Accumulation, metabolites formation and elimination behavior of rac-glufosinate-ammonium and glufosinate-P in zebrafish (Danio rerio)

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ABSTRACT

An efficient trace detection method for the determination of residues of the glufosinate enantiomers and metabolites in zebrafish by HPLC-Q-Exactaive Orbitrap Mass Spectrometry was developed. After the purification of dichloromethane and Oasis PRiME HLB SPE column, the recovery ranges from 77% to 104%, with RSD < 10.03%. The limits of quantitation in zebrafish were 0.006-0.02 mg/kg. The results revealed zebrafish absorbed glufosinate slowly, reaching a steady state in 10-14 days, and the bioaccumulation factor (BCF) of D/L-glufosinate-ammonium was less than 0.3. L-glufosinate-ammonium accumulated preferentially in zebrafish. The residue of the metabolite N-acetyl glutamate (NAG) was smaller than that of 3-methyl phosphonic acid (MPP). D/L-glufosinate-ammonium had an elimination half-life of less than 2.3 days during the elimination phase. The bioaccumulation and elimination behavior of glufosinate-ammonium in zebrafish aquatic system was shown in this work, which offered scientific data for assessing the food safety of rac-glufosinate-ammonium and glufosinate-P (pure L-glufosinate-ammonium) in fish.

Introduction

The herbicide glufosinate-ammonium is non-selective. (2-amino-4-(hydroxymethyl phosphoryl) butyrate) is the chemical name for glufosinate-ammonium. Figure S 1 (in Supporting Information) shows the glufosinate-ammonium structural formula. The racemate of L- and D-enantiomers is glufosinate-ammonium. The active herbicidal ingredient is L-enantiomer, while the D-enantiomer is inactive (Hoerlein, 1994; Meiji, 1981). Fruit trees, medium crops, vegetables, and non-cropland can all benefit from glufosinate-ammonium (Bellinder, Lyons, Scheckler, & Wilson, 1987). Glufosinate has become the world’s second-largest herbicide-resistant genetically modified crop herbicide (Nolte, 2004). Most glufosinate-ammonium products on the market, on the other hand, are racemic. Meiji Pharmaceutical Co., Ltd. of Japan first registered the pure L-glufosinate-ammonium enantiomer glufosinate-P in 2014, making the pure L-glufosinate-ammonium enantiomer glufosinate-P a commercial pesticide.

R-enantiomers, S-enantiomers, and racemates all behave differently in soil, plants, and other non-target organisms, resulting in varied half-lives, degradation properties, and toxicity (Liu, Diao, Di, & Zhou, 2014; Kania-Korwel & Lehmler, 2013; Dong, Li, Chankvetadze, Cheng, Xu, Liu, Li, Chen, Bertucci, Tedesco, Zanasi, & Zheng, 2013; Ye, Zhao, Liu, & Liu, 2010; Ulrich, Morrison, Goldsmith, & Foreman, 2012). So, researchers are interested in learning more about the differences in behavior between rac-glufosinate-ammonium and glufosinate-P (pure L-glufosinate ammonium). According to Yue et al, rac-glufosinate-ammonium is substantially more toxic to zebrafish embryos than L-glufosinate-ammonium, hence they advocate using glufosinate-P (pure L-glufosinate ammonium) instead (Yue, Kong, Shen, Cui, & Zhao, 2020). In the research on the enantioselective degradation and chiral stability of glufosinate-ammonium in soil and water samples, our team discovered that L-glufosinate-ammonium degrades quicker than rac-glufosinate-ammonium in non-sterile soil and water samples. So, research into the bioaccumulation behavior of L-glufosinate-ammonium and racemic glufosinate-ammonium in organisms is currently lacking.

In agriculture, glufosinate-ammonium is frequently used. It is a severe hazard to water systems and human habitats because it is absorbed in the soil by organic matter and soil clay, spreading groundwater and surface runoff during rainfall (Lin, Liu, Fang, Qiu, & Yu, 2012). The precursor and metabolites of glufosinate-ammonium can be found in livestock after feeding them glufosinate-ammonium-containing feed (JMPR, 1999). The definition of the glufosinate residue for animal
commodities by many organisations (e.g. the European Union and Codex Alimentarius Commission) and countries (e.g. China and Japan) is the sum of glufosinate, N-acetyl-glufosinate (NAG) and 3-methylphosphinicopropionic acid (MPP) (Fig. S1), calculated as glufosinate. There has been no research on glufosinate-ammonium absorption and degradation in aquatic organisms.

