Transformation of the Herbicide [^{14}C]Glufosinate in Soils

Allan E. Smith

The degradation of 2 μg/g [^{14}C]glufosinate (DL-homoalan-4-ylmethylphosphinic acid) was studied in clay, clay loam, and sandy loam soils at 85% field capacity and at 20 °C. Over a 4-week period the soils were extracted and analyzed for transformation products by radiochemical and gas chromatographic techniques. In all soils there was release of [^{14}C]carbon dioxide and formation of [^{14}C]-3-(hydroxymethylphosphinyl)propionic acid (MPPA) as major degradation products. Within 21 days, about 55% of the applied ^{14}C herbicide had been transformed to MPPA in the sandy loam and 19% to [^{14}C]carbon dioxide. After 28 days, approximately 45% of the ^{14}C herbicide had been transformed to MPPA in the clay and clay loam and 10% released as [^{14}C]carbon dioxide. At all samplings, other ^{14}C transformation products appeared to be insignificant.

Glufosinate-ammonium (Figure 1, **a**), the ammonium salt of DL-homoalan-4-ylmethylphosphinic acid, is currently being evaluated worldwide, at rates up to 1 kg/ha, as a postemergence treatment for the nonselective control of annual and perennial broadleaf weeds and grasses in a variety of agricultural situations.

The herbicide (Figure 1, **a**) and the free amino acid referred to here as glufosinate, although it is also known as phosphinothricin (Bayer et al., 1972), are nonvolatile, soluble in water and methanol, and insoluble in nonpolar solvents. Herbicidal action results from interference with photosynthesis by inhibition of glutamine synthetase, the enzyme that catalyzes the combination of glutamic acid and ammonia (Leason et al., 1982; Fraser and Ridley, 1984).

In moist Saskatchewan soils, [^{14}C]glufosinate is biologically transformed to unidentified degradation product(s) that are further degraded to [^{14}C]carbon dioxide (Smith, 1988). At 20 °C, the half-life values for the ^{14}C herbicide in three prairie soils were 3–7 days and, at 10 °C, 8–11 days. Over a 90-day incubation period at 20 °C, between 28 and 55% of the applied radioactivity was released from the treated soils as [^{14}C]carbon dioxide (Smith, 1988). Unpublished research (Gildemeister, 1986) has confirmed that MPPA (Figure 1, **b**; 3-(hydroxymethylphosphinyl)-propionic acid) is a major degradation product of glufosinate-ammonium in soils.

The present studies were undertaken to isolate and quantify ^{14}C transformation products formed during the breakdown of [^{14}C]glufosinate in clay, clay loam, and sandy loam soils at 85% of field capacity and at 20 °C. Over a 4-week period, the treated soils were extracted and analyzed for ^{14}C transformation products by radiochemical techniques. Soil-based residues of glufosinate and MPPA were also quantified gas chromatographically.

MATERIALS AND METHODS

Soils. The three soils used in these studies were a clay (Typic Boroll), a clay loam (Udic Boroll), and a sandy loam (Typic Boroll), the composition and physical characteristics of which are summarized in Table I.

Soil samples were collected, during October 1987, from the 0–10-cm soil horizons of fallow areas that had not been cropped for several years. The soils were stored at laboratory temperature, in wooden boxes, and passed through a 2-mm screen prior to use in early 1988.

Chemicals. [^{14}C]Glufosinate (as its hydrochloride), labeled in the two $-CH_2-$ carbon atoms and with a specific activity of 54.4 μCi/mg and a radiochemical purity of over 99%, was provided by Hoechst Aktiengesellschaft, Frankfurt am Main, FRG, as were analytical samples of glufosinate-ammonium and MPPA. The [^{14}C]glufosinate salt was dissolved in water to give a solution containing 0.151 MBq/mL and 63 μg of glufosinate/mL. An aqueous solution of nonradioactive glufosinate-ammonium was also prepared containing the equivalent of 1.0 mg of glufosinate/mL.

