Simultaneous Esterification and Acylation of Pesticides for Analysis by Gas Chromatography. 1. Derivatization of Glyphosate and (Aminomethyl)phosphonic Acid with Fluorinated Alcohols–Perfluorinated Anhydrides

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Glyphosate [*N*-(phosphonomethyl)glycine] and its major metabolite, (aminomethyl)phosphonic acid, were fully functionalized for analysis by gas chromatography and mass spectrometry by reaction with mixtures of fluorinated alcohols and perfluorinated anhydrides. All phosphonic and carboxylic acid groups were esterified and all amino groups were acylated; derivatization was effected at 100 °C for 1 h. Average recoveries from potable water samples were 95, 90, and 104% respectively for glyphosate at the 10, 50, and 100 ppb levels respectively; (aminomethyl)phosphonic acid recoveries averaged 106, 99, and 114% at the 10, 50, and 100 ppb levels, respectively.

Glyphosate (GLYPH), *N*-(phosphonomethyl)glycine, is an extremely effective nonselective postemergence herbicide with an increasing number of international applications. The major metabolite in plants, water, and soil is (aminomethyl)phosphonic acid (AMPA; Sprankle et al., 1978).

High performance liquid chromatography involving either postcolumn derivatization (Moye and St. John, 1980; Moye et al., 1983) or precolumn derivatization (Moye and St. John, 1980) has been used for the quantitation of GLYPH and AMPA. However, many laboratories do not have the multiplicity of pumps required for the postcolumn fluorogenic labeling or are unfamilar with techniques for precolumn fluorogenic labeling.

Gas chromatography, which could take advantage of the specificity of the flame photometric detector or the extreme sensitivity of the electron capture detector would seem to be an alternative, but both published procedures (Pesticide Analytical Manual, 1980; Guinivan et al., 1982) require a double derivatization. Furthermore, one of them (Pesticide Analytical Manual, 1980) suffers from the additional drawback of employing diazomethane, a highly toxic, carcinogenic, and explosive reagent.

Earlier, we reported a single-step derivatization of GLYPH and AMPA suitable for GC analysis (Moye and Deyrup, 1984). The derivatization was effected with *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA). In order to obtain high yields of the GLYPH derivative, it was necessary to coat the glass by exposing the glass surface to a dilute solution of phosphoric acid in ethanol. Although this procedure worked well with concentrated solutions (0.4 mg/mL) of GLYPH, it was subsequently found that the yields were very poor below 50 ppb. Coating the tube with phosphoric acid increased yields markedly, but frequent low yields were still observed at the 10 ppb level.

When this probelm proved to be insurmountable, we turned our attention to another derivative. We have found that a mixture of a fluorinated alcohol, such as trifluoroethanol (TFE) and a perfluorinated anhydride, such as trifluoroacetic anhydride (TFAA) converts both GLYPH and AMPA in a single step to derivatives which are suitable for GC analysis with flame photometric or electron capture detection. Similar reagents have been found to derivatize hydroxyl groups, amino groups, carboxylic acids, phenols, and amides (Watson, et al., 1974; Wilk and Orlowski, 1975; Degen and Schneider, 1983; Brooks, et al., 1974), but the reaction with GLYPH and AMPA represents the first derivatization of a phosphonic acid, as well as being the first application to the derivatization of a trifunctional molecule (carboxylic acid, amine, and phosphonic acid).

MATERIALS AND METHODS

Instrumentation. A Hewlett Packard 5840A gas chromatograph equipped with a flame photometric detector (250 °C) operated in the phosphorus mode and a ^{63}Ni electron capture detector (300 °C) was used for all measurements. A 1.8 m × 2 mm i.d. silanized glass column was packed with Ultra-Bond 20 SE on 80/100 mesh support (Ultra Scientific, Hope, RI). An oxygen trap was installed in front of the column to prevent its deterioration. Column temperature was held at 150 °C for the analysis of GLYPH and 140 °C for the analysis of AMPA. Carrier gas flow (N_2) was 27.6 mL/min. Other detector gases were: H_2, 200 mL/min; air, 50 mL/min; O_2, 20 mL/min.

