Extraction of Glyphosate Herbicide from Soil and Clay Minerals and Determination of Residues in Soils[1]

Carl J. Miles* and H. Anson Moye

Extraction of the herbicide glyphosate [N-(phosphonomethyl)glycine] from clay minerals and two soils was studied by a batch equilibrium technique. Experiments showed that, for most of the sorbent/solvent systems studied, the amount of herbicide extracted increased as pH increased, suggesting that sorption occurs through ion exchange and hydrogen bonding. Alkaline solutions of kaolinite, iron oxide, and two Calvin silt loam soil samples showed resorption of glyphosate after an initial desorption step. Determination of glyphosate residues in soils was performed by derivatization of soil extracts with 9-fluorenylmethyl chloroformate and analysis by HPLC with fluorometric detection. Glyphosate was successfully recovered from sandy soils at 0.5 ppm by triplicate extraction with 0.1 M KH_2PO_4 and 30-fold concentration. For soils with high clay content, acceptable recovery at 1.0 ppm was achieved with triplicate extraction using 0.2 M KOH.

Glyphosate [N-(phosphonomethyl)glycine], the active ingredient of Roundup and Rodeo (Monsanto), is a nonselective, postemergence herbicide that has found widespread agricultural and domestic use. Many investigators have found the *Pesticide Analytical Manual* (PAM) methods (FDA, 1980) for measuring glyphosate residues on crops and other vegetation to be inadequate; consequently new methods have been developed (Moye and St. John, 1980; Guinivan et al., 1982; Moye et al., 1983; Glass, 1983; Bardalaye et al., 1985). A sensitive and precise method for measuring residues in a broad spectrum of soils has not yet been demonstrated.

Low and irreproducible recoveries from many soils are believed to result from sorption of glyphosate to soil clays and organic matter. It has been shown to bind strongly to clay minerals (Sprankle et al., 1975a; Hance, 1976), and sorption is influenced by pH and the nature of the saturating cation on the clay (McConnell and Hossner, 1986; Glass, 1987). There is also evidence that glyphosate binds to soil minerals in a manner similar to inorganic phosphate (Sprankle et al., 1975b; Hance, 1976). Shoval and Yariv (1979) reported that glyphosate binds to montmorillonite by water bridges between the carboxylic and phosphonic acid groups on glyphosate and the exchangeable cations on the clay. Close correlation between glyphosate sorption and soils with high amounts of organic matter and iron and aluminum has led some investigators to propose binding by an organic matter–metal–glyphosate complex (Nomura and Hilton, 1977; Hensley et al., 1978).

Although the PAM method (FDA, 1980) describes a procedure for analysis of glyphosate residues in soil, several workers have reported low and irreproducible recoveries in soil samples using this method. Glass (1983) analyzed soils by alkaline extraction, followed by cleanup using flocculation with $CaCl_2$ and anion exchange. Samples were analyzed by HPLC precolumn fluorogenic labeling with 9-fluorenylmethyl chloroformate. Recoveries were poor and ranged from 19% to 55% at the 25–200 ppm fortification level. Lundgren (1986) analyzed soils for glyphosate

and its major metabolite, (aminomethyl)phosphonic acid (AMPA), by extraction with triethylamine followed by cleanup with anion exchange. Samples were analyzed by HPLC using precolumn chromogenic labeling with 1-fluoro-2,4-dinitrobenzene. Recoveries were good at the 1.43 ppm level except for "organogenic" soils, which gave recoveries of 56% and 55% for glyphosate and AMPA.

The objectives of this study were two-fold: (1) to determine the extent of sorption/desorption of glyphosate to several soils and clay materials; (2) to develop an analytical method for the determination of glyphosate in soils with high amounts of clay. Sorption/desorption experiments were designed to aid in the development of an analytical technique for glyphosate residues in soil. Thus, many questions concerning the behavior of glyphosate in the soil environment are not addressed in this investigation.

EXPERIMENTAL SECTION

Two samples (1, 2) of a Calvin silt loam soil (loamy-skeletal, mixed, mesic Typic Dystrochrepts) were obtained from the Pacific Northwest Forest and Range Experimental Station (USDA), Corvallis, OR. These soils were passed through a 2-mm sieve to remove twigs and stones, thoroughly mixed, and refrigerated in glass jars until use. Placid fine sand (sandy, siliceous, hyperthermic Typic Humaquept; virgin and fertilized) and Pomona sand (sandy, siliceous, hyperthermic Ultic Haplaquod) soil samples were obtained from the Soils Department, University of Florida, and were treated similarly to the Calvin silt loam except the sands were air-dried. Physical analysis of soils was performed by standard methods (Jackson, 1958). Clay minerals were obtained from Wards Scientific (Rochester, NY). Ferric oxide (goethite, FeOOH) and aluminum oxide (boehmite, AlOOH) were prepared by the method described by Anderson and Arlidge (1962). Clay minerals were saturated with sodium ions, washed with deionized water to remove excess salt, and freeze-dried. Surface area measurements were made by an ethylene glycol monoethyl ether method (Carter et al., 1965).