Table I. Composition and Physical Characteristics of Soils

soil	composition, % clay	silt	sand	organic content	field capacity, %	pH
clay	70	25	5	4.2	40	7.7
clay loam	30	40	30	11.7	35	6.0
sandy loam	10	25	65	4.0	20	7.6

Degradation Studies. Duplicate samples (50 g) of the three soils at 85% of their field capacities were weighed into polystyrene foam cartons and placed in 2-L Mason jars fitted with spring-clip lids. The jars were placed in a darkened incubator at 20 ± 1 °C for 7 days. There were no moisture losses during this equilibration period. The soils were then treated with [^{14}C]glufosinate hydrochloride solution (100 μL, 15.1 kBq, 6 μg of glufosinate) and with glufosinate-ammonium solution (93 μL, 93 μg acid equivalent) so that the moist soil treatments contained 2 μg/g glufosinate. This concentration is approximately equal to a rate of 1 kg/ha, assuming that, in the field, the herbicide is located in the top 5 cm of soil. The soils were carefully mixed to distribute the chemical in the soil and the cartons replaced in the Mason jars. In each jar was placed a 50-mL beaker containing a 20-mL glass vial filled with 0.2 N aqueous sodium hydroxide (15 mL) solution to absorb [^{14}C]carbon dioxide evolved (Soulas et al., 1984). Following treatment, the jars were reincubated, in the dark, at 20 ± 1 °C. Samples (1 mL) of the sodium hydroxide were analyzed for radioactivity at regular intervals, at which time the absorbing vials were replaced with others containing fresh sodium hydroxide. During the incubation period there were negligible moisture losses from the soils.

Duplicate sandy loam treatments were extracted and analyzed after 1 h and then after 3, 7, 14, and 21 days, while duplicate samples of the treated clay were analyzed after 1 h and then after 3, 7, 14, and 28 days. Clay loam replicates were taken for analysis after 1 h and 7, 14, and 28 days. The cumulative amounts of [^{14}C]carbon dioxide released at each sampling time were calculated as a percentage of the total radioactivity originally applied to the soils.

Extraction and Analysis. The procedure for the extraction and analysis of the soils is summarized in Figure 2. The soils from the cartons were placed in a 250-mL glass-stoppered flask, and sufficient water was added so

Agriculture Canada Research Station, Box 440, Regina, Saskatchewan, S4P 3A2 Canada.

0021-8561/89/1437-0267$01.50/0 Published 1989 by the American Chemical Society

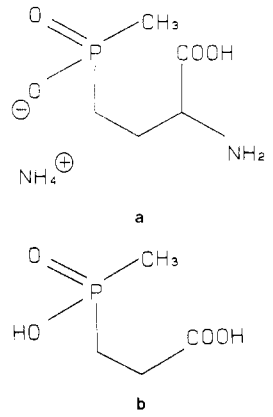

Figure 1. Structures of glufosinate-ammonium (**a**) and MPPA (**b**).

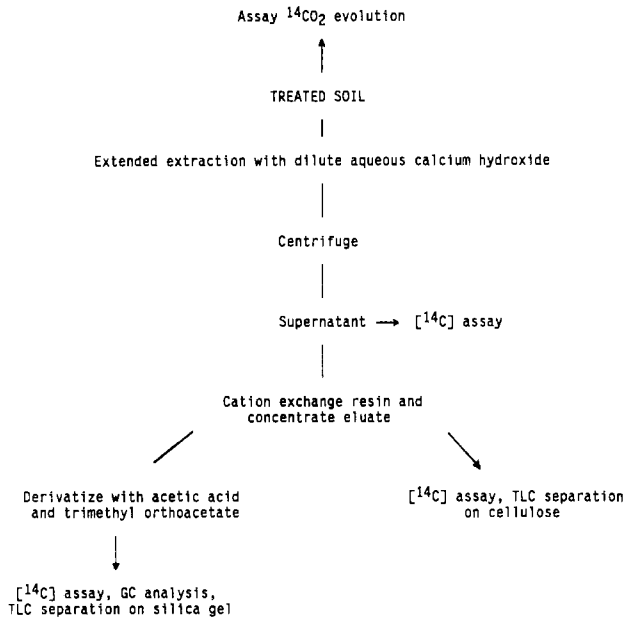

Figure 2. Outline of procedures used for the extraction and analysis of [^{14}C]glufosinate and its transformation products from soils.

that the total volume of water added, together with the water present in the soil sample, was 100 mL. Calcium hydroxide was added to each flask (300, 600, and 800 mg to the sandy loam, clay, and clay loam slurries, respectively), and the flasks were shaken on a wrist-action shaker for 1 h. The soils then remained in contact with the alkaline extractant overnight before being shaken for a further 1-h period. After centrifugation at 3500 rpm for 10 min, supernatant (3 mL) was examined for extractable radioactivity.