Because glufosinate-ammonium is a strongly polar compound that is easily soluble in water, is not volatile, and lacks chromophores and fluorescent groups (Li et al., 2015; Oulkar, Hingmire, Goon, Jadhav, Ugare, Thekkumpurath, & Banerjee, 2017; Pinto, Soares, & Ferreira, 2018), glufosinate residues in the environment and food samples are mostly derivatised and then detected. Motojyuku et al. (2008) determined glufosinate-ammonium in human serum by gas chromatography-mass spectrometry (GC-MS) using N-methyl-N-( tert-butyl dimethylsilyl) trifluoroaceticamide as derivatisation reagent. By using a derivatisation step along with 9-fluorenylmethylloxycarbonyl chloride (FMOC-CI), the determination of glufosinate-ammonium residues in tea and water samples was optimised and reported by high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) (Pinto et al., 2018; Lin et al., 2012; Hanke, Singer, & Hollender, 2008). The sample preparation methods for pesticide determination in fat-containing matrices usually involve different analytical steps for the extraction, isolation and fractionation of the target pesticides from the bulk of the investigated matrix (Li, Chen, Zhang, Li, Zhou, & Ma, 2011; Tca et al., 2016). It could be predicted that the residue analysis method of glufosinate and its metabolites in fat-containing samples will be a challenging task. There has yet to be a method for determining glufosinate-ammonium residues in fish or comparable animal products. In this study, we developed a technique for detecting glufosinate-ammonium enantiomers and their metabolites (MPP, MPA and NAG) residues in zebrafish. The bio-concentration and elimination behavior of racemic glufosinate-ammonium and glufosinate-P (pure L-glufosinate ammonium) in the aquatic organism Danio rerio were investigated in this way, which offered scientific data for assessing the food safety of rac-glufosinate-ammonium and glufosinate-P (pure L-glufosinate ammonium) in fish.

Materials and methods

Chemicals and reagents

Dr. Ehrenstorfer GmbH provided the glufosinate racemate standard (97.5%); Shanghai Maclean Biochemical Technology Co (Shanghai, China) provided racemic glufosinate (97% purity); Shandong Pesticide Research Institute (Shandong, China) provided glufosinate-P (original powder; pure L-glufosinate ammonium) (93% purity, e.e 98.3%); Toronto Research Chemicals provided 95.0% of the sodium N-acetyl glufosinate; Wako Pure Chemical Industry Co., Ltd. supplied MPP (99.7%); and TLC Pharmaceutical Standards Ltd. provided the 2-methylphosphinic-acetic acid (MPA) (99.0%); Thermo Fisher Scientific (Waltham, MA, USA) provided the LC grade formic acid. All other chemicals and solvents are analytical grade, purchased from Jinshan Chemical Reagent Co., Ltd (Chengdu, China). Distilled water is purchased from Watsons Group (Hong Kong, China). Diatomaceous earth purchased from Tianjin Xiehe Haopeng Chromatography Technology Co., Ltd (Tian Jin, China). Oasis PRIME HLB cartridge (200 mg) were purchased from Waters Technology Co., Ltd (Massachusetts, USA).

Experimental apparatus and analysis condition of HPLC-HRMS

Dionex UltiMate 3000 SRSLC HPLC Injection system is equipped with SRD-3600 solvent degasser, HPG-3400RS binary pump, WPS-3000TR5 autosampler, and TCC-3000R constant temperature column box. A high-resolution mass spectrometer (Q-Exactive) is equipped with ESI ion source and Orbitrap Mass Spectrometry, purchased from Thermo Fischer Technologies Co., Ltd (Waltham, MA, USA). CROWNPAK CR (+) column with Silica gel coated with chiral crown ether (150 mm × 4.6 mm, 5 µm) bought from Daicel (Tokyo, Japan). The determination conditions of HPLC-Q-Exactive Orbitrap Mass Spectrometry refer to the method established in the early stage of this research group (Jia, Xu, Long, Ge, Chen, Hu, & Zhang, 2019), and the specific conditions and parameters are shown in Table S 1 (supporting information). In this method, the parentions (M + H+ ) were selected for quantitative analysis with the exact ion mass (182.05767, 139.01547, 153.03112 and 224.06624 m/z) for glufosinate, MPA, MPP and NAG, respectively.

