# Development of an Analytical Method for the Determination of Glyphosate and (Aminomethyl)phosphonic Acid Residues in Soils by Nitrogen-Selective Gas Chromatography

Dibyendu N. Roy* and Samir K. Konar

An analytical method has been developed for the determination of glyphosate and its metabolite (aminomethyl)phosphonic acid residues in soils. The herbicide and its metabolite were extracted with deionized water in the presence of phosphoric acid. The compounds in the extract were derivatized with a mixture of trifluoroacetic anhydride and trifluoroethanol and quantified by gas chromatography using a nitrogen–phosphorus detector. The limits of detection of glyphosate and its metabolite were 0.05 and 0.01 $\mu g\ g^{-1}$.

Glyphosate (GLYPH), *N*-(phosphonomethyl)glycine, the active ingredient of the commercial herbicide Roundup (Monsanto Co., St. Louis, MO) is known for its extensive use for controlling both annual and perennial herbaceous plants. It is also used for control of woody plants and undesirable low-quality trees. (Aminomethyl)phosphonic acid (AMPA) has been reported to be the principal metabolite in plants, water, and soils (Sprankle et al., 1978).

A complete review on the analysis of GLYPH and AMPA has recently been published (Bardalaye et al., 1984). Because both compounds are exceptionally polar and thereby highly soluble in water but insoluble in most organic solvents, their extraction and quantification at residue levels are very difficult. The existing gas chromatographic (GC) method (*Pesticide Analytical Manual*, 1977) is very lengthy and laborious, utilizing cleanup with two different ion-exchange columns and a double-derivatization procedure. High-performance liquid chromatography (HPLC) is another method for quantitation of GLYPH and AMPA that includes either postcolumn derivatization (Moye and St. John, 1980; Moye et al., 1983; Lundgren, 1986) or precolumn derivatization (Moye and St. John, 1980). But many laboratories do not have the facility required for this type of pre- or postcolumn fluorogenic labeling.

A mixture of trifluoroacetic anhydride (TFAA) and trifluoroethanol (TFE) has been known to convert both GLYPH and AMPA in a single-step reaction to derivatives that are sufficiently volatile for GC analysis (Deyrup et al., 1985). In the present paper, a method has been described for the determination of GLYPH and its metabolite AMPA. The compounds were extracted from two soil types and their organic matter and derivatized with a mixture of TFAA and TFE prior to analysis by gas chromatography.

## EXPERIMENTAL SECTION

**Apparatus and Reagents.** A Shimadzu GC-9A (Shimadzu Corp., Kyoto, Japan) chromatograph equipped with a nitrogen–phosphorus detector was used. The chromatographic column was Ultra-bond 20SE on 80/100-mesh support (Ultra Scientific, Hope, RI): 1.8-m glass; 3-mm i.d. Glyphosate (98%) and AMPA (94%) were supplied by Monsanto. Trifluoroacetic anhydride (TFAA) and trifluoroethanol (TFE) were purchased from Aldrich Chemical Co. Anhydrous sodium sulfate was heated at 140 °C overnight prior to use. All organic solvents used were pesticide grade (Caledon Laboratories, Georgetown, Ontario, Canada).

Faculty of Forestry, University of Toronto, Toronto, Ontario, Canada M5S 1A1.

**Table I. Soil Characteristics**[a]

| substrate | pH | % OC | % MC | particle size | | |
|---|---|---|---|---|---|---|
| | | | | % clay | % silt | % sand |
| clay mineral | 4.43 | 0.00 | 0.85 | 87.60 | 12.40 | 0.00 |
| sand mineral | 4.72 | 0.34 | 0.55 | 7.60 | 10.00 | 82.40 |
| organic | 4.48 | 29.47 | 8.26 | | | |

[a] Key: OC = organic carbon; MC = moisture content. Soil classification (Jotcham, 1985): clay of the Ryland series Orthic Humic Gleysol type; sand of the Abitibi series Orthic Humo-Ferric Podzol type.

