stable-labeled isotopes are being monitored simultaneously with the naturally abundant compounds helps in the identification process since the retention time of the stable-labeled isotope and that of the naturally abundant compound are identical during sample analysis. The lack of specificity of the technique presented here has turned out to be a significant advantage for quantitative work for several reasons: (1) It eliminates the cleanup step and allows concentration of reaction extracts to 0.2 mL even in the absence of silica gel cleanup; thus, the method detection limit achieved by the SIM GC/MS technique presented here is comparable to that reported by Lee and Chau (1983) for GC/EC. (2) Interfering peaks from reactions of PFBBr with itself or with acetone under the catalytic influence of K_2CO_3 are not detected since only certain ions are being monitored.

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Registry No. Dicamba, 1918-00-9; 2,4-D, 94-75-7; H_2O, 7732-18-5.

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A New Method for the Determination of Glyphosate and (Aminomethyl)phosphonic Acid Residues in Soils

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A new method has been developed and used to analyze soils for the phosphonic acid herbicide glyphosate and its metabolite (aminomethyl)phosphonic acid. The compounds were extracted with aqueous triethylamine solution. The phosphonic acids in the extract were derivatized with 1-fluoro-2,4-dinitrobenzene and quantified with ion-pair HPLC using tetraethylammonium bromide as counterion reagent. *N*-(Phosphonomethyl)-β-alanine was used as internal standard. Minimum detectable quantities were 0.05 $\mu g \cdot g^{-1}$ for glyphosate and 0.1 $\mu g \cdot g^{-1}$ for the metabolite.

Glyphosate, *N*-(phosphonomethyl)glycine (GLYPH), the active ingredient of the commercial herbicide Roundup, is used extensively for controlling many annual and perennial weeds. (Aminomethyl)phosphonic acid (AMPA) has been shown to be the major metabolite of GLYPH in plants and soils (Sprankle et al., 1978).

The literature on the analysis of GLYPH and AMPA residues has recently been fully reviewed (Bardalaye et al., 1984). Residues in soils have been determined quantitatively by gas chromatography (GC) (Pesticide Analytical Manual, 1977) and by high-performance liquid chromatography (HPLC) (Glass, 1983). The former method is very tedious and includes cation- and anion-exchange chromatography and a two-step derivatization procedure prior to the analysis. The HPLC method, which only determines GLYPH, suffers from a high limit of detection.

1-Fluoro-2,4-dinitrobenzene is a well-known reagent for the derivatization of primary and secondary amines in aqueous solutions (Edwards, 1977), and the high molar absorption of the 2,4-dinitrophenyl (DNP) derivatives in the UV–visible region offers a sensitive and selective quantification of the derivatives. In our procedure, GLYPH and AMPA were extracted from three soil types and analyzed as DNP derivatives by reversed-phase ion-pair HPLC. The new compound *N*-(phosphonomethyl)-β-alanine was synthesized for use as internal standard (IS).

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EXPERIMENTAL SECTION

Chemicals. GLYPH (97.9% purity) was obtained from Monsanto Chemical Co. AMPA was synthesized according to the method of Oleksyszyn and Subotkowska (1980) and recrystallized from aqueous ethanol.

The internal standard *N*-(phosphomethyl)-β-alanine was prepared as GLYPH (Pfiegel et al., 1977). Cold (0 °C) aqueous 37% formalin (8.6 g, 0.1 mol) was added to an ice-cooled solution of β-alanine (8.9 g, 0.1 mol) and sodium hydroxide (4.0 g, 0.1 mol) in water (40 mL) with continuous stirring. After 10 min, diethyl phosphite (13.8 g, 0.1 mol) was added and the mixture was stirred on a boiling water bath for 2 h. Concentrated hydrochloric acid (60 mL, 0.7 mol) was added. The mixture was stirred on the water bath for another 2 h and then evaporated to dryness at reduced pressure at 40 °C. The residue was boiled with 90% aqueous ethanol (200 mL). After cooling, the precipitate was filtered off and dissolved in hot water (250 mL). The solution was heated with charcoal and filtered. Acetone (250 mL) was added, and the mixture was cooled. Filtration of the product and recrystallization from aqueous ethanol yielded *N*-(phosphonomethyl)-β-alanine: 3.2 g (17%); mp 221–222 °C; IR (KBr) ν_{max} 3600–2200, 1718, 1582, 1480, 1445, 1425, 1390, 1340, 1285, 1250, 1220, 1185, 1095, 1062, 1020, 920 cm^{-1}; ^1H NMR (D_2O) δ 2.87 (t, 2 H, $J_{H,H}$ = 6.5 Hz), 3.24 (d, 2 H, $J_{P,H}$ = 12.7 Hz), 3.46 (t, 2 H, $J_{H,H}$ = 6.6 Hz). Anal. Calcd for $C_4H_{10}NO_5P$ C, 26.2; H, 5.5; N, 7.7. Found: C, 26.1; H, 5.6; N, 7.7.