Electronic balance (XSE-105) purchased from Mettler Toledo International Trade Co., Ltd (Shanghai, China). Ql-901 vortex mixer obtained from Jiangsu Haimen Kylin Medical Equipment Factory (Jiangsu, China). High-speed refrigerated centrifuge (ROTINA 380R) was prepared by Hettich Scientific Instrument Company (Hamburg, Germany). German adjustable micropipette was provided by Eppendorf (Hamburg, Germany). Dissolved oxygen analyzer (Hanna-9373S) was supplied by Shanghai Bilang Instrument Manufacturing Co., Ltd (Shanghai, China).

Zebrafish husbandry and chronic exposure and depuration experiment

The zebrafish, which weighed around 0.10 g and measured about 2-3 cm, were obtained from the China Zebrafish Resource Center in Wuhan, China. It was acclimated to 24-hour aeration and dechlorination tap water for 10–14 days before the experiment. The zebrafish is cultured in a fish facility (50 L) with a temperature of 25–27 ° C, a pH of 7.6–7.8, and a 14-/10-hour light/dark cycle. Domestication resulted in a mortality rate of less than 5%. The hardness is 210 ± 25 µg/mL (CaCO3), and the dissolved oxygen concentration is up to 80% of the air saturation value. Keep the fish healthy by feeding them twice a day with commercially available fish food and changing the water every 2 days.

Before the chronic exposure and depuration experiment, we first conducted a 96-hour acute toxicity test for both agents (rac-glufosinate-ammonium and glufosinate-P) and found that the lethality of both agents was less than 50% at a concentration of 100 µg/mL, which is considered low toxicity for zebrafish. Laboratory test results show that the half-life of glufosinate-ammonium in sterile buffer solutions of pH 5, 7 and 9 and natural water exceeds 400 days (Jia et al., 2019; Niu, Gui, & Zhu, 2010). The estimated environmental expected concentration (EEC) value of glufosinate in the Canadian environment is 1 µg/mL (Marvin, 1998). Therefore, for the evaluation of the bioconcentration and elimination behaviour of the two agents in zebrafish, the concentration was set as 2 and 10 µg/mL, which is slightly higher than the environmental concentration.

The bioconcentration and elimination of glufosinate-ammonium were conducted in accordance to the rules and regulations required by OECD guideline 305 (OECD, 2012). The absorption kinetics are studied using the bio-concentration test, and the depuration kinetics are determined using the removal test. For bioaccumulation exposure testing, use a semi-static system (14 days). The two exposure concentrations of rac-glufosinate-ammonium and glufosinate-P used in the experiment (2 µg/mL and 10 µg/mL) were determined. The aqueous solution used in the absorption experiment was dilutes in dechlorinated aerated tap water with the mother liquor of rac-glufosinate-ammonium and glufosinate-P. In the chronic exposure experiment, 190 active zebrafish were placed in a glass aquarium with 40 L of the test solution and a zebrafish-free glufosinate-ammonium aqueous solution as a control. Every three days, change the water. In 0–14 days, the bioaccumulation phase begins. On the 2 h, 1, 3, 5, 7, 10, and 14 days, remove 14 zebrafish from each culture tank and euthanize them with an aqueous solution containing ethyl 3-amino benzoate. Rinse the dead zebrafish twice with clean water and store at –80 ° C in the refrigerator. All remaining zebrafish were removed 14 days after the last bioaccumulation sample was taken, rinsed twice with clean water, and then placed in 20 L of glufosinate-ammonium-free water for 14–21 days for the depuration experiment. On days 14, 18, 15, 17, 19, and 21, samples (14 zebrafish) were obtained and refrigerated at –80 °C. We did all incubation experiments in

2
The preparation of standard solutions

Pure water was used to make a standard stock solution containing rac-glufosinate-ammonium (302.96 μg/mL), D-glufosinate-ammonium: L-glufosinate-ammonium = 1:1), MPP (212.85 μg/mL), NAG (222.3 μg/mL), and MPA (241.5 μg/mL). For each analyte, a standard mixture was prepared and serially diluted with water to 0.002, 0.004, 0.01, 0.1, and 0.2 μg/mL. We kept all solutions at 4 °C in the dark. By evaporating 1 mL of each concentration standard solution at 55 °C and dissolving in a 1 mL blank extract, a 0.002–0.2 μg/mL mixed matrix compatible standard of D-glufosinate-ammonium, L-glufosinate-ammonium, MPP, NAG, and MPA are obtained.