**Soils.** Two soil types (clay and sand) typical of the northern Ontario forest and their organic matters were used for recovery experiments. The characteristics of the soils are given in Table I.

**Extraction and Cleanup.** To a subsample (5 g) of a finely ground, homogenized soil in a 250-mL screw-cap bottle was added concentrated phosphoric acid (0.5 mL). The bottle was capped and shaken manually for 2 min. Deionized water (100 mL) was added followed by the addition of chloroform (50 mL) and the resulting slurry quantitatively transferred to a small domestic blender. After being blended for 2 min, the solution was filtered under suction, the extract transferred to a separatory funnel, and the residue rinsed twice with water (2 × 40 mL) and chloroform (50 mL). The aqueous fraction was washed first with hexane (50 mL) and then with ethyl acetate (50 mL). Both hexane and ethyl acetate washings were discarded. Darco (G-60) charcoal (1 g) was added to the aqueous fraction which was filtered under suction.

The filtrate was concentrated to ~5 mL in vacuo at 60 °C and refiltered through a Millipore filter (0.45 $\mu$m; Millipore, Waters). After filtration, the pH was adjusted to 0.5 with phosphoric acid and the sample was evaporated to dryness in vacuo at 60 °C. The dried samples were stored under vacuum in a desiccator containing phosphorus pentoxide.

**Derivatization.** The derivatization reactions utilized in this procedure are based on those as described by Deyrup et al. (1985). The flask containing the residues of GLYPH and AMPA from the previous extraction was equipped with a Claisen condenser and an anhydrous calcium chloride guard tube. A gentle stream of dry nitrogen was passed through the system. TFAA (2 mL) followed by TFE (1 mL) was added. The mixture was then refluxed for 90 min in an oil bath at 80 °C. The excess reagents were removed by a gentle stream of nitrogen at 40 °C. The derivatives were cooled in an ice–water bath, water (5 mL) was added, and the contents were transferred to a 125-mL separatory funnel with water (5 mL) and then chloroform (60 mL) as rinse. After the mixture was shaken ~1 min, the chloroform layer was collected and the

0021-8561/89/1437-0441$01.50/0 

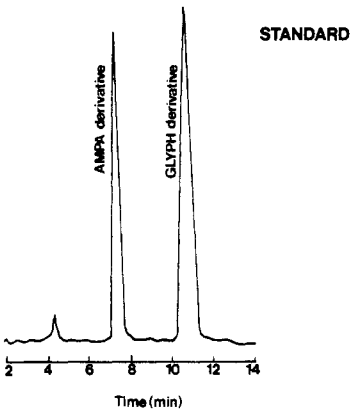


**Figure 1.** Chromatogram of a derivatized standard containing glyphosate (10.56 ng) and AMPA (4.05 ng).

aqueous layer was extracted two more times with chloroform (2 × 60 mL). The combined chloroform extract was dried over anhydrous sodium sulfate (2-cm bed) followed by the removal of the solvent in a vacuum rotary evaporator at 60 °C. The residue was dissolved in ethyl acetate and injected into the gas chromatograph. The derivatized sample was stable for at least 2 weeks.

**Gas Chromatographic Calibration.** For the calibration of the instrument, separate solutions containing 10, 5, 2, 1, 0.5, 0.1, and 0.05 $\mu g\ mL^{-1}$ GLYPH and AMPA were prepared in water. Aliquots of these solutions were transferred to vials, evaporated at 80 °C under nitrogen, and derivatized as described above. A calibration chart was prepared by plotting peak areas against mass of analyte to determine a linear detector response over the operational range (50–10 000 pg for glyphosate and 10–30 000 pg for AMPA). Certain unknown factors were found to cause the detector's sensitivity to change with time although the ratio of response increase to an increase in mass of analyte remained the same. It was decided to sandwich unknown samples between two identical sets of standards of GLYPH and AMPA and to accept a fluctuation in response of ±10% between the two standards. Values ranging between 50 and 200% of the average of those of the standards were used for quantification.

