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ABSTRACT

Glyphosate-based herbicides (GBHs) are the most-used herbicides worldwide. Concerns about their toxicity and ecotoxicity have motivated scientists to assess their potential effects on animals, as well as their toxicokinetic parameters in rats and humans. However, to our knowledge, such data have not been produced for avian models. In this study, toxicokinetic parameters for glyphosate and AMPA were calculated after one unique dietary exposure (40 mg of glyphosate equivalent per kg) and one unique intravenous injection of a GBH, in hens and roosters respectively. Non compartmental analysis was used to show the evolution of glyphosate and AMPA plasma concentrations over time. After one unique intravenous injection of a glyphosate-based herbicide, glyphosate and AMPA were quickly eliminated from plasma and were poorly distributed ($Vss_{glyphosate}$ = 0.30 L/kg). Their terminal half-lives are 4.7 h and 8.10 h, respectively. After dietary exposure, glyphosate and AMPA followed a 6 h absorption phase followed by a 42 h elimination phase. They were poorly distributed ($Vss_{glyphosate}$ = 0.00562 L/kg), and their maximum concentrations (Cmax) were 21285 μg/L and 108 μg/L, respectively. Their terminal elimination half-lives were 8.94 h and 6.93 h, respectively. Taken together, this study provides new data on the elimination rate and approximate biological half-life range of glyphosate in birds.

1. Introduction

Growing populations and the resulting increases in food demand have led farmers to look for strategies to increase their productivity (Gill et al., 2018). The use of fertilizer increases the growth of crops, which can then be effectively harvested using mechanical harvesting machines. Unwanted weeds can be removed with powerful herbicides. The most commonly used of these is glyphosate, or N-(phosphonomethyl)glycine (Gill et al., 2018). This broad-spectrum herbicide owes its effectiveness to its ability to inhibit EPSPS (5-enolpyruvylshikimate-3-phosphate synthase), an enzyme from the shikimate pathway exclusively expressed in plants and in some microorganisms (Schönbrunn et al., 2001). The resulting cancellation of the shikimate pathway leads to a default in an aromatic amino acid and, more generally, a dysregulation of various metabolic pathways in the plant, which eventually dies (Bradberry et al., 2004). When spread in the environment, glyphosate has two main fates. The vast majority of it is degraded by soil microbial communities into aminomethyl phosphonic acid (AMPA), its main metabolite. Some is also turned into sarcosine, then glycine. Both pathways ultimately end with mineralization into CO_2, phosphonic acid, and NH_4^+ (Bai and Ogbourne, 2016). It is worth noticing that the European Food Safety Authority (EFSA) warns of the high persistence of glyphosate and AMPA, which have a DT50 (time required for the chemical concentration under defined conditions to decline to 50% of the amount at application) reaching hundreds of days and being variable depending on the aerobic/anaerobic status of the soil (Martins-Gomes et al., 2022).

Confusion between glyphosate and glyphosate-based herbicide (GBH) must be avoided. Indeed, glyphosate needs wetting agent to better penetrate plant systems (Bradberry et al., 2004). The addition of organic co-formulants (such as surfactants) is therefore essential to allow glyphosate to exert its full herbicide power (Bradberry et al., 2004; Kim et al., 2013; Mesnage et al., 2019). The resulting formulation is called a GBH; this is the actual product spread on fields. Studying the impact of GBHs on animal systems is therefore more relevant than studying glyphosate alone, but it does not affirm that the observed effects are due to the action of glyphosate, since they are likely caused by

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the co-formulants or by their combination.

Previous studies performed in our laboratory on broiler hens show that dietary GBH intake results in a significant increase in embryo mortality and developmental defects (Estienne et al., 2022). These data are in good agreement with a recent study showing that pesticide use is one of main causes for the decline of most bird populations (Rigal et al., 2023). We also confirmed that glyphosate is poorly biotransformed in this model, as in previous studies using other animal models (Williams et al., 2000). Still, we observed a disturbance in cecal bacteria populations, with possible metabolic repercussions (Fréville et al., 2022). However, to our knowledge, data about the toxicokinetics (TK) of glyphosate and AMPA in birds have not been yet obtained. Such data have been produced in rats after a single oral administration, showing a poor oral bioavailability of glyphosate (about 23%), which is still distributed in the organism via blood circulation (Anadón et al., 2009). Glyphosate was biotransformed to AMPA in rats, and AMPA represented 6.5% of the parent compound plasma concentrations.