Mass spectra were collected on a Hewlett-Packard Model 5985 or a Finnigan Model 4021 equipped with a 30 m × 0.2 mm DB5NFC silica capillary column (J and W Scientific).

Glassware and Reagents. Reactions and dilutions were carried out in 15 mm × 125 mm borosilicate glass culture tubes with Teflon lined screw caps (Fisher Scientific). Halogenated alcohols and anhydrides (trifluoroethanol, pentafluoropropanol, trifluoroacetic anhydride, and heptafluorobutyric anhydride (TFE, PFP, TFAA, and HFBA)) were obtained from SCM/PCR Specialty Chemicals (Gainesville, FL). Glassware was soaked in methanolic KOH before use and rinsed with deionized water.

Derivatizations. A 10-μL volume of the pesticide (GLYPH, AMPA) in water was added to a culture tube and the water evaporated at 100 °C with a stream of dry nitrogen. The tube was removed and allowed to cool to room temperature, 100 μL of the anhydride was added, and 50 μL of the alcohol was then added. The tubes were capped, and except for the kinetics curve studies, heated at 100 °C for 1 h.

The reagents were removed at 25 °C with a stream of dry nitrogen, and the residue was dissolved in 200 μL of ethyl acetate which had been stored over 4A molecular sieve, making it ready for GC analysis.

Kinetics Curves. The effect of reaction time and temperature was determined for GLYPH and AMPA by

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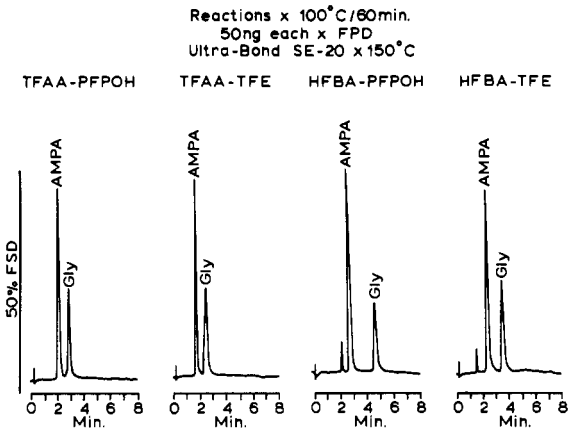


Figure 1. Chromatograms of derivatives of AMPA and GLYPH with various mixtures of alcohols and anhydrides. See text for abbreviations.

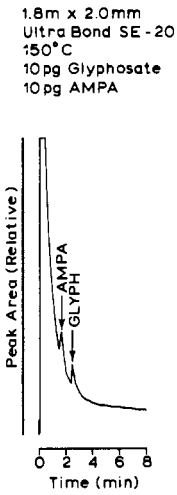


Figure 2. Chromatogram of 10 pg each of TFE–TFAA derivatives of GLYPH and AMPA with electron capture detection.

using TFAA and TFE as reagents. Ten micrograms of pesticide was derivatized in 100 μL of TFAA and 50 μL of TFE. Samples were prepared in duplicate. Reactions were carried out at 60, 80, and 100 °C in a heating block accurate to within 1 °C and were terminated at 15, 30, 60, and 90 min.

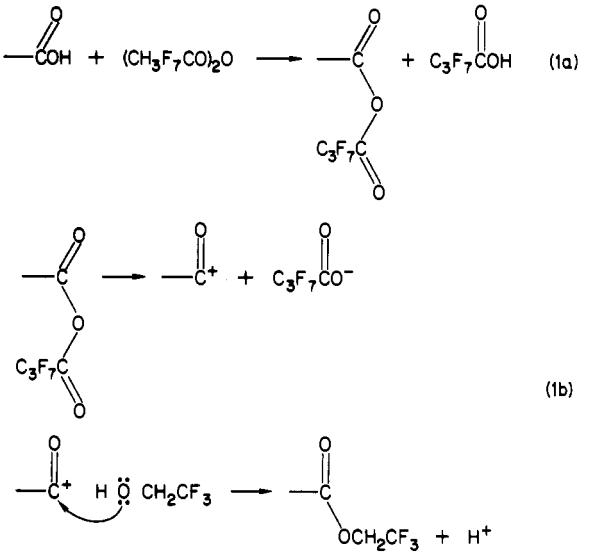
Analytical Curves. From 0.5 to 50 μL of a stock solution containing GLYPH and AMPA (0.4 μg/mL) was added to duplicate culture tubes. Water was removed with a stream of dry N_2, and 100 μL of TFAA and 50 μL of TFE were added. The tubes were incubated at 100 °C for 60 min, the reaction was terminated, the reagent was removed, and the residue was dissolved in 1 mL of ethyl acetate.

