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Applying Non-steady-state Compartmental Analysis to Investigate the Simultaneous Degradation of Soluble and Sorbed Glyphosate (*N*-(Phosphonomethyl)glycine) in Four Soils

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Abstract: The decomposition behaviour of glyphosate in four Victorian soils was investigated at two temperatures using non-steady-state compartmental analysis. At 25°C, glyphosate degradation was shown numerically to be derived from two different sources where the rate of release from each source behaved in accordance with first-order kinetics. Over the first 40 day period for each of the soils, glyphosate was derived simultaneously from the labile and non-labile phase, whilst after the first 40 days, glyphosate was derived solely from the non-labile phase. At this temperature, the amount of glyphosate partitioned into the labile phase ranged from 24·1 to 34·5%, whilst the amount partitioned into the sorbed, non-labile phase ranged from 67·2 to 74·9%. The half-lives for glyphosate within each phase was calculated and ranged from six to nine days for the labile phase to 222–835 days for the non-labile phase. Glyphosate appeared to be more strongly held in the acidic Rutherglen soil than in the alkaline soils studied, and this was thought to be related to the substantially lower pH and higher Fe content of the acidic soil. At 10°C, glyphosate was shown numerically to be derived from two different sources for two of the soils. However, for the two remaining soils, glyphosate appeared to be derived either from a single phase or from two phases at either the same rate or at differential rates where the rate of release from one phase was sufficiently fast to mask the rate of release from the other. At this temperature, more glyphosate was partitioned into the non-labile phase of the Walpeup and Rutherglen soils than at 25°C. However, the rate of release of glyphosate from this phase increased for the Walpeup soil relative to that at 25°C, but decreased substantially for the Rutherglen soil. This suggests that different mechanisms for the binding of glyphosate into the non-labile phase may exist between soils. © 1998 SCI.

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Key words: glyphosate; degradation; sorption strength; desorption; compartmental analysis; soil

1 INTRODUCTION

Degradation of herbicides and pesticides in soil is frequently assumed to follow first-order reaction kinetics. However, attempts to linearise the apparent first-order kinetic function using a logarithmic transformation of the decomposition product have frequently failed, suggesting that, for these cases, the decomposition function is not first-order. Several hypotheses to explain these phenomena have been offered and include: reaction kinetics of an order higher than one,[1] processes in addition to decomposition affecting the catabolism of the pesticide,[2] sorption processes influencing availability of the substrate for decomposition,[3] and the heterogeneity

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or spatial variability of soils.[4] Catabolism of the herbicide glyphosate in soil frequently conforms to an apparent non-first-order decay paradigm.[5–10] Nomura and Hilton[3] suggested that the shape of the decomposition curve related the strength of binding of glyphosate to the soil.

Compartmentation is a theory first proposed by Hamaker and Goring[1] which provides an excellent conceptual image of the factors affecting pesticide behaviour in soil, particularly the dependent relationship between adsorption and the availability of pesticides for biodegradation. The theory suggested that pesticides exist in soil either in the labile phase as soluble or readily exchangeable pesticide, which is weakly held and is, as a result, chemically and biologically active, or in the non-labile phase, as material chemically bound (adsorbed) to soil solids (Fig. 1) and not biologically available.

An assumption of this model is that decomposition of substrate can occur only from the labile phase. This assumption may be inappropriate for pesticides whose major mode of decomposition is chemical hydrolysis, such as the sulfonylureas, but is probably valid for compounds in which the mode of degradation is largely microbial. This assumption is particularly valid for glyphosate, as Rueppell *et al.*[11] contended that the slowdown in glyphosate degradation after seven days reflected adsorption by the substrate and hence reduced availability for decomposition. Each phase in the model depicts a combination of different forms of binding, which are common in relative binding strength. Therefore material in each of the two phases maintains a degree of exclusivity and obeys kinetics which are likely to be phase-dependent, approximately conforming to first-order rate law. However, despite this phase exclusion, pesticide can still move between the two phases, probably to maintain equilibrium. Therefore a pesticide disappearance curve may depict the activity of a combination of phases of the pesticide in soil, in this instance, a two-stage, first-order rate response for a hypothetical two-stage, first-order event.[12]

A procedure was described in Hamaker and Goring[1] to estimate the kinetics of these two phases by obtaining tangents from the initial and steady-state sections of a semi-logarithmic pesticide disappearance curve, and the steady-state intercept. This technique has been used to characterise the nature of persistence of trifluralin,[12,13] prometryne, carbaryl and metribuzin in soil.[12] However, the technique of Hamaker and Goring[1] was not developed in a sound mathematical manner, as the maximal and minimal slopes of a semi-logarithmic plot of the pesticide disappearance curve over time were used to determine the kinetics of the labile and the non-labile phases in a manner which assumed absolute exclusion between the two phases. This technique emphasised this outcome, since the intersection between the two tangents was assumed to be the point where the influence of one phase ceased and that of the other commenced. As a result, this procedure was unable to separate the influence that one phase may have on the other: for example, how desorption from the non-labile phase may influence apparent disappearance of substrate from the labile phase.

A more appropriate mathematical procedure has been developed to determine the kinetics of two phases occurring simultaneously. This procedure, termed Non-Steady-State Compartmental analysis,[14] uses curve peeling to strip numerically the influence of activity in one phase from other phases, allowing the kinetics of each of the identified phases to be quantified. This technique is commonly used by cellular physiologists.[15] For example Thornton[16] attempted to identify barriers to the uptake of root-applied copper into the leaves of ryegrass plants. This technique shows a particular applica-

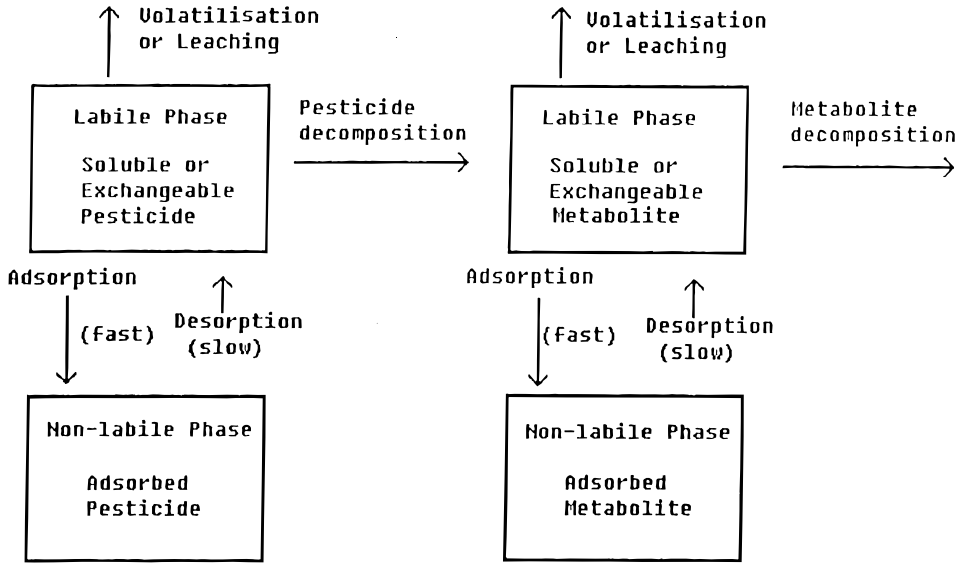

Fig. 1. Two-compartment model for soil degradation of pesticides (from Ref. 1).

bility to pesticide residue chemistry, allowing us to develop a better appreciation of factors which influence pesticide biodegradation in soil.

The purpose of this paper is to investigate the use and application of curve peeling and non-steady-state compartmental analysis to explain the apparent non-first-order decomposition of glyphosate in four Victorian soils at two temperatures.

2 MATERIALS AND METHODS

2.1 Soils

Soils used in this study were surface (0–15 cm) samples from Victoria, Australia selected for their wide range of chemical and physical properties and for their important use in cropping systems.

Some physicochemical properties of each soil are given in Table 1. Particle-size analysis was determined using the pipette method,[17] following oxidation of organic matter with peroxide and dispersion with Calgon. Organic carbon was determining using the Walkley–Black procedure using a correction factor of 1·3.[18] Cation exchange capacity was determined by extracting with neutral ammonium acetate and quantification of NH_4^+ using semi-micro steam distillation,[19] exchangeable potassium and sodium by extraction with neutral ammonium acetate and quantification with flame photometry,[20] exchangeable calcium and magnesium by EDTA titration,[21] and exchangeable iron by extraction with neutral ammonium acetate and quantification by colorimetry.[22]

2.2 Soil preparation

After collection, soils were immediately refrigerated (4°C) and kept this way until they were used. Soils stored for more than two weeks after collection were discarded. When required for use, soils were laid out thinly on a plastic sheet and air dried overnight at room temperature. After air drying, a subsample was taken and oven dried to determine air-dry moisture content. Air-dried soil was then mixed, sieved gently to pass through a 2-mm sieve prior to being weighed into incubation vessels. Microbial respiration of the soils pre- and post air drying, and following wetting to field capacity was not determined, as it was assumed that passive air drying at ambient temperatures for a short time period would be unlikely to have any major effect on overall microbial activity. This assertion has been confirmed with recent work which has shown that respiration by soil biomass in a sandy loam did not differ markedly when kept in a moist state from that when subjected to repeated wetting and drying cycles.[23]

2.3 Glyphosate

[*Methyl*-^{14}C]glyphosate (sp. act. 1·93 MBq μmol^{-1}; radio-chemical purity 95·6%; Amersham) was diluted with unlabelled analytical grade glyphosate in deionised water to give a concentration in water of 136 μmol or 23 μg ml^{-1} and a specific activity of 273·3 kBq μmol^{-1}. This concentration was verified by ECD-GLC analysis using a previously reported procedure.[24]

2.4 Flow-through apparatus

The flow-through apparatus used in these decomposition studies has been previously reported,[25] except with the following changes. In this study a modified Erlenmeyer flask was used with a side air inlet set in the lower wall of the flask such that carbon-dioxide-free air flushed directly over the top of the soils to improve the efficiency of flushing the [^{14}C]carbon dioxide evolved from the soil. Silica gel moisture traps prepared from disposable 1-ml plastic syringe casings stoppered at either end with non-adsorbent cotton wool were filled with anhydrous moisture-indicating silica gel. The silica gel moisture traps were changed on a weekly basis for both incubation temperatures.

TABLE 1
Soil Chemical Properties

Soil[a]	*pH* (*1 : 5 H_2O*)	*Silt*	*Clay*	*Organic carbon*	*CEC*[b]	*Exchangeable*				*Fe* (*ppm*)
		($g\ kg^{-1}$)			($CEQ\ kg^{-1}$)	*Ca*	*Mg*	*K*	*Na*	
Walpeup SL	7·3	11	64	6·7	4·5	4·2	0·3	0·5	0·1	25·5
Wimmera C	8·4	145	466	11·3	45·8	41·8	5·8	1·7	0·4	1·1
Culgoa SiCL	8·4	26	168	15·6	25·7	27·8	3·3	1·2	0·1	1·9
Rutherglen L	5·3	118	214	13·1	5·9	4·6	1·2	0·4	0·1	26·0

[a] Letters following soil names denote soil texture class: C, clay; L, loam; LS, loamy sand; SiCL, silty clay loam.
[b] CEC: cation exchange capacity; centimoles of negative charge per kilogram of soil.

2.5 Metabolism of glyphosate in soil

Metabolic studies were carried out by weighing 10 g of air-dried soil into a 100-ml side-inlet Erlenmeyer incubation vessel (four replicates per soil). Deionised water was added to the soil such that when 1 ml of [^{14}C]glyphosate solution was added to the soil, the moisture content of the soil would be at 75% of field capacity. The flasks were then sealed and left at room temperature overnight in the case of the 25°C incubation study or cooled at 10°C overnight for the same period in the case of the 10°C study, to allow the soils to equilibrate in regard to moisture and temperature. After equilibration, [^{14}C]glyphosate diluted with non-radioactive material (sp. act. 273·3 kBq μmol^{-1}) in 1 ml of deionised water was added to the soil such that 37 kBq of [^{14}C]glyphosate was added per vessel; thus the final concentration of glyphosate was 2300 ng g^{-1} air-dry soil. The incubation vessels were immediately put into the incubator and connected to the flow-through apparatus.

The incubation vessels were flushed with moist, carbon-dioxide-free air flowing at 80 ml vessel^{-1} min^{-1} for 15 min automatically every 3 h. The [^{14}C]carbon dioxide evolved from the decomposing glyphosate was trapped with ethylene glycol monomethyl ether + ethanolamine (3 + 1 by volume; 12 ml) in scintillation vials covered with laboratory film. The scintillation trap vessels were removed and a toluene + ethylene glycol monomethyl ether (2 + 1 by volume; 10 ml) scintillation cocktail[26] was added. In the experiment conducted at 25°C, traps were removed from the apparatus and counts were made every 24 h for four days, and then at two- to three-day intervals for a total of 70 days. For the 10°C experiment, traps were removed and counts were made every 24 h for three days and then every three to four days for a total of 80 days.

2.6 Scintillation counting

Samples were counted for a period of 5 min or until the equivalent of 10 000 counts was recorded, whichever was the longer. The efficiency of counting was determined twice using the Compton (H number) quench curve from which disintegrations per minute (DPM) were calculated.

2.7 Data handling

Cumulative decomposition of [^{14}C]glyphosate was calculated for each soil by measuring the [^{14}C]carbon dioxide evolved from the soil over a particular time interval and adding this value to the amounts of [^{14}C]carbon dioxide previously evolved. Subtracting the cumulative value from the amount of glyphosate initially added allowed relationship between the amount of glyphosate remaining in soil and time to be established (Fig. 2a).

At the completion of the experimental period, the amounts of glyphosate remaining for each time interval were transformed logarithmically to determine if decomposition was first-order. For most soil–

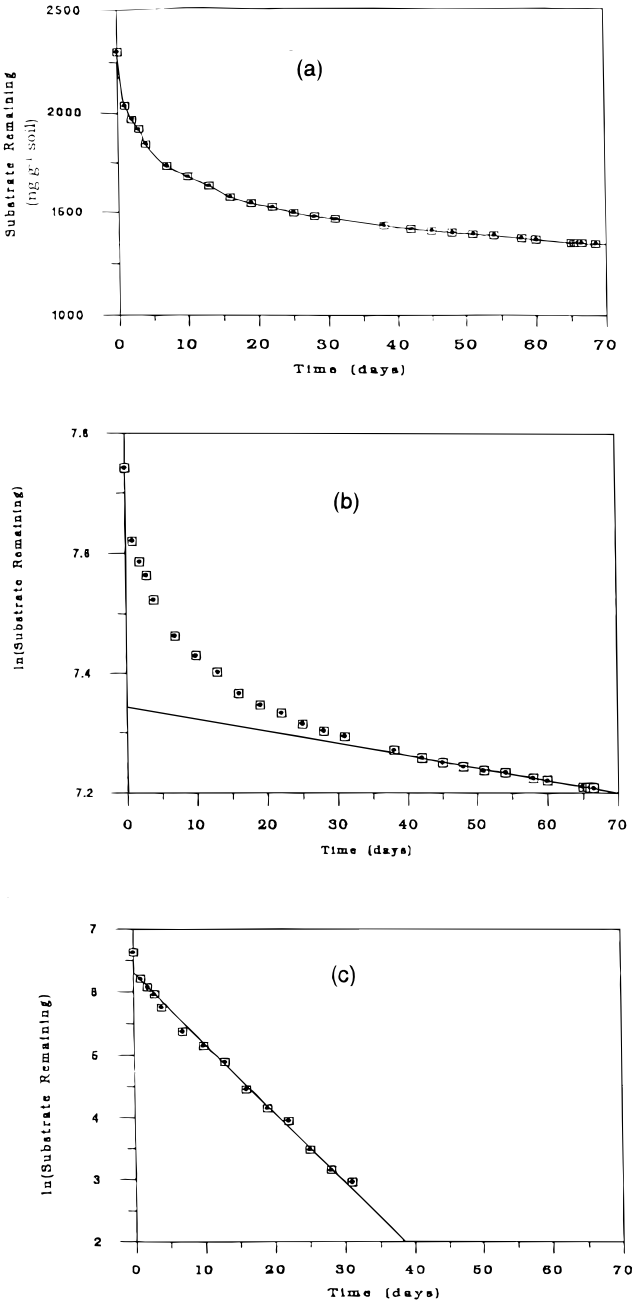

Fig. 2. Generalised glyphosate decomposition curves showing the technique of curve stripping. (a) normal glyphosate decomposition curve at 25°C (Walpeup loamy sand). (b) Log-normal transformed glyphosate decomposition curve where the final linear phase is attributed to the slowest exchanging compartment. (c) log-normal transformed glyphosate decomposition curve of all glyphosate in the system except that from the slowest exchanging phase. In (c) the linear nature of the curve suggests that further stripping is unnecessary.

temperature combinations, this transformation did not linearise the entire decomposition curve, hence analysis using non-steady-state compartmental analysis was attempted. This involved dividing the semi-logarithmic disappearance curve into its two components; the initial curvilinear phase and the final linear (steady-state) phase (Fig. 2b). The final linear phase was assumed to represent mineralisation of glyphosate derived from the most slowly available source or compartment. The regression of this relationship yields k, which describes the rate of release of glyphosate derived from this source, while the y-intercept (C_0) estimates the amount of glyphosate initially partitioned into this phase. Assuming the sorption of glyphosate, when applied to soil, occurs rapidly, and that the desorption of glyphosate from this phase commences immediately following adsorption, then the influence of glyphosate derived from the non-labile phase can be separated numerically from the glyphosate partitioned in the labile phase. This involves using the regression describing glyphosate desorption from the non-labile phase to predict the amount of glyphosate desorbed for each time interval during the curvilinear phase. These predicted values are converted to their real number form, and subtracted (stripped) from the original curve. The resultant values represent the concentration of glyphosate eluted from all other phases which exchange glyphosate at a rate faster than the rate of desorption from that non-labile phase at that point in time. These values are then transformed logarithmically, and if the plot of the transformed data over time is linear, no further stripping is required. However, if the plot is curvilinear, further peeling is repeated. In this study, only one strip of the data set was required (Fig. 2b, 2c).

2.8 Statistical analysis

All non-transformed and natural logarithm transformed data were tested for homogeneity of variance using Bartlett's test,[27] and in both cases were found to be homogeneous. Logarithmic transformation was used to linearise the shape of the decomposition curves so that the first-order kinetics of the identified phases could be determined. The slopes of the regression lines were compared following the procedure for two independent slopes as described in Howell.[28]

3 RESULTS AND DISCUSSION

In this paper, it is assumed that measured evolution of [^{14}C]carbon dioxide reflects the decomposition of [^{14}C]glyphosate. This assumption is made following on from previous work of Eberbach[29] which showed that the loss of extractable glyphosate (triethylamine soluble) at 25°C occurred at the same rate as did the evolution of [^{14}C]carbon dioxide as shown here. This suggests that, once a molecule of glyphosate begins decomposition, its catabolism is rapid and complete. In addition, other work showed that aminomethylphosphonic acid (AMPA) is only a transitory intermediate of glyphosate metabolism,[29,30] supporting the suggestion of rapid catabolism of glyphosate. This work will be discussed further in a subsequent publication.[31]

3.1 Metabolism at 25°C

Decomposition of [^{14}C]glyphosate under aerobic conditions in the four soils tested varied as shown in Fig. 3. For each soil, rapid decomposition of the herbicide occurred during the first day. This was followed by a gradual reduction in the rate of decomposition occurring over the next 30–40 days until an apparent steady rate of decomposition was achieved, which continued until the termination of the experiment on day 70. The pattern of decomposition reported here is similar to patterns observed in other reports.[3,5–9,11] However, the establishment of steady-state took considerably longer in this study than has been observed by others.[3,5,6,8,11]

Transforming logarithmically the cumulative amount of glyphosate decomposed as a function of time did not linearise the decomposition curve for any of the soils (Fig. 4). This suggested that decomposition at this temperature did not obey first-order reaction kinetics, and was consistent with observations of others regarding degradation of trifluralin, prometryne, carbaryl and metribuzin.[12,13] However, this transformation improved the linearity of the steady-state portion of the curve for all soils as evidenced by a marked improvement in the R^2 of the regression.

Other field and laboratory-based studies investigating the disappearance of glyphosate and other herbicides in soil have shown similar onset of steady-state conditions.[2,3,8,12,32] Hence we assume here that the onset of steady-state is not an artefact of incubation conditions in the present study but is likely instead to reflect the influence of certain soil factors, particularly adsorption, which restrict over time the availability of glyphosate for decomposition.

Using the regression of the steady-state section of the curve, the influence of the steady-state condition was stripped from the initial portion of the curve using non-steady-state compartmental analysis. For all soils, a plot of a logarithmic transformation of the data points remaining after NSSCA was linear with respect to time (Fig. 5). The data points remaining after the curve strip corresponded to decomposition of glyphosate derived from a more available source, assumed here to represent the labile phase. The linearity of this function suggested also that decomposition of glyphosate from this phase obeyed first-order reaction kinetics.

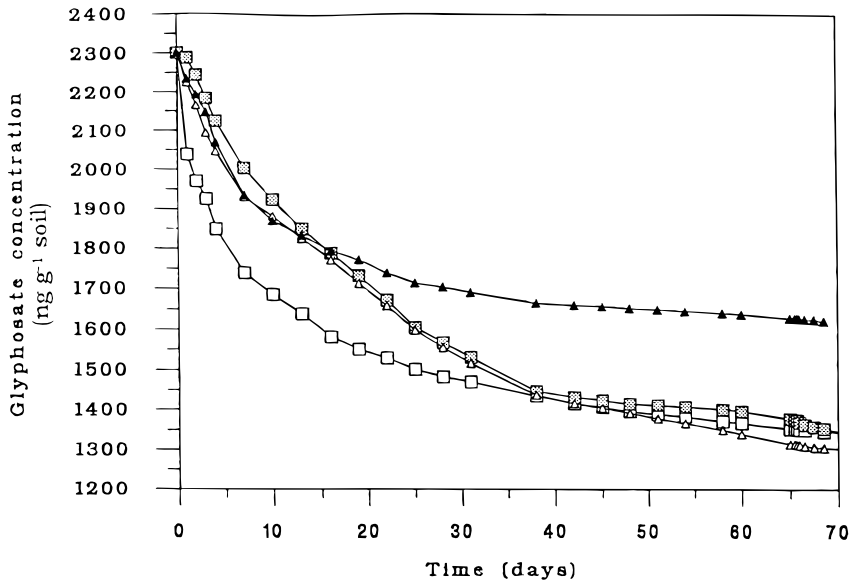

Fig. 3. Changes in the concentration of glyphosate in four Victorian soils incubated at 25°C for 70 days. (□) Walpeup, (▨) Wimmera, (△) Culgoa, (▲) Rutherglen.

Regression of the functions representing the steady-state and the assumed labile phase for each soil were in each case highly significant ($P < 0{\cdot}0001$; Table 2).

For each soil, the slope of the function describing the decay of glyphosate derived from the labile and non-labile phases were compared and found to be significantly different ($P < 0{\cdot}01$). This further confirmed the suggestion that decomposing glyphosate was derived from two different phases, and is numerically consistent with the concept of the two-compartment model proposed by Hamaker and Goring.[1] Therefore, in this paper, we postulate that the glyphosate disappearance curve is composed in the first instance of decomposing glyphosate derived from two phases simultaneously; soluble glyphosate (labile) and glyphosate desorbed into the soluble pool from the sorbed (non-labile) phase. However, in the later portion of the curve, the readily available glyphosate has decayed, such that decomposing glyphosate now is derived only from the non-labile phase. The linearity of the labile and non-labile glyphosate decomposition curves (Figs 4 & 5 respectively), as separated using NSSCA, mathematically supports this suggestion. Hence from these curves, better understanding of glyphosate decomposition and *in-situ* desorption in relation to certain soil factors and conditions can be made.

Using regression analysis, the kinetics of glyphosate decomposition derived from each phase for each of the soils was determined (Table 2). The amount of glyphosate partitioned into either phase for each soil was

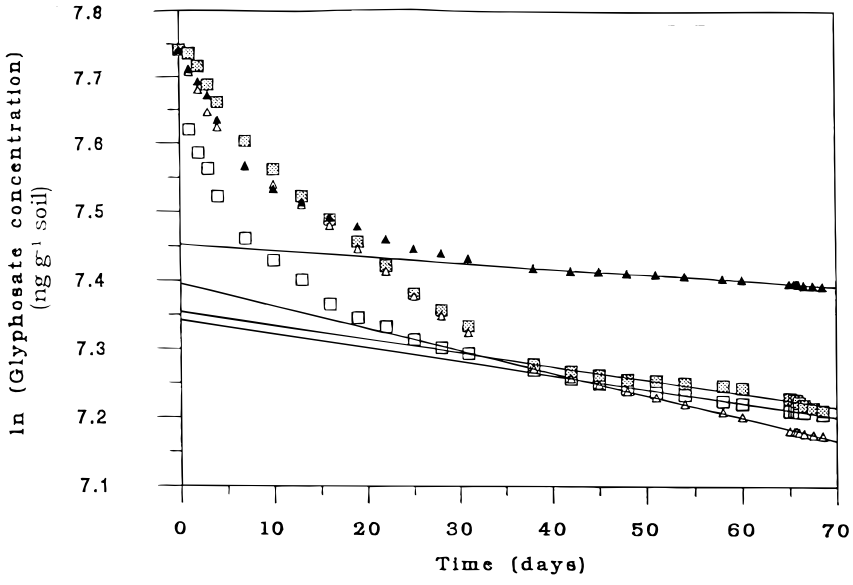

Fig. 4. Changes in the log-normal transformation of concentration of glyphosate in four Victorian soils incubated at 25°C for 70 days. Lines of best fit were fitted using simple linear regression analysis. (□) Walpeup, (▨) Wimmera, (△) Culgoa, (▲) Rutherglen.

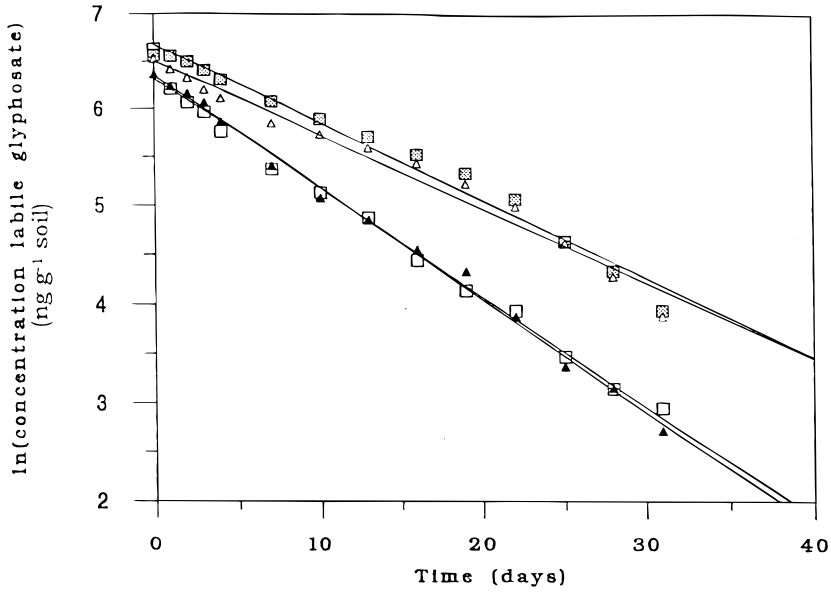

Fig. 5. Log-normal relationship of concentration of labile glyphosate as a function of time, incubated at 25°C. Lines of best fit were fitted using simple linear regression analysis. (□) Walpeup, (▨) Wimmera, (△) Culgoa, (▲) Rutherglen.

derived by solving the regression for time zero, obtaining C_0. The partitioning of glyphosate between the labile and non-labile phases in each of the soils examined was similar, with 25–35% of the material decomposed coming from the labile phase and the remaining material coming from the non-labile phase for the Wimmera, Culgoa and Rutherglen soils. The preference of glyphosate for the non-labile phase is consistent with the findings of others.[3,8] The prediction of the size of the labile phase of the Walpeup soil was thought to be underestimated as the flux of carbon dioxide within the first 24 h was very rapid (Fig. 3) and was sufficiently large to bias the regression analysis.

The half-lives of glyphosate from each phase were calculated from the rate constants for decomposition of glyphosate derived from each compartment and were within the same order of magnitude for each of the soils examined. Half-lives for glyphosate in the labile phase were quite similar; between six and nine days for the four soils studied. Conversely, the half-lives for decomposition of non-labile glyphosate varied widely (Table 2). This suggested that different strengths of binding existed within the non-labile phase for each of the four soils investigated. Assuming that estimates of half-lives reflect binding strength, then the strength of binding of non-labile glyphosate decreased according to the following order: Rutherglen loam > Wimmera clay > Walpeup loamy sand > Culgoa silty clay loam.

Previously, studies have shown that sorption of glyphosate is influenced by numerous soil factors, most

TABLE 2
Regression Equations, Adjusted R^2, Rate Constants, Calculated Pool Size and Half-Lives of the Decomposition of Glyphosate Derived from Two Phases in Four Victorian Soils at 25°C

Soil type	*Phase*	*Regression equation*	R^2 (*Adj*)	*Rate constant ng day*$^{-1}$	*Pool size*[a] (%)	*Half-life* (*days*)
Walpeup	Labile	$\ln y = 6{\cdot}32 - 0{\cdot}112x^b$	0·991***[c]	−0·111	24·1	6
	Non-labile	$\ln y = 7{\cdot}34 - 0{\cdot}0021x$	0·994***	−0·0021	67·2	338
Wimmera	Labile	$\ln y = 6{\cdot}68 - 0{\cdot}080x$	0·980***	− 0·0801	34·5	9
	Non-labile	$\ln y = 7{\cdot}34 - 0{\cdot}0016x$	0·973***	−0·0016	66·8	432
Culgoa	Labile	$\ln y = 6{\cdot}50 - 0{\cdot}076x$	0·976***	−0·076	29·1	9
	Non-labile	$\ln y = 7{\cdot}39 - 0{\cdot}0031x$	0·996***	−0·0031	70·3	222
Rutherglen	Labile	$\ln y = 6{\cdot}35 - 0{\cdot}115x$	0·995***	−0·115	24·9	6
	Non-labile	$\ln y = 7{\cdot}45 - 0{\cdot}0008x$	0·998***	−0·0008	74·9	835

[a] Pool size (amount partitioned into each phase) expressed as a percentage of the amount of glyphosate originally applied.
[b] Equation in the form of $\ln y = a + bx$ where y = the loss of glyphosate from that particular phase; a = intercept (or pool size); b = gradient of the regression (rate constant); and x = time (days).
[c] *** Indicates significant at a probability of less than 0.1%.

particularly soil pH,[33,34] and levels of exchangeable Fe.[35] It also shows some correlation with the amount of clay and of montmorillonite.[33–36]

Glyphosate's particular sensitivity to soil pH may be due to the zwitterionic nature of this compound. Sprankle *et al.*[37] showed that, as soil pH decreased, glyphosate became progressively less negatively charged. Hence, as glyphosate dissociates from the divalent to the monovalent anionic form at a pH of 5·6 (pKa 5·6), then a substantial proportion of glyphosate in the Rutherglen soil (pH 5·2) is likely to be in the monovalent form, while in the divalent anionic form in the other three alkaline soils. We postulate that the differences in the charge on the molecule as a result of soil pH may influence the manner in which the compound is adsorbed or the strength of adsorption in acid relative to neutral-alkaline soils. Recent work with imazethapyr agrees with this suggestion. Imazethapyr is an anion at soil pH values between 5 and 9 but protonated and hence non-charged at pH values below 5.[38] The extent of adsorption of this molecule reflects its charge, such that, in soils with a pH of less than 5, considerably more imazethapyr is adsorbed relative to higher pH soils and mineral solids.[39,40]

In the present study, degradation of labile glyphosate in the Walpeup and Rutherglen soils, and in the Wimmera and Culgoa soils appeared similar (Fig. 5). A statistical comparison of the rates of degradation of these pairs of soils confirmed this observation ($P < 0{\cdot}05$). However, when data for each pair were combined, the two pairs were shown to be significantly different ($P < 0{\cdot}05$). To assist in explaining this phenomenon, previous studies have shown that high concentrations of exchangeable Fe in soil significantly reduce the phytotoxicity of acropetal imbibed glyphosate.[35,37] We suspect that, in the present study, the higher concentrations of exchangeable Fe in the Walpeup and Rutherglen soils (25·5 and 26 mg kg^{-1} respectively) may have influenced the rate of decomposition of labile glyphosate in these soils relative to the Wimmera and Culgoa soil (1·1 and 1·9 mg kg^{-1} respectively). The mechanism by which this occurs is as yet unclear.

3.2 Metabolism at 10°C

Aerobic decomposition of [^{14}C]glyphosate at 10°C for the four soils examined varied as shown in Fig. 6. Similar to decomposition of glyphosate at 25°C, degradation of the compound in each soil was more rapid on the first day than over the ensuing incubation period. The rate of decomposition of glyphosate in the Rutherglen and Walpeup soils decreased gradually over the first 20 to 40 days respectively until a steady-state of decomposition was achieved. The rate of decomposition of glyphosate in the Wimmera and Culgoa soils was, however, constant over the entire experimental period.

3.2.1 Metabolism in the Walpeup and Rutherglen soils

As at 25°C, NSSCA was used to determine the partitioning of glyphosate into the two phases and to determine the kinetics of glyphosate decomposition in the two phases (Figs 7 and 8; Table 3). For both soils, regression analyses of the curve products were highly significant ($P < 0{\cdot}001$). It is interesting to note that, for the two soils, at this temperature less glyphosate was partitioned into the soluble phase, while more was adsorbed (Table 3). This suggests that glyphosate sorption is an exothermic reaction, and is thermodynamically consistent with sorption behaviour of many other herbicides.[41] However, in this study, the rate of decay for soluble glyphosate increased slightly at this tem-

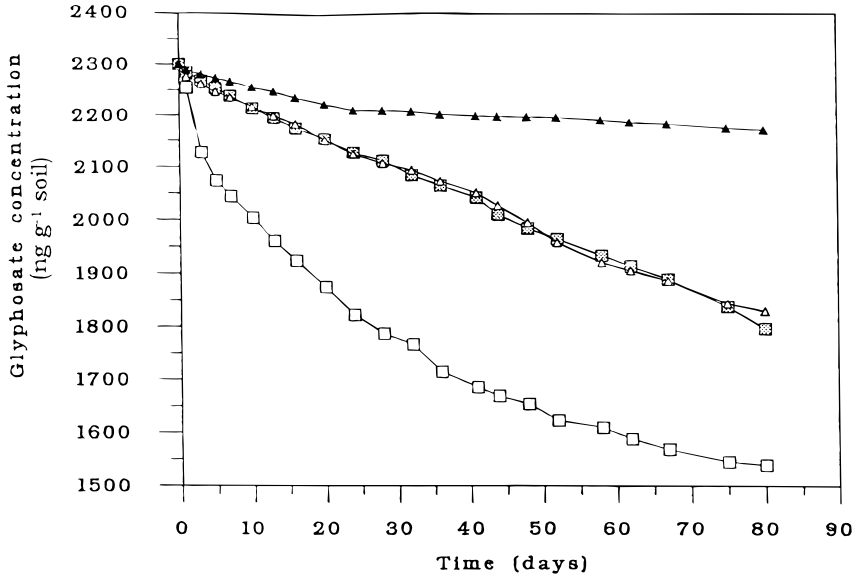

Fig. 6. Changes in the concentration of glyphosate in four Victorian soils incubated at 10°C for 80 days. (□) Walpeup, (▨) Wimmera, (△) Culgoa, (▲) Rutherglen.

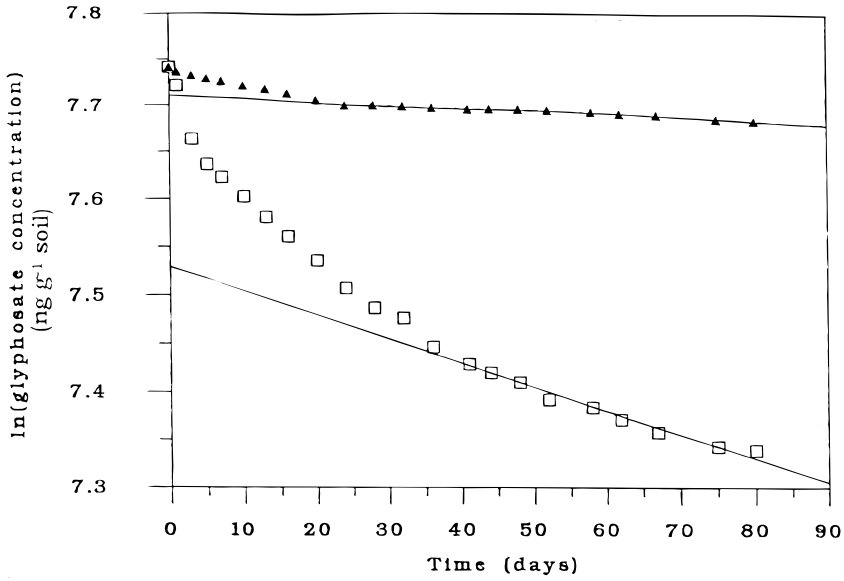

Fig. 7. Changes in the log-normal transformation of the concentration of glyphosate in two Victorian soils incubated at 10°C for 80 days. Lines of best fit were fitted using simple linear regression analysis. (□) Walpeup, (▲) Rutherglen.

perature for both soils, while the decay rate constant for sorbed glyphosate decreased for the Walpeup soil and increased for the Rutherglen soil (significant at $P < 0{\cdot}05$). Since the rates of decay were higher at the beginning of the incubation and lower later in both soils, it can only be assumed that the temperature was also affecting the rate of desorption and not simply co-metabolic activity, suggesting that the rate of desorption and perhaps strength of binding are temperature-dependent. Scheuner,[41] in a review of sorption and desorption processes, suggested that sorption may be the sum of numerous physical and chemical binding processes acting on a particular sorbate in a particular soil, and that temperature may affect these different binding processes differently. Given also the zwitterionic nature of glyphosate,[37] this compound is predominantly in the divalent anionic form in the alkaline Walpeup soil, while in the acidic Rutherglen soil, it is present as a monovalent anion. Hence, it is likely that the apparent inconsistency in the present study in relation to the effect of temperature of sorption may be due to different suites of binding processes being responsible for adsorbing glyphosate in the two soils. Similar observations have been made of the sorptive behaviour of imidazolinone herbicides in alkaline relative to acid soils, which support this notion.[38]

3.2.2 *Metabolism in Wimmera and Culgoa soils*

While loss of glyphosate from Wimmera and Culgoa soils appeared to continue steadily over the incubation

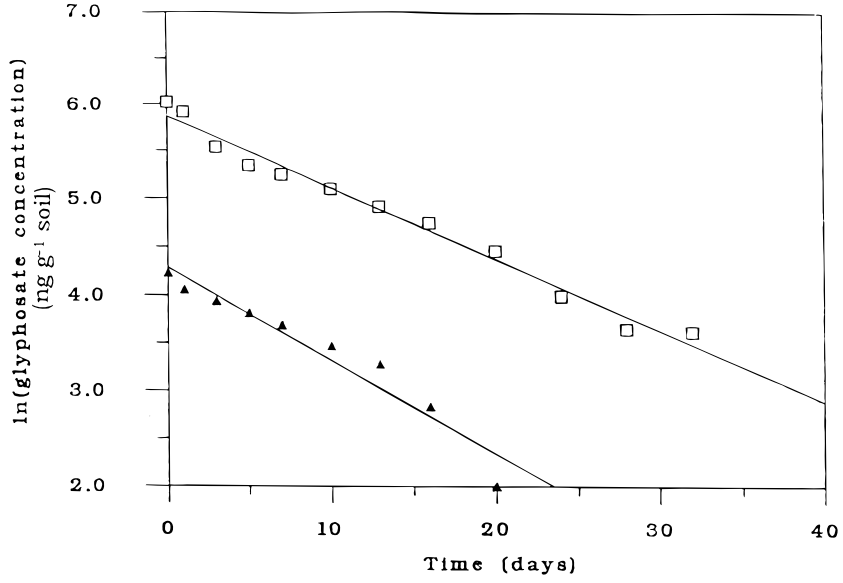

Fig. 8. Log-normal relationship of concentration of labile glyphosate as a function of time, incubated at 10°C. Lines of best fit were fitted using simple linear regression analysis. (□) Walpeup, (▲) Rutherglen.

TABLE 3
Regression Equations, Adjusted R^2, Rate Constants, Calculated Pool Size and Half-Lives of the Decomposition of Glyphosate Derived from Two Phases in Four Victorian Soils at 10°C

Soil type	*Phase*	*Regression equation*	R^2 (*Adj*)	*Rate constant ng day*$^{-1}$	*Pool size*[a] (%)	*Half-life* (*days*)
Walpeup	Labile	$\ln y = 5{\cdot}87 - 0{\cdot}0695x$[b]	0·981***[c]	−0·0695	15·4	10
	Non-labile	$\ln y = 7{\cdot}53 - 0{\cdot}0025x$	0·990***	−0·0025	80·9	280
Wimmera		$\ln y = 7{\cdot}74 - 0{\cdot}0029x$	0·998***	−0·0029	99·8	238
Culgoa		$\ln y = 7{\cdot}73 - 0{\cdot}00284x$	0·991***	−0·00284	99·0	244
Rutherglen	Labile	$\ln y = 4{\cdot}29 - 0{\cdot}0977x$	0·938***	−0·0977	3·2	7
	Non-labile	$\ln y = 7{\cdot}71 - 0{\cdot}0003x$	0·998***	−0·0003	97·0	2260

[a] Pool size (amount partitioned into each phase) expressed as a percentage of the amount of glyphosate originally applied.
[b] Equation in the form of $\ln y = a + bx$ where y = the loss of glyphosate from that particular phase; a = intercept (or pool size); b = gradient of the regression (rate constant); and x = time (days).
[c] *** Indicates significant at a probability of less than 0.1%.

period (Fig. 6), transforming this data logarithmically improved the linearity of the relationship (Fig. 9). Therefore the decomposition of glyphosate in these soils appeared consistent with first-order kinetics. The predicted rate constants and half-lives of glyphosate decomposition from these soils are given in Table 3. The linear rate of loss of glyphosate from these soils at 10°C in contrast to its two-phase nature at 25°C suggested that glyphosate may be derived from only one phase at 10°C. This, however, seems unlikely. It is more likely that, in these soils, glyphosate is derived simultaneously from the soluble and adsorbed phases, but at similar rates, such that the two phases are indistinguishable using NSSCA. Data from the Walpeup soil support this notion. Desorption from the alkaline Walpeup soil increased as temperature decreased. Hence, if the binding mechanisms in these three alkaline soils are similar, then it is possible that, as in the Walpeup soil, as temperature decreases, the rate of desorption in the Wimmera and Culgoa soils increases. But in these soils, it increases so substantially that desorption now occurs

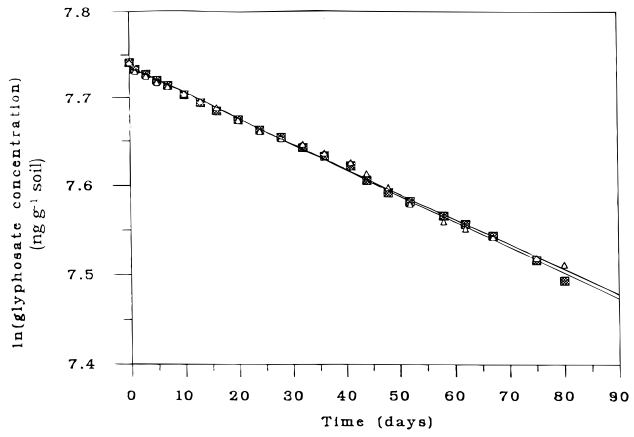

Fig. 9. Changes in the log-normal transformation of the concentration of glyphosate in two Victorian soils incubated at 10°C for 80 days. Lines of best fit were fitted using simple linear regression analysis. (▨) Wimmera, (△) Culgoa.

at a rate faster than co-metabolic decomposition. Evidence to support this assertion will be reported in a further publication.[31] Similar observations to these have been reported by Zimdahl and Gwynn,[2] who showed that, at 30°C, trifluralin degradation followed the two-stage pattern of decomposition, but at 15°C the pattern of decomposition of the herbicide followed first-order kinetics. They suggested that two rate processes may be operative in the decomposition of trifluralin, but that at 15°C the effect of these was either absent or masked due to the effect of low temperature on degradative processes.

Our observations from the present study suggest that, in alkaline soils, glyphosate is more extensively adsorbed at lower temperatures, but the strength of binding is weaker than at higher temperatures. Similarly sorption of phosphate by soils has shown some degree of temperature dependence[42,43] where desorption decreases as temperature increases.[43] Hence, sorption of glyphosate in alkaline soils and sorption of phosphate may behave similarly. Conversely, the effect of decreasing incubation temperature on the acidic Rutherglen soil was to reduce the rate of release of glyphosate from both sources, particularly from the sorbed phase. While it is possible that the response of this soil was simply due to a property peculiar to this soil, it is also possible that this phenomenon could have been due to the acidic nature of the soil which caused glyphosate to be bound more strongly than in the alkaline soils. The rate of release of sorbed glyphosate and the effect of incubation temperature on this reaction will be discussed further in a subsequent publication.

4 CONCLUSION

The present study using NSSCA showed numerically that the kinetics of glyphosate decay in soils is first-order, but that the availability of glyphosate for decom-

position was affected by the rate of desorption. In addition, this technique showed that temperature plays a vital role in influencing the behaviour of glyphosate, affecting the partitioning of glyphosate into soluble and sorbed forms, the rate of desorption and the rate of co-metabolic decay.

The findings of this work may have commercial implications. Our results suggest that glyphosate is less strongly bound in some alkaline soils at lower temperatures. It could be speculated that in these soils, the onset of cool or cold conditions may enhance glyphosate desorption, such that sufficient concentrations may be present in the soluble phase to damage susceptible plant species. However in acid soils under the same environmental conditions, desorption is likely to have decreased relative to warmer conditions. Hence under these conditions, it would be extremely unlikely that sufficient glyphosate would be available in the solution form to damage even the most sensitive plant species.

We are currently undertaking further research to investigate the effect of soil pH, Fe mineral content, temperature, and competing ion species on glyphosate sorption and its availability for acropetal uptake.

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