Portions of the aqueous supernatant were passed through a H^+ cation-exchange resin to remove calcium and other soil cations and to convert salts of glufosinate, and any other acidic degradation products, to their respective free acids. The resin was prepared by adding Dowex 50W-X8 cation-exchange resin (100–200 mesh) in the H^+ form (6.0 g; Bio-Rad Laboratories, Richmond, CA) to a chromatography column (40 × 1.4 cm (i.d.)) having a 250-mL reservoir. After the resin was washed successively, at a rate of 5 mL/min, with 50-mL portions of acetone, distilled water, and 1.0 N hydrochloric acid, the column was finally washed with a further 50-mL portion of distilled water. All washings were discarded. Aqueous supernatant (50 mL, equivalent to 25 g of moist soil) was percolated through the resin at a flow rate of about 5 mL/min and the eluate collected in a 500-mL round-bottom flask. As soon as the level of extract reached the level of the column, the column was rinsed with two portions (5 mL each) of distilled water. The column was finally percolated with 140 mL of distilled water at a flow rate of about 5 mL/min. The entire column eluate (200 mL) was evaporated to dryness at 50 °C on a rotary evaporator and the residue redissolved in distilled water (20.0 mL), an aliquot (1 mL) of which was examined for radioactivity.

Some of the above extract (10 mL, equivalent to 12.5 g of moist soil) was transferred to a 50-mL glass tube. Two glass beads were added, and the water was evaporated to dryness at 50 °C on a rotary evaporator. To the residue were added glacial acetic acid (1 mL) and trimethyl orthoacetate (4 mL). The tube was fitted with an air condenser and the whole heated under reflux conditions, in a well-ventilated fume hood, for 2 h. These conditions convert amino groups to aminoacetyl derivatives and also methylate both hydroxyl and carboxylic groups (Gildemeister, 1986). After cooling, the condenser and tube were rinsed with ethyl acetate and the volume of extract was adjusted to 25 mL. Portions of this extract (1 mL) were assayed for radioactivity and also analyzed by gas chromatography. The remaining solution was evaporated, at 25 °C, to approximately 0.5 mL and examined by silica gel thin-layer chromatographic and radiochemical techniques for the presence of ^{14}C-containing compounds.

The remaining postcolumn extracts (about 9 mL) were transferred to a 50-mL tube and evaporated at 50 °C to approximately 0.5 mL. These evaporated extracts were then subjected to chromatographic separation, on cellulose TLC plates, and then radiochemical analysis.

Thin-Layer Chromatography. For the evaporated postcolumn extracts, TLC plates precoated with 0.1 mm of cellulose (E. Merck, Darmstadt, FGR) were used. The plates were developed to a height of 8 cm above the origin with a mixture of 2-propanol–acetone–1 N hydrochloric acid (30:7.5:12.5), as previously reported (Smith, 1988). With the derivatized extracts, TLC plates coated with silica gel 60F-254 (Merck) were developed to a height of 10 cm above the origin with a mixture of chloroform and glacial acetic acid (70:30).

After development, the plates were air-dried in a fume hood and radioactive compounds detected and quantified on a Model 2832 Berthold automatic TLC-linear analyzer (Labserco Ltd., Oakville, ON, Canada). Typical radiochromatogram scans are displayed in Figure 3. With the cellulose chromatographic system, the mean R_f value of [^{14}C]glufosinate was 0.45, while ^{14}C transformation product(s) extracted from the soils appeared as a band with an average R_f of 0.85. On the silica gel surfaces, derivatized [^{14}C]glufosinate appeared as a streak with an R_f between 0.0 and 0.3. A compound with an R_f value of 0.35, which always accounted for less than 2% of the applied radioactivity, was considered to be an artifact resulting from the derivatization process, since traces of this compound were observed on all chromatograms, including those of derivatized [^{14}C]glufosinate standards. A major product with an average R_f of 0.55 and a minor product with a mean R_f of 0.72 were also separated on silica gel (Figure 3).