Soils. Three soils, A, B, and D, described earlier by Glad et at. (1980), were used. Soil A was a clay loam, pH 6.4, B a sand, pH 6.6, and D an organogenic soil, pH 6.4. They were air-dried at room temperature before use. The water contents, determined after drying at 105 °C overnight, were 2.3% for soil A, 0.5% for soil B, and 12.2% for soil D.

Extraction and Ion Exchange. To the soil sample (3.5 g) in a 50-mL screw-cap plastic centrifuge tube was added an aliquot (1 mL) of the GLYPH-AMPA standard solution. The sample stood at 8 °C overnight. The concentration of the standard ranged from 0 to 35 $\mu g \cdot mL^{-1}$. A 2.5 ppm IS solution (3.0 mL) was added. After 1 h at room temperature, the sample was extracted by shaking for 15 min with aqueous 0.1 M triethylamine (30 mL) and then centrifuged at 2300*g* for 10 min. The supernatant was filtered through cotton wool into a 100-mL Erlenmeyer flask containing 7 mL of Dowex 1-X8, 50–100 mesh, Cl^- form. The resin was pretreated with 0.5 M hydrochloric acid and washed with water until the washings were neutral. The flask was shaken for 2 min and the solution decanted and discarded. The resin was washed twice with water (2 × 20 mL). The phosphonic acids were liberated by shaking for 2 × 2 min with 0.1 M hydrochloric acid (2 × 10 mL). The acid solution was filtered through cotton wool into a 50-mL conical flask and evaporated to dryness under reduced pressure at 40 °C. Water (1 mL) was added, and the evaporation was repeated.

Derivatization and Workup. The evaporated residue was dissolved in saturated sodium tetraborate solution (1 mL) and DNP reagent (2 mL, 3.75 mg 1-fluoro-2,4-dinitrobenzene/1 mL of 96% ethanol) was added. The reaction was carried out in the dark at room temperature for 1 h whereafter 0.1 M sodium phosphate buffer, pH 3.2 (3 mL), was added and the mixture transferred to a screw-cap glass vial containing sodium chloride (1 g). The contents (pH ~5) were extracted with ethyl acetate (2 × 5 mL), and the organic phase was removed with a Pasteur pipet and discarded. The aqueous phase was acidified by addition of 25% phosphoric acid solution (0.2 mL) and extracted with ethyl acetate (2 × 5 mL). The organic phase was dried over anhydrous sodium sulfate, filtered through cotton wool into a 50-mL conical flask, evaporated to dryness at reduced pressure, and dissolved in 0.1 M phosphate buffer, pH 3.2 (0.5 mL). The contents were extracted by shaking the flask twice with ethyl acetate (2 × 1 mL). The organic phase was removed with a Pasteur pipet and discarded, and the aqueous phase was evaporated to dryness.

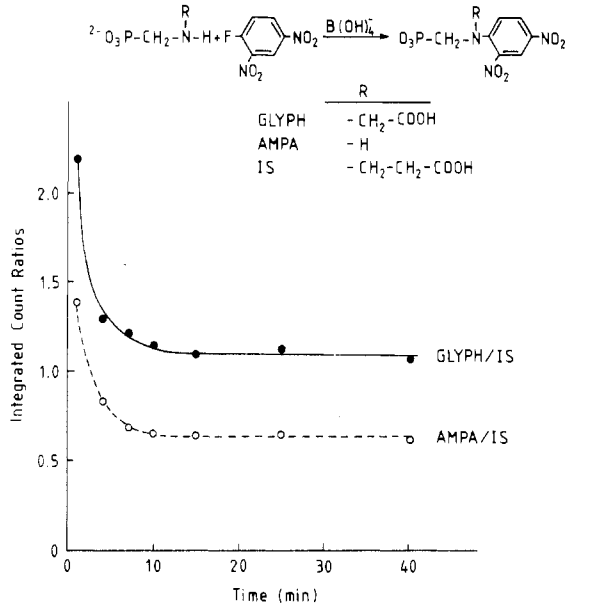

Figure 1. Kinetics of the reaction between 1-fluoro-2,4-dinitrobenzene and GLYPH, AMPA, or IS at 5 ppm in saturated sodium tetraborate solution expressed as a plot of integration ratios (GLYPH/IS, AMPA/IS) vs. time.