X-ray diffraction analyses were performed on a General Electric XRD 700 instrument equipped with a copper target and scanned at 2° in 2θ/min. Radiation was generated at the Kα wavelength and attenuated by a nickel filter. Small portions of each clay were resaturated separately with K^+ and Mg^{2+} and dried onto glass plate mounts for X-ray analysis. After each Mg^{2+}-saturated clay was analyzed, the clays were treated with a 10% solution

Department of Agricultural Biochemistry, University of Hawaii at Manoa, Honolulu, Hawaii 96822 (C.J.M.), and Pesticide Research Laboratory, Department of Food Science and Human Nutrition, University of Florida, Gainesville, Florida 32611 (H.A.M.).

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of glycerol in ethanol, air-dried, and reanalyzed. After each K^+-saturated clay was analyzed, the slide was heated for 4 h at 300 °C, cooled, and reanalyzed. Reheating this sample to 500 °C for 4 h more, cooling, and reanalyzing completed the X-ray diffraction studies.

Extraction Studies. All extraction experiments employed N-(phosphono[^{14}C]methyl)glycine (glyphosate; sp act. 9.3 mCi/mM, 98% pure; Monsanto). A stock solution was prepared by dissolving 0.91 mg of labeled glyphosate in 25.0 mL of deionized water (36.4 μg/mL). Ten microliters of this solution was added to 2.0 g of soil (0.182 ppm) or 0.2 g of clay (1.82 ppm) in a polypropylene centrifuge tube, mixed well, capped, and allowed to sorb for 24 h at room temperature (23–27 °C). All extraction experiments were done in duplicate. Subsequently, 10.0 mL of 0.1 M HF, 0.1 M KOH, 0.1 M NH₄OH, 0.1 M KH₂PO₄, or deionized water was added to each, and the tubes were shaken intermittently for 24 h. After centrifugation at 5000 rpm for 15 min, 0.5 mL of supernatant was transferred from each tube to a scintillation vial, and 15 mL of Aquasol 2 (New England Nuclear) was added and the solution mixed well. After an initial 15-min equilibration period, ^{14}C activity was measured on a Searle Analytic 92 liquid scintillation counter using a 10-min counting time. Counting efficiency was determined by a quench curve. Desorption isotherms were constructed by adding different amounts of [^{14}C]glyphosate (0.182–3.64 ppm) to both Calvin silt loam soil samples (in duplicate) and following the procedure above using deionized water and 0.1, 0.2, and 0.5 M KOH as solvents.

Sorption/Desorption Kinetic Studies. Experiments were designed to determine the rate of sorption and desorption of glyphosate to the clays and soils studied. For the desorption kinetic studies, [^{14}C]glyphosate was added to the sorbent (0.182 ppm level) and allowed to sorb for 24 h. After either deionized water or 0.1 M KOH was added to each sorbent and mixed, supernatant (0.1 mL) was removed at various time periods (1, 3, 6, 24, 48, 72, 140 h) and ^{14}C activity counted as described above. For sorption kinetic studies, [^{14}C]glyphosate was added to the sorbents (0.182 ppm) and allowed to sorb for various periods of time (0, 3, 6, 24, 48, 72, 120 h) before a single extraction (24 h) was performed with either deionized water or 0.1 M KOH. ^{14}C activity was counted on 0.5-mL aliquots of each of these solutions.

Since changes in glyphosate concentration with time could also be due to degradation to AMPA, aliquots of each of the sorption kinetics experiment samples were analyzed at the end of the experiment by thin-layer chromatography (TLC) using the method of Pavoni (1978) (silica gel, 6:2:2 BuOH–HOAc–H₂O).

Determination of Glyphosate in Soils. Triplicate samples of each Calvin silt loam soil (2.0 g) were placed in centrifuge tubes and fortified with glyphosate (2 μg/2 g = 1.0 ppm; 20 μg/2 g = 10.0 ppm) 24 h before extraction. Controls were also analyzed. Each sample was extracted by adding 10 mL of 0.2 M KOH and shaking for 15 min. After centrifugation at 5000 rpm for 20 min, the supernatants were filtered through Whatman no. 1 paper. This extraction procedure was repeated two more times, the filtrates were combined, and glyphosate was determined by HPLC of fluorescent derivatives.

Triplicate samples of the Pomona and Placid sands and Calvin silt loam soil sample 2 (25 g) were fortified with 0.5 ppm glyphosate. Control and fortified samples were extracted by adding 100 mL of phosphate solution (0.1 M H₃PO₄, 0.1 M KH₂PO₄, or 0.1 M K₂HPO₄), shaking 15 min, centrifuging at 5000 rpm for 20 min, and filtering the

Table I. Physical–Chemical Properties of Soil Constituents

constituent	cation exchange[a] capacity, mequiv/100 g	av surface area,[a] m²/g	measd surface area, m²/g
organic matter	200–400	500–800	
vermiculite	100–150	600–800	
montmorillonite	80–150	600–800	513
illite	10–40	65–100	120
chlorite	10–40	25–40	
kaolinite	3–15	7–30	56
metal oxides and hydroxides	2–6	100–800	617 (Fe)
			418 (Al)
bentonite			746
silica (Merck Si60)		550	744
clay 1[b]			208
clay 2[b]			175

[a] Bailey and White (1970). [b] Clays isolated from the two Calvin silt loam soil samples used in the sorption experiments described: 1 was mostly vermiculite with traces of quartz; 2 was montmorillonite and traces of kaolinite.

supernatants with Whatman No. 1 paper. The extraction was repeated two more times and the combined filtrates were concentrated to 10 mL by rotary evaporation before analysis of glyphosate by HPLC of fluorescent derivatives.