The R_f value of MPPA could not be determined directly since this compound has no ultraviolet absorption and undergoes no reaction with reagents normally sensitive to phosphorus groupings.

Gas Chromatographic Analysis. Derivatized glufosinate and MPPA extracts were analyzed using a Hewlett-Packard 5730A gas chromatograph equipped with a

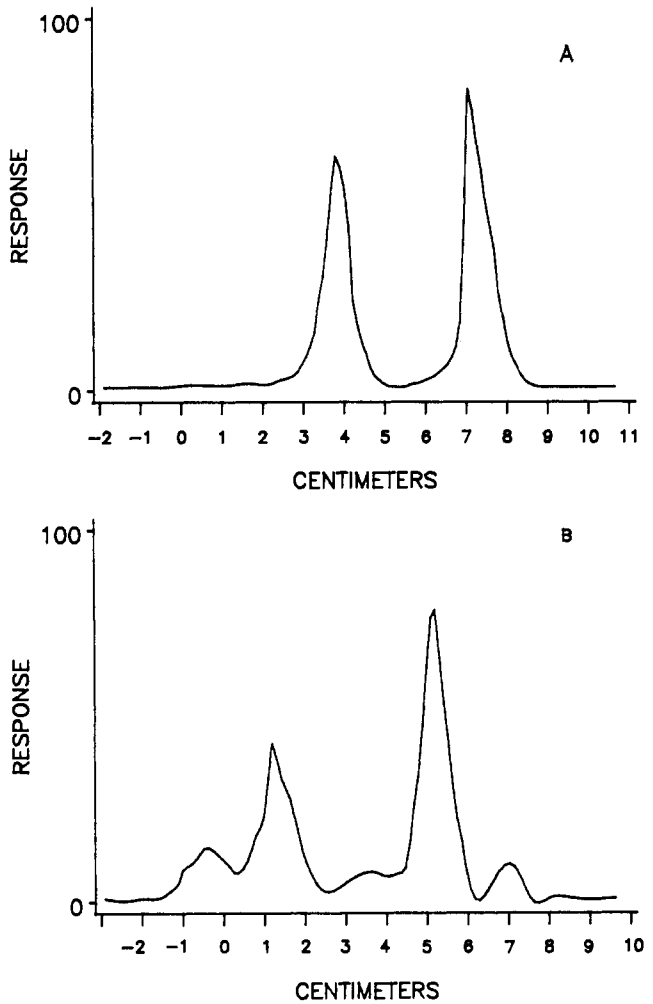

Figure 3. A. Radiochromatogram scan of extracts from a sandy loam incubated 7 days with [14C]glufosinate and separated on cellulose surfaces (8.0 cm) with 2-propanol–acetone–1 N hydrochloric acid (30:7.5:12.5) as developing solvent. B. Radiochromatogram scan of derivatized extracts from a sandy loam incubated 7 days with [14C]glufosinate and separated on silica gel surfaces (10.0 cm) with chloroform and acetic acid (70:30) as developing solvent.

nitrogen–phosphorus flame ionization detector operated at 15 V. The megabore column (15 m × 0.53 mm (i.d.)) was of fused silica coated with 1.5 μm of OV-17. Column carrier gas was helium at a flow rate of 5 mL/min, while flow rates of hydrogen and air through the detector were 3 and 50 mL/min, respectively. All samples (2 μL) were injected using a Varian Series 8000 autosampler. With injection port and detector temperatures of 250 and 300 °C, and a column temperature of 205 °C, the retention time of the derivatized glufosinate was 11.2 min. With a column temperature of 140 °C, the retention time of the derivatized MPPA was 11.5 min.