Pre-treatment and residue analysis of target analytes in zebrafish

Chop 1.0 g of zebrafish and place it in a 10 mL plastic centrifuge tube with 5 mL methanol–water (methanol:water = 10: 90) extractant with 0.5% formic acid. The solution was then vortexed for 2 min, sonicated for 10 min, then centrifuged for 5 min at 8000 rpm at 4 °C. In a 50-mL centrifuge tube, collect all the supernatants. To the residue, add 5 mL of methanol–water (methanol:water = 10:90) extractant containing 0.5% formic acid, vortex for 2 min, sonicate for 10 min, and centrifuge for 5 min at 4 °C at 8000 rpm. Combine the supernatants twice more, then add 20 mL dichloromethane and 1 g diatomaceous earth. The solution was then vortexed for 3 min before being centrifuged for 5 min at 4 °C at 8000 rpm. Before HPLC - high-resolution mass spectrometry (HRMS) residue analysis, pass 1 mL of centrifugal supernatant through an Oasis PRIME HLB (200 mg) cleanup column, filter through a 0.22 μm membrane, and transfer to an autosampler vial.

Because glufosinate-ammonium is soluble in water, 1 mL of water was taken directly from the culture tank to assess the glufosinate-ammonium level. A 0.22 μm membrane was used to filter the water sample before HPLC-HRMS analysis.

Method validation

The linearity, accuracy, precision, limit of quantification (LOQ), the limit of detection (LOD), and matrix effect (ME) of the devised method are all evaluated. The linearity of the solvent and matrix-matched calibration curves for D/L-glufosinate-ammonium, MPP, NAG, and MPA was validated at concentrations between 0.002 and 0.2 μg/mL. Five replicates of the spiked samples at three levels of glufosinate-ammonium and three metabolites in zebrafish matrix were prepared on three different days. The relative standard deviation (RSD) obtained from repeatability (intra-day) and reproducibility (inter-day) experiments is used to evaluate stability. The LOQ values are usually expressed in terms of concentrations that produce 10 times the signal-to-noise ratio (S/N), and the LOD value is determined using a signal-to-noise ratio of 3:1.

Statistical analysis

The stereoselective bioaccumulation of glufosinate-ammonium in zebrafish is measured using the enantiomeric ratio, which is expressed as the enantiomeric fraction (EF). Calculate EF using Equation (1).

\[
EF = \frac{C_{D}-\text{glufosinate}}{C_{D}-\text{glufosinate} + C_{L}-\text{glufosinate}}
\]

Where \( C_{D}-\text{glufosinate} \) and \( C_{L}-\text{glufosinate} \) is the D- and L-ammonium glufosinate concentrations (mg/kg), respectively; the range of EF values is 0 to 1, with a value of 0.5, indicating racemic.

Fitting the data to a first-order kinetics model estimated the dissipation kinetics of glufosinate-ammonium in zebrafish. Equation (2) was used to obtain the degradation rate constant \( k \), and Equation (3) was used to calculate the half-life \( t_{1/2} \), days.

\[
C = C_0 e^{-kt}
\]

\[
t_{1/2} = \ln2/k = 0.693/k
\]

Where \( C_0 \) and \( C \) are the initial concentration of stereoisomers in zebrafish (mg/kg) and the concentration of stereoisomers (mg/kg) at time \( t \) (days), respectively. \( k \) is the elimination rate constant.

The bio-concentration factor (BCF) of fish is defined as the ratio of the concentration of the measured substance in fish (CB) and the water, the bioaccumulation factor (BCF) is determined by Equation (4).

\[
BCF = \frac{C_{B}}{C_{W}}
\]

Where \( C_{B} \) (mg/kg) and \( C_{W} \) (mg/kg) are the average concentrations of enantiomers in zebrafish and water, respectively.

ME is determined based on the slope ratio of the matrix-matched calibration curve/pure water calibration curve 5 (Gosetti, Mazzucco, Zampieri, & Gennaro, 2010).

\[
ME(\%) = 100 \times \frac{K_{\text{matrix}}}{K_{\text{solvent}}}
\]

Where \( K_{\text{matrix}} \) is the slope of the matrix calibration curve in the matrix, and \( K_{\text{solvent}} \) is the slope of the standard curve in the solvent. ME = 100% is considered to have no matrix effect; ME > 100% has an enhanced matrix effect; and ME less than 100% has an inhibited matrix effect.