Determination of glyphosate in soil extracts and standard solutions was achieved by reaction with 9-fluorenylmethyl chloroformate (FMOCCl), resulting in the formation of a fluorescent derivative that was separated by anion-exchange liquid chromatography (Moye and Boning, 1978; Miles et al., 1986). Derivatives were formed by mixing 0.1 mL of 0.2-μm filtered sample or standard, 0.9 mL of 0.025 M borate buffer (pH 9), 0.9 mL of HPLC-grade acetone, and 0.1 mL of 0.01 M FMOCCl (Aldrich) in acetone. After 20-min reaction at room temperature, the mixture was extracted with 3 × 1 mL aliquots of ethyl ether or ethyl acetate.

The chromatographic system consisted of an Altex Model 110A pump, a Rheodyne 7125 injector (200-μL loop), a 0.4 × 25 cm Alltech NH₂ column, an Aminco spectrophotofluorometer (E_x 270 nm; E_m 315 nm) equipped with a 50-μL flow cell, and a Soltec strip chart recorder (50 mV). Mobile phase consisted of a 75% (v/v) mixture of 0.05 M KH₂PO₄ (pH 6.0) in acetonitrile (Fisher HPLC grade) delivered at 1.0 mL/min. Measurement of glyphosate was performed by comparing peak heights of samples to an external standard curve of at least three standards.

RESULTS AND DISCUSSION

All minerals except kaolinite had a relatively clean X-ray spectrum and showed no uncharacteristic peaks under all conditions tested. Kaolinite showed traces of quartz and gibbsite. The clay fraction from Calvin silt loam soil 1 showed the presence of mostly vermiculite and traces of quartz while the clay fraction from Calvin silt loam soil 2 was dominated by montmorillonite with small amounts of kaolinite.

Surface areas of the clay minerals examined ranged from 56 to 746 m²/g, and the values were close to the average surface areas determined by Bailey and White (1970; Table I). Bentonite, a smectite mineral from volcanic ash, should have properties similar to those of montmorillonite.

Solvents were selected to cover a broad pH range and to optimize recovery of soils with high clay content. Since there is evidence that glyphosate complexes with iron (Hance, 1976; Nomura and Hilton, 1977), hydrofluoric acid was chosen because fluoride is known to strongly complex with iron. Potassium phosphate (monobasic) was chosen because there is evidence that glyphosate binds to soils

Table II. Fraction of [^{14}C]Glyphosate Extracted with Various Solvents and the pH of Each System[a]

| solvents | Calvin silt loam | | iron oxide | aluminum oxide | montmorillonite | kaolinite | silica | illite | bentonite |
	soil 1	soil 2							
deionized water	0.02	<0.01	0.03	0.41	0.57	0.01	0.92	0.18	>0.99
	4.0	5.0	2.9	4.2	8.5	6.5	6.8	7.6	9.4
0.1 M HF	0.18	0.08	0.01	0.03	0.75	0.69	0.94	0.03	0.84
	2.9	4.5	2.3	4.6	2.0	2.2	1.9	2.0	2.3
0.1 M KH$_2$PO$_4$	0.24	0.16	0.33	0.46	0.87	0.76	0.93	0.19	>0.99
	3.8	4.8	5.3	5.8	4.9	4.8	4.7	4.7	5.5
0.1 M NH$_4$OH	0.27	0.30	0.17	0.18	0.86	0.90	0.75	0.92	>0.99
	6.8	9.2	10.1	10.3	10.8	10.8	10.0	10.6	11.2
0.1 M KOH	0.23	0.30	0.94	0.89	0.94	0.94	0.94	>0.99	>0.99
	6.7	10.0	12.7	13.0	12.9	11.4	11.4	12.7	13.0
0.2 M KOH	0.26	0.77							
	9.9	12.1							
0.5 M KOH	0.24	0.73							
	13.2	13.6							

[a]Average of duplicate samples.

through the phosphonic group and inorganic phosphate competes for these sites (Sprankle et al., 1975b; Hance, 1976). Ammonium hydroxide was chosen because it is the solvent used in the current standard method for measuring glyphosate residues in soil (FDA, 1980). Finally, potassium hydroxide was used because it is a strong base that would raise the pH very high and make glyphosate completely anionic.