All gas chromatographic standards were freshly prepared by derivatizing glufosinate and MPPA acids (25 μg), as described, at the same time as the soil extracts were being derivatized. The standard solutions thus contained the equivalent of 1 ng of the respective acids/μL and were then diluted with ethyl acetate as necessary. Data were plotted and integrated on a Varian Vista 402 chromatography work station.

Confirmation of MPPA as Transformation Product. The ethyl acetate solutions containing derivatized soil extracts from the 7-day incubation, in the case of the sandy loam, and the 28-day treatments of the clay and clay loam were evaporated barely to dryness at 25 °C, and the

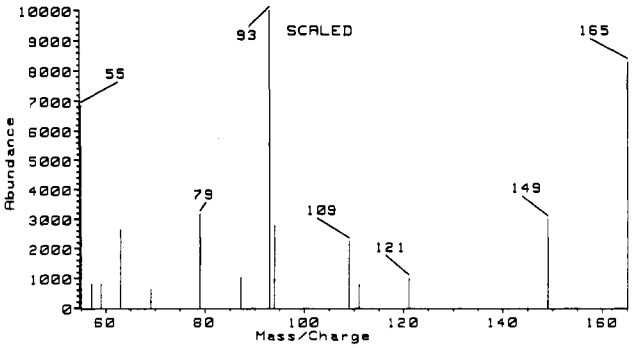

Figure 4. Mass spectrum of dimethylated MPPA obtained by GC–MS.

residue was taken up in 1 mL of acetone. Aliquots (2 μL) were then injected, with a Hewlett-Packard 7673A automatic sampler, into a Hewlett-Packard 5890A gas chromatograph equipped with a 5970 mass ion detector and scanned from 10 to 200 amu. The injector was operated in the splitless mode, with a purge time of 0.8 min. Injector temperature was 240 °C, with a transfer line temperature of 280 °C. The column was of fused silica (25 m × 0.2 mm) coated with 0.33 μm of HP-1 Ultra; carrier gas was helium with a gas linear velocity of 25.3 cm/s. Initially the column was held at 70 °C for 1 min after injection and then increased at a rate of 5 °C/min to a final temperature of 250 °C.

Under the above conditions, dimethylated MPPA had a retention time of approximately 15.7 min, and the mass spectrum (Figure 4), with major ions at *m/e* 165, 149, 121, 109, 93, and 79, was identical with that provided by Hoechst Aktiengesellschaft (Gildemeister, 1986).

Radioactivity Determination. The radioactivity in the various solutions was measured using a Packard Tri-Carb 300C liquid scintillation spectrometer, with counting efficiencies being determined using an external ^{226}Ra standard. Scinti Verse II (15 mL; Fisher Scientific Co., Fair Lawn, NJ) was used as liquid scintillation solution.

RESULTS AND DISCUSSION

It was assumed that both ammonium and hydrochloride salts of glufosinate would dissociate in the neutral soils to the anionic form and then reassociate with inorganic soil cations prior to undergoing breakdown. A similar dissociation of the dimethylamine salts of 2,4-D and dicamba is known to occur in Saskatchewan soils (Grover and Smith, 1974).

The results of the transformation experiments conducted with [14C]glufosinate in the clay, clay loam, and sandy loam are summarized in Tables II–IV. There was release of [14C]carbon dioxide from all soils with more rapid evolution from the sandy loam (19% after 21 days) than from the clay and clay loam (about 10% after 28 days). These losses were very similar to those noted previously (Smith, 1988) from the same soils under identical conditions. Extractable radioactivity from the three soils, at all sampling times, was quantitatively eluted from the cation-exchange resin. In addition, there was no ^{14}C lost during the subsequent evaporation of the postcolumn aqueous extracts or during derivatization (Tables II–IV). With time, there was an increase in the nonextractable radioactivity from the treated soils (Tables II–IV). This loss was attributed to radioactivity being incorporated into soil microbial biomass and into the fulvic, humic, and humin soil fractions as previously reported (Smith, 1988).

