phytotoxic response which overshadows the slower effect on the shikimate pathway. However, this situation may be reversed in the case of many terrestrial species possibly due to differences in the phytotoxicity of TMS among species and/or differences in the penetration and translocation properties of TMS in terrestrial species. Additional studies in which SC-0224 is compared directly with TMS and glyphosate on terrestrial species are needed to resolve this situation conclusively.

ACKNOWLEDGMENTS

This work was supported, in part, by Stauffer Chemical Co. and the Virginia Agricultural Experiment Station. We thank Stauffer Chemical Co. and Monsanto Agricultural Products Co. for providing technical and formulated SC-0224 and glyphosate, respectively.

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