Reproducibility of Derivatization. Two microliters of a stock solution containing 1 mg/mL each of GLYPH and AMPA was added to each of 5 tubes, the water was evaporated with a stream of dry N_2, and 100 μL of TFAA and 50 μL TFE were added. The tubes were capped and incubated at 100 °C for 1 h after which the reagents were removed with a stream of N_2 at 25 °C and 200 μL of ethyl acetate was added. One-microliter aliquots were injected into the gas chromatograph equipped with a flame photometric detector.

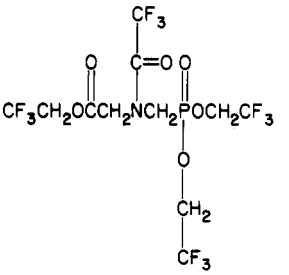
Recoveries from Water. Deionized laboratory water (20 mL) was placed in a 50-mL pear-shaped flask and fortified with GLYPH and AMPA at the 10, 50, and 100 ppb levels (n = 3). The flasks were rotary evaporated to dryness at 25 °C and incubated with 0.70 mL of TFAA and 0.35 mL of TFE in a sandbath at 100 °C for 1 h after sealing with parafilmed stoppers. At the end of the incubation the derivatives were dissolved in 1.0 mL of ethyl acetate and 1 μL was injected into the gas chromatograph with electron capture detection. Recoveries were determined by comparing responses to those obtained by diluting samples prepared at concentrations used in the "kinetics curves" section, previously described.

RESULTS AND DISCUSSION

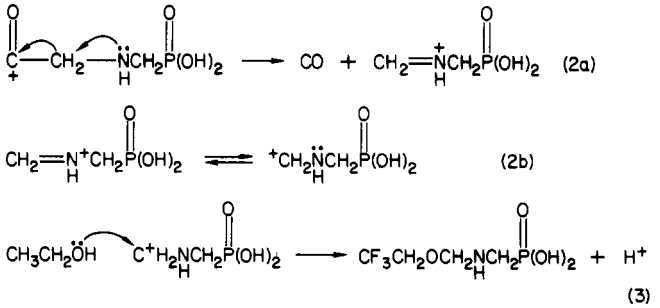
The esterification of the carboxylate that we observed of both GLYPH and AMPA has been reported to proceed through formation of a mixed anhydride, heterolysis to form the acylium ion and then nucleophilic attack by the alcohol (Watson et al., 1974; Bourne et al., 1954; Parish and Stock, 1965; see eq 1). When TFAA–TFE is used the



following derivative for GLYPH results (see subsequent discussion of mass spectral data). This pathway was



substantiated by our observation that when GLYPH is derivatized with TFE–HFBA we observe a product having a molecular ion of m/z 563, which appears on the chromatogram just before the fully derivatized GLYPH (molecular ion m/z 591). Such a product could very well be due to the decarbonylation of the acylium ion produced by heterolysis of the GLYPH derivative (eq 1) and is shown in eq 2. This peak can be seen eluting just before