Extraction Studies. In the discussion that follows, the term sorption is used where others might use adsorption. Sorption includes any process that binds a chemical to a surface while adsorption implies a strictly physical holding process (Smith et al., 1977). Physical adsorption includes van der Waals forces and hydrogen bonding while chemisorption includes coordination complexation, ion exchange, and protonation or charge transfer. Although the experiments described in this section examine the sorption/desorption equilibrium process of glyphosate/sorbent systems, the term extraction is used instead of sorption/desorption to simplify the following discussion and better describe the objectives of this study.

The fraction of glyphosate extracted and the pH of each of the various clay/soils and different solvents were determined (see Table II). Two generalizations can be made upon examination of the data in Table II: (1) Extraction efficiency increases with increasing pH. (2) Extraction efficiency increases with increasing ionic strength of solvent. Exceptions to this pH effect are aluminum oxide, montmorillonite, and silica, which sorb glyphosate stronger in ammonium hydroxide (pH ≈10) than in phosphate (pH ≈5). This difference may be due to the cations (NH$_4^+$ vs K$^+$) or anions (OH$^-$ vs PO$_4^{3-}$) and not due solely to the effect of hydrogen ion activity.

Glyphosate is zwitterionic with pK_a values of 2.0, 2.6, 5.6, and 10.6 (see Figure 1). Thus, at pH 2 and lower it has a net positive charge; from pH 2 to 2.6 it has a net charge of zero (pH$_{ZPC}$; pH of zero point of charge), and above pH 2.6 it has a net negative charge that increases with increasing pH. Most oxides are also zwitterionic, which accounts for both cation- and anion-exchange capacity. The pH$_{ZPC}$ values of silica, kaolinite, iron oxide, and aluminum oxide are 2.0, 4.6, 7.8, and 8.2 (Stumm and Morgan, 1981), respectively, indicating that at more acidic conditions than the pH$_{ZPC}$ they have a net positive charge (anion exchanger) and a net negative charge (cation exchanger) at more alkaline conditions than the pH$_{ZPC}$.

On the basis of these properties, one would expect less ion exchange between glyphosate and clay minerals at either pH extreme because at very acidic conditions both clay and glyphosate would be positively charged and thus repel each other while at very alkaline conditions both
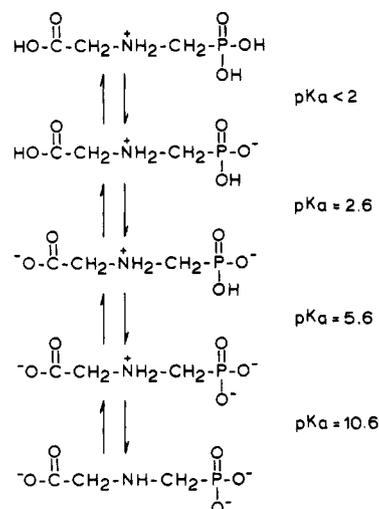

Figure 1. Proposed dissociation pathway and ionization constants for glyphosate (Sprankle et al., 1975a).

would be negatively charged and also repelled. Thus, one would expect maximum attraction at intermediate-pH conditions. This trend was not observed, and as previously discussed, there was a direct relationship between pH and extraction of glyphosate for most of the clay minerals examined. At low pH values, glyphosate could be held by hydrogen bonding to the hydrated surface (McConnell, 1984). Also, precipitated complexes of Fe^{3+} and Al^{3+} can account for the increased sorption at acidic conditions.

The cation-exchange capacity (CEC) decreases in the order montmorillonite > illite > kaolinite > metal oxides (Table I), and surface areas decrease in the order metal oxides ≈ montmorillonite > illite > kaolinite. Sorption of glyphosate should follow these trends if it is nonspecific. These trends were not observed in any of the solvent systems examined but even appear to be reversed in some cases. For example, montmorillonite and bentonite have high CEC and surface areas but have a relatively low affinity for glyphosate. This suggests that sorption of glyphosate to clays occurs by a specific sorption mechanism.

The effect of ionic strength on extraction efficiency is less evident than the effect of pH. Both Calvin silt loam soil samples, montmorillonite, and kaolinite show lower extraction efficiencies in deionized water than any of the other solvents examined, suggesting that increased ionic strength increases extraction efficiency by increasing competition for active sites (see Table II). The extraction data for illite, bentonite, and iron and aluminum oxide indicate that the concentration of hydronium ions is more significant to sorption of glyphosate on these materials

Table III. Physical and Chemical Analyses of Calvin Silt Loam Soil Samples[a]

soil	% organic matter	% sand	% silt	% clay	% Fe
1	80	16	49	35	2.0
2	46	36	37	27	3.3

[a] Results are the average of triplicate analyses.
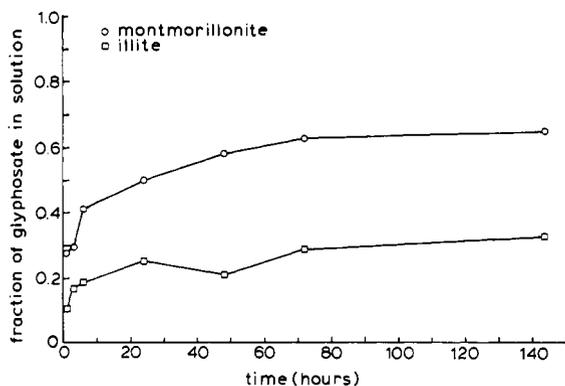
Figure 2. Kinetics of glyphosate desorption from montmorillonite and illite in deionized water.