Thin-layer chromatographic and radiochemical analyses indicated (Tables II–IV) that, in the three soils, ^{14}C transformation products were formed possessing higher R_f

Table II. Radioactivity, Glufosinate, and MPPA Recovered over a 28-Day Period from a Clay Treated with 2 μg/g [^{14}C]Glufosinate following Incubation at 20 °C and 85% Field Capacity

	% of applied [^{14}C]glufosinate recovered[a]				
	0 days	3 days	7 days	14 days	28 days
radioact released as CO_2	0	1	3	5	12
aq extr radioact	96	88	81	84	69
post cation resin radioact	100	88	80	83	65
radioact in deriv extr	96	87	74	81	63
mean	97	88	78	83	66
total radioact recovered	97	89	81	88	78
[^{14}C]glufosinate[b]	95	59	41	30	12
^{14}C transformn products[b]	2	29	37	53	54
deriv [^{14}C]glufosinate[c]	97	61	44	38	17
deriv [^{14}C]1[c]	0	25	32	42	45
deriv [^{14}C]2[c]	0	2	2	3	4
glufosinate by GC	88	66	50	43	12
MPPA (glufosinate equiv) by GC	<5	25	29	41	37

[a] Average of duplicate samples. [b] Separated on cellulose surfaces. [c] Separated on silica gel surfaces.

Table III. Radioactivity, Glufosinate, and MPPA Recovered over a 28-Day Period from a Clay Loam Treated with 2 μg/g [^{14}C]Glufosinate following Incubation at 20 °C and 85% Field Capacity

	% of applied [^{14}C]glufosinate recovered[a]			
	0 days	7 days	14 days	28 days
radioact released as CO_2	0	2	3	9
aq extr radioact	86	80	80	71
post cation resin radioact	90	82	84	67
radioact in deriv extr	90	79	83	65
mean	89	80	82	68
total radioact recovered	89	82	85	77
[^{14}C]glufosinate[b]	87	46	39	17
^{14}C transformn products[b]	2	34	43	51
deriv [^{14}C]glufosinate[c]	86	52	47	26
deriv [^{14}C]1[c]	3	26	35	42
deriv [^{14}C]2[c]	<1	2	<1	<1
glufosinate by GC	93	50	54	24
MPPA (glufosinate equiv) by GC	<5	20	37	33

[a] Average of duplicate samples. [b] Separated on cellulose surfaces. [c] Separated on silica gel surfaces.

values than [^{14}C]glufosinate (cf. Figure 3).

On silica gel, the derivatized extracts were separated into three ^{14}C compounds. The ^{14}C material with R_f 0.0–0.3 was considered to be derivatized [^{14}C]glufosinate since known standards and the extracts recovered from all soils after 1 h revealed a similar chromatographic pattern. Compound 1 (R_f 0.55) was the major ^{14}C transformation product, with 40–45% of the applied radioactivity being in such a form in the clay and clay loam after 28 days (Tables II and III). In the sandy loam, approximately 50% of the applied ^{14}C had been transformed to compound 1 within 14 days (Table IV). Compound 2 (R_f 0.72) was a minor ^{14}C transformation product, and amounts detected were always <5% of the applied radioactivity. Since the soil concentrations were so small, no attempts were made to further characterize compound 2.

As reported earlier (Smith, 1988) cellulose surfaces separated ^{14}C transformation products (R_f 0.85) from [^{14}C]glufosinate (R_f 0.45). However, there was no separation of compound 1 and compound 2 (cf. Figure 3).

Gas chromatographic analysis confirmed that the amounts of glufosinate recovered from all soils at all sampling times were similar to those determined by radiochemical analysis of soil extracts following separation on

Table IV. Radioactivity, Glufosinate, and MPPA Recovered over a 21-Day Period from a Sandy Loam Treated with 2 μg/g [^{14}C]Glufosinate following Incubation at 20 °C and 85% Field Capacity

	% of applied [^{14}C]glufosinate recovered[a]				
	0 days	3 days	7 days	14 days	21 days
radioact released as CO_2	0	2	7	13	19
aq extr radioact	101	92	86	74	68
post cation resin radioact	105	94	85	73	65
radioact in deriv extr	–[b]	97	82	71	64
mean	103	94	84	73	66
total radioact recovered	103	96	91	86	85
[^{14}C]glufosinate[c]	102	52	34	14	3
^{14}C transform products[c]	1	42	50	59	63
deriv [^{14}C]glufosinate[d]	100	56	38	20	11
deriv [^{14}C]1[d]	3	35	43	49	51
deriv [^{14}C]2[d]	<1	3	3	4	4
glufosinate by GC	93	60	44	18	10
MPPA (glufosinate equiv) by GC	<5	27	44	63	56

[a] Average of duplicate samples. [b] Not determined. [c] Separated on cellulose surfaces. [d] Separated on silica gel surfaces.

cellulose and silica gel surfaces (Tables II–IV).

At all sampling dates, the amounts of dimethyl-MPPA in the soil extracts, as determined by gas chromatographic analysis, were very similar to those found for compound 1 by radiochemical analysis of the derivatized extracts (Tables II–IV). Confirmation for the structural identity of compound 1 was provided by mass spectral analysis of the methylated products derived from the 7-day sandy loam incubations and from the 28-day clay and clay loam soil treatments. In all soils, the product with the same gas chromatographic retention time as dimethyl-MPPA also had a mass spectrum identical with that of the authentic compound (Figure 4).

These studies indicate that, besides [^{14}C]carbon dioxide, MPPA is the major transformation product of glufosinate in moist nonsterile soils and that it is rapidly formed as glufosinate undergoes breakdown in the soils (Tables II–IV). At all samplings, the amounts of glufosinate and MPPA recovered from the treated soils, as determined by the specific gas chromatographic procedure, together with the small amounts of compound 2 isolated, accounted for nearly all of the radioactivity extracted from the soils by the aqueous solvent. This indicates that significant quantities of water-soluble ^{14}C transformation products containing carbon atoms derived from the C3 or C4 atoms of glufosinate are not formed during the microbial breakdown of the herbicide in the soils under test.

The soil transformations of other herbicides containing phosphorus and nitrogen groups have been reported. Fosamine (ethyl hydrogen (aminocarbonyl)phosphonate) undergoes biochemical deethylation in moist soils to carbamoylphosphonic acid (Han, 1979). From the present studies there was no evidence of any significant analogous demethylation of the phosphinico moiety of glufosinate. Glyphosate [*N*-(phosphonomethyl)glycine], whose amino acid grouping more closely resembles that of glufosinate, is biologically degraded in the soil to (aminomethyl)-phosphonic acid via a mechanism involving removal of a $-CH_2COOH$ grouping (Nomura and Hilton, 1977; Rueppel et al., 1977). In the soils under study, the transformation of glufosinate to MPPA appears to be by oxidative deamination.

From the previous investigations with [^{14}C]glufosinate (Smith, 1988), conducted over a 90-day period, evolution of [^{14}C]carbon dioxide from treated soils continued at a constant rate even after 95% of the ^{14}C herbicide had been

degraded. Thus, [^{14}C]MPPA must undergo degradation in the soil with release of [^{14}C]carbon dioxide.

ACKNOWLEDGMENT

Thanks are due to Hoechst Aktiengesellschaft, Frankfurt am Main, FRG, for the generous gift of chemicals. Thanks are also due to A. Aubin for technical assistance and to L. Kerr for determining the mass spectra.

LITERATURE CITED

Bayer, E.; Gugel, K. H.; Hägele, K.; Hagenmaier, H.; Jessipow, S.; König, W. A.; Zähner, H. Phosphinothricin und Phosphinothricin-Alanyl-Alanin. *Helv. Chim. Acta* **1972**, *55*, 224–239.

Fraser, A. R.; Ridley, S. M. Kinetics for Glutamine-Synthetase Inhibition by Phosphinothricin and Measurement of Other Enzyme Activities *In Situ* in Isolated Asparagus Cells Using a Freeze-Thaw Technique. *Planta* **1984**, *161*, 470–474.

Gildemeister, H., Hoechst Aktiengesellschaft, Frankfurt, FRG, 1986, personal communications.

Grover, R.; Smith, A. E. Adsorption Studies with the Acid and Dimethylamine Forms of 2,4-D and Dicamba. *Can J. Soil Sci.* **1974**, *54*, 179–186.

Han, J. C. Y. Stability of [^{14}C]Fosamine Ammonium in Water and Soils. *J. Agric. Food Chem.* **1979**, *27*, 564–571.

Leason, M.; Cunliffe, D.; Parkin, D.; Lea, P. J.; Miflin, B. J. Inhibition of Pea Leaf Glutamine Synthetase by Methionine Sulphoxime, Phosphinothricin and Other Glutamine Analogues. *Phytochemistry* **1982**, *21*, 855–857.

Nomura, N. S.; Hilton, H. W. The Adsorption and Degradation of Glyphosate in Five Hawaiian Sugarcane Soils. *Weed Res.* **1977**, *17*, 113–121.

Rueppel, M. L.; Brightwell, B. B.; Schaefer, J.; Marvel, J. T. Metabolism and Degradation of Glyphosate in Soil and Water. *J. Agric. Food Chem.* **1977**, *25*, 517–528.

Smith, A. E. Persistence and Transformation of the Herbicide [^{14}C]Glufosinate-Ammonium in Prairie Soils under Laboratory Conditions. *J. Agric. Food Chem.* **1988**, *36*, 393–397.

Soulas, G.; Chaussod, R.; Verguet, A. Chloroform Fumigation Technique as a Means of Determining the Size of Specialized Soil Microbial Populations: Application to Pesticide-Degrading Microorganisms. *Soil. Biol. Biochem.* **1984**, *16*, 479–501.

Received for review May 9, 1988. Accepted September 21, 1988.

REVIEW

Insect Neuropeptides: Potential New Insect Control Agents

Julius J. Menn* and Alexej B. Bořkovec

Insect neuropeptides provide exciting new approaches to insect management. The last two decades have seen the emergence of the first chemically identified insect neurohormones, their synthesis, and beginning understanding of their mode of action. The identification of many additional neuropeptides that regulate all aspects of insect development and reproduction can be expected in the near future. Identification of functionally critical neuropeptides and their precursors (proneurohormones) may provide models for designing highly selective insect control agents; these agents may act as antagonists of proneurohormone-processing enzymes or inhibitors of neuropeptide-degrading enzymes. It is also anticipated that through genetic engineering manipulations it will become possible to deliver neuropeptide genes into host cells with successful transformation and subsequent expression via potent expression vectors such as the insect baculoviruses.

EVOLUTION OF NEUROPEPTIDE RESEARCH IN INSECTS

The proposition that neurons, the cellular components of the animal nervous system, produce substances that control the biochemical and physiological processes at distant parts of the body appeared revolutionary when it was first made 70 years ago (Kopec, 1917, 1922). Nevertheless, the hypothesis was confirmed (Scharrer, 1928), and its gradual acceptance in vertebrate as well as invertebrate physiology opened a new area of investigation concerned with the origin, nature, and function of chemical messengers used by the central nervous system to communicate with its subordinate organs. In terms of their chemical structures, most, if not all of these messengers are now believed to be oligopeptides or small protein molecules; but the exact mechanisms by which they function are not known. The generic designation of neuropeptides as "neurohormones" may not always be accurate but will be used here interchangeably as is the common practice.

Kopec formulated his great hypothesis on the basis of laboratory experiments with insects (gypsy moth, *Lymantria dispar*), so it may seem ironic that more than 50 years elapsed before the first insect-derived neuropeptide, proctolin, was isolated and identified (Starratt and Brown, 1975) and that only now are attempts being made, in our laboratory (Masler et al., 1986), to characterize Kopec's original "brain hormone", the gypsy moth prothoracicotropic hormone (PTTH).

The reasons for the slow progress in isolating insect neuropeptides are primarily twofold: They are present in insects in almost unimaginably small quantities that must be measured in femtomoles (1×10^{-15} mol), and they are often highly labile to one or more of the conditions or substances used in the isolation process (e.g., heat, freezing, and organic solvents). Moreover, the richest source of neuropeptides, the neuroendocrine organs, are often microscopic so that thousands or even millions of insects have to be used in the initial extraction. The advent of high-

U.S. Department of Agriculture—Agricultural Research Service, Beltsville, Maryland 20705.