Toxicology Letters 190 (2009) 91–95

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Toxicokinetics of glyphosate and its metabolite aminomethyl phosphonic acid in rats

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ARTICLE INFO

Article history:
Received 6 May 2009
Received in revised form 2 July 2009
Accepted 6 July 2009
Available online 14 July 2009

Keywords:
Glyphosate
AMPA
Plasma disposition
Rats

ABSTRACT

The toxicokinetics of glyphosate after single 100 mg kg^{-1} intravenous (i.v.) and 400 mg kg^{-1} oral doses were studied in rats. Serial blood samples were obtained after i.v. and oral administration. Plasma concentrations of glyphosate and its metabolite amiomethyl phosphonic acid (AMPA) were determined by HPLC method. After i.v. and oral administration, plasma concentration–time curves were best described by a two-compartment open model. For glyphosate, the elimination half-lives ($T_{1/2\beta}$) from plasma were 9.99 h after i.v. and 14.38 h after oral administration. The total plasma clearance was not influenced by dose concentration or route and reached a value of 0.995 l h^{-1} kg^{-1}. After i.v. administration, the apparent volume of distribution in the second compartment (V_2) and volume of distribution at steady state (V_{ss}) were 2.39 and 2.99 l kg^{-1}, respectively, suggesting a considerable diffusion of the herbicide into tissues. After oral administration, glyphosate was partially and slowly absorbed with a T_{max} of 5.16 h. The oral bioavailability of glyphosate was found to be 23.21%. Glyphosate was converted to AMPA. The metabolite AMPA represented 6.49% of the parent drug plasma concentrations. The maximum plasma concentrations of glyphosate and AMPA were 4.62 and 0.416 μg ml^{-1}, respectively. The maximum plasma concentration of AMPA was achieved at 2.42 h. For AMPA, the elimination half-life ($T_{1/2\beta}$) was 15.08 h after oral administration of glyphosate parent compound.

1. Introduction

Glyphosate [N-(phosphonomethyl)glycine] is a widely used non-selective post-emergence herbicide (Fig. 1). It has been marketed since 1974 and its use is likely to increase further as it is one of the first herbicides against which crops have been genetically modified to increase their tolerance; glyphosate has been used for the control of the illicit crops coca and poppy (Solomon et al., 2007). Glyphosate inhibits plant growth through interference with the production of essential aromatic amino acids. Glyphosate is primarily a competitive inhibitor of the critical enzyme of the shikimate pathway, 5-enolpyruvylshikimate-3-phosphate synthase (Steinrücken and Amrhein, 1980), which is responsible for the synthesis of an intermediate in the biosynthesis of phenylalanine, tyrosine and tryptophan (Mousdale and Coggins, 1984; Rubin et al., 1984; Malik et al., 1989). The resulting reduction in protein synthesis causes termination of growth and eventually, cellular disruption and death. The effectiveness of glyphosate as a phytotoxin is due in part to its low molecular weight and high solubility in water, which aid its rapid absorption and translocation by plant tissues.

Since glyphosate competitively inhibits 5-enolpyruvylshikimate-3-phosphate synthase, an enzyme which is absent in animals, it is selectively toxic to plants and relatively non-toxic to mammals (acute oral rat LD_{50} ~5.6 g kg^{-1}, Street et al., 1979; Monsanto, 1989). Based upon animal studies, some investigators suggest that glyphosate may enhance adenosine triphosphatase activity and uncouple mitochondrial oxidative phosphorylation (Bababunmi et al., 1979; Olorunsogo et al., 1979; Olorunsogo and Bababunmi, 1980; Olorunsogo, 1982) although this has been disputed by Tominack (1999). Glyphosate is a phosphorous-containing compound, at high concentrations *in vitro*, it has been shown to inhibit acetylcholinesterase (El Demerdash et al., 2001), although there is no evidence for significant acetylcholinesterase inhibition in mammals *in vivo*. Cutaneous exposure to a glyphosate-containing herbicide has been postulated as contributing to Parkinsonism (Barbosa et al., 2001). The authors proposed that glyphosate may have contributed to the neurological pathology by virtue of its chemical similarity with glycine, a co-factor required for activation of the N-methyl-D-aspartase (NMDA) receptor, which controls excitatory actions in the central nervous system and is also involved

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doi:10.1016/j.toxlet.2009.07.008

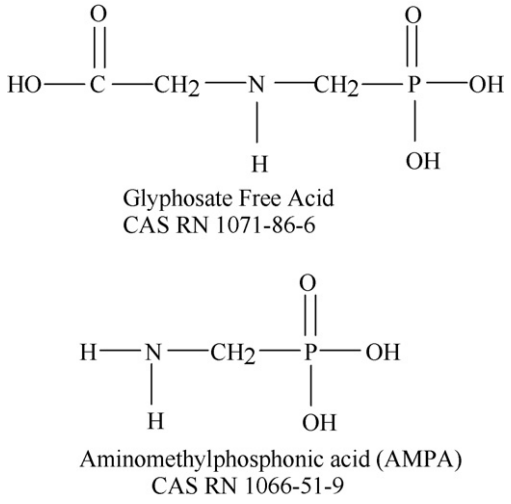

Fig. 1. Chemical structures of glyphosate and its metabolite AMPA.

in memory and learning. However, glyphosate doses not possess NMDA activity clinically (Bradberry et al., 2004). Exposure to pesticides has been suggested to increase the risk of Parkinson's disease, but the mechanisms responsible for this association are not clear. Our preliminary results in rats treated orally with glyphosate are consistent with a loss of dopamine to a level about 50% below that of controls in frontal cortex, hippocampus, hypothalamus and striatum tissues (Anadón et al., 2008). Loss of dopamine is a cardinal sign of Parkinson's disease (Hornykiewicz and Kish, 1987).

Glyphosate is predominantly degraded in the environment by microorganisms (Sprankle et al., 1975; Rueppel et al., 1977; Mueller et al., 1981) and through some limited metabolism in plants and mammals (Ghassemi et al., 1982; Newton et al., 1984; Brewster et al., 1991). Glyphosate is metabolized by several bacteria in soil to give sarcosine which is then converted to glycine and ammonia by sarcosine oxidase. The alternative metabolic pathway involves the formation by glyphosate oxidoreductase of aminomethyl phosphonic acid (AMPA) (Fig. 1), which is also a minor metabolite detected in colon tissue in rats (Brewster et al., 1991). Glyphosate ultimately breaks down to innocuous natural substances such as carbon dioxide and phosphonic acid.

The existing knowledge of the toxicokinetics of glyphosate is limited and mainly derivated from one animal study performed primarily to assess the distribution and nature of glyphosate-derived radioactivity in tissues following a 10 mg kg^{-1} dose (Brewster et al., 1991). Other previous toxicokinetic studies of glyphosate in rats have been conducted by researchers from Monsanto Company (Colvin and Miller, 1973; Ridley and Mirley, 1988; Howe et al., 1988) and by National Toxicology Program (NTP, 1992). Dates from these unpublished investigations have been reviewed by Williams et al. (2000). To date, no others toxicokinetic studies have been reported. Because the information regarding kinetic profile improves the scientific basis for risk decisions, the objective of this research was to examine the oral bioavailability and disposition of glyphosate in rats.

2. Materials and methods

2.1. Chemicals

Glyphosate [N-(phosphonomethyl)glycine]; molecular formula $C_3H_8NO_5P$ CAS RN 107-83-6, purity 95% (w/w), AMPA, molecular formula CH_6NO_3P CAS RN 1066-51-9, purity 99% (w/w), and 9-fluorenylmethylchloroformate (FMOC-Cl) were purchased from SIGMA CHEMICAL CO., St Louis, MO, USA. All other chemicals were of the highest quality grade and obtained from commercial sources.

2.2. Animals and experimental design

The study was undertaken in accordance with the ethics requirements and authorized by the official ethical committee of our university. Adult male Wistar rats (Charles River Inc., Margate, Kent, UK) each weighing 200–210 g were used. The animals were individually housed in polycarbonate cages with sawdust bedding and were maintained in environmentally controlled rooms (22 ± 2 °C and 50 ± 10% relative humidity) with a 12 h light/dark cycle (light from 08.00 to 20.00 h). Food (A04 rodent diet, Panlab SL) and water were available ad libitum. The rats were divided into two groups of 80 animals each, one group (Group 1) received glyphosate orally and the other group (Group 2) intravenously. Group 1 rats were deprived of food for 12 h before the single oral administration of 400 mg kg^{-1} body weight but were allowed water ad libitum. Glyphosate was administrated by gavage in a volume of 0.5 ml corn oil/rat. Group 2 rats were given a single i.v. injection of 100 mg kg^{-1} body weight into the lateral tail vein (in 0.1 ml glycerol formal/rat; Sanderson, 1959). Doses of glyphosate were selected based on preliminary investigations that indicated these doses make sure that there was sufficient compound in the plasma samples to be above the level of limit of quantification (LOQ) of the analytical method. All animals were killed by cervical dislocation (eight animals at each time) and then exanguination at 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 h after oral and i.v. administration of glyphosate. Blood samples were withdrawn and collected in heparinized tubes. Plasma was separated by centrifugation and stored frozen at −80 °C until analyzed.

2.3. Assay of glyphosate and AMPA

Glyphosate and its metabolite AMPA concentrations in plasma were measured by high-performance liquid chromatography. The plasma samples were subjected to derivatization using FMOC-Cl as derivatizing agent. Briefly, frozen each plasma sample was thawed at room temperature and then 100 μl of plasma was mixed with 100 μl of acetonitrile, vortex mixed for 1 min and centrifuged at 10,000 × *g* for 30 min in a refrigerated laboratory centrifuge (RC-5B, Sorvall, Newton, CT). After centrifuging, 100 μl of clear supernatant was transferred to clean glass tube and was then derivatized by adding 100 μl of 1.25 mM borate buffer (pH 9) followed by 100 μl of 10 mM FMOC-Cl reagent and allowing the reaction to take place for 30 min at 25 °C temperature. Derivatized sample was filtered through a 0.45 μm syringe filter and finally an aliquot was directly injected into the HPLC system.

Plasma concentrations of glyphosate and its metabolite AMPA were measured in our laboratory using an Agilent Technologies liquid chromatograph system (Palo Alto, CA, USA), Mod. 1100 series LC-FD system consisting of vacuum degasser, a quaternary solvent pump, an autosampler with a column oven and fluorescence detector and a computer equipped with HP Chemstation software. The HPLC separation was performed using a 4 μm particle size Synergi MAX-RP column (4.6 mm i.d. × 250 mm) protected by Synergic Max-RP security guard cartridge (3 mm i.d. × 4 mm) (Phenomenex) with a volume of injection of 20 μl. The mobile phase was 20 mM ammonium formate (A) and acetonitrile (B). A gradient solvent programme with following conditions was applied: (i) 0–5 min (A–B, 85:15, v/v); (ii) 5–10 min (A–B, 80:20, v/v); (iii) 10–13 min (A–B, 78:22, v/v); (iv) 13–17 min (A–B, 70:30, v/v); (v) 17–20 min (A–B, 50:50, v/v); (vi) 20–25 min (A–B, 0:100, v/v); (vii) 25–38 min (A–B, 85:15, v/v). Flow rate was performed at 1.0 ml min^{-1}. For glyphosate, the fluorescence detection was performed at an excitation wavelength of 240 nm and an emission wavelength of 320 nm. For AMPA, the fluorescence detection was performed at an excitation wavelength of 250 nm and an emission wavelength of 620 nm. Peak areas in the sample chromatograms were quantitated by external standard technique using solutions of glyphosate and AMPA reference standards. The system worked at 45 °C temperature. Results for the method are linear over the calibration range of 0.025–20.00 μg ml^{-1} as determined by use of the linear least squares regression procedure. The method linearity correlation coefficient was 0.999 for derivatized glyphosate and 0.995 for derivatized AMPA, in concentrations ranging from 0.025 to 20.00 μg ml^{-1}. Using blank plasma fortified at 0.05, 0.3 and 1 μg ml^{-1}, glyphosate and AMPA recoveries ranged from 76% to 86% and 67% to 75% (n = 6), respectively. Within-day and day-to-day precision were <5.5% for glyphosate and for AMPA. Interference of endogenous compounds was verified on blank plasma from untreated rats which provided the specificity of the method. The LOQ of glyphosate and AMPA was 0.025 μg ml^{-1} (calculated as the lowest fortification level where analytes were detected with a signal-to-noise ratio >3). The quantification of glyphosate and AMPA could be well done with this method; therefore the use of the internal standard technique was not necessary.

2.4. Data analysis

The mean plasma concentration versus time data were sequentially fitted to 1-, 2- and multiple-compartment models, using the computer program WinNonlin (Version 5.0.1; Pharsight Corporation, Mountain View, CA, USA). The model was determined for best fit on the basis of a smaller value for the Akaike's Information Criterion (Yamaoka et al., 1978). The 2-compartment model was the best fit for the two animal groups. This model was used to establish toxicokinetic characteristics. Plasma curves of glyphosate after a single i.v. and oral administration and those of AMPA (the main metabolite in plasma) after a single oral administration of

glyphosate were fitted to the following exponential equations:

$$C = A_1\,e^{-\alpha t} + A_2\,e^{-\beta t}\ \text{(i.v.)}$$

$$C = A_1\,e^{-\alpha t} + A_2\,e^{-\beta t} - A_3\,e^{-K_a t}\ \text{(oral)}$$

where C is the plasma concentration of drug; A_1, A_2 and A_3 are mathematical coefficients (i.e. A_1 and A_2 are the plasma concentrations extrapolated to time zero of the first and second elimination phases of drug and A_3 for the absorption phase); α is the hybrid rate constant for the distribution phase; β is the hybrid rate constant for the elimination terminal phase (i.e. α and β are the slopes of the first and second elimination phases of the drug disposition); and K_a the first-order absorption rate constant and t is the time. Absorption half-life ($T_{1/2a}$), half-life of α phase ($T_{1/2\alpha}$), half-life of β phase ($T_{1/2\beta}$), distribution rate constants for transfer of the drug from the central to the peripheral compartment (K_{12}) and from the peripheral to the central compartment (K_{21}), and the elimination rate constant (K_{10}) were calculated by use of standard equations as described (Wagner, 1975, 1976). After i.v. and oral administration, the area under the concentration–time curves (AUC) was calculated as follows:

$$\text{AUC} = \left(\frac{A_1}{\alpha}\right) + \left(\frac{A_2}{\beta}\right);\ \text{or}$$

$$\text{AUC} = \left(\frac{A_1}{\alpha}\right) + \left(\frac{A_2}{\beta}\right) - \left(\frac{A_3}{K_a}\right)$$

Total plasma clearance (CL) was calculated, using the following formula:

$$\text{CL} = \frac{\text{dose kg}^{-1}}{\text{AUC}};\ \text{or}$$

$$\text{CL} = \frac{(\text{dose kg}^{-1})(F)}{\text{AUC}}$$

where bioavailability (F) is $(\text{dose}_{iv} \times \text{AUC}_{oral})/(\text{dose}_{oral} \times \text{AUC}_{iv})$.

Mean residence time (MRT) (only for i.v. administration) was calculated as follows:

$$\text{MRT} = \left[\left(\frac{A_1}{\alpha^2}\right) + \left(\frac{A_2}{\beta^2}\right)\right]\left(\frac{1}{\text{AUC}}\right)$$

Volume of distribution in the central compartment (V_1) was determined as follows:

$$V_1 = \frac{\text{dose kg}^{-1}}{A_1 + A_2};\ \text{or}$$

$$V_1 = \frac{\text{dose kg}^{-1}\,F}{A_1 + A_2}$$

Apparent volume of distribution in the second compartment (V_2) was determined as follows:

$$V_2 = V_1\left(\frac{K_{12}}{K_{21}}\right);\ \text{or}$$

$$V_2 = V_1 F\left(\frac{K_{12}}{K_{21}}\right)$$

Volume of distribution at steady state (V_{ss}) (only for i.v. administration) was determined as follows:

$$V_{ss} = \text{MRT} \times \text{CL}$$

Maximum drug plasma concentration (C_{max}) after oral administration and the time at which C_{max} was achieved (T_{max}) was determined directly from the concentration versus time curve.

3. Results

Mean plasma concentrations of glyphosate after i.v. administration and those of glyphosate and AMPA after oral administration of glyphosate are presented in Fig. 2. Analysis of plasma concentration versus time curves indicated a biphasic decrease after i.v. and oral administration. Good fit of the observed data for a two-compartment open model was obtained. Values of the relevant kinetic variables that described absorption and disposition kinetics of glyphosate in rats are presented in Table 1. The kinetic parameters of AMPA after oral administration of glyphosate are summarized in Table 2.

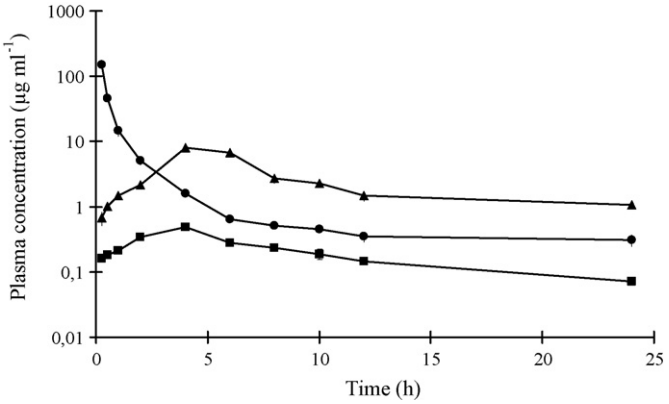

Fig. 2. Mean plasma concentrations of glyphosate after single oral (▲) administration of 400 mg kg^{-1} of body weight and after single i.v. (●) administration of 100 mg kg^{-1} of body weight and mean plasma concentrations of AMPA (■) after single oral administration of glyphosate (400 mg kg^{-1} body weight). Data represent mean ± S.D. values for eight rats. Symbols without bars indicate that S.D. is within the symbols.

Table 1
Toxicokinetic parameters for glyphosate in rats after single oral and i.v. administration.

Parameter[a]	Dose	
	i.v. (100 mg kg^{-1} body weight)	Oral (400 mg kg^{-1} body weight)
A_1 (μg ml^{-1})	164.96	12.50
A_2 (μg ml^{-1})	1.26	3.40
A_3 (μg ml^{-1})	–	15.88
α (h^{-1})	2.00	0.166
β (h^{-1})	0.069	0.048
K_a (h^{-1})	–	0.303
$T_{1/2\alpha}$ (h)	0.345	4.17
$T_{1/2\beta}$ (h)	9.99	14.38
$T_{1/2a}$ (h)	–	2.29
V_1 (l kg^{-1})	0.602	5.84
V_2 (l kg^{-1})	2.39	2.32
V_{ss} (l kg^{-1})	2.99	–
K_{12} (h^{-1})	0.334	0.035
K_{21} (h^{-1})	0.084	0.088
K_{10} (h^{-1})	1.65	0.091
AUC (mg h l^{-1})	100.24	93.26
F (%)	–	23.21
MRT (h)	3.01	–
CL (l h kg^{-1})	0.995	0.995
C_{max} (μg ml^{-1})	166.22	4.62
T_{max} (h)	–	5.16

[a] Toxicokinetic parameters were calculated from the mean concentration–time curve.

Table 2
Toxicokinetic parameters of AMPA after a single oral administration of glyphosate (400 mg kg^{-1} body weight).

Parameter[a]	
$T_{1/2\alpha}$ (h)	2.94
$T_{1/2\beta}$ (h)	15.08
K_{12} (h^{-1})	0.073
K_{21} (h^{-1})	0.105
K_{10} (h^{-1})	0.103
AUC (mg h l^{-1})	6.05
C_{max} (μg ml^{-1})	0.416
T_{max} (h)	2.42

[a] Toxicokinetic parameters were calculated from the mean concentration–time curve.

After i.v. administration of glyphosate, a rapid distribution phase and a slower elimination phase were observed, with a half-life of distribution α phase ($T_{1/2\alpha}$) of 0.345 h and a half-life of elimination β phase ($T_{1/2\beta}$) of 9.99 h (Table 1). The central volume of distribution (V_1) was 0.602 l kg^{-1}. The apparent volume of distribution in the second compartment (V_2) and at steady state V_{ss} and clearance (CL) values were 2.39 l kg^{-1}, 2.99 l kg^{-1} and 0.995 l h^{-1} kg^{-1}, respectively (Table 1).

When administered orally, the herbicide was slowly and poorly absorbed. The half-life of oral absorption ($T_{1/2a}$) was 2.29 h. Bioavailability (F) of glyphosate after oral administration was 23.21%. The maximal plasma concentration of glyphosate (C_{max} = 4.62 μg ml^{-1}) was estimated 5.16 h after oral administration (Table 1). Glyphosate was distributed more slowly after oral than i.v. dosing (distribution half-lives, $T_{1/2\alpha}$, 4.17 and 0.345 h, respectively). The elimination half-life ($T_{1/2\beta}$) of glyphosate after oral dose was somewhat greater than that calculated after i.v. dose. The elimination half-lives were 14.38 and 9.99 h, respectively, indicating a slow final disappearance of the herbicide from blood (Table 1).

A fraction of glyphosate was metabolized to AMPA after oral administration of glyphosate. This metabolite represented 6.49% of the parent drug plasma concentrations, as calculated by use of the ratio between AUC for AMPA and AUC for glyphosate after oral administration of glyphosate. The plasma concentration of AMPA (0.416 μg ml^{-1}) peaked at 2.42 h after oral administration of glyphosate. The $T_{1/2\beta}$ of AMPA after oral glyphosate administration was 15.08 h (Table 2).

There were no adverse effects from glyphosate administration noted during this study.

4. Discussion

Glyphosate is a broad spectrum, post-emergent herbicide active by plant translocation. Glyphosate is used in both agriculture and forestry. It is also used for control of plant above the surface in aquatic environments, parks and road verges. In plants and some microorganisms glyphosate inhibits the shikimic acid pathway, causing a deficit in aromatic amino acids. Absence of this pathway may account for its low toxicity in animals. Ataxia, breathing difficulties and occasionally convulsions, preceded death in rats receiving lethal doses of glyphosate (EPA, 1980). After ingestion in humans, mild poisoning symptoms may include stomach cramps, vomiting, nausea and diarrhoea, and mouth and throat pain. Moderate poisoning is associated with gastrointestinal tract ulceration, hypotension, and hepatic and renal damage. Severe poisoning is characterized by respiratory and renal failure, seizures, coma and eventually death. Dermal and conjunctival irritation may follow topical exposures (EPA, 1980). Through production and general use glyphosate may come in contact with human. Improper use of this agent can potentially lead to high glyphosate levels in the environment. The limited information available on the kinetics of glyphosate (Brewster et al., 1991) makes it difficult to interpret toxicological findings and to make risk assessment for glyphosate, topics that are still under debate.

Risk assessment is largely extrapolation of experimental data from animal experimentation to human situations. Laboratory animals, particularly rodents, are largely used for toxicity evaluation. When several factors like differences in species sensitivity determined by genetic and other factors are considered, toxicokinetic variables are of major importance and should be considered in extrapolation of laboratory data to humans, since changes in various kinetic parameters are either easily determined experimentally or can be predicted with a reasonable accuracy. It is apparent that the principles of pharmacokinetics are indispensable in the overall assessment of human health risk. By using pharmacokinetic principles, it is possible to estimate the total body burden after an exposure and how much time would be needed to completely eliminate the chemical from the body. Distribution, elimination and metabolism data are very important to be extrapolated from experimental animals to humans. Extrapolation between species is easily done if differences in physiological process related them in these species are understood (Sharma and Coulombe, 1996). Toxicokinetic characteristics combined with toxicodynamic patterns must be considered in the use safety evaluation of glyphosate. To the best of our knowledge, the present paper is the first to report in rats the plasma disposition of glyphosate using a selective HPLC analytical method to determine the levels of glyphosate and its metabolite AMPA in biological fluids in order to evaluate its pharmacokinetics. The validation parameters used show that the method is reliable and sensitive and allow an adequate characterization of the disposition of glyphosate in rats. In the study reported here the kinetics of glyphosate after a single i.v. (100 mg kg^{-1}) and oral (400 mg kg^{-1}) administration were determined in rats. Plasma disposition of glyphosate after i.v. and oral administration in rats as well as disposition of AMPA after oral administration of glyphosate were best described by use of a two-compartment open model. Disappearance of glyphosate from plasma was characterized by an initial rapid distribution phase followed by a slower elimination phase. Brewster et al. (1991) also reported that [^{14}C]-glyphosate elimination from tissues followed a two-component decay process consisting of a relatively short α phase followed by a much longer β phase. The elimination half-lives ranged from 20 h in the small intestine to over 90 h in the bone (Brewster et al., 1991).

After i.v. administration of 100 mg kg^{-1}, the distribution phase of glyphosate was fast ($T_{1/2\alpha}$ = 0.345 h) and with a high value of volume of distribution at steady state (V_{ss} = 2.99 l kg^{-1}) which indicate that glyphosate is extensively distributed in extravascular tissues. The values of apparent volume of distribution in the second compartment (2.39 and 2.32 l kg^{-1} after i.v. and oral administration) also indicate that glyphosate easily penetrated all tissues, in agreement with data reported for Brewster et al. (1991). The elimination half-life ($T_{1/2\beta}$) calculated after i.v. administration was 9.99 h. The $T_{1/2\beta}$ of glyphosate increased by 44% (to 14.38 h) after oral administration. This suggests that in rats the plasma disposition of glyphosate after oral administration is conditioned by the absorption process.

Glyphosate was slowly and poorly absorbed through the gastrointestinal tract in rats as reflected by an absorption half-life ($T_{1/2a}$) of 2.29 h, a maximal plasma concentration (C_{max}) of 4.62 μg ml^{-1} and a T_{max} of 5.16 h after oral dose of 400 mg kg^{-1}. This T_{max} is comparable to previous studies using [^{14}C]-glyphosate, where glyphosate-derived radioactivity appeared to reach maximal tissue concentrations at 6.3 h after oral administration (Brewster et al., 1991). The oral bioavailability of glyphosate was 23.21% in rats, which was slower to those of studies in which [^{14}C]-glyphosate administered at the oral dose of 10 mg kg^{-1} approximately 30–36% of the dose was absorbed (Ridley and Mirley, 1988; Howe et al., 1988; Brewster et al., 1991). However, this result was close to the NTP study (NTP, 1992) which showed that approximately 19–23% of the administered 1000 mg kg^{-1} dose was absorbed as determined by urinary excretion data. Colvin and Miller (1973) also reported previously a poor oral absorption of ^{14}C-labeled glyphosate. When a single oral dose of glyphosate (6–9 mg kg^{-1}) was administered to New Zealand white rabbits, an 80% of the material appeared in the feces (Colvin and Miller, 1973). The low bioavailability of glyphosate may be caused by biliary excretion or glyphosate degradation at the site of absorption.

Glyphosate is poorly metabolized in rats. AMPA is the main metabolite in rats. The rate of elimination of AMPA ($T_{1/2\beta}$ = 15.08 h) after oral glyphosate administration was similar to that of glyphosate ($T_{1/2\beta}$ = 14.38 h). The metabolite AMPA represented 6.49% of the parent drug plasma concentrations. A similar metabolic characterization was previously indicated by Brewster et al. (1991).

The production of this metabolite could have been the result of intestinal microbial action (Rueppel et al., 1977; Mueller et al., 1981). These results agree with that of other Monsanto studies where AMPA was found to be formed as the sole metabolite in very low levels (Howe et al., 1988) and they suggest that glyphosate does not induce its own metabolism. In fat, at high levels glyphosate produced a moderate inhibition of microsomal monooxygenases (Hietanen et al., 1983) and it had little effect on peroxisomal β oxidation and GSH activity (Vainio et al., 1983). Nevertheless, the impact of glyphosate on xenobiotic-metabolizing enzymes in mammalian organisms is still highly unexplored.

In summary, the present study indicates that when given orally, glyphosate was absorbed and likely distributed throughout the body by the circulation blood. Although the bioavailability was low, glyphosate and its metabolite AMPA were eliminated from plasma slowly and therefore would be diffused to target tissues to exert systemic effects. The toxicokinetic characteristics of glyphosate identified in this study warrant further research on possible mechanisms of toxicity of this herbicide.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This work was supported by the Ministerio Educación y Ciencia Projects AGL2005-05320-C02-02 and Consolider-Ingenio 2010 Programme, FUN-C-FOOD CSD2007-0623, Madrid (Spain).

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