the AMPA peak in the chromatograms of Figure 1 when

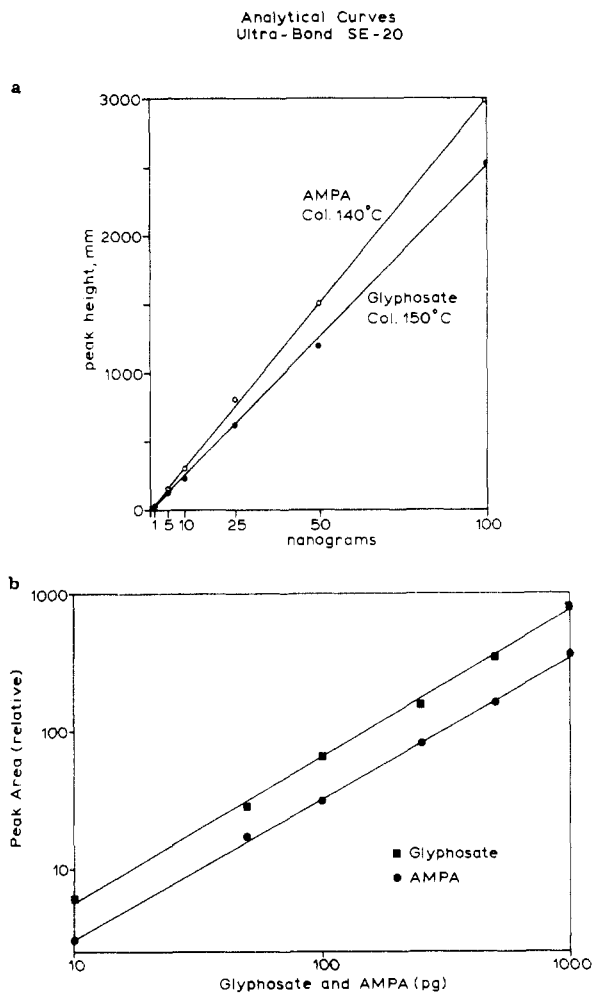


Figure 3. Analytical curves for AMPA and GLYPH with the flame photometric detector (a) and the electron capture detector (b).

HFBA is used as the anhydride; why such a decarbonylation product is not observed when TFAA is used is open for speculation. The chromatograms in Figure 1 also illustrate that as the molecular weight of the derivatives is increased the retention times increase as well as the resolution between GLYPH and AMPA. They also illustrate that the decarbonylation is not a result of TFE but rather HFBA, since no decarbonylation is observed for either of the TFAA derivatives. The excellent sensitivity of the electron capture detector is shown in Figure 2 as 10-pg amounts of AMPA and GLYYPH are easily seen and resolved. Analytical curves for the flame photometric and electron capture detectors are shown in Figure 3. The curves are linear over two decades with r^2 values of 0.999 throughout.

By obtaining both electron impact (EI) and chemical ionization (CI) mass spectra the expected structures of derivatives were confirmed for both AMPA and GLYPH by using the TFE–TFAA derivatives. Additionally, the TFE–HFBA derivative of GLYPH was also confirmed, along with the decarbonylation product. When the TFE–TFAA derivative of GLYPH was examined by positive CI with methane as the reagent gas the M + H^+ ion was observed to be the base peak (m/z 512); another strong ion was observed at m/z 492, corresponding to loss of HF. Adduct ions at 540 and 552 confirm that 512 is the M + H^+ ion. An ion at 412 is indicative of the $C(O)CH_2N[C(O)CF_3]CH_2P(O)(OCH_2CF_3)_2$ fragment. Negative CI with methane revealed additional ions at 370 for $N[C(O)CF_3]CH_2P(O)(OCH_2CF_3)_2$ and at 245 for $P(O)(OCH_2CF_3)_2$.

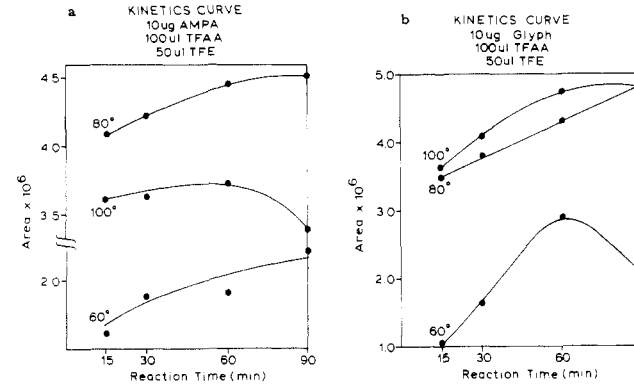


Figure 4. Kinetics curves for AMPA (a) and GLYPH (b) at various temperatures.