Figure 3. Kinetics of glyphosate desorption from montmorillonite and illite in 0.1 M KOH.

Figure 4. Kinetics of glyphosate desorption from kaolinite and iron oxide in 0.1 M KOH.

Figure 5. Kinetics of glyphosate desorption from Calvin silt loam soil samples 1 and 2 in 0.1 M KOH.

than is the total ion concentration.

Specific sorption for glyphosate on the clay minerals and soils studied was calculated on a weight/weight (nmol/g) and weight/area ($nmol/m^2$) basis. Most of these values follow the same trend as the fraction sorbed (Table II), but sorption per unit area ($nmol/m^2$) accents the effect that certain surfaces have on sorption of glyphosate. Kaolinite showed the most pronounced difference, indicating a specific type of sorption. Sprankle et al. (1975a) and McConnell and Hossner (1986) found that most of the clay minerals they studied sorbed about 50-fold more glyphosate (nanomoles of glyphosate/gram of sorbent) than this study, but they used about 50-fold more pesticide. These results suggest that the maximum sorption capacity of these clays was not reached in this study.

Physical and chemical analyses of the two Calvin silt loam soil samples differed significantly for most of the parameters measured (Table III), although both had a high percentage of clay, organic matter, and iron, all of which are potential sorption media for glyphosate. Recovery or extraction of glyphosate from these two samples was poor with both 0.1 M KOH and 0.1 M NH_4OH, the solvent used in the PAM method (see Table II). Nomura and Hilton (1977) found 0.5 M NaOH to be a superior extractant to 0.5 M NH_4OH for recovery of [^{14}C]glyphosate from several Hawaiian soils. Since NH_4OH interferes in the FMOCCl reaction, KOH was examined further as an extractant.

Desorption isotherms (Freundlich; $n = 1$) of glyphosate from the Calvin silt loam soil samples were linear from 0.18 to 3.64 ppm, and duplicate measurements compared well. This indicates that desorption (extraction) was linear over the range of glyphosate concentrations examined for these soil samples. With alkaline solvents, Calvin silt loam soil sample 1 showed significantly more sorption of glyphosate than soil sample 2. Soil sample 1 was mostly vermiculite while soil 2 was mostly montmorillonite, and it is possible that these different kinds of clay are responsible for the differing extent of sorption. Extraction of glyphosate from montmorillonite was good in alkaline solutions (Table II), but extraction of glyphosate from vermiculite was not investigated.

Kinetics of Sorption and Desorption. Experiments designed to determine the rate of glyphosate desorption from clays and soils showed that most of the systems
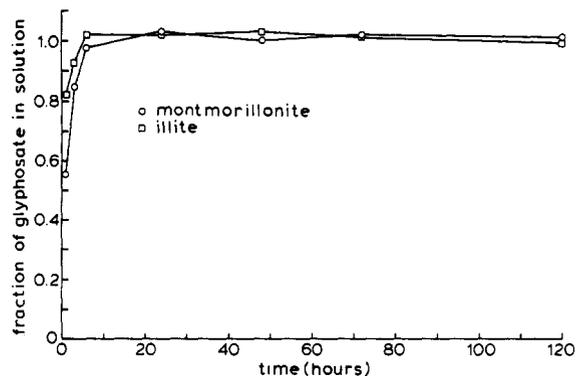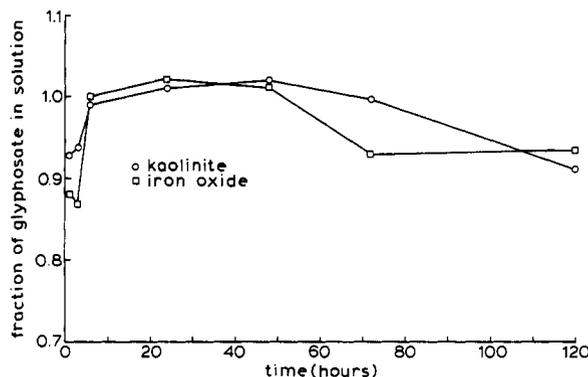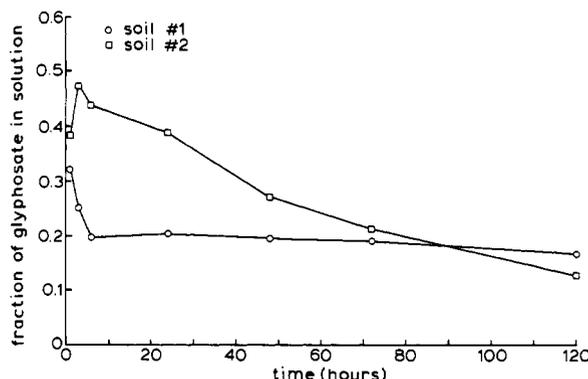
studied reached steady-state solution concentrations in 6 h or less. Exceptions were the desorption of glyphosate from montmorillonite and illite in deionized water. Solution concentrations of ^{14}C were still increasing at 140 h although concentration changes were small after 72 h (see Figure 2). Plots indicated a two-step desorption process: initially a rapid exchange of "labile" glyphosate followed by a slower exchange of "nonlabile" or strongly bound herbicide (Karickhoff and Morris, 1985). Only the first step of this process was observed in alkaline solutions of the same clay minerals (see Figure 3).

Further evidence that equilibrium is not rapidly achieved or maintained in glyphosate/sorbent systems was observed in the behavior of glyphosate on kaolinite and iron oxide in alkaline solutions (Figure 4). Steady-state concentrations were achieved in about 24 h, but after 48 h concentrations decreased, indicating resorption or degradation of the herbicide (loss as $^{14}CO_2$). Similar behavior was also observed for the two test soils (especially soil 2)

in alkaline solutions (Figure 5). Microbial degradation is unlikely for the clays because there was no lag phase of microbial growth and microbe populations should be very low because samples were freeze-dried. Also, TLC analysis showed no significant degradation to the metabolite AMPA. Resorption could take place as the dry clay environment changes with time (e.g., hydration). Resorption of phosphate ion has been observed on silty clay soil (Munns and Fox, 1976) and lepidocrocite (Madrid and de Arambarri, 1985) and was ascribed to molecular rearrangement at the sorbent surface resulting in a new sorbed phase.

Experiments designed to determine the rate of glyphosate sorption to clays and soils showed that there were only small changes in the amount of sorbed glyphosate over the time period studied (0–120 h). This indicates that sorption between glyphosate and the soils and clays studied is rapid.

These results indicate that the process of achieving equilibrium between glyphosate and some clay minerals is slow and that, in some cases, up to 6 h is needed to reach equilibrium conditions. This is especially important for recovery of residues from field soil samples.

Determination of Glyphosate in Soils. Extraction studies indicated that desorption of glyphosate from soils increased with increasing pH. However, there are both advantages and disadvantages of alkaline extracting conditions. Glyphosate is stable in very alkaline solutions, and the FMOCCl/glyphosate reaction is favored in slightly alkaline conditions. Unfortunately, basic solutions are the best extractants for humic substances, making these organics potential interferences. Nonetheless, successful determination of glyphosate in soil first requires a successful extraction followed by cleanup steps and analytical modifications required to achieve a workable method.

Extraction of the two Calvin silt loam soil samples with solutions of increasing KOH concentration showed that maximum recovery of glyphosate occurred at about pH 4 for soil 1 and between pH 10 and 12 for soil 2 (Table II). Both soil samples had a large amount of base-neutralizing capacity, which required that sufficient base be added to raise the pH and achieve successful recovery of glyphosate. Concentrations of KOH greater than 0.2 M did not increase recovery of glyphosate.

Initial residue recovery studies focused on direct analysis (no cleanup) of the alkaline extracts of the two Calvin silt loam soils. All recovery experiments were performed in at least triplicate and were started by fortification of a portion of soil (2.0 g) with glyphosate 24 h before extraction. This step better represents what occurs in actual environmental residues than when extraction was done immediately after fortification.

Percent recovery of glyphosate from Calvin silt loam soil sample 1 was 119 ± 18.1% at the 1.0 ppm fortification concentration and 93 ± 0.8% at 10 ppm fortification concentration ($n = 3$). Percent recovery of glyphosate from Calvin silt loam soil sample 2 was 108 ± 13.9% at 1.0 ppm fortification concentration and 86 ± 12.8% at 10 ppm fortification concentration ($n = 3$). Peaks much smaller than those observed in the 1.0 ppm fortified soil samples would be difficult to measure accurately, and this approaches the practical method detection limit (see Figure 6).

Extraction of these two soil samples using longer shaking times (up to 24 h) and higher temperatures (up to 60 °C) was performed to increase the extraction efficiency of glyphosate. Both experiments resulted in increased interferences that obscured the glyphosate–FMOC peak in the HPLC/fluorometric chromatograms. Acceptable re-
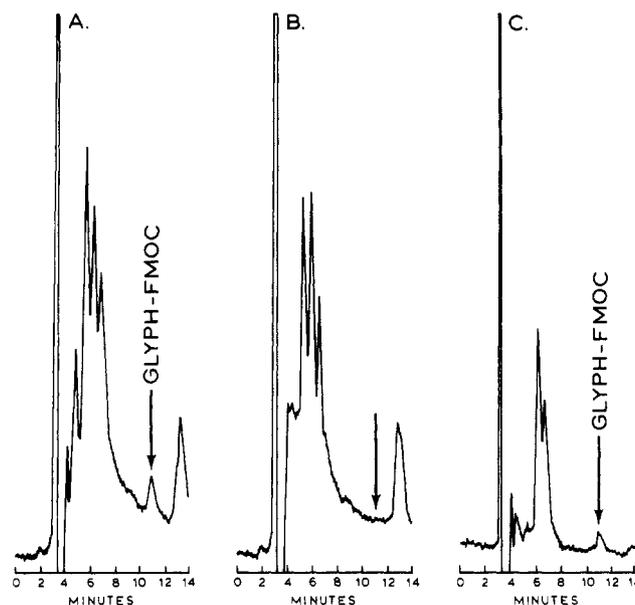

Figure 6. Chromatograms of Calvin silt loam soil sample 2 extracts and glyphosate standard derivatized with FMOCCl: (A) soil sample 2 fortified at 1.0 ppm; (B) soil sample 2 control; (C) a 0.1 µg/mL standard of glyphosate. Mobile phase is a 75% mixture of 0.05 M KH_2PO_4 (pH 6) in acetonitrile at 1.0 mL/min; Alltech NH_2 column; spectrophotofluorometric detection at E_x 270, E_m 315.

Table IV. Characteristics and Percent Recovery of Glyphosate from Different Soils Using Three Extractions with 0.1 M KH_2PO_4 and No Sample Cleanup[a]

soil	pH	% clay	% OM[b]	PO_4 (µg P/g)[c]	% rec
Pomona sand	5.3	0.5	2.5	2.8	100 ± 8
Placid fine sand[d]	4.5	3.6	4.0	59	86 ± 7
Placid fine sand[e]	4.5	3.6	4.0	6.8	80 ± 15
Calvin silt loam 2	acid	27	16.2		35 ± 11

[a] Average of triplicate determinations; 25-g samples fortified to 0.5 ppm. [b] Organic matter. [c] Mehlich I extractable phosphate. [d] Fertilized soil. [e] Virgin soil.

coveries of glyphosate from these soil samples with three, 15-min extractions at room temperature make these modifications unnecessary.

These results indicate that glyphosate can be readily determined in soil down to the 1 ppm level by simple extraction, derivatization, and HPLC analysis. Quantitative recovery of glyphosate from the organic-rich soil extracts indicates that quantitative derivatization of glyphosate can take place in complex environmental samples.

Sorption of glyphosate to clays has been reported to take place through the phosphonic acid moiety (Sprankle et al., 1975b), and Hance (1976) concluded that inorganic phosphate excludes glyphosate from soil sorption sites. Applying this principle, we extracted the Pomona sand, both Placid fine sands, and Calvin silt loam 2 with various phosphate solutions.

All extracts using 0.1 M H_3PO_4 formed a gel that inhibited filtration and concentration; consequently these extracts were not analyzed. All extracts using 0.1 M K_2HPO_4 were colored by coextracted humic substances. These extracts had chromatograms with high backgrounds, making it difficult to determine low concentrations of glyphosate accurately. Extracts using 0.1 M KH_2PO_4 gave acceptable results except for the Calvin silt loam (Table IV). The estimated method detection limit was about 0.1 ppm on a 25-g sample. The Placid fine sand with the higher amount of extractable phosphate (previously added

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as fertilizer) had a higher mean recovery of glyphosate than its counterpart, suggesting that inorganic phosphate competes for soil binding sites with glyphosate. The results show that the percent recovery decreases as the percentage of clay or organic matter increases. This is further evidence that clay or organic matter is sorbing glyphosate. Extraction with 0.1 M KH_2PO_4 is an acceptable method for determination of glyphosate in soils with low to moderate clay contents.

SUMMARY

Extraction efficiency of the herbicide glyphosate from soils and several clay minerals using various solvents was found to increase as pH increased. Iron oxide and illite had the highest sorption per unit weight of sorbent while kaolinite had the largest sorption per unit area of clay. The degree of sorption did not correlate with the cation-exchange capacity or surface area of the sorbents, indicating that sorption was specific as opposed to general. Our research and that of others indicate that the mechanism of glyphosate sorption is H-bonding and ion exchange. Extraction studies showed that maximum recovery of glyphosate residues on soils with high clay and organic matter content would be achieved with use of 0.2 M KOH. Desorption kinetic studies showed that glyphosate concentrations in solution reached equilibrium in 6 h or less for most of the sorbents studied. Exceptions were aqueous solutions with montmorillonite and illite, which did not reach equilibrium until about 3 days. Alkaline solutions with kaolinite, iron oxide, and the two Calvin silt loam soil samples showed resorption of glyphosate after an initial desorption step. Sorption kinetic studies showed that glyphosate was rapidly sorbed to the clays and soils studied. For soils with low to moderate amounts of clay and organic matter, extraction with 0.1 M KH_2PO_4 and 30-fold concentration gave acceptable recoveries with 0.5 ppm of fortified glyphosate (25-g sample). For soils with high amounts of clay and organic matter, extraction with 0.2 M KOH gave acceptable recoveries down to 1.0 ppm (2-g sample). Direct derivatization of these soil extracts with 9-fluorenylmethyl chloroformate and analysis by HPLC with fluorometric detection were rapid and gave good resolution from interferences.

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Registry No. Glyphosate, 1071-83-6; iron oxide, 1332-37-2; aluminum oxide, 1344-28-1; montmorillonite, 1318-93-0; kaolinite, 1318-74-7; silica, 7631-86-9; illite, 12173-60-3.

LITERATURE CITED

Anderson, G.; Arlidge, E. Z. "The Adsorption of Inositol Phosphates and Glycerophosphate by Soil Clays, Clay minerals, and Hydrated Sesquioxides in Acid Media". *J. Soil Sci.* **1962**, *13*, 216–224.

Bailey, G. W.; White, J. L. "Factors Influencing the Adsorption, Desorption, and Movement of Pesticides in Soil". *Residue Rev.* **1970**, *32*, 29–92.

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: Woburn, MA, 1985; pp 263–285.

Carter, D. L.; Heilman, M. D.; Gonzalez, C. L. "Ethylene Glycol Monoethyl Ether for Determining Surface Area of Silicate Minerals". *Soil Sci.* **1965**, *100*, 356–360.

Food and Drug Administration *Pesticide Analytical Manual*; FDA: Washington, DC, 1980.

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

Glass, R. L. "Adsorption of Glyphosate by Soils and Clay Minerals". *J. Agric. Food Chem.* **1987**, *35*, 497–500.

Guinivan, R. A.; Thompson, N. P.; Wheeler, W. B. "Derivatization and Cleanup Improvements in Determination of Residues of Glyphosate and Aminomethylphosphonic Acid in Blueberries". *J. Assoc. Off. Anal. Chem.* **1982**, *65*, 35–39.

Hance, R. J. "Adsorption of Glyphosate by Soils". *Pestic. Sci.* **1976**, *7*, 363–366.

Hensley, D. L.; Beuerman, D. S. N.; Carpenter, P. L. "The Inactivation of Glyphosate by Various Soils and Metal Salts". *Weed Res.* **1978**, *18*, 287–291.

Jackson, M. L. *Soil Chemical Analysis*; Prentice-Hall: New York, 1958.

Karickhoff, S. W.; Morris, K. R. "Sorption Dynamics of Hydrophobic Pollutants in Sediment Suspensions". *Environ. Toxicol. Chem.* **1985**, *4*, 469–479.

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.

Madrid, L.; de Arambarri, P. "Adsorption of Phosphate by Two Iron Oxides in Relation to Their Porosity". *J. Soil Sci.* **1985**, *36*, 523–530.

McConnell, J. S. "pH Dependent Adsorption Isotherms of Glyphosate". Ph.D. Dissertation, Texas A&M University, 1984; p 121.

McConnell, J. S.; Hossner, L. R. "pH-Dependent Adsorption Isotherms of Glyphosate". *J. Agric. Food Chem.* **1986**, *33*, 1075–1078.

Miles, C. J.; Wallace, L. R.; Moye, H. A. "Determination of Glyphosate Herbicide and (Aminomethyl)phosphonic Acid in Natural Waters by Liquid Chromatography Using Pre-column Fluorogenic Labeling with 9-Fluorenylmethyl Chloroformate". *J. Assoc. Off. Anal. Chem.* **1986**, *69*, 458–461.

Moye, H. A.; Boning, A. J., Jr. "A Versatile Fluorogenic Labelling Reagent for Primary and Secondary Amines: 9-Fluorenylmethyl Chloroformate". *Anal. Lett.* **1979**, *12*, 25–35.

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 Postcolumn Fluorogenic Labeling". *J. Agric. Food Chem.* **1983**, *31*, 69–72.

Munns, D. N.; Fox, R. L. "The Slow Reaction Which Continues After Phosphate Adsorption: Kinetics and Equilibrium in Some Tropical Soils". *Soil Sci. Soc. Am. J.* **1976**, *40*, 46–51.

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

Pavoni, G. "Ricerca di Glifosate e del sno Metabolita, l'Acido Aminometilfosonico per TLC". *Boll. Chim. Unione Ital. Lab. Prov.* **1978**, *9*, 157–161.

Shoval, S.; Yariv, S. "The Interaction between Roundup (Glyphosate) and Montmorillonite. Part 1. Infrared Study of the Sorption of Glyphosate by Montmorillonite". *Clays Clay Miner.* **1979**, *27*, 19–28.

Smith, J. H.; Mabey, J. H.; Bohonos, N.; Holt, B. R.; Lee, S. S. "Environmental Pathways of Selected Chemicals in Freshwater Systems. Part 1: Background and Experimental Procedures"; EPA 600/7-77/113; U.S. EPA: Athens, GA, 1977; p 329.

Sprankle, P.; Meggitt, W. F.; Penner, D. "Rapid Inactivation of Glyphosate in Soils". *Weed Sci.* **1975a**, *23*, 224–228.

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

Stumm, W.; Morgan, J. J. *Aquatic Chemistry*; Wiley: New York, 1981; p 780.