The present study therefore aims to determine toxicokinetic parameters such as absorption, half-life, or clearance for glyphosate and AMPA in hens and roosters, after unique oral and intravenous administration of GBH.

2. Material and methods

2.1. Ethical issues

All experimental procedures were performed in accordance with the French national guidelines for the care and use of animals for research purposes (certificate of authorization to experiment on living animals APAFIS number 21549–2019071809504554v3, Approval Date: 6 November 2021, Ministry of Agriculture and Fish Products; notice of ethics committee of Val de Loire N◦19).

2.2. Animals

Animals were divided into two experiments: Experiment 1 involved a single dietary exposure to a GBH in adult hens and monitoring of plasma glyphosate concentration for 48 h, and Experiment 2 involved a single intravenous injection of GBH into adult roosters and monitoring of plasma glyphosate concentration for 24 h. The GBH concentration used for both experiments was chosen to correspond to 40 mg of glyphosate equivalent per kg of body weight, corresponding to approximatively half the NOAEL (no-observed adverse-effect level; 100 mg glyphosate/kg body weight/day) reported by the European Food Safety Authority (EFSA). In studies previously performed in our laboratory, several adverse effects were detected in chicken after exposure to a similar dose (Estienne et al., 2022; Fréville et al., 2022). The experimental design is summarized in Fig. 1.

For Experiment 1, 4 ROSS 308 roosters were obtained at 1 day of age from a local hatchery (Boye Accouvage La Villonniere, 79310 La Boissière en Gatine, France) and reared at "Pôle Expérimental Avicole de Tours" (INRAE, Nouzilly, France) under traditional breeding conditions. At 36 weeks old, the roosters were given a single intravenous injection of a solution of GBH diluted in physiological serum (a GBH dose equivalent to 40 mg glyphosate/kg body weight) through the left alar vein and fasted for the following 48 h. GBH was totally dissolved in the resulting solution. Plasma samples were collected from the occipital venous sinus before injection and several times after (0, 15, and 30 min; 1, 2, 4, 6, 8, 10, and 24 h) during the following 24 h.

For Experiment 2, 20 ROSS 308 hens were obtained at 1 day of age from a local hatchery (Boye Accouvage La Villonniere, 79310 La Boissière en Gatine, France) and reared at "Pôle Expérimental Avicole de Tours" (INRAE, Nouzilly, France) under traditional breeding conditions. At 36 weeks old, 10 hens were exposed to GBH via their feed (a GBH dose equivalent to 40 mg glyphosate/kg body weight), and 10 hens were fed a regular diet without GBH exposure. Animals in the first group had free access to the GBH-enriched food for 30 min, the time needed for all the feed to be eaten. After that, the animals no longer had access to food. Plasma samples were collected several times from the occipital venous sinus before and after the end of feed access (0 and 30 min; 4, 8, 12, 24, 30, and 48 h).