**Gas Chromatograhpic Analysis.** The operating parameters of the gas chromatograph were as follows: detector temperature, 250 °C; injector temperature, 250 °C; column temperature, 150 °C; gas flow rate, nitrogen 50 mL/min (ultra high purity), hydrogen 4 mL/min (pure), and air 175 mL/min (high purity). The approximate retention times are 11 min for GLYPH and 8 min for AMPA. A chromatogram of the derivatized standard sample containing GLYPH and AMPA is shown in Figure 1.

**Fortification.** Aliquots of prespray residue free soil were placed in 250-mL screw-capped bottles. Appropriate aliquots of previously prepared solutions containing both GLYPH and AMPA were added to provide fortification levels of 1, 0.48, and 0.096 ppm for GLYPH and 0.5, 0.18, and 0.035 ppm for AMPA. The bottles were capped and shaken manually to ensure uniform mixtures and then kept in a freezer at −20 °C for 24 h prior to extraction. The fortified samples were then extracted following the procedure described under extraction and cleanup. The recovery efficiencies for GLYPH and AMPA from organic matter and soil are shown in Table II. Chromatograms of the blank and the fortified samples are shown in Figure 2.

## RESULTS AND DISCUSSION

**Use of Phosphoric Acid.** It has been reported that adsorption of GLYPH in soil occurs through the phosphonic acid moiety, and that competes for binding sites with inorganic phosphates (Sprankle et al., 1975). Phosphate level in the soil plays an important role in determining the quantity of GLYPH adsorbed. As the concentration level of phosphate increases, glyphosate adsorption decreases whereas pH has little or no effect on adsorption (Sprankle et al., 1975). The addition of phosphoric acid to soil prior to extraction gave higher recoveries of GLYPH and AMPA than those obtained without the phosphoric acid treatment. Results showed that the recoveries of GLYPH and AMPA were poor in the case of mineral soils (Table II). Similar lower recoveries of GLYPH of about 20% in clay and 55% in sand loam soils have been reported by Glass (1983). This type of observation indicated that the composition of soil could be the key factor for obtaining the higher or lower recovery of GLYPH.

**Table II. Recoveries of GLYPH and AMPA from Fortified Soils**

| sample | added, ppm ($\mu g/g$) GLYPH | AMPA | recovery, % GLYPH | AMPA |
|---|---|---|---|---|
| organic (2)[a] | 1.000 | 0.500 | 70.27 ± 1.74[b] | 61.15 ± 2.94 |
| | 0.480 | 0.180 | 76.05 ± 0.64 | 63.95 ± 3.75 |
| | 0.096 | 0.035 | 87.15 ± 3.61 | 70.00 ± 2.12 |
| clay (2) | 1.000 | 0.500 | 54.34 ± 0.87 | 48.74 ± 1.35 |
| | 0.480 | 0.180 | 50.75 ± 2.32 | 51.28 ± 0.91 |
| | 0.096 | 0.035 | 51.30 ± 1.98 | 50.25 ± 1.06 |
| sand (2) | 1.000 | 0.500 | 50.17 ± 3.20 | 46.23 ± 1.49 |
| | 0.480 | 0.180 | 47.23 ± 2.00 | 40.54 ± 0.89 |
| | 0.096 | 0.035 | 45.63 ± 1.59 | 41.15 ± 3.04 |

[a] Number of determinations per concentration. [b] Average and standard deviation.

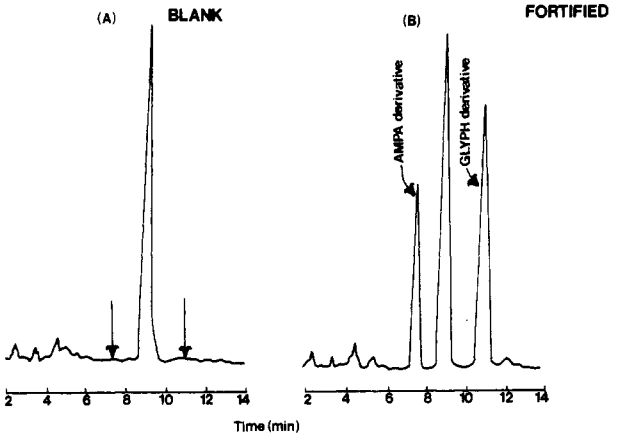


**Figure 2.** Chromatograms of (A) derivatized residue free organic matter extract (blank) and (B) fortified residue free organic matter (containing glyphosate (0.48 $\mu g\ g^{-1}$) and AMPA (0.18 $\mu g\ g^{-1}$)).

**Derivatization.** The plausible reaction mechanism of the single-step derivatization reaction involving GLYPH and AMPA and a mixture of TFAA and TFE has been discussed (Deyrup et al., 1985). The proposed structures of the derivatized product of both the compounds have also been confirmed by our laboratory from both electron impact (EI) and chemical ionization (CI) mass spectra. Structures of the derivatized products of GLYPH and AMPA are shown in Figure 3.

**Recovery from Fortified Soils.** Recovery results of GLYPH and AMPA (Table II) indicated that this method gives quite satisfactory results in the case of organic matter. The average recovery was 75% for GLYPH and 66% for AMPA. Recovery efficiencies for both the compounds in clay and sand soils could be improved after some modification of the method currently being investigated. The

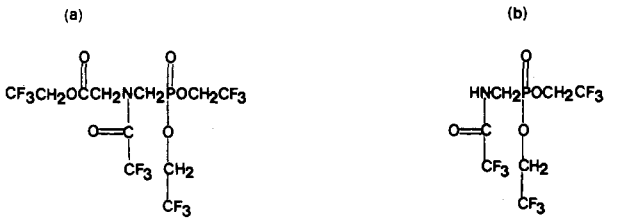


**Figure 3.** Structures of derivatized (a) glyphosate and (b) AMPA.

limits of detection for GLYPH and its metabolite AMPA were 0.05 and 0.01 μg/g, respectively. At present this method is being utilized to analyze GLYPH and AMPA in different substrates, and the results will be presented in subsequent communications.

Overall this method is very economical and less laborious than existing methods.

ACKNOWLEDGMENT

We are thankful to D. A. Charles and Dr. S. Banerjee, Faculty of Forestry, University of Toronto, for their assistance. We are also thankful to Monsanto, Canada, for supplying glyphosate and metabolite AMPA. The study was financed by a PRUF grant from the Canadian Forestry Service, Natural Sciences and Engineering Research Council of Canada, and Faculty of Forestry, University of Toronto.

**Registry No.** GLYPH, 1071-83-6; AMPA, 1066-51-9.

LITERATURE CITED

Bardalaye, P. C.; Wheeler, W. B.; Moye, H. A. Analytical Techniques of Glyphosate Residue Analysis. In *The Herbicide Glyphosate*; Grossbard, E., Atkinson, D., Eds.; Butterworths: London, 1984; pp 263–285.

Deyrup, C. L.; Chang, S. M.; Weintraub, R. A.; Moye, H. A. Simultaneous Esterification and Acylation of Pesticides for Analysis by Gas Chromatography. 1. Derivatization of Glyphosate and (Aminomethyl)phosphonic Acid with Fluorinated Alcohols—Perfluorinated Anhydrides. *J. Agric. Food Chem.* **1985**, *33*, 944–947.

Glass, R. L. Liquid Chromatographic Determination of Glyphosate in Fortified Soil and Water Samples. *J. Agric. Food Chem.* **1983**, *31*, 280–282.

Jotcham, J. Environmental Properties of Triclopyr. M.Sc. Dissertation, University of Guelph, 1985.

