Bull. Environ. Contam. Toxicol. (1993) 50:499–505

Environmental Contamination and Toxicology

Degradation of ^{14}C-Glyphosate in Saskatchewan Soils

Allan E. Smith and Andrew J. Aubin

Agriculture Canada, Research Station, Regina, Saskatchewan, S4P 3A2, Canada

Glyphosate (N-(phosphonomethyl)glycine), the active ingredient in several herbicide formulations, is a non-selective, post-emergent herbicide used in a variety of crop and non-crop situations. Field rates of glyphosate range from 0.34 to 1.2 kg/ha (ai) for control of annual species and 1.12 to 4.48 kg/ha (ai) for perennials (Humburg et al. 1989).

Glyphosate is a non-volatile herbicide that is relatively immobile in soil, with little potential for leaching (Torstensson 1985). Its degradation in soil is due to microbiological processes (Sprankle et al. 1975; Nomura and Hilton 1977; Rueppel et al. 1977; Moshier and Penner 1978). The major soil metabolite of glyphosate has been identified as aminomethylphosphonic acid which also undergoes breakdown in the soil (Nomura and Hilton 1977; Rueppel et al. 1977). Chemical analysis of residues in soils is both complex and time consuming. Consequently, most laboratory degradation studies have been conducted with ^{14}C-glyphosate with the rate of $^{14}CO_2$ evolution being used as an indication of herbicide breakdown (Torstensson 1985). A summary of such studies has revealed half-life values ranging from 3 days to 22.8 years (Torstensson 1985)

No information is available on the degradation and fate of glyphosate in Saskatchewan soils, and with its increasing use in chemical fallow cropping systems, some data are necessary. The following study was undertaken to investigate the breakdown of ^{14}C-glyphosate in three Saskatchewan soils, under laboratory conditions using a closed system, by measuring amounts of $^{14}CO_2$ evolved over a 90-day period. After 90 days, solvent extractable radioactivity and non-extractable ^{14}C associated with the soil was also quantified.

Address correspondence to Dr. Allan E. Smith

MATERIALS AND METHODS

Soils used in these studies were a clay from the Regina Research Station, a clay from Indian Head and a loamy sand from White City, Saskatchewan. The soils were collected immediately prior to commencement of the studies from the 0- to 10-cm of field soils when the moisture was <10% field capacity. After sieving (2-mm mesh) the soils were stored in plastic sacks at 4 ± 1°C. The physical characteristics of these soils have been previously described (Smith and Aubin 1990).

Glyphosate, labeled with ^{14}C in the phosphonomethyl position, and having a specific activity of 2.12 MBq/mg and a radiochemical purity >95% was obtained from Sigma Chemical Co., St. Louis, MO. The ^{14}C-herbicide was dissolved in distilled water (10 mL) to give a solution containing 185 KBq/mL (0.09 mg glyphosate/mL). An aqueous solution containing non-radioactive glyphosate (2 mg/mL) was also prepared.

Samples (50 g) of all three soils at 85% of their field capacities were weighed into 175-mL polystyrene foam cartons and placed in 2-L Mason jars fitted with spring-clip lids. The jars were placed in an incubator, in the dark at 20 ± 1°C, for 7 days. After equilibration, the soils were treated with ^{14}C-glyphosate solution (100 μL, 9 μg herbicide) and non-radioactive glyphosate solution (25 μL, 50 μg) so that the moist soil treatments contained 1.2 μg/g herbicide and 18.5 KBq. The soils were thoroughly stirred to ensure mixing. In each jar was placed a 50-mL beaker containing a 20-mL glass vial filled with 0.2M aqueous sodium hydroxide (15 mL) to absorb ^{14}C-carbon dioxide evolved. After treatment, the jars were re-incubated at 20 ± 1°C in the dark. Samples (1 mL) of the sodium hydroxide solution were analyzed for radioactivity at regular intervals, at which time the absorbing vials were changed. During the 90-day incubation period there were negligible losses of water from the soils by evaporation. The cumulative amounts of ^{14}C-carbon dioxide released were calculated as a percentage of the total radioactivity originally applied to the soils. Presence of ^{14}C-carbonate in the trapping vials was confirmed by precipitation as barium ^{14}C-carbonate (Harvey et al. 1985) and at all sampling times radioactivity in the sodium hydroxide trapping solution was attributable to $^{14}CO_2$. After 90 days, the soils were extracted to determine the amounts of solvent extractable radioactivity present and also the amounts of ^{14}C associated with the soils in a non-extractable form.

The soil from each carton was transferred to a 250-mL glass-stoppered flask, to which was added extraction

solvent containing water (100 mL), o-phosphoric acid (85%, 4 g) and calcium chloride (1 g) so that the total volume of extractant together with the water present in the soil samples was 100 mL. After a 1-hr shake on a wrist-action shaker, the flask and contents were allowed to remain in contact for an 18-hr period before being shaken for a further 1-hr period. Following centrifugation (2000 X g for 5 min), aliquots of the supernatant (4 mL) were analyzed for radioactivity remaining.

The soil residues following solvent extraction were collected and successively washed with the extraction solvent (100 mL), water (200 mL), methanol (100 mL) and finally acetone (100 mL). All washings were discarded since they contained negligible amounts of radioactivity. After drying for 2 hr at 90°C, aliquots (1.0 g) of the weighed soils were combusted (see below) to determine radioactivity remaining.

The ^{14}C present in the sodium hydroxide and soil extraction solvent was measured using a Packard Tri-Carb, Model 1900TR Liquid Scintillation Spectro- photometer. Scintillation solution was Scinti-Verse II (15 mL, Fisher Scientific Co., Fair Lawn, N.J.). Counting efficiences were determined using a ^{133}Ba external standard. Radioactivity in the solvent extracted soils was measured following combustion in a Harvey Biological Material Oxidizer, Model OX-500 (Harvey Instrument Corp., Hillsdale, N.J.). The evolved $^{14}CO_2$ was absorbed in Harvey Carbon 14 Cocktail (15 mL). $^{14}CO_2$ released from soils fortified with ^{14}C-glyphosate immediately before combustion, was equivalent to over 95% of the applied ^{14}C.

RESULTS AND DISCUSSION

With time there was a steady evolution of $^{14}CO_2$ from all three soils (Fig. 1) so that after the 90 days of incubation between 69 and 75% of the radioactivity from the applied ^{14}C-glyphosate had been released (Fig. 1 and Table 1). The times for release of 50% of the applied radioactivity as $^{14}CO_2$ was 30 ± 2 days from the Indian Head clay, 37 ± 2 days from the White City loamy sand and 40 ± 2 days from the Regina clay. As cautioned (Torstensson 1985), the liberation of $^{14}CO_2$ does not necessarily reflect the actual rate of decomposition of ^{14}C-glyphosate. In the radioactive herbicide used in the present studies the ^{14}C label was in the phosphonomethyl carbon atom. This carbon atom is also present in aminomethylphosphonic acid, the major soil metabolite. Thus, the $^{14}CO_2$ evolved could have been released both by the direct metabolism of ^{14}C-glyphosate and/or by metabolism of ^{14}C-containing degradation products. Hence the half-life values for glyphosate in the soils under

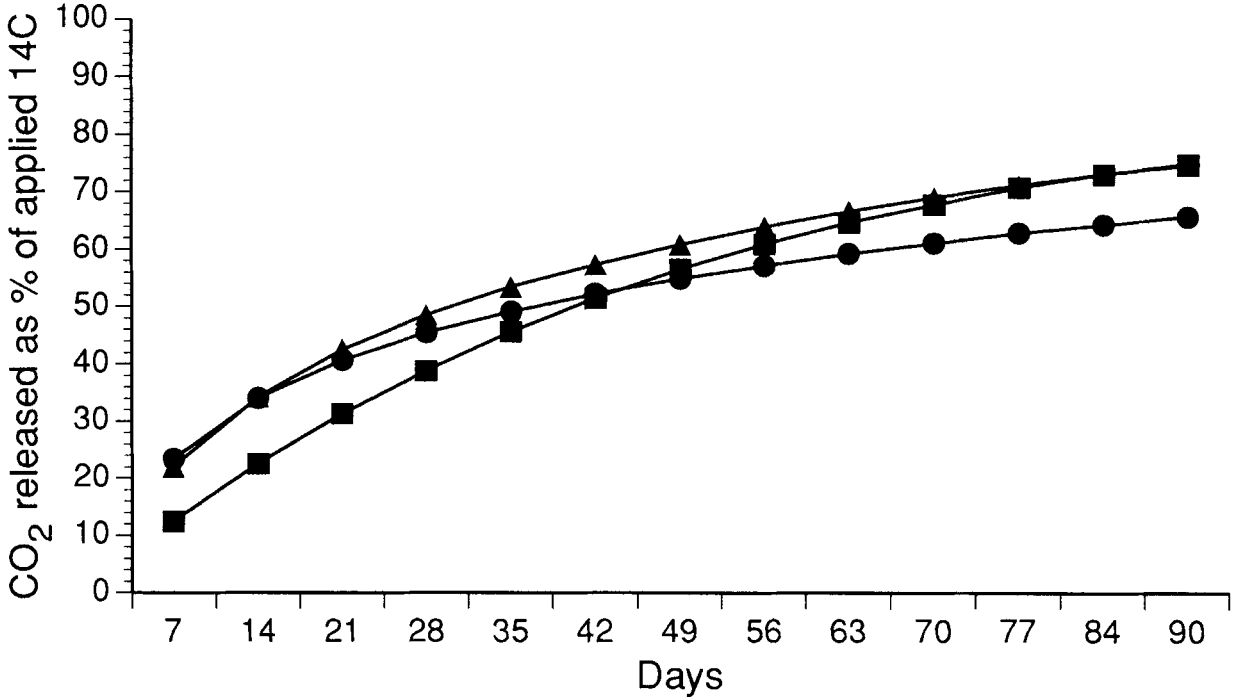

Figure 1. Cumulative release of 14C-carbon dioxide from an Indian Head clay (▲), a Regina clay (■) and a White City loamy sand (●) treated with 14C-glyphosate at a rate of 1.2 µg/g, following incubation at 20 ± 1 °C and 85% of field capacity. Each point is an average from 3 replicate soils with standard deviations <1.

Table 1. Radioactivity recovered after 90 days from soils treated with 1.2 μg/g ^{14}C-glyphosate following incubation at 20 ± 1°C and 85% of field capacity

	Recovered ^{14}C (% of applied)*		
	Indian Head (clay)	Regina (clay)	White City (loamy sand)
Released as $^{14}CO_2$	75 ± 1	75 ± 2	68 ± 1
Solvent extractable	9 ± 0	7 ± 0	16 ± 0
Soil non-extractable**	12 ± 1	14 ± 2	7 ± 0
Total radioactivity	96 ± 2	96 ± 2	91 ± 1

* Mean and standard deviation from 3 replicates.
** Via soil combustion.

study will be less than the 30 to 40 days indicated above. Such half-life values are similar to those reported by others under laboratory conditions (Hance 1976; Rueppel et al. 1977; Nomura and Hilton, 1977; Moshier and Penner 1978).

After 90 days of incubation, solvent extractable radioactivity ranged from 7% in the clay from Regina to 16% in the loamy sand from White City (Table 1). Extraction of glyphosate from soils using aqueous solutions containing phosphoric acid have been reported (Roy and Konar 1989). Unpublished studies from the Regina Research Station have shown that the aqueous extraction solvent containing phosphoric acid and calcium chloride is efficient, and gives reproducible ^{14}C-glyphosate recoveries in the range of 82 to 90%, from air-dried samples of the same soils used in this study, 28 days after fortification. The high phosphoric acid content of the extraction solvent precluded thin-layer, or high pressure liquid, chromatographic analysis of the extracts. It must therefore be surmized that the solvent extractable radioactivity contains such compounds as undegraded ^{14}C-glyphosate and ^{14}C-containing metabolites.

After 90 days, 7 to 14% of the initial ^{14}C remained in the soil in a non-extractable form (Table 1) but no attempts were made to further characterize this radio-activity. At the end of the experiment between 91 and 96% of the applied radioactivity could be accounted for (Table 1).

Field persistence studies with glyphosate have been almost exclusively conducted in forest soils where half-life values ranged from 29 to 60 days (Newton et al. 1984; Feng and Thompson 1990). Persistence studies with glyphosate under Canadian boreal forest conditions in

Ontario indicated that residues were reduced to less than 10% of that applied after 78 days (Roy et al. 1989). While a study conducted in a coastal B.C. forest watershed indicated that 13 to 18% of appled glyphosate residues could be detected after 360 days (Feng and Thompson 1990). No other Canadian soil persistence data are available.

Under the present study conditions it was observed that glyphosate was metabolized in moist non-sterile Saskatchewan soils with a half-life of less than 30 to 40 days. Since glyphosate shows little herbicide activity when applied to the soil (Hance 1976; Torstensson 1985) there is little likelihood of soil bound residues causing unexpected crop damage.

REFERENCES

Feng JC, Thompson DG (1990) Fate of glyphosate in a Canadian forest watershed. 2. Persistence in foliage and soils. J Agric Food Chem **38**:1118-1125

Hance RJ (1976) Adsorption of glyphosate by soils. Pestic Sci **7**:363-366..

Harvey J, Dulka JJ, Anderson JJ (1985) Properties of sulfometuron methyl affecting its environmental fate: Aqueous hydrolysis and photolysis, mobility and adsorption on soils, and bioaccumulation potential. J Agric Food Chem **33**:590-596

Humburg NE, Colby SR, Hill ER, Kitchen LM, Lym RG, McAvoy WJ, Prasad R (1989) Herbicide Handbook of the Weed Science Society of America. 6th ed, Weed Science Society of America, Champaign, Illinois

Moshier LJ, Penner D (1978) Factors influencing microbial degradation of ^{14}C-glyphosate to $^{14}CO_2$ in soil. Weed Sci **26**:686-691

Newton M, Howard KM, Kelpsas BR, Danhaus R, Lottman CM, Dubelman S (1984) Fate of glyphosate in an Oregan Forest Ecosystem. J Agric Food Chem **32**:1144-1151

Nomura NS, Hilton HW (1977) The adsorption and degradation of glyphosate in five Hawaiian sugarcane soils. Weed Res **17**:113-121

Roy DN, Konar SK (1989) Development of an analytical method for the determination of glyphosate and (aminomethyl)phosphonic acid in soils by nitrogen-selective gas chromatography. J Agric Food Chem **37**:441-443

Roy DN, Konar SK, Banerjee S, Charles DA, Thompson DG, Prasad R (1989) Persistence, movement, and degradation of glyphosate in selected Canadian boreal forest soils. J Agric Food Chem **37**:437-440

Rueppel ML, Brightwell BB, Shaefer J, Marvel JT (1977) Metabolism and degradation of glyphosate in soil and water. J Agric Food Chem **25**:517-528

Smith AE, Aubin AJ (1990) Degradation studies with ^{14}C-fenoxaprop in prairie soils. Can J Soil Sci **70**:343-350
Sprankle PL, Meggitt WF, Penner D (1975) Adsorption, mobility, and microbial degradation of glyphosate in the soil. Weed Sci **23**:229-234
Torstensson L (1985) Behaviour of glyphosate in soils and its degradation. In: Grossbard E, Atkinson D (eds) The Herbicide Glyphosate. Butterworths, London, pp 137-150

Received June 30, 1992; accepted October 30, 1992.