

Extraction of [¹⁴C]Glyphosate from Saskatchewan Soils

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The extraction of ¹⁴C from air-dried samples of three soils fortified 14 days previously with [¹⁴C]-glyphosate was compared using 10 different solvent systems and an extended-shaking procedure. ¹⁴C recoveries exceeding 73% were reproducibly achieved with 0.35 M H₃PO₄ (and 0.09 M with respect to CaCl₂), 0.1 M NaOH, and 0.5 M NH₄OH. With the phosphoric acid and ammonium hydroxide extractants, highest recoveries from a loamy sand were recorded with a soil/solvent ratio of 1:5 or 1:10. With the aqueous sodium hydroxide, highest recoveries were achieved from all soils using a soil/solvent ratio of 1:2.5. Recoveries of ¹⁴C from all three soils, using 0.1 M NaOH, were not significantly different whether fortified 14, 28, or, in the case of a clay, 56 days previously with [¹⁴C]glyphosate. Some loss of extractable radioactivity was noted in two of the soils after 56 days.

INTRODUCTION

Glyphosate [*N*-(phosphonomethyl)glycine], the active ingredient in the formulation Roundup, is a nonselective, postemergent herbicide used in a variety of crop and non-crop situations at rates of 0.34–1.12 kg/ha. Many analytical methods for the extraction and determination of glyphosate in soils have been presented (*Pesticide Analytical Manual*, 1980; Glass, 1983; Lundgren, 1986; Miles and Moye, 1988; Thompson et al., 1989; Roy and Konar, 1989; Eberbach and Douglas, 1991). Since the herbicide is amphoteric, the chemical analysis of residues in soils is both complex and time-consuming. Following solvent extraction, anionic and cationic exchange resins are necessary for cleanup prior to high-pressure liquid chromatographic analysis; extracted residues can also be derivatized and quantified gas chromatographically (*Pesticide Analytical Manual*, 1980; Glass, 1983; Lundgren, 1986; Miles and Moye, 1988; Thompson et al., 1989; Roy and Konar, 1989; Eberbach and Douglas, 1991). The analysis of glyphosate and its major degradation product, AMPA [(aminomethyl)phosphonic acid], in soil and plant tissue has been reviewed (Bardalaye et al., 1985).

Glyphosate adsorbs strongly to soil colloids (Sprankle et al., 1975a,b; Hance, 1976; Glass, 1987; Miles and Moye, 1988), thus making its extraction difficult. As well, it has also been shown that when herbicide residues come into contact with soil under field conditions for extended periods of time, aging, or weathering, occurs, making these residues even more resistant to solvent extraction (Smith, 1981; Smith and Milward, 1983).

For the majority of analytical procedures used for glyphosate analysis in soils, methods were validated by extraction of soils fortified with glyphosate for only 24 h or less. Because of this short aging period, adsorptive processes would not be completed when the soils were extracted.

The assessment of extraction procedures for glyphosate from soil is difficult given the complex and time-consuming cleanup and detection procedures. Several analysts have used ¹⁴C-labeled pesticides to compare extraction procedures following various equilibration periods (Chiba and Morley, 1968; Saha et al., 1969; Lichtenstein et al., 1977). Thus, the use of [¹⁴C]glyphosate would give an accurate and simple means of comparing recoveries of the herbicide from fortified soils using various solvent systems, extraction procedures, and aging periods.

The objectives of the present study were to compare the

recoveries of [¹⁴C]glyphosate from three air-dried Saskatchewan soils that had been previously fortified for at least 14 days, using a number of different solvent systems and extraction conditions. The effects of soil/solvent ratio and extraction time were also investigated for selected solvent systems.

MATERIALS AND METHODS

Soils. Soils from three sites in Saskatchewan were used in this study: a Typic Boroll clay from Regina, a Udic Boroll clay from Indian Head, and a Typic Boroll loamy sand from White City. All soils were collected from the 0–5-cm soil horizon, air-dried to constant weight (~10% field capacity), and screened through a 2-mm sieve. The physical characteristics of these soils have already been described (Smith and Aubin, 1990).

Chemicals. Glyphosate, labeled with ¹⁴C in the phosphonomethyl position, with a specific activity of 2.12 MBq/mg (1 MBq = 27 μCi) and a radiochemical purity >95% (Sigma Chemical Co., St. Louis, MO) was dissolved in distilled water (10 mL) to give a solution containing 185 kBq/mL and 0.09 mg of glyphosate/mL.

Reagents. Chemical reagents used in the study were all of ACS grade and obtained from Fisher Scientific Co. (Fair Lawn, NJ).

Fortification of Soils. Air-dried samples (5 g) of all soil types were weighed into 125-mL glass-stoppered Erlenmeyer flasks and treated with portions of the [¹⁴C]glyphosate solution (25 μL, 4.62 kBq, 2.2 μg, 0.44 μg/g). This concentration is approximately equal to a rate of 0.22 kg/ha, assuming that, in the field, the herbicide is distributed in the top 5 cm of soil (Smith, 1988). The flask and contents were thoroughly shaken on a wrist-action shaker for 10 min. This ensured distribution of both [¹⁴C]glyphosate and water throughout the soil, thus minimizing herbicide degradation resulting from small amounts of localized moisture. Under these dry conditions, [¹⁴C]glyphosate would not be expected to degrade to AMPA for the duration of the studies. Following treatment, all soils were incubated in the dark at 20 ± 1 °C until extraction.

Effect of Extraction Solvents on Recovery. Following 14 days of incubation, three replicate samples of all three soils were extracted with 50 mL of the 10 solvent systems, listed in Table I, using an extended-shaking procedure. The soils were first shaken for 1 h on a wrist-action shaker and then allowed to remain in contact with the extraction solution overnight before being shaken for a further 1-h period. Sample extracts were then centrifuged at 3000 rpm for 5 min or, in the case of sodium hydroxide extractant, at 10 000 rpm for 10 min, and a 4-mL aliquot (1 mL in the case of the NaOH and HCl extracts) of the clear supernatant was analyzed for radioactivity.

Effect of Shaking Procedures on Recovery. The recoveries of [¹⁴C]glyphosate from triplicate samples of the treated soils,

Table I. Extraction of ^{14}C from Air-Dried Soils (5 g) 14 Days after Fortification with [^{14}C]Glyphosate (0.44 $\mu\text{g/g}$) Using Different Solvent Systems (50 mL) with an Extended-Shaking Procedure

solvent system	% of applied radioactivity ^a		
	Regina (clay)	Indian Head (clay)	White City (loamy sand)
0.35 M H_3PO_4 and 0.09 M with respect to CaCl_2	85.2 \pm 0.4	86.2 \pm 0.4	90.7 \pm 0.4
0.1 M NaOH	77.6 \pm 1.2	81.1 \pm 0.3	89.3 \pm 1.0
0.5 M NH_4OH	72.8 \pm 0.8	76.5 \pm 0.2	88.5 \pm 1.7
0.1 M K_2HPO_4	44.1 \pm 0.4	54.2 \pm 0.7	73.0 \pm 1.2
0.1 M KH_2PO_4	12.6 \pm 0.1	19.6 \pm 0.7	47.6 \pm 0.8
0.1 M HCl	18.9 \pm 0.7	24.5 \pm 0.3	37.9 \pm 0.3
0.1 M triethylamine and 0.09 M with respect to CaCl_2	7.7 \pm 0.3	12.2 \pm 0.1	24.1 \pm 0.3
0.18 M CaCl_2	6.4 \pm 0.2	7.6 \pm 0.4	9.5 \pm 0.2
$\text{Ca}(\text{OH})_2$ (1.0 g) in water (50 mL)	2.6 \pm 0.2	4.2 \pm 0.1	7.7 \pm 0.2
$\text{CH}_3\text{CN}/\text{H}_2\text{O}$ /acetic acid (40:7.5:2.5 v/v/v)	1.0 \pm 0.1	1.8 \pm 0.1	0.8 \pm 0.1

^a Mean and standard deviation from three replicates.

14 days after fortification, were compared using aqueous 0.35 M phosphoric acid (and 0.09 M with respect to CaCl_2), 0.1 M NaOH, and 0.5 M NH_4OH as extractants in conjunction with a simple 1-h shake or the extended shake (see above). Following extraction, the centrifuged extracts were examined for radioactivity.

Effect of Soil/Solvent Ratio on Recovery. The recoveries of [^{14}C]glyphosate from triplicate samples of the Regina clay and Indian Head clay, treated 14 days prior to extraction, were compared using 0.1 M NaOH and soil solvent ratios of 1:2.5, 1:5, and 1:10 in conjunction with the extended-shaking procedure. After extraction, the centrifuged extracts were assayed for radioactivity. Recoveries of the ^{14}C -labeled herbicide from the White City loamy sand were analogously compared using the aqueous phosphoric acid containing CaCl_2 , 0.1 M NaOH, and 0.5 M NH_4OH as extractants.

Effect of Time on Recovery Using 0.1 M NaOH as Extractant. The recovery of [^{14}C]glyphosate from triplicate samples of all three soils 14, 28, and 56 days after initial fortification was determined using 0.1 M NaOH, with an extended shaking, and a soil/solvent ratio of 1:2.5. Centrifuged extracts (1 mL) were analyzed for ^{14}C as described.

Measurement of Radioactivity. Radioactivity in the various extracts was measured with a Packard Tri-Carb 1900TR liquid scintillation analyzer using a ^{139}Ba external standard for the determination of counting efficiencies. Scintillation solution was Scinti-Verse II (15 mL, Fisher Scientific).

RESULTS AND DISCUSSION

For the initial comparison of extraction solvents a soil/solvent ratio of 1:10 was selected since similar ratios have been used in other glyphosate analytical studies (*Pesticide Analytical Manual*, 1980; Lundgren, 1986; Miles and Moye, 1988; Roy and Konar, 1989; Thompson et al., 1989; Eberbach and Douglas, 1991). The extended-shaking procedure was employed since it has been shown to result in the recovery of greater amounts of herbicide residues from aged field soils than a 1-h extraction time (Smith, 1981; Smith and Milward, 1983). For glyphosate, both acidic and alkaline extraction solutions have been used. These include phosphoric acid (Roy and Konar, 1989), ammonium hydroxide (*Pesticide Analytical Manual*, 1980; Miles and Moye, 1988; Thompson et al., 1989), triethylamine (Lundgren, 1986; Eberbach and Douglas, 1991), and sodium or potassium hydroxides (Glass, 1983; Miles and Moye, 1988). In addition to the aforementioned extractants, solvent systems based on aqueous monobasic and dibasic potassium phosphate were tried since it has been reported (Sprankle et al., 1975b; Hance, 1976) that inorganic phosphate may compete with glyphosate for sorption sites on soil. An aqueous suspension of calcium hydroxide was used since this extractant, in conjunction with the extended shaking, has proved to result in efficient and reproducible recoveries from soils of the herbicide glufosinate (DL-homoalan-4-ylmethylphosphinic acid), which like glyphosate contains nitrogen- and phosphorus-

Table II. Comparison of the Extraction of ^{14}C from Air-Dried Soils (5 g) 14 Days after Fortification with [^{14}C]Glyphosate (0.44 $\mu\text{g/g}$) Using Three Solvent Systems (50 mL) and Two Shaking Procedures

solvent system	% of applied radioactivity ^a	
	extended shake	1-h shake
Regina (Clay)		
0.35 M H_3PO_4 and 0.09 M with respect to CaCl_2	85.2 \pm 0.4a	84.9 \pm 1.8a
0.1 M NaOH	77.6 \pm 1.2a	73.3 \pm 2.0b
0.5 M NH_4OH	72.8 \pm 0.8a	73.0 \pm 3.8a
Indian Head (Clay)		
0.35 M H_3PO_4 and 0.09 M with respect to CaCl_2	86.2 \pm 0.4a	85.6 \pm 1.7a
0.1 M NaOH	81.1 \pm 0.3a	82.6 \pm 1.3a
0.5 M NH_4OH	76.5 \pm 0.2a	81.0 \pm 0.4b
White City (Loamy Sand)		
0.35 M H_3PO_4 and 0.09 M with respect to CaCl_2	90.7 \pm 0.4a	86.3 \pm 1.0b
0.1 M NaOH	89.3 \pm 1.0a	87.9 \pm 1.3a
0.5 M NH_4OH	88.5 \pm 1.7a	82.1 \pm 0.2b

^a Mean and standard deviation from three replicates; means within a row followed by a common letter are not significantly different at the 0.05 level according to Duncan's multiple range test.

containing groups (Smith, 1988). Other extractants such as aqueous calcium chloride, dilute hydrochloric acid, and aqueous acidic acetonitrile were also included in the study.

The ^{14}C recovery data for the three soils, 14 days after fortification, using 10 solvent systems indicate that in all cases extraction was reproducible though variable (Table I). It was observed that the solvent systems containing calcium chloride or calcium hydroxide resulted in less colored solutions than the other extractants. For all solvent systems, except for the aqueous acidic acetonitrile where <2% of the applied radioactivity was recovered, [^{14}C]glyphosate extraction was lowest from the Regina clay and highest from the White City loamy sand. Of the extraction solvents, those based on phosphoric acid, sodium hydroxide, and ammonium hydroxide resulted in recoveries of between 72.8 and 90.7% of the applied radioactivity (Table I) with excellent reproducibility as indicated by the low standard deviations. Thus, these solvent systems were selected for further study.

Although the extended-shaking procedure did tend to recover significantly greater amounts of radioactivity from soils fortified 14 days prior to extraction, the differences were not very pronounced compared to a 1-h shaking (Table II). Thus, the extended shake significantly improved recoveries compared with the 1-h shake with the Regina clay using aqueous sodium hydroxide and the White City loamy sand using both the phosphoric acid and ammonium hydroxide extractants. Differences were not significant in five cases, and the 1-h shake proved

Table III. Extraction of ¹⁴C from Air-Dried Soils (5 g) 14 Days after Fortification with [¹⁴C]Glyphosate (0.44 μg/g) Using Selected Solvent Systems with an Extended-Shaking Procedure and Different Soil/Solvent Ratios

solvent system	% of applied radioactivity ^a at soil/solvent ratio of		
	1:2.5	1:5	1:10
Regina (Clay)			
0.1 M NaOH	80.9 ± 1.1a	76.3 ± 1.7b	77.6 ± 1.2b
Indian Head (Clay)			
0.1 M NaOH	92.7 ± 0.9a	85.3 ± 1.2b	81.1 ± 0.3c
White City (Loamy Sand)			
0.35 M H ₃ PO ₄ and 0.09 M with respect to CaCl ₂	85.2 ± 1.6a	90.0 ± 2.6b	90.7 ± 0.4b
0.1 M NaOH	95.6 ± 2.1a	94.1 ± 1.6a	89.3 ± 1.0b
0.5 M NH ₄ OH	80.5 ± 1.3a	84.6 ± 2.9b	88.5 ± 1.7b

^a Mean and standard deviation from three replicates; means within a row followed by a common letter are not significantly different at the 0.05 level according to Duncan's multiple range test.

Table IV. Extraction of ¹⁴C from Air-Dried Soils (5 g) 14, 28, and 56 Days after Fortification (DAF) with [¹⁴C]Glyphosate (0.44 μg/g) Using 0.1 M NaOH (12.5 mL) and an Extended-Shaking Procedure

soil	% of applied radioactivity ^a		
	14 DAF	28 DAF	56 DAF
Regina (clay)	80.9 ± 1.1a	79.7 ± 0.5a	78.8 ± 3.6a
Indian Head (clay)	92.7 ± 0.9a	89.2 ± 1.7a	76.1 ± 3.9b
White City (loamy Sand)	95.6 ± 2.1a	92.3 ± 1.2a	84.9 ± 4.0b

^a Mean and standard deviation from three replicates; means within a row followed by a common letter are not significantly different at the 0.05 level according to Duncan's multiple range test.

significantly better only in the case of the Indian Head clay using ammonium hydroxide. In all cases, except one, the standard deviations of recoveries (Table II) were lower using the extended procedure, indicating more reproducible recoveries.

It has been noted (Smith, 1981; Smith and Milward, 1983) that the extended-shaking procedure can recover greater amounts of herbicide residues than a shorter shake from field soils which received treatments of individual herbicides 12 months previously. During the weathering process, in the field study, the herbicides in the soils were exposed to hot dry summer conditions and long cold winter temperatures that probably resulted in a greater sorption of the herbicide residues to soil colloids and therefore a greater recalcitrance to extraction (Smith and Milward, 1983). Although such weathering would not occur during the 14 days at 20 °C encountered in the present study, the extended shake was used throughout the rest of the investigations.

As noted, many analytical procedures for glyphosate use a high soil/solvent ratio. Recoveries of [¹⁴C]glyphosate after 14 days (from the White City sandy loam) were dependent upon soil/solvent ratio when extracted with aqueous phosphoric acid and the 0.5 M NH₄OH solutions (Table III) with significantly greater amounts being recovered at 1:10 than at 1:2.5. In contrast, with all three soils, extraction with 0.1 M sodium hydroxide at a soil/solvent ratio of 1:2.5 resulted in recoveries that were equal to or greater than those achieved using ratios of 1:5 and 1:10. Thus, by being able to use such a low ratio, extraction solvent volumes can be kept to a minimum for resin cleanup.

It was therefore concluded that for the extraction of [¹⁴C]glyphosate from the three soils 0.1 M NaOH at a soil/solvent ratio of 1:2.5 was the solvent of choice. A time study (Table IV), using 0.1 M NaOH as extractant and

accompanied by the extended-shaking procedure, indicated that the recovery of ¹⁴C from all air-dried soils after 28 days at 20 °C was not significantly different from that after 14 days. After 56 days in air-dried Regina clay, the recovery of ¹⁴C was still 79% and not significantly changed. However, radioactivities recovered from the Indian Head clay and White City loamy sand 56 days after fortification were 76 and 85%, respectively, and significantly lower than the recoveries after 28 days. Whether this is due to increased adsorption to soil colloids or chemical or biological breakdown is unknown.

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Received for review January 8, 1992. Accepted April 23, 1992.

Registry No. NaOH, 1310-73-2; NH₄OH, 1336-21-6; H₃PO₄, 7664-38-2; CaCl₂, 10043-52-4; glyphosate, 1071-83-6.