For statistical analysis, use IBM SPSS 16.0 and Microsoft Excel 2016. The differences were determined using a one-way analysis of variance (ANOVA), with a difference of p less than 0.05 regarded as statistically significant.

Results and discussion

Extraction and purification

Previous research extracted glufosinate from several types of samples using pure, ammoniacal, or acidic water (Oulkar et al., 2017; Li et al., 2015; Jia et al., 2019; Pinto et al., 2018), with excellent results. Because glufosinate is very soluble in water, the extracting solvent for glufosinate in all the samples was almost always an aqueous solution. An aqueous solution containing 10% methanol was added to fully extract glufosinate from fish tissues. As shown in Fig. S2 (Supporting Information), adding 0.5% formic acid to the extract improves the extraction effect, showing the peak of L-glufosinate-ammonium, especially at low glufosinate concentrations in zebrafish. So, methanol–water (methanol:water = 10:90) extractant with 0.5% formic acid was used to extract glufosinate from zebrafish. To investigate the effect of the extraction solvent volume on extraction efficiency of five target compounds in zebrafish, various volumes (e.g. 5, 10 and 5 + 5 mL) were investigated. As shown in Fig. 1 A, good recoveries (81% to 95%) of five analytes were achieved when the sample was extracted two times with 5 mL of extractant. Fig. 1 B showed the variation of analytes’ recovery versus the extraction time. With an increment in the extraction time from 5 to 10 min, the recoveries of analytes increased. However, when the extraction time reached 15 min, the growth of recoveries was not obvious. Therefore, 10 min was adopted. The polar extract was purified by liquid–liquid extraction to reduce some fats and phospholipids in zebrafish. Dichloromethane, ethyl acetate and n-hexane were chosen for the liquid extraction.

Dichloromethane, ethyl acetate and n-hexane were investigated. The recoveries of the five target analytes spiked were 68% to 75% without using liquid extraction to reduce some fats and phospholipids in zebrafish. To investigate the effect of the extraction solvent volume on extraction efficiency of five target compounds in zebrafish, various volumes (e.g. 5, 10 and 5 + 5 mL) were investigated. As shown in Fig. 1 A, good recoveries (81% to 95%) of five analytes were achieved when the sample was extracted two times with 5 mL of extractant. Fig. 1 B showed the variation of analytes’ recovery versus the extraction time. With an increment in the extraction time from 5 to 10 min, the recoveries of analytes increased. However, when the extraction time reached 15 min, the growth of recoveries was not obvious. Therefore, 10 min was adopted. The polar extract was purified by liquid–liquid extraction to reduce some fats and phospholipids in zebrafish.

Dichloromethane, ethyl acetate and n-hexane were chosen for the liquid extraction.
We employed diatomaceous earth to adsorb insoluble particles in this work. Finally, the sample was purified using Oasis PRiME HLB cartridge solid-phase extraction (SPE). The Oasis PRiME HLB SPE is a water-wettable sorbent made up of a macroporous copolymer of two monomers: lipophilic divinylbenzene and hydrophilic N-vinylpyrrolidone polymerized in a specific ratio. Oasis PRiME HLB SPE has been shown to remove phospholipid-disrupting matrices from plasma, urine, meat, and other biological samples in recent years (Liu, Wang, Zhang, & Shen, 2019; Zhai et al., 2018). The water-wettable feature of the Oasis HLB sorbent, in particular, allows the SPE purification process to be simplified by eliminating the conditioning and equilibration steps. Oasis PRiME HLB SPE was chosen to remove phospholipids and proteins from the zebrafish samples in this study. We determined that heavy variation can occur in the analyte peak when the extraction supernatant of zebrafish was not purified by the Oasis PRiME HLB SPE column (Fig. S3 in Supporting Information).

Method validation

For each analyte in zebrafish, calculate the regression equation, ME, LOQ, and LOD, and summarize in Table S 2 (Supporting information). The linear correlation between D-glufosinate-ammonium, L-glufosinate-ammonium, MPP, NAG, and MPA is good ($R^2 \geq 0.9915$). The LOQ for the glufosinate enantiomers and metabolites were 0.006–0.02 mg/kg, while the LODs of five target analytes in zebrafish and water is 0.002–0.006 mg/kg. Matrix effects, such as matrix augmentation or matrix restraint, are typical issues in HPLC-MS, and they can affect the method’s reproducibility and accuracy (Gosetti et al., 2010). D-glufosinate-ammonium, L-glufosinate-ammonium, NAG, MPP, and MPA have substrate effects of 6%, 19%, 4%, 10%, and 7% in zebrafish extract, respectively. The matrix effect values of the five substances in zebrafish and culture water are far less than 100%, and all five substances show significant substrate inhibition according to Formula (5) and the judgement criterion for ME. The linear equations and matrix effects of the five substances in zebrafish and culture water are shown in Tables S2 and S3 (Supporting information), respectively. Therefore, each analyte is quantified using the corresponding zebrafish matrix or water matrix standard to eliminate matrix effects and obtain more accurate results.

As indicated in Table 1, repeatability (intraday) and reproducibility (inter-day) are presented as relative standard deviation (RSD), which is used to measure the method’s accuracy. At spiked levels of 0.05–1 mg/kg, this technique yields a satisfactory mean recovery rate of between 77 and 104%. In zebrafish, all five analytes have good precision, with RSD values of less than 10.03%. The results show that this method’s sensitivity, accuracy, and precision suit the experimental requirements. Figure S 4 (Supporting information) shows a typical chromatogram of glufosinate-ammonium and its metabolites.

Concentration of glufosinate-ammonium in the exposure solution

In the exposure medium, the concentrations of rac-glufosinate-ammonium and glufosinate-P were measured (Fig. S5 Supporting information). Every 3 days, we replaced the exposed solution with a new aqueous solution containing glufosinate-ammonium during the incubation period. Each sampling point in Fig. S 5 (Supporting information) represents the concentration of the two enantiomers in the water before the solution is changed. The low-concentration D-glufosinate-ammonium and L-glufosinate-ammonium treatment groups remained at 1.00 ± 0.05 and 1.00 ± 0.04 μg/mL, respectively, when exposed to rac-glufosinate-ammonium, while the high-concentration treatment group remained at 5 ± 0.17 and 5 ± 0.15 μg/mL. So, the glufosinate-ammonium concentration remains constant during the incubation period. The EF values of glufosinate-ammonium at high and low concentrations were 0.5 ± 0.03 and 0.5 ± 0.03, respectively. In the presence of fish, the concentration of D/L-glufosinate-ammonium in water in the rac-glufosinate-ammonium exposure treatment group is equal, based
Bioaccumulation and elimination of rac-glufosinate-ammonium in zebrafish

As shown in Fig. 2, the quantity of the glufosinate-ammonium enantiomer from rac-glufosinate-ammonium in zebrafish was investigated. It covers bio-concentration for 0–14 days and removal for 14–21 days. The bioaccumulation of D-glufosinate-ammonium and L-glufosinate-ammonium increased with exposure time in the low treatment group (2 µg/mL). D-glufosinate-ammonium levels increased from 0.007 µg/mL on day 0 and peaked at 0.21 µg/mL on day 14. L-glufosinate-ammonium concentrations increased from 0.014 µg/mL on day 0 to 0.036 µg/mL on day 1, reaching a peak of 0.17 µg/mL at the end of the exposure experiment in the high concentration treatment group (10 µg/mL). The concentration of L-glufosinate-ammonium is always greater than the concentration of D-glufosinate-ammonium in the two-dose exposure bioaccumulation tests. From 10 to 14 days, both the low and high concentration treatment groups reached a concentration plateau.

At the end of the 14-day bioaccumulation, the BCF (as shown in Fig. 56) values of the low-concentration group D/L-glufosinate-ammonium were 0.18 and 0.22, respectively, while the BCF values of the high-concentration treatment group were 0.15 and 0.17, respectively. The low concentration treatment group’s BCF was slightly higher than the high concentration treatment group.

The EF in the low treatment group (as shown in Fig. S7, in Supporting Information) was 0.71–0.76 during the incubation period, while it was 0.69–0.71 in the high treatment group. Enantioselective bioaccumulation is indicated by a deviation of the EF value from 0.5, showing that L-glufosinate-ammonium is preferentially enriched in zebrafish. L-glufosinate-ammonium had a higher BCF value than D-glufosinate-ammonium during the bioaccumulation phase. These findings suggest zebrafish collect L-glufosinