Degradation of Glyphosate in the Aquatic Environment: An Enzymatic Kinetic Model That Takes into Account Microbial Degradation of both Free and Colloidal (or Sediment) Particle Adsorbed Glyphosate

Mark F. Zaranyika* and Munyaradzi G. Nyandoro

Chemistry Department, University of Zimbabwe, P.O. Box MP 167, Mount Pleasant, Harare, Zimbabwe

The kinetics of the degradation of the herbicide glyphosate in distilled water and river water containing river sediment were investigated over a period of 72 days. No appreciable degradation of glyphosate was observed in distilled water, while rapid degradation occurred in the river water plus sediment from the outset, suggesting that the degradation is mainly microbial. An immediate 35% loss from solution of glyphosate due to adsorption to suspended sediment particles and deposition to the bottom sediment was observed in the river water plus sediment experiment. Subsequently, two linear rates of degradation were observed in the water phase of this experiment: an initial rapid degradation followed by a slower breakdown. An enzymatic kinetic model is presented showing that the rate of degradation of glyphosate (G) is given by $-d(\Delta G)/dt = k_2[G_B] + k_6[GC_B]$, where k_6 and k_2 are the rate constants for sediment or colloidal particle absorbed glyphosate (GC) and the unadsorbed glyphosate (G), respectively, and the subscript B denotes microflora-bound.

INTRODUCTION

Glyphosate [*N*-(phosphonomethyl)glycine, CAS Registry No. 1071-83-6] is a postemergence nonselective broad spectrum herbicide extensively used in agriculture for the control of many annual and perennial weeds. Glyphosate is essentially nontoxic to mammals and birds, but fish and invertebrates are more sensitive to the herbicide [Weed Science Society of America (WSSA), 1983]. Recommended field application rates range from 0.34 to 4.48 kg of active ingredient/ha (WSSA, 1983). Rates of 1.8–2.2 kg/ha are recommended for the control of aquatic weeds (British Crop Protection Council, 1978).

The rate of glyphosate degradation in soil samples or soil suspended in distilled water has been found to correlate with the respiration of the sample (Ruepped et al., 1977; Sprankle et al., 1975a,b; Torstensson and Aamizepp, 1977). Since respiration is a measure of the microbial activity of the sample, the degradation of glyphosate in the soil and water environment is thought to be mainly microbial.

The degradation of glyphosate in the soil environment has been studied by Nomura and Hilton (1977) and by Hance (1976). These studies showed that the degradation of glyphosate in the soil environment involves an initial rapid degradation followed by a prolonged and slower breakdown. Nomura and Hilton (1977) suggested that this may arise from the early rapid metabolism of free glyphosate by microorganisms, followed by a slower metabolism of glyphosate adsorbed onto soil particles.

Carlisle and Trevors (1978) reviewed the use, mode of action, and degradation of the herbicide and concluded that the half-life of glyphosate in the soil environment varied considerably, ranging from less than a week to years, and appears to depend in part on the extent of soil binding and level of microbial activity. pH was found to have little effect on soil binding (Sprankle et al., 1975a,b; Hance, 1976) or rate of degradation (Moshier and Penner, 1978).

The aim of the present work was to carry out laboratory studies to elucidate further the kinetics of the degradation of the herbicide. Experimental conditions were selected to simulate as closely as possible those to be found in the natural aquatic environment. Thus, the laboratory experiments were conducted using river water and river sediment contained in plastic drums covered with clear perforated plastic and exposed to sunlight.

EXPERIMENTAL PROCEDURES

Equipment. The following were used: a high-performance liquid chromatograph, Shimadzu LC-4A system, was equipped with a 10-cm cell, a variable UV detector, and chart recorder; separations were made using a C_{18} column (0.8 × 10 cm); white plastic tanks, 150-L capacity, were used for the degradation experiments.

Materials. The following were used: Round-up (Monsanto Agricultural Products) containing 395 g of glyphosate/L, supplied by the Zimbabwe Fertilizer Co.; ethyl acetate, sodium dihydrogen phosphate buffer, tetraethylammonium bromide (AR grade), acetonitrile (HPLC grade); distilled water; river water and sediment collected from the Mukuvisi River, Harare, Zimbabwe (the river had never been treated with glyphosate); 2,4-dinitrochlorobenzene (reagent grade); sodium bicarbonate and sodium hydroxide (AR grade). The glyphosate standard was obtained by precipitation from Round-up and recrystallization from ethanol, melting point 199 °C (literature 200 °C).

Procedure. One lot each of 100 L of river water and distilled water was charged into separate 150-L plastic tanks, and the levels were marked. To the tank containing river water was added 1.93 kg of the sediment from the Mukuvisi River. Thirty-eight milliliters of Round-up was then added to each tank (to give a solution containing approximately 150 ppm of glyphosate), and then contents were thoroughly mixed. Samples for analysis at zero time were taken immediately after the mixing. The new levels of water in the tanks were marked. The tanks were then covered with transparent perforated polythene and left exposed to the sun on the roof of the University of Zimbabwe Chemistry Department building. Thereafter, samples were taken periodically over a period of 72 days, each time compensating for evaporation prior to sampling and marking the new level of water after each sampling. Sediment samples were scooped from the bottom of the tank before any agitation of the tank.

Once collected, the samples were frozen in plastic bottles until required for analysis, whereupon they were thawed and mixed thoroughly before extraction and derivatization of the glyphosate for HPLC analysis.

Extraction, Derivatization, and HPLC Analysis. Water samples were filtered through Whatman No. 1 filter paper,

* Author to whom correspondence should be addressed.

0021-8561/93/1441-0838$04.00/0

Table I. Degradation of Glyphosate in (A) Distilled Water, (B) River Water Containing Sediment, and (C) Sediment

	analysis (ppm)					
	A		B		C	
day of sampling	[G]	$-\Delta$[G]	[G]	$-\Delta$[G]	[G]	$-\Delta$[G]
before charging	0.0	0	0.0	0	0.0	
0	160.0	0	96.8	63.2	3230	
3	159.6	0.4	87.5	72.5		
6	159.6	0.4	82.7	77.3		
16	149.1	10.1	58.9	101.1		
23	150.6	9.4	55.6	104.4	2150	1080
37	151.1	8.9	52.9	107.1		
47	151.1	8.9	40.5	119.5	1740	1490
58	153.9	6.1	47.5	112.5	1640	1590
72	153.9	6.1	45.0	115.0	1350	1880

discarding the first 50 mL (to avoid any losses through adsorption of glyphosate on cellulose). Four milliliters of the filtered sample was pipetted into a 30-mL test tube and 2 mL of a 4% $NaHCO_3$ solution was added, followed by 2 mL of a 0.05 g/mL solution of 2,4-dinitrochlorobenzene in ethanol, and the contents were mixed by shaking vigorously. Glyphosate standards for the calibration curve were prepared in the same way. The test tubes were then incubated at 70 °C in the dark for exactly 3 h with agitation every 15 min. (Preliminary studies had shown that derivatization was complete under these conditions.)

Sediment samples were extracted after the excess water in the sample was drained by suction from a Büchner funnel through a Whatman No. 1 filter paper and air-drying for 3 h. The glyphosate was extracted from the sediment using the method of Miles and Moye (1988), but using sodium hydroxide instead of ammonia as suggested by Nomura and Hilton (1977). After extraction from the sediment, the glyphosate was derivatized as above. In both cases the 2,4-dinitrophenyl derivative was liquid–liquid extracted into ethyl acetate. The ethyl acetate extract was filtered through a 0.45-μm filter. Eight microliters was injected into the HPLC and eluted with 0.04 M sodium dihydrogen phosphate buffer (pH 4.0), containing 0.01 M tetraethylammonium bromide, as mobile phase at a flow rate of 1.0 mL/min. UV detection was done at 340 nm. Table I and Figures 1 and 2 show the concentration of glyphosate obtained for the different samples. The amount of glyphosate lost by degradation, $-\Delta G$, was calculated and plotted as a function of time in Figure 3. Glyphosate was not detected when blank determinations on the river water and sediment were done.

RESULTS AND DISCUSSION

A heavy green algal growth developed in the river water plus sediment experiment after 3 days. The algal growth disappeared after 58 days. No algal growth was observed in the distilled water experiment. Since no growth was observed in the distilled water experiment, the algal growth is attributed to the presence of nutrients in the river water which were used up after 58 days.

The immediate loss from solution of 63.2 ppm of glyphosate observed when the tank for the river water plus sediment experiment was charged with Round-up (see Table I) is attributed to adsorption of glyphosate onto suspended sediment particles which then settled to the bottom of the tank. This was confirmed by analysis of the sediment which showed that on day 0 5.26 g of the 15.0 g of glyphosate added to the tank was in the sediment, leaving 97.5 ppm in solution [experimental figure is 96.8 ppm (see Table I)]. Strong binding of glyphosate by soil particles was reported previously by Hance (1976).

Little degradation of glyphosate was observed in the distilled water experiment throughout the period of the investigations (see Figure 1), while rapid degradation occurred in the river water plus sediment experiment from the outset, confirming earlier findings that the degradation of glyphosate in the aquatic environment is mainly

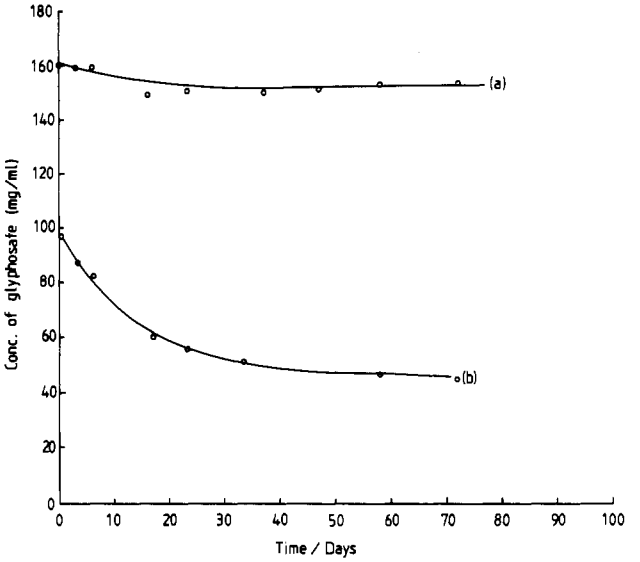

Figure 1. Concentration of glyphosate as a function of time: (a) in distilled water and (b) in the water phase of the river water containing sediment experiment.

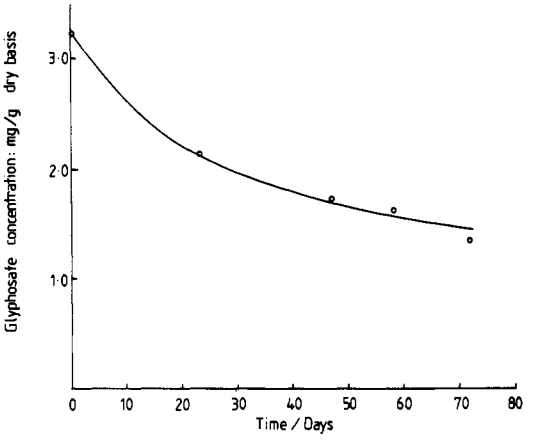

Figure 2. Concentration of glyphosate in the sediment phase as a function time.

microbial as discussed under Introduction. Figures 1 and 2 show that the rate of decrease of glyphosate both in the water phase and in the sediment phase is initially fast but decreases asymptotically with time. These results are in agreement with those obtained by Nomura and Hilton (1977) for the degradation of glyphosate in the soil environment.

Figure 3 shows that both (a) the fast and slow rates of loss of glyphosate in the water phase and (b) the rate of loss of glyphosate in the sediment phase are linear. These results suggest that the degradation of "free" and "adsorbed" glyphosate occurs by a similar mechanism (i.e., both are microbial), while the fact that rates of degradation are linear points to steady-state kinetics. Similar constant rates have been reported by Nomura and Hilton (1977) for the degradation of glyphosate from three different soil types. Since chemical degradation and photodecomposition appear to be minor routes of glyphosate decomposition (Carlisle and Trevors, 1988), below we discuss a possible enzymatic kinetic model which is consistent with the observed kinetics. Microbial degradation of glyphosate was demonstrated previously (Carlisle and Trevors, 1988, Rueppel et al., 1977; Sprankle et al., 1975a,b).

Proposed Enzymatic Kinetic Model. Microbial degradation of glyphosate in either the water or sediment phase may be represented by the following steps:

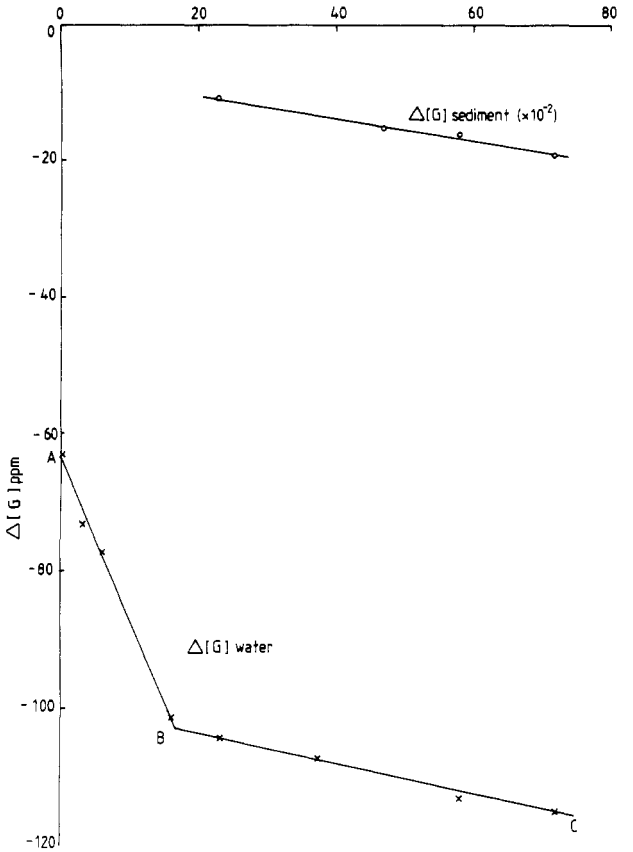

Figure 3. Loss of glyphosate (−Δ[G]) as a function of time (days) in the water phase and the sediment phase of the river water plus sediment experiment.

$$G_F + B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} G_B \qquad (1)$$

$$G_B + E \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} GE \xrightarrow[\text{fast}]{k_3} P + E \qquad (2)$$

$$G_F + C \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} GC_F \qquad (3)$$

$$GC_F + B \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} GC_B \qquad (4)$$

$$GC_B + E \underset{k_{-6}}{\overset{k_6}{\rightleftharpoons}} C + GE \xrightarrow{k_3} P + E \qquad (5)$$

where G is free glyphosate, GE is the glyphosate–enzyme complex, E is enzyme, P represents products, C is a colloidal particle, and GC is the glyphosate–colloidal particle complex. The subscripts F and B denote "free" and "microbial-bound", respectively. It can be shown that at steady state (with respect to GE) the rate of formation of product is given by

$$\frac{dP}{dt} = \frac{k_3[E](k_2[G_B] + k_6[GC_B])}{k_{-2} + k_{-6} + k_3} \qquad (6)$$

The degradation of glyphosate occurs inside cells of microflora, and it can be assumed that inside such cells $[E] \gg [G_B]$ and $[E] \gg [GC_B]$; i.e., E is in large excess, and we may assume that $[E] = 1$. We may assume further that $k_3 \gg k_{-2} + k_{-6}$. With these assumptions the rate equation simplifies to

$$dP/dt = k_2[G_B] + k_6[GC_B] \qquad (7)$$

The presence of a steady state means that in eqs 1 and 4 G_F and GC_F are in excess compared to the concentration of microflora in the medium, so that G_B and GC_B are constant. Thus, eq 7 can be expressed as

$$dP/dt = k_2' + k_6' \qquad (8)$$

where

$$k_2' = k_2[G_B] \qquad k_6' = k_6[GC_B] \qquad (9)$$

Equation 8 is consistent with Figure 3: k_2' corresponds to the slope of the initial rapid rate of degradation in the water phase (A–B in Figure 3), while k_6' corresponds to the slope of the slower rate of degradation of colloidal particle adsorbed glyphosate in the water phase (B–C in Figure 3) or the rate of degradation of glyphosate adsorbed onto sediment particles (D–E in Figure 3). The actual rate of degradation observed will depend on the values of $[G_B]$ and $[GC_B]$ in each phase. These will depend on the microbial count in the system under study.

Estimation of the Values of k_2, k_6, $[G_B]$, and $[GC_B]$. The quantity measured during these experiments was the total glyphosate, G_T, remaining at any instant, where

$$[G_T] = [G_F] + [GC_F] + [G_B] + [GC_B] \qquad (10)$$

Hence

$$[G_B] = [G_T] - [GC_F] - [G_F] - [GC_P] \qquad (11)$$

$$[GC_B] = [G_T] - [G_F] - [GC_F] - [G_B] \qquad (12)$$

As discussed above, $[GC_B]$ and $[G_B]$ are constant, and $[G_F]$ and $[GC_F]$ are both unity. Invoking these assumptions and putting $P = -\Delta G$, where $\Delta G = G_0 - G_T$ and G_0 is the initial concentration of glyphosate, i.e., at $t = 0$ (t = time), and substituting eq 11 into eq 7 yield

$$-d(\Delta G)/dt = k_2[G_T] + K \qquad (13)$$

Similarly

$$-d(\Delta G)/(dt) = k_6[G_T] + K' \qquad (14)$$

where K and K' are constants. Values of $-\Delta G$ and $-\Delta(\Delta G)/\Delta t$ obtained for the water phase and the sediment phase of the river water plus sediment experiment are shown in Tables II and III, respectively.

Figures 4 and 5 show the graphs of $-\Delta[\Delta G]/\Delta t$ vs $[G_T]$ for the water and sediment phases, respectively. These figures confirm the validity of eqs 13 and 14; i.e., the rate of degradation of glyphosate is a linear function of the total concentration of glyphosate in the medium.

It is interesting to note that according to Figure 4 $k_2 = k_6$. We therefore conclude that the difference in the rates of degradation of free and colloidal particle adsorbed glyphosate in the water phase is due to the difference in the steady-state concentration of G_B and $(GC)_B$ in eq 7. According to this interpretation, the adsorption of glyphosate onto colloidal particles and the binding of both free and adsorbed glyphosate are all fast processes. However, the adsorption process is slightly favored so that both G_B forms are formed simultaneously, but $[G_B] > [(GC)_B]$ simply because formation of $(GC)_B$ is a two-step process. The final numerical steady-state concentrations of G_B and $(GC)_B$ will depend on the microflora count in the medium.

Enzymatic degradation is often described in terms of the Michaelis–Menten kinetics which relates the initial rate of reaction to the initial concentration of the substrate for a given constant concentration of the enzyme. According to the Michaelis–Menten model, the initial rate

Table II. Kinetic Data for the Degradation of Glyphosate in the Water Phase of the River Water plus Sediment Experiment[a]

t	G_0	G_T	$-\Delta G$	$-\Delta(\Delta G)$	Δt	$-\Delta(\Delta G)/\Delta t$	$\bar{G}_T$
0	160	99.0	61				
				8	2	4.0	95
2		91.0	69				
				6	2	3.0	88
4		85.0	75				
				5	2	2.5	82.5
6		80.0	80				
				4.5	2	2.25	77.5
8		75.5	84.5				
				3.7	2	1.85	73.65
10		71.8	88.2				
				3.3	2	1.65	70.15
12		68.5	91.5				
				3.0	2	1.5	67.0
14		65.5	94.5				
				2.7	2	1.35	64.15
16		62.8	97.2				
				4	4	1.0	60.8
20		58.8	101.2				
				3	4	0.75	57.15
24		55.5	104.2				
				2.6	4	0.65	54.35
28		53.2	106.8				
				1.7	4	0.43	52.35
32		51.5	108.5				
				1.0	4	0.25	51.00
36		50.5	109.5				
				1.5	4	0.38	49.75
40		49.0	111				
				1.0	8	0.13	8.5
48		48.0	112				
				1.0	12	0.08	47.5
60		47	113				

[a] G_0 = initial concentration of glyphosate. G_T = concentration at any time t (in days). $\bar{G}_T$ = average concentration during sampling interval.

Table III. Kinetic Data for the Degradation of Glyphosate in the Sediment Phase of the River Water plus Sediment Experiment[a]

t	G_0	G_T	$-\Delta G$	$-\Delta(\Delta G)$	Δt	$-\Delta(\Delta G)/\Delta t$	$\bar{G}_T$
0	3230	3230	0				
				280	4	70	3090
4		2950	280				
				250	4	62.5	2830
8		2700	530				
				180	4	45	2610
12		2520	710				
				160	4	40	2440
16		2360	870				
				120	4	30	2300
20		2240	990				
				120	4	30	2180
24		2120	1110				
				90	4	22.5	2080
28		2030	1200				
				80	4	20	1990
32		1950	1280				
				80	4	20	1910
36		1870	1360				
				70	4	17.5	1840
40		1800	1430				
				50	4	12.5	1780
44		1750	1480				
				50	4	12.5	1730
48		1700	1530				
				50	4	12.5	1680
52		1650	1580				
				50	4	12.5	1630
56		1600	1630				
				40	4	10	1580
60		1560	1670				
				40	4	10	1540
68		1520	1710				

[a] G_0 = initial concentration of glyphosate. G_T = concentration at any time t (in days). $\bar{G}_T$ = average concentration during sampling interval.

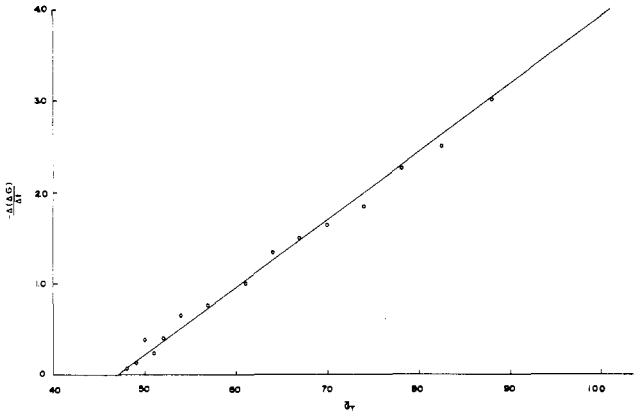

Figure 4. Graph of $-\Delta(\Delta G)/\Delta t$ vs $\bar{G}_T$ for the degradation of glyphosate in the water phase of the river water plus sediment experiment.

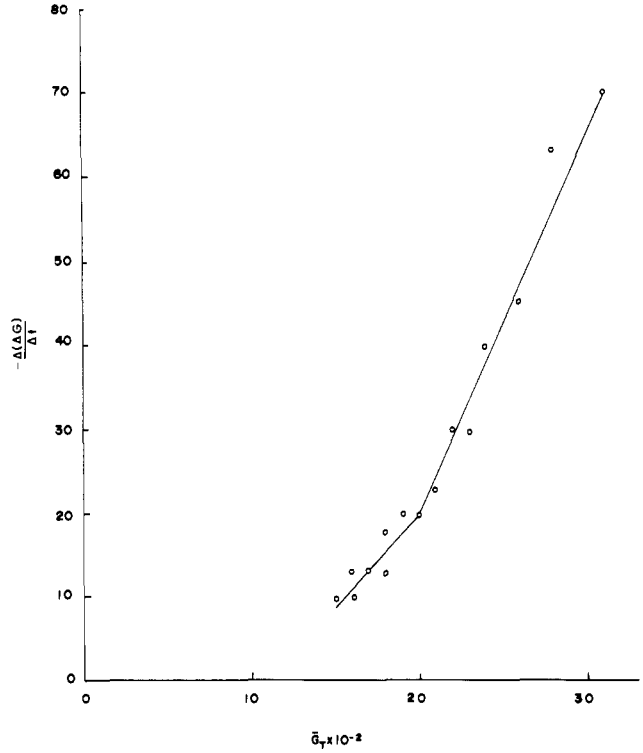

Figure 5. Graph of $-\Delta(\Delta G)/\Delta t$ vs $\bar{G}_T$ for the degradation of glyphosate in the sediment phase of the river water plus sediment experiment.

of reactions becomes constant when the concentration of the substrate is in excess to that of the enzyme. A similar situation is possible with microbial binding of the substrate; i.e., if the substrate concentration is in excess of the microbial concentration, a steady-state situation obtains and a constant rate of reaction will be observed. The results discussed above are consistent with such a situation. This was possible because of the restricted volume of water and sediment used in the experiments.

In natural aquatic systems such as rivers and lakes, the glyphosate applied will be dispersed into a large volume of water. The supply of microorganisms to bind the glyphosate in the water phase of the system is essentially unlimited under these conditions. The net result will be that the microbial concentration will be in excess of the glyphosate concentration, so that all of the glyphosate

Table IV. Estimated Rates of Degradation of Glyphosate in the Water and Sediment Phases, Estimated Rate Constants, and Concentration of Microbial-Bound Glyphosate

phase	state of glyphosate (free, G; adsorbed, GC)	rate of degradation[a] k_2'	rate of degradation[a] k_6'	rate constant[b] k_2	rate constant[b] k_6	concn of microbial-bound glyphosate, ppm $[G_B]$	concn of microbial-bound glyphosate, ppm $[(GC)_B]$
water	free (G)	2.325		0.071		31.75	
	colloidal particle adsorbed (GC)		0.222		0.071		2.13
sediment	colloidal particle adsorbed (GC)		25.0		0.045		555

[a] $dp/dt = k_2'$ or k_6'. [b] Units: ppm per day per ppm of microbial-bound glyphosate.

will be present as enzyme complexed (or microorganism bound) and colloidal (or sediment) particle adsorbed glyphosate, and $[G_F]$ will be zero. Under these conditions $[G_B]$ is no longer a constant and eq 8 becomes

$$dP/dT = k_2[G_B] + k_6' \quad (15)$$

The values of the rate constants k_2 (or k_6) and steady-state concentrations of microflora-bound glyphosate, G_B and $(GC)_B$, in the water and sediment phases obtained from Figures 5 and 3 are summarized in Table IV. The numerical values of k_2 and k_6 will depend on the type of microorganism which binds the glyphosate molecules. Several microorganisms are likely to be involved in binding glyphosate and each type, i, will have different $k_{2(i)}$ and $k_{6(i)}$ values. The overall rate constants k_2 ($=\sum k_{2(i)}$) and k_6 ($=\sum k_{6(i)}$) will depend on the relative concentration of the different microorganisms which bind the glyphosate in a given aquatic environment. The type and concentration of the different microorganisms will vary in different compartments of a given system, as well as from one system to another. Thus, k_2 and k_6 should be determined for each given aquatic system or compartment of a given aquatic system.

The numerical values of k_2 and k_6 will also depend on the temperature and pH of the medium inasmuch as these affect the activity of microorganism.

Equation 3 accounts for the adsorption/desorption equilibrium between the glyphosate and colloidal (or sediment) particles. Equation 4 assumes that $k_5 \gg k_{-4}$ so that for all intents and purposes the microorganisms bind the colloidal particle glyphosate complex. In the present case the validity of this assumption is confirmed by the fact that k_2 and k_6 are numerically equal as discussed above.

Conclusions. From the foregoing discussion we conclude that the degradation of glyphosate in the aquatic environment can be explained in terms of an enzymatic kinetic model which takes into account microbial degradation of both free and colloidal (or sediment) particle adsorbed glyphosate. Provided the concentration of glyphosate in the medium is in excess of the microflora present that can bind immediately, the glyphosate will be lost at a constant rate which depends on (i) whether the glyphosate undergoing degradation is free or adsorbed onto colloidal particles, (ii) the microflora count of the specific medium, and (iii) the colloidal particle content, in the case of degradation of glyphosate in water.

ACKNOWLEDGMENT

We acknowledge the courtesy of Mr. D. Lowe and the Zimbabwe Fertilizer Co. in providing the sample of Round-up used in these experiments.

LITERATURE CITED

British Crop Protection Council. In *Weed Control Handbook, Vol. II: Recommendations*, 8th ed.; Fryer, J. D., Makepeace, R. J., Eds.; Blackwell Scientific Publications: Oxford, U.K., 1975; Chapter 10.

Carlisle, S. M.; Trevors, J. T. Glyphosate in the environment. *Water, Air Soil Pollut.* **1988**, *39*, 409–420.

Hance, P. J. Adsorption of glyphosate by soils. *Pestic. Sci.* **1976**, *7*, 363–366.

Miles, C. J.; Moye, H. A. Extraction of Glyphosate Herbicide from Soil and Clay Minerals and Determination of Residues in Soils. *J. Agric. Food Chem.* **1988**, *36*, 486–491.

Moshier, L. J.; Penner, D. Factors Influencing Microbial Degradation of ^{14}C-Glyphosate to $^{14}CO_2$ in Soil. *Weed Sci.* **1978**, *26*, 686–691.

Nomura, N. S.; Hilton, H. W. The adsorption and degradation of glyphosate in five Hawaiian sugarcane soils. *Weed Res.* **1977**, *17*, 113–121.

Rueppel, M. L.; Brightwell, B. B.; Schaeffer, J.; Marvel, J. T. Metabolism and Degradation of Glyphosate in Soil and Water. *J. Agric. Food Chem.* **1977**, *25*, 517–528.

Sprankle, P.; Meggitt, W. F.; Penner, D. Adsorption, Mobility and Microbial Degradation of Glyphosate in the Soil. *Weed Sci.* **1975a**, *23* (3), 229–234.

Sprankle, P.; Meggitt, W. F.; Penner, D. Rapid Inactivation of Glyphosate in the Soil. *Weed Sci.* **1975b**, *23* (3), 224–228.

Torstensson, N. T. L.; Aamisepp, A. Detoxification of Glyphosate in Soil. *Weed Res.* **1977**, *17*, 209–212.

Weed Science Society of America. In *Herbicide Handbook*; Weed Science Society of America: Champaign, IL, 1983.

Received for review September 16, 1992. Accepted January 22, 1993.