Table 1. Recoveries of GLYPH and AMPA from 20 mL of Water

final concn, μg/L	mean % recovery[a] GLYPH	AMPA
10	95 (93, 97)	106 (103, 110)
50	90 (88, 93)	99 (96, 102)
100	104 (109, 99)	114 (122, 106)

[a] Individual values of duplicate samples are shown in parentheses. Duplicates were analyzed by EC-GC following chemical derivatization of GLYPH and AMPA with TFAA–TFE.

By EI, the molecular ion was observed at m/z 511 with the base peak being m/z 113, an unidentifiable fragment. When the TFE–TFAA derivative of AMPA was examined by EI a base peak of m/z 126 was observed, due to the $CF_3C(O)NHCH_2$ fragment; at m/z 246 the $(CF_3CH_2O)_2P$-OH fragment was observed. A molecular ion at m/z 371 was also observed.

Kinetics curves for both GLYPH and AMPA are shown in Figure 4. For GLYPH, as would be expected, the 100 °C incubation produced the highest yields, reaching a maximum in 60 min and remaining constant. Contrarily, AMPA was observed to give higher yields of derivative at 80 °C, nearly reaching a maximum in 60 min. Consequently, a 90-min incubation at 80 °C would be optimum; we used a 100 °C incubation for 60 min to conserve analysis time.

Excellent reproducibility of replicate derivatizations of both GLYPH and AMPA was obtained (n = 5) with a mean relative standard deviation for AMPA of 0.06 and for GLYPH of 0.05. Recoveries from fortified laboratory grade water are shown in Table I. They were efficient and consistent even at the 0.01 ppm level. No reasonable explanation exists for the values above 100%, although random errors could account for part of it.

We have found the fluorinated alcohol–perfluorinated anhydride derivatization approach to be superior to the MTBSTFA reagent in that no special coating of the glassware is needed and much better recoveries are obtained at low levels. It also eliminates the need for the hazardous diazomethane. The ability to vary chromatographic retention by selecting various alcohols is also attractive, particularly when potential interferences may be present. In addition, the incorporation of halogens into the analyte molecule gives the analyst the choice of employing either flame photometric or electron capture for detection. If high sensitivity is not a requirement then the flame photometric detector would be the choice since it is much more selective.

We are presently examining this reagent for its applicability to other types of multifunctional pesticides and anticipate that it will find broad use, particularly in

screening procedures for pesticide mixtures in a wide variety of substrates.

Registry No. GLYPH, 1071-83-6; AMPA, 1066-51-9; TFE, 75-89-8; PFP, 28302-70-7; TFAA, 407-25-0; HFBA, 336-59-4; $CF_3CH_2OC(O)CH_2N[C(O)CF_3]CH_2P(O)(OCH_2CF_3)_2$, 97280-52-9; H_2O, 7732-18-5.

LITERATURE CITED

Bourne, E. J.; Randles, J. E. B.; Stacey, M.; Tatlow, J. C.; Tedder, J. M. *J. Am. Chem. Soc.* **1954**, *76*, 3206.
Brooks, J. B.; Alley, C. C.; Liddle, J. A. *Anal. Chem.* **1974**, *46*, 1930.
Degen, P. H.; Schneider, W. *J. Chromatogr.* **1983**, *277*, 361.
Guinivan, R. A.; Thompson, N. P.; Wheeler, W. B. *J. Assoc. Off. Anal. Chem.* **1982**, *65*, 35.
Moye, H. A.; Deyrup, C. L. *J. Agric. Food Chem.* **1984**, *32*, 192.
Moye, H. A.; Miles, C. J.; Scherer, S. J. *J. Agric. Food Chem.* **1983**, *31*, 69.
Moye, H. A.; St. John, P. A. *ACS Symp. Ser.* **1980**, No. 136, Chapter 7.
Parish, R. C.; Stock, L. M. *J. Org. Chem.* **1965**, *30*, 927.
"Pesticide Analytical Manual"; Food and Drug Administration: Washington, D.C., 1980; Pesticide Registration Section 180.364.
Sprankle, P.; Sandberg, C. L.; Meggitt, W. F.; Penner, D. *Weed Sci.* **1978**, *26*, 673.
Watson, E.; Wilk, S.; Roboz, J. *Anal. Biochem.* **1974**, *59*, 441.
Wilk, S.; Orlowski, M. *Anal. Biochem.* **1975**, *69*, 100.

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Degradation of the Tri-*n*-butyltin Species in Water and Sediment from Toronto Harbor

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Contamination of water and sediment in Toronto Harbor by the highly toxic tri-*n*-butyltin species (Bu_3Sn^+) and its less toxic degradation products, the di-*n*-butyltin species (Bu_2Sn^{2+}), *n*-butyltin species ($BuSn^{3+}$), and inorganic tin, is demonstrated. At some locations the concentration of Bu_3Sn^+ in water is high enough to warrant concern with regard to chronic toxicity to sensitive organisms. The Bu_3Sn^+ species (i) is bound fairly strongly to sterile Toronto Harbor sediment and the half-life of desorption is at least 10 months at 20 °C, (ii) can be taken up from sediment and degraded by oligochaetes, and (iii) is degraded by a sequential debutylation pathway at 20 °C in Toronto Harbor water and water–sediment mixtures with half-lives of 5 and 4 months, respectively. On the basis of this and earlier work it is concluded that the main factors limiting the persistence of the tri-*n*-butyltin species in aquatic ecosystems are photolysis in water and biological degradation in water and sediment, and with the temperatures and sunlight intensities prevalent in Canada, the half-life is likely to be at least a few to several months.

Organotin compounds are used in three main ways, viz., as stabilizers for poly(vinyl chloride), as catalysts, and as pesticides (Zuckerman et al., 1978). Organotin compounds are a class of compounds about which more information is sought under Canada's Environmental Contaminants Act (Canada Department of Environment and Department of National Health and Welfare, 1979) regarding toxicology and environmental fate. We have been attempting to determine the aquatic environmental occurrence, persistence, and fate of the highly toxic tri-*n*-butyltin species (Bu_3Sn^+), which is used as an antifouling agent in some paints for boats, ships, and docks, as a general lumber preservative, and as a slimicide in industrial cooling water (Davies and Smith, 1980). Recently we reported the occurrence of Bu_3Sn^+ and its less toxic degradation products Bu_2Sn^{2+}, $BuSn^{3+}$, and inorganic tin in water (Maguire et al., 1982) and sediment (Maguire, 1984) at 30 locations in Ontario. Although inorganic tin was practically ubiquitous, the three butyltin species were usually found in the water and sediment of harbors, marinas, and other areas of heavy boating and shipping traffic, which reflects the antifouling use of Bu_3Sn^+ noted above.

Our earlier work suggested that Bu_3Sn^+ might be moderately persistent in water. For example, it neither volatilized nor lost butyl groups over a period of at least two months in the dark at 20 °C. Bu_3Sn^+ did undergo slow ($t_{1/2} > 89$ d) sunlight photolytic degradation, at least partially by stepwise debutylation to inorganic tin (Maguire et al., 1983). In addition, it underwent 50% conversion to Bu_2Sn^{2+} by a green alga, *Ankistrodesmus falcatus*, over a 4-week period at 20 °C, but at algal cell concentrations up to one hundred times higher than what might be expected in Lake Ontario or Toronto Harbor (Maguire et al., 1984). The sequential debutylation observed in the above experiments appears to be a general phenomenon, observed in mammals (Kimmel et al., 1977), bacteria and fungi (Barug, 1981), and soil (Sheldon, 1978; Barug and Vonk, 1980).

This article discusses the adsorption of Bu_3Sn^+ to sediment and the degradation of Bu_3Sn^+ in water and sediment from nearby Toronto Harbor, which, as will be shown below, is contaminated with butyltin species and inorganic tin.

For brevity, each of the *n*-butyltin species is referred to in this article as though it existed only in cationic form (e.g., Bu_3Sn^+). This formalism is not meant to imply exact

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