Lundgren, L. N. A New Method for the Determination of Glyphosate and (Aminomethyl)phosphonic Acid Residues in Soils. *J. Agric. Food Chem.* **1986**, *34*, 535–538.

Moye, H. A.; St. John, P. A. A Critical Comparison of Pre-column and Post-column Fluorogenic Labeling for the HPLC Analysis of Pesticide Residues. In *Pesticide Analytical Methodology*; Harvey, J., Jr., Zweig, G., Eds.; ACS Symposium Series 136; American Chemical Society: Washington, DC, 1980; pp 89–102.

Moye, H. A.; Miles, C. J.; Scherer, S. J. A Simplified High-Performance Liquid Chromatographic Procedure for the Determination of Glyphosate Herbicide and (Aminomethyl)-phosphonic Acid in Fruits and Vegetables Employing Post-column Fluorogenic Labeling. *J. Agric. Food Chem.* **1983**, *31*, 69–72.

*Pesticide Analytical Manual*; FDA: Washington, DC, 1977.

Sprankle, P.; Meggitt, W. F.; Penner, D. Adsorption, Mobility, and Microbial Degradation of Glyphosate in the Soil. *Weed Sci.* **1975**, *23*, 229–234.

Sprankle, P.; Sandberg, C.; Meggitt, W. F.; Penner, D. Separation of Glyphosate and Possible Metabolites by Thin-Layer Chromatography. *Weed Sci.* **1978**, *26*, 673–674.

Received for review March 8, 1988. Accepted August 31, 1988.

# Determination of Persistence, Movement, and Degradation of Hexazinone in Selected Canadian Boreal Forest Soils

Dibyendu N. Roy,* Samir K. Konar, Douglas A. Charles, Joseph C. Feng, Raj Prasad, and Robert A. Campbell

One flat and one slope site for each type of soil (sand, clay) were chosen near Matheson, Ontario, to study the persistence, movement, and degradation of hexazinone [3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1*H*,3*H*)-dione] after spraying at a rate of 4 kg of active ingredient (AI)/ha. Soils at three depths were collected and analyzed for residues of hexazinone and its metabolites A [3-(4-hydroxycyclohexyl)-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1*H*,3*H*)-dione] and B [3-cyclohexyl-6-(methylamino)-1-methyl-1,3,5-triazine-2,4(1*H*,3*H*)-dione]. The time required for hexazinone residues to remain consistently below 50% of the highest amount recovered was 43 days in both clay and sand soils. Results indicated that hexazinone had very limited potential to leach vertically through the soil column. The mobility study showed that there was no evidence of lateral movement of the herbicide either in runoff water or through subsurface flow. Metabolites A and B were found within a range of 0–32% and 0–50% of hexazinone concentration.

The ongoing search for herbicides with the desired characteristics of short half-life, little or no toxicity to humans and wildlife, and low environmental impact possessing a broad range of activity has resulted in the introduction of a group of triazine-based herbicides to the market. Hexazinone [3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1*H*,3*H*)-dione] (Figure 1) is the active ingredient of Du Pont's Velpar (formerly DPX-3674) and conforms to the above criteria. It has a potential for use in site preparation, conifer release, and nursery stock production in the boreal forest regions of Canada. The operational use of Velpar in these regions will be extremely important as this is an area of major economic significance

Faculty of Forestry, University of Toronto, Toronto, Ontario, Canada M5S 1A1 (D.N.R., S.K.K., D.A.C.), Northern Forestry Centre, Edmonton, Alberta, Canada T6H 3S5 (J.C.F.), Forest Pest Management Institute, Sault Ste. Marie, Ontario, Canada P6A 5M7 (R.P.), and Pest Management Section, Ontario Ministry of Natural Resources, Sault Ste. Marie, Ontario, Canada P6A 5N5 (R.A.C.).

0021-8561/89/1437-0443$01.50/0 