HPLC Analysis. The evaporated residue was dissolved in (0.5 mL) water, and 50 μL of the solution was injected to a NOVA-PAK C_{18} Radial-PAK cartridge, 0.8 × 10 cm (Waters). The mobile phase consisted of 0.02 M tetraethylammonium bromide, 0.05 M sodium dihydrogen phosphate (pH 3.2 with phosphoric acid)–acetonitrile, 5/1. The composition of the mobile phase was changed to 1/1 for a 1-min period between 7 and 8 min after injection. The next sample was injected 16 min after the first one. The flow rate was 1.0 mL/min, and the absorbance at 405 nm was measured with a single-wavelength UV detector. All peaks were integrated and expressed as unit areas.

Kinetics. To a standard sample containing 5 μg each of GLYPH, AMPA, and IS in 1.0 mL of saturated sodium tetraborate solution was added 2 mL of DNP reagent. The reaction was stopped by addition of 3 mL of 0.1 M phosphate buffer, pH 3.2. The sample was worked up and analyzed as above. The experiment was performed at different reaction times, and the ratios (GLYPH/IS, AMPA/IS) vs. reaction time were plotted (Figure 1).

Recovery (Soil). A 5.0-μg portion each of GLYPH and AMPA in 1.0 mL of water was added to 3.5 g of air-dried soil. The samples were kept at 8 °C overnight, extracted with aqueous triethylamine, and worked up as described above. The residue was dissolved in 1.0 mL of saturated sodium tetraborate solution containing 5.0 μg of IS. DNP reagent (2 mL) was added, and the mixture was reacted 1 h, worked up, and analyzed as above. As a reference, 1.0 mL of the 5.0 ppm GLYPH–AMPA solution was mixed with 30 mL of 0.1 M aqueous triethylamine and treated and analyzed as the soil samples. The recoveries were calculated by comparing the ratios (GLYPH/IS, AMPA/IS) for the soil and reference analyses (Table I).

Table I. Recoveries of GLYPH and AMPA from Fortified Soils (1.43 μg of GLYPH and AMPA/1 g of Air-Dried Soil)

sample	recoveries, % GLYPH	AMPA
clay loam soil (2)[a]	90	83
sand soil (2)	93	90
organogenic soil (2)	56	55

[a] Number of samples.

Table II. Reproducibility (Mean Value, Standard Deviation, and Relative Standard Deviation) of Integration Ratios (GLYPH/IS, AMPA/IS) for Fortified Soil Samples at 1.00 ppm GLYPH and AMPA and 2.14 ppm IS

sample	mean value (dev, %) GLYPH/IS	AMPA/IS
clay loam soil (6)[a]	0.65 ± 0.02 (3.6)	0.43 ± 0.02 (4.7)
sand soil (5)	0.65 ± 0.01 (2.6)	0.48 ± 0.02 (3.7)
organogenic soil (6)	0.59 ± 0.03 (4.4)	0.49 ± 0.02 (4.6)

[a] Number of samples.

Recovery (Ion Exchanger). The recoveries of GLYPH and AMPA from the anion-exchange resin were calculated by comparing the results from the analyses of the reference sample with those from the analyses of a standard sample consisting of 5.0 μg each of GLYPH, AMPA, and IS in 1.0 mL of saturated sodium tetraborate solution. On the latter analyses the ion-exchange step was omitted.

RESULTS AND DISCUSSION

Extraction of the Phosphonic Acids. It has been postulated that GLYPH is bound through the phosphonic acid moiety to clay and organic matter in the soil (Sprankle et at., 1975). Alkaline conditions seem to favor its extraction and 0.5 M ammonia (Pesticide Analytical Manual, 1977) and 0.1 M sodium hydroxide (Glass, 1983) have been used. Aqueous 0.1 M triethylamine gave, at an early stage of this work, higher recoveries of GLYPH and AMPA from soil than these two former media. Later work, however, when the method was refined, yielded similar recovery figures for the three media. This investigation confirmed that the recovery of GLYPH is strongly dependent on soil type (Table I). Earlier, Glass (1983) reported a recovery of about 20% for a clay loam and 55% for a sandy loam. The use of the internal standard compound *N*-(phosphonomethyl)-β-alanine, structurally similar to GLYPH and AMPA, diminishes the influence on the analytical results from the recoveries (Tables I and II); the largest difference between the soils is more than 38% without and less than 13% with the use of internal standard for both GLYPH and AMPA.

Anion-Exchange Workup. A simple batchwise procedure was used. This technique facilitates the handling of many samples at the same time. The recovery of GLYPH and AMPA in this step was 51% and 54%, respectively, which agrees well with the recovery (58%) reported for GLYPH from a similar column procedure with the same resin (Brønstad and Friestad, 1976). Prior to derivatization it is important to evaporate the acid eluate from the resin since traces of hydrochloric acid in the evaporated residue have a negative influence on the DNP reaction.

Derivatization. The amino group in the phosphonic acids reacted smoothly at room temperature with 1-fluoro-2,4-dinitrobenzene in a 2/1 mixture of ethanol and saturated sodium tetraborate solution. For standard samples a constant level for the ratios of the integrals (GLYPH/IS, AMPA/IS) was reached after 20 min, (Figure 1) but to be on the safe side, the reaction time for the soil samples was increased to 1 h. Before HPLC analysis, the following extraction scheme was employed. First, excess reagent was extracted from the reaction mixture at approximately pH 5, and subsequently, after acidification, the undissociated DNP derivatives of the phosphonic acids were extracted into ethyl acetate. Sodium chloride was added to the reaction mixture to facilitate phase separation and to increase the extraction yield. Some impurities still remained, however, especially in samples from organogenic soil. By an additional partition between phosphate buffer, pH 3.2, and ethyl acetate most of these impurities were extracted into the organic phase. Since the DNP derivatives in the aqueous phase decompose slowly in daylight, the samples were stored in the dark and direct sunlight was avoided during the derivatization.

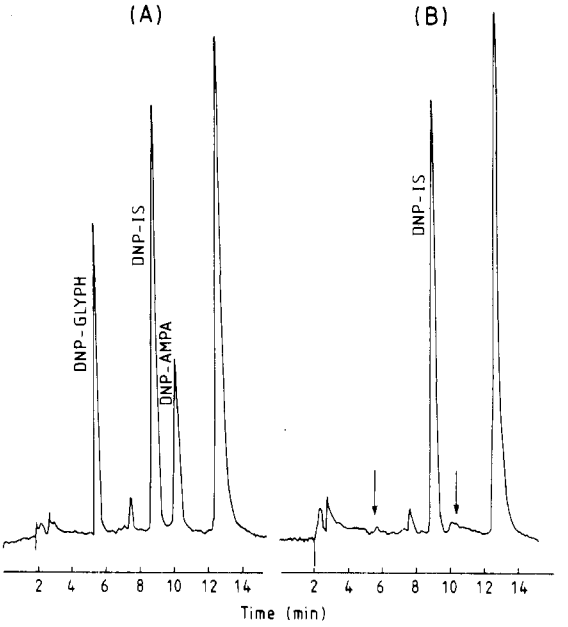

Figure 2. Chromatograms of DNP derivatives of extractives from organogenic soil samples: (A) fortified soil (1.0 μg of GLYPH and AMPA/1 g of air dried soil); (B) blank, for (A) and (B) the soils were fortified at 2.5 μg of IS/1 g of soil.

HPLC Determination. The samples were analyzed on a reversed-phase column and quantified by photometry at 405 nm. Although the sensitivity to DNP-AMPA was higher at 365 nm, there was an increase in the background noise and thus the higher wavelength was preferred. An ion-pair HPLC method with tetraethylammonium bromide as ion interacting reagent was used. This method, widely used for organic ions (Snyder and Kirkland, 1979), makes it possible to control the retention by manipulation of parameters such as pH, the composition of the mobile phase, and the concentration and type of counterion. From Figure 2, it appears that the DNP derivatives of GLYPH, AMPA, and IS are completely separated within 11 min and with insignificant amount of interfering compounds. The impurities appearing at 13 min are derived from the 1-min washing step, which prevents them from interfering with the DNP phosphonic acids in the subsequent analyses. The limits of detection for GLYPH and AMPA were 0.05 and 0.1 ppm, respectively, which although other soils were analyzed, seems to be a considerable improvement over the figures (5-50 ppm) for GLYPH reported by Glass (1983). The relative standard deviation at 1 ppm was less than 5% for both GLYPH and AMPA (Table II). The high degree of reproducibility is attributed to the use of an internal standard. For determinations of GLYPH and AMPA in fortified organogenic soil in the concentration range 0–10 ppm, a four-point standard curve showed a

0.998 linear coefficient for both compounds ($\mu g \cdot g^{-1}$, soil vs. integration ratios GLYPH/IS or AMPA/IS). The analyses of one single sample starting from the soil extraction takes about 3 h. The process can easily be adapted for simultaneous analyses of many samples, and routinely eight samples a day have been analyzed.

This procedure offers a simple, sensitive, and reproducible determination of GLYPH and AMPA at residue levels in soils of very different character in a single operation. It is also applicable to water samples and most probably, perhaps after some modification, to other types of materials.

Registry No. Glyphosphate, 1071-83-6; $NH_2CH_2PO_3H_2$, 1066-51-9.

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Characterization of Volatile Compounds Generated from the Reactions of Aldehydes with Ammonium Sulfide

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Volatile compounds generated from the reactions of selected aldehydes with ammonium sulfide were characterized by GC–MS. The mass spectral data from 2-pentyl-3,5-dibutylpyridine, 3-methyl-5-butyl-1,2,4-trithiolane, and 3-methyl-5-pentyl-1,2,4-trithiolane allowed us to identify these compounds in fried chicken or french-fried potato flavor.

INTRODUCTION

Previous studies in our laboratory reported the identification of 130 compounds from fried chicken flavor (Tang et al., 1983) and 427 compounds from french-fried potato flavor (Carlin, 1983). Due to the lack of reference mass spectral data a large number of compounds from these studies remain unidentified.

The compounds identified in fried chicken and french-fried potato flavors were postulated to form primarily through the nonenzymatic browning reactions, degradation of sugars, thermal and oxidative decomposition of lipids, and lipid–protein interactions. In addition, the structures of several compounds identified suggested that they were formed from reactions between degradation products.

Shu et al. (1980, 1981, 1985) examined reactions between degradation products. They characterized the volatile compounds generated from the reaction of isovaleraldehyde and ammonium sulfide. Mass spectral data from this work allowed us to identify 3,5-diisobutyl-1,2,4-trithiolane and 2-isobutyl-3,5-diisopropylpyridine in fried chicken flavor (Hartman et al., 1984).

We subsequently conducted our own reaction experiments in order to understand the generation of volatile compounds in fried chicken and french-fried potato flavors and to assist in the identification of unknown mass spectra from these previous studies. This paper reports the characterization of volatile compounds generated from the reactions of selected aldehydes with ammonium sulfide.

EXPERIMENTAL SECTION

Preparation of Reaction Mixtures. *I. Reaction of Pentanal or Hexanal with Ammonium Sulfide.* Pentanal or hexanal was mixed with aqueous 22% ammonium sulfide (Mallinckrodt, Inc., St. Louis, MO) in a 1:1 molar ratio at room temperature for 2 h. The reaction mixture was vacuum steam distilled (0.3 mmHg). The distillate was condensed by a four-stage cold-finger trapping sequence cooled with dry ice–acetone slurries (−70 °C). The aqueous distillate was saturated with NaCl and extracted with redistilled diethyl ether. The ether extracts were back-washed with water and dried over anhydrous sodium sulfate. The ether extracts were concentrated on a spinning-band distillation apparatus.

II. Reaction of Pentanal, Isopentanal, or Hexanal with Acetaldehyde and Ammonium Sulfide. Pentanal, isopentanal, or hexanal was mixed with acetaldehyde and aqueous 22% ammonium sulfide in a 1:1:2 molar ratio at room temperature for 2 h. The reaction mixture was vacuum steam distilled, extracted with diethyl ether, dried, and concentrated as previously described.

III. Reaction of β-Mercaptoacetaldehyde and Ammonium Sulfide. β-Mercaptoacetaldehyde in the form of its

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