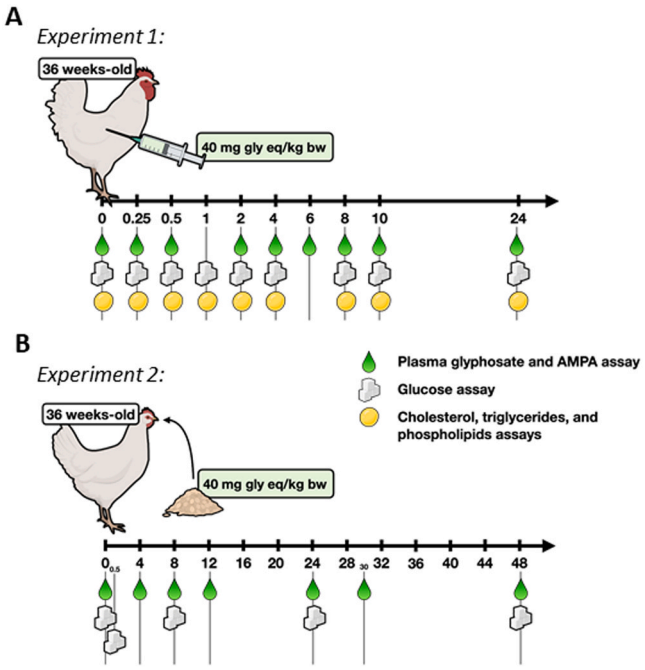

Fig. 1. Experimental design applied to the animals. For experiment 1 (A), ROSS 308 roosters (36 weeks-old; n = 4) were injected with a single dose of GBH (Glyphosate-Based Herbicide; 40 mg glyphosate/kg body weight). For experiment 2 (B), ROSS 308 hens (36 weeks-old; n = 10) were exposed a single time to GBH via their feed (GBH hens; 40 mg glyphosate/kg body weight). Their controls (36 weeks old; n = 10) were fed with a regular diet without exposure to GBH (CT hens). Blood plasma from the animals from experiment 1 and experiment 2 were regularly sampled for glyphosate, AMPA, glucose and cholesterol, triglycerides and phospholipids assays during the following 24 h and 48 h respectively.

2.3. Chemicals

Gallup Super 360, called GBH within this text, was obtained from Axereal (Monnaie, France); it contained 360 g/L Gly (485.8 g/L isopropylamine salt). It was mixed with the diet (whose composition is shown in Supplementary Table 1), which contains low measurable glyphosate and AMPA (0.21 mg/kg feed for Gly and undetectable levels for AMPA as determined by Phytocontrol, Nimes, France). The mix was done in accordance with the directives of the Departmental Directorate for the Protection of Populations. Mixing was carried out by a technician with a "Certiphyto" certification for the handling of phytosanitary products, as recommended by French law.

2.4. Plasma samples

In Experiment 1, blood samples (1 mL) were collected from the occipital sinuses of 4 injected roosters into heparinized tubes at 0, 15, and 30 min and 1, 2, 4, 6, 8, 10, and 24 h after the GBH intravenous injection. Blood plasma was recovered after centrifugation at 5000 g for 10 min at 4 °C and stored at −20 °C until use.

In Experiment 2, blood samples (1 mL) were collected from the occipital sinuses of 10 dietary exposed hens and 10 non-exposed animals into heparinized tubes at 0 and 30 min and 4, 8, 12, 24, 30, and 48 h after feeding.

2.5. Glyphosate and AMPA assays in plasma

Glyphosate and AMPA concentrations were measured in the plasma from 20 hens and 4 roosters after a derivatization reaction using FMOC-Cl (9- fluorenylmethyl chloroformate), in collaboration with Dr. S El Balkhi (Service de Pharmacologie, Toxicologie et Pharmacovigilance, Limoges, France), as previously described (Serra et al., 2021). Higher and lower limits of quantification (LOQ) were 0.1 and 10 µg/L, respectively. 100 µL of blood plasma per sample was necessary to perform the analysis. Quality controls for both glyphosate and AMPA were systematically prepared and analyzed at concentrations of 0.5 and 5 µg/L. These controls were examined after the calibration points and after every set of 10 samples. For glyphosate, the average bias was 87%, with a standard deviation of 9% and a coefficient of variation of 10%. For AMPA, the average bias stood at 99%, accompanied by a standard deviation and coefficient of variation both at 19%.

2.6. Plasma glucose, triglycerides, phospholipids, and cholesterol assays

Plasma concentrations of glucose, triglycerides, phospholipids, and cholesterol were determined by enzymatic assay using specific kits (glucose: GOD-POD, reference MG981780, ThermoScientific, Asnières sur Seine, France; triglycerides: reference LP80519, Biolabo SAS, Maizy, France; phospholipids: reference 99105, Biolabo SAS, Maizy, France; cholesterol: reference 80106, Biolabo SAS, Maizy, France). Measurements were performed according to the manufacturers' protocols. The intra-assay and inter-assay coefficients of variation (CV) for each assay averaged $< 15\%$.

2.7. Cortisol assay in roosters' plasma

Plasma cortisol levels were measured using an IBL Cortisol ELISA kit (Immuno-Biological Laboratories, Germany), and absorbance was measured at 450 nm, with 630 nm as the reference wavelength. The lower detection limit for cortisol was 0.05 ng/mL, and the intra-assay and inter-assay coefficients of variation (CV) for each assay averaged $< 10\%$.

2.8. Testosterone assay in roosters' plasma

Testosterone concentrations in blood plasma were determined using commercial ELISA assays from Cayman Chemicals, following the manufacturer's instructions. The sensitivity of this assay was 0.01 ng/mL. The intra-assay and inter-assay coefficients of variation (CV) for each assay averaged $< 10\%$.

2.9. Data analyses

GraphPad Prism® software (Version 8) was used for all analyses, except for pharmacokinetic analyses. Data were tested for homogeneity of variance by Bartlett's test and for normal distribution by Shapiro-Wilk test. GraphPad Prism® software (Version 8) was used to create all histogram graphs, and Rstudio was used to create all curve graphs. One-way ANOVA was performed with Tukey-Kramer multiple comparisons tests or Dunnett's multiple comparisons tests as appropriate. Data were presented as means ± SD, with $p < 0.05$ considered significant. Pharmacokinetic non-compartmental analyses were done using PKanalix (Version 2020R1; Antony, France: Lixoft SAS, 2020, http://lixoft.com/products/PKanalix/). Integrals were realized using the linear log trapezoidal method. Terminal half-lives (from final slope) were calculated on the last three time points.

3. Results

There were no clinical signs of adverse effects from GBH administration noted in this study.

3.1. Experiment 1: Glyphosate and AMPA plasma concentrations rapidly decrease after intravenous GBH injection

Glyphosate and AMPA plasma concentrations (µg/L) after a single intravenous (IV) GBH injection (40 mg of glyphosate equivalent/kg of bodyweight) are shown in Figs. 2-A and 2-B, respectively. Fifteen minutes after IV injection, the concentration of glyphosate ranges between $1.3*10^4$ and $1.7*10^4$ µg/L. It quickly drops during the first eight hours of the experiment, then declines slowly. AMPA follows a similar pattern, with a rapid decrease during the six first hours after IV injection of GBH, followed by a slow decline. Nevertheless, the two molecules, on a logarithmic scale (not shown), do not rigorously describe a bi- (or even a tri-) exponential decay; therefore, the data were analyzed using the non-compartmental approach (Bulitta, n.d.). Glyphosate's and AMPA's area under the curve extrapolated to infinity (AUCinf), total clearance (CLt), volume of distribution at steady state (Vss) and terminal half-life ($T_{1/2}$) are given in Table 1 for each individual and its mean with coefficient of variation (CV). Mean glyphosate AUCinf is 49,333 µg.h/L (for the tested dose of 40 mg/kg). Mean glyphosate total clearance is 0.081 L/h/kg bw. Glyphosate volume of distribution at steady state (Vss) is 0.30 L/kg bw. Terminal glyphosate half-life ($T_{1/2}$) is 4.7 h. AMPA AUC infinite (AUCinf) is 104 µg.h/L. AMPA represents 0.32% of the parent compound plasma concentrations (based on their AUCinf corrected by their molecular weights). AMPA terminal half-life ($T_{1/2}$) is 8.10 h in mean.

3.2. Experiment 2: Glyphosate and AMPA concentrations in plasma peak 6 h after dietary administration

Glyphosate and AMPA plasma concentrations (µg/L) after a unique oral GBH administration (40 mg of glyphosate equivalent/kg of bodyweight) are given in Figs. 3-A and 3-B. Plasma concentrations of glyphosate and AMPA peak 6 h after glyphosate administration. Glyphosate concentrations quickly drop from the 6th hour to the 24th hour, when they return to almost zero (Fig. 3-A). AMPA concentrations peak 6 h after administration and immediately return to near zero from the 12th hour to the end of the experiment. Glyphosate's and AMPA's main toxicokinetic parameters are given in Table 2. After oral administration (40 mg/kg), glyphosate Cmax is 21285 µg/L, glyphosate AUCinf is 366760 h.µg/L, apparent glyphosate total clearance (CLt/F) is 0.000436 L/h/kg bw, apparent glyphosate volume of distribution at steady state (Vss/F) is 0.00562 L/kg bw, and glyphosate oral terminal half-life ($T_{1/2}$) is 8.94 h. On the other hand, AMPA Cmax is 108 µg/L, and AMPA AUCinf is 582 h.µg/L. AMPA represents 0.24% of the parent compound, and its terminal half-life ($T_{1/2}$) is 6.93 h.

3.3. Blood glucose levels increase after intravenous GBH injection

Cholesterol, triglycerides, phospholipids, and glucose levels were measured in the animals that were injected with one unique intravenous GBH injection (40 mg of glyphosate equivalent/kg of bodyweight). Results are given in Figs. 4-A, 4-B, 4-C, and 4-D, respectively. Glucose concentrations continuously increased ($P < 0.05$) for the first 10 h after the injection, then stabilized 24 h after the injection. Triglycerides levels were stable until the 4th hour after injection, after which levels dropped slightly ($P < 0.05$) until the 10th hour after injection. Cholesterol and phospholipids levels remained unaffected during the 24 h after injection ($P > 0.05$). Cortisol concentration over time was measured to ensure that the aforementioned variations did not result from stress induced by puncture. Results are shown in Fig. 4-E. Cortisol levels were stable ($P > 0.005$). Testosterone levels over time are given in Fig. 4-F. Mean testosterone level is higher during the 24th hour after injection.

3.4. Blood glucose levels increase after dietary GBH exposure

Glucose levels were measured in the plasma from animals that were

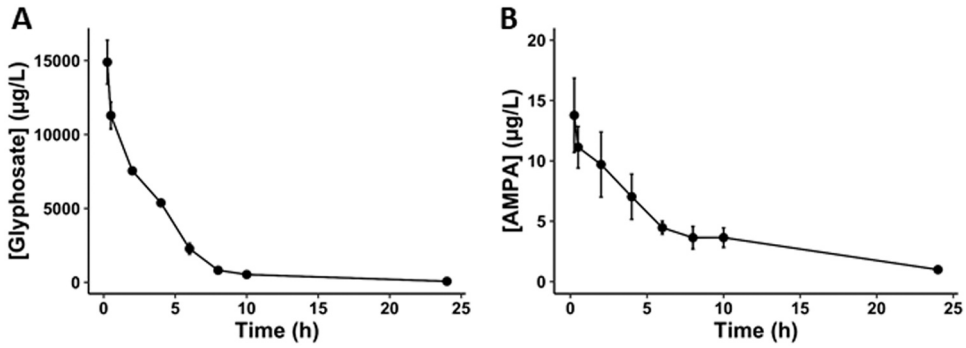

Fig. 2. Glyphosate (A) and AMPA (B) plasma concentration in roosters after a single GBH intravenous injection (40 mg glyphosate/kg body weight; n = 4). Results are represented as mean ± SD.

Table 1
Non compartmental analysis of plasma Glyphosate and AMPA concentrations after unique intravenous injection of 40,000 µg/kg of bodyweight of GBH in 4 roosters.

Parameter	Unit	Rooster 1	Rooster 2	Rooster 3	Rooster 4	Mean (CV in %)
Body weight	kg	4.37	4.81	4.27	4.32	4.44 (5.6%)
Glyphosate AUC infinite (AUCinf)	µg.h/L	46136	54851	47282	49035	49333 (7.8%)
Glyphosate Total clearance (CLt)	L/h/kg bw	0.087	0.073	0.084	0.082	0.081 (7.4%)
Glyphosate Volume of distribution at steady-state (Vss)	L/kg bw	0.32	0.30	0.30	0.28	0.30 (5.5%)
Glyphosate Terminal half-life (T1/2)	h	5.1	4.7	4.5	4.3	4.7 (7.7%)
AMPA AUC infinite (AUCinf)	µg.h/L	110	118	95	92	104 (12.1%)
AMPA percentage (on AUCinf)	%	0.36	0.33	0.31	0.28	0.32 (10.4%)
AMPA Terminal half-life (T1/2)	h	9.4	7.8	8.6	6.6	8.10 (14.7%)

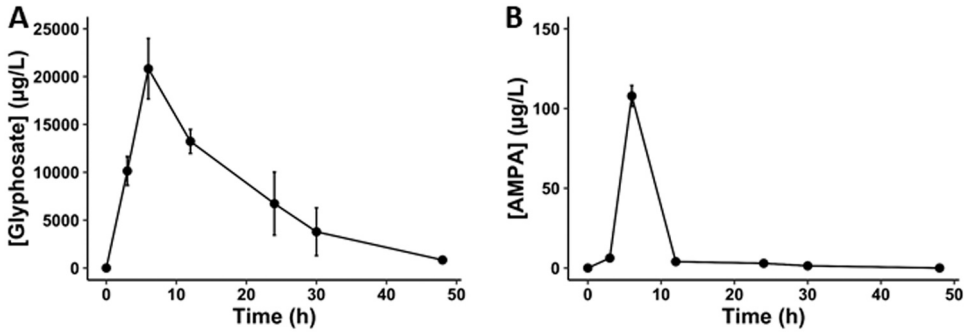

Fig. 3. Glyphosate (A) and AMPA (B) plasma concentration in hens after a single dietary GBH exposure (40 mg glyphosate/kg body weight; n = 10). Results are represented as mean ± SD.

orally exposed to GBH (40 mg of glyphosate equivalent/kg of body weight). Results are shown in Fig. 5. Glucose concentrations continuously increased during the 48 h of monitoring and were significantly higher ($P < 0.0001$) in GBH-exposed animals than in control animals.

4. Discussion

Glyphosate-based herbicides, and by extension glyphosate itself, are not supposed to adversely affect humans and animals in any way, since the target of glyphosate is an enzyme expressed only in plants and some microorganisms (Schönbrunn et al., 2001). However, some studies show possible adverse effects after GBH administration in various models (Gill et al., 2018). Furthermore, some data show that human populations are widely exposed, mainly via their diet, as well as through the respiratory tract for occupational populations (Grau et al., 2022). Zhang et al. (2020) measured various concentrations of urinary glyphosate in occupational and non-occupational populations. Although other recent studies claim that non-occupational populations are weakly exposed, they still show non-negligible levels of contamination in horticulture, farm, and glyphosate production workers (Connolly et al., 2017; Zhang et al., 2020; Faniband et al., 2021; Kohsuwan et al., 2022).

Concerns about animals exposure have also grown, as animals are very likely to be exposed via their feed (Van Eenennaam & Young, 2017). Previous works in our laboratory have identified several toxic outcomes following dietary GBH exposure in chickens, including embryo mortality, oxidative stress induction, and microbiome disruptions (Estienne et al., 2022; Fréville et al., 2022). Information about the behavior of glyphosate inside the body should therefore allow a better understanding of the mechanisms behind these effects. Such data are already available for rats as laboratory animals for human risk assessment (Anadón et al., 2009), but as far as we know, they were not explored yet for chickens. We chose to work with a dose of 40 mg of glyphosate equivalent per kg bw, corresponding to approximately half the NOAEL (no-observed-adverse-effect level; 100 mg glyphosate/kg body weight/day) reported by the European Food Safety Authority (EFSA). After IV injection of 40 mg of glyphosate equivalent in GBH, glyphosate and AMPA are rapidly eliminated from the plasma. Glyphosate and AMPA concentrations decrease rapidly during the minutes

Table 2
Non compartmental analysis of plasma Glyphosate and AMPA concentrations after unique GBH oral ingestion via the feed of 40 mg/kg of body weight in 10 laying hens.

Parameter	Unit	Hen A	Hen B	Hen C	Hen D	Hen E	Hen F	Hen G	Hen H	Hen I	Hen J	Mean (CV in %)
Bodyweight	kg	4.115	4.810	4.270	3.660	2.825	3.795	3.855	3.210	4.350	3.915	3.880 (14.7%)
Glyphosate Cmax	µg/L	25432	15954	23456	22341	19745	16578	18764	22546	24576	23456	21285 (15.6%)
Glyphosate AUC infinite (AUCinf)	h.µg/L	453726	423031	329965	313185	320841	358321	383898	353551	345160	385921	366760 (12.3%)
Apparent Glyphosate Total clearance (CLt/F)	L/h/kg bw	0.00035	0.0004	0.00051	0.00047	0.00054	0.00042	0.0004	0.00036	0.0005	0.00041	0.000436 (14.9%)
Apparent Glyphosate Volume of distribution at steady-state (Vss/F)	L/kg bw	0.0052	0.0039	0.0064	0.0052	0.0079	0.0053	0.0051	0.0062	0.0062	0.0048	0.00562 (19.5%)
Glyphosate Terminal half-life (T1/2)	h	10.19	6.71	8.66	7.67	10.09	8.72	8.77	11.93	8.52	8.22	8.94 (16.3%)
AMPA Cmax	µg/L	118	106	103	109	110	98	100	105	118	109	108 (4.4%)
AMPA AUC infinite (AUCinf)	h.µg/L	630	566	568	557	595	547	595	566	617	577	582 (4.6%)
AMPA percentage (on AUCinf)	%	0.21	0.20	0.26	0.27	0.28	0.23	0.23	0.24	0.27	0.22	0.24 (10.9%)
AMPA Terminal half-life (T1/2)	h	10.32	16.17	5.25	4.58	5.12	5.46	2.96	4.21	11.29	3.97	6.93 (61.1%)

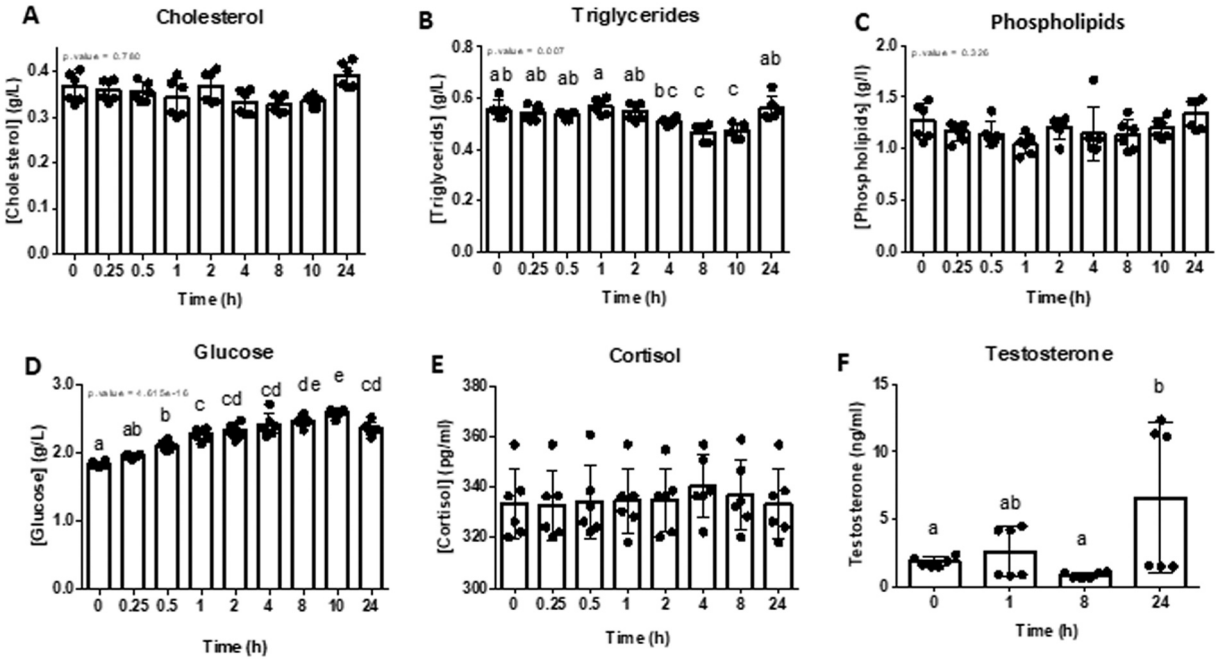

Fig. 4. Cholesterol (A), triglycerides (B), phospholipids (C) and glucose (D), cortisol (E) and testosterone (F) concentrations in roosters plasma after a single GBH intravenous injection (40 mg glyphosate/kg body weight; n = 4). Results are represented as mean ± SD. Letters indicate statistical significance after one-way ANOVA ($p < 0.05$).

following the injection, which suggest the possibility of fast distribution. However, the volume of distribution at steady state is pretty low (Vss = 0.30 L/kg) compared to that observed in rats (Vss = 2.99 L/kg, after IV injection of 100 mg/kg of glyphosate 95% v/v). Glyphosate and AMPA should therefore be more weakly distributed in the peripheral tissues (Anadón et al., 2009) of chickens [as Vss gives an estimate of glyphosate distribution in tissues (White, 2017)]. We measured the half-life values after IV injection for glyphosate ($T_{1/2}$ = 4.7 h) and AMPA ($T_{1/2}$ = 8.10 h). The half-life value for glyphosate after IV injection ($T_{1/2}$ = 4.7 h) is much shorter than that measured in rats [9.99 h for glyphosate, (Anadón et al., 2009)] and shorter than what we measured after oral administration of the same dose of GBH ($T_{1/2}$ = 8.40 h). Half-life value for AMPA after IV injection is greater than the value after oral administration ($T_{1/2}$ = 6.93 h).

Species, dosage, and mode of administration strongly influence toxicokinetic parameters (Zhang et al., 2020). The AMPA metabolite represented 6.49% of the parent compound after oral administration in rats (Anadón et al., 2009); this proportion was further reduced here in chickens to 0.24%. This extremely low metabolism of glyphosate to AMPA was also found in our intravenous study, at a similar rate of 0.32%. It is interesting that this biotransformation, usually attributed to the microbiota (Rueppel et al., 1977), is found at the same level after an intravenous treatment, i.e. without passing through the microbiota. However, Rueppel et al. refers to a microbial biotransformation in soil and in animals, but enzyme responsible of this biotransformation has never been identified. This could be an important gap to fill in future investigations for interspecies comparisons. Although this metabolite appears to be minor in mammals and birds, little is known about the

Fig. 5. Glucose concentration in hens plasma after a single dietary GBH exposure (GBH; 40 mg glyphosate/kg body weight; n = 10) or after exposure to the same diet without glyphosate (CT; n = 10). Results are represented as mean ± SD. Two-way ANOVA; ****p < 0.0001.

clearance of glyphosate in animals. This makes inter-species comparisons difficult for this parameter, which is 0.995 L/h/kg in rats (Anadón et al., 2009) and more than ten times lower in chickens (0.081 L/h/kg). Inter-species differences are more difficult to interpret, as details of elimination mechanisms (hepatic, renal, and others) are unknown. However, the hydrophilicity of glyphosate suggests that renal filtration is a major route of elimination, which could be attenuated by binding to plasma proteins.

Glyphosate is poorly bioavailable and poorly absorbed in chicken and other models, as up to 80% of the administered glyphosate is found in feces (JMPR 2004). Furthermore, with the oral absorption rate being low, the elimination rate could be faster, making the absorption rate the limiting factor in the kinetics and thus influencing the half-life of the molecule (Kuan et al., 2023). This phenomenon is called flip-flop in pharmacokinetics (Toutain and Bousquet-Melou, 2004). Glyphosate and AMPA display a rapid distribution phase which peaks at 6 h, after which glyphosate displays a longer elimination phase, consistent with that observed in rats (Anadón et al., 2009). However, the glyphosate maximum concentration (Cmax) in chickens was 21,290 μg/L. This is four times the Cmax measured in rats (4.62 μg/mL), while the oral dose administered is ten times lower (Anadón et al., 2009). We can hypothesize that the surfactants present in the GBH formulation we used influence the toxicokinetic parameters of glyphosate. This variation could also be explained by inter-species differences.

To further complete the above-mentioned observations, we decided to perform several metabolic and endocrinologic dosages. It has been shown that oral administration of glyphosate in rats results in increased blood glucose levels, which are linked to insulin resistance and elevated insulin levels (Prasad et al., 2022). Glycemia increases in dietary exposed animals from the first half hour after exposure, and its difference from the control value continues growing throughout the 48 h of glycemia monitoring. Injected animals showcase a constant and significant increase in circulating blood glucose levels during the 10 first hours of exposure (while the animals are fasting), which seems to start restoring after 24 h of exposure. Measurements of insulin levels in plasma could help identify the process leading to this increase, but in the absence of a real control, we cannot confirm that the link between the GBH injection and the glucose rise is not merely correlational. GBHs have also been linked to endocrine disorders (Muñoz et al., 2021). Previous works in our laboratory showed that dietary exposure to GBH increases testosterone and estradiol levels in roosters' plasma (Serra et al., 2021). We measured plasma testosterone levels in the hours after injection and observed an increase 24 h later. Though this increase is statistically significant, testosterone levels increased in only three out of the six tested animals. The question arises as to why testosterone levels are stable in some animals and not in others. It could be explained by a disruption of testosterone producing cells, though this more likely would have led to reduced plasma levels (Muñoz et al., 2021). We are again unable to confirm that this observation is not simply correlational.

5. Conclusion

This study characterizes the main toxicokinetic parameters related to glyphosate and AMPA after GBH administration in chickens. The data show that dietary glyphosate and AMPA are quickly absorbed and eliminated. Their concentrations in plasma are almost zero 48 h after ingestion, and their low volume of distribution at steady state indicates that they are poorly distributed in peripheral tissues. Considering that co-formulants are suspected to be more toxic that glyphosate itself, it might be useful to assess their toxicokinetic parameters. However, the lack of available information about the exact composition of most GBHs makes it difficult to study those parameters. Experimental protocols including models of different ages and sexes would also be helpful, as most mortal poisoning cases involve older men (Roberts et al., 2010).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.toxlet.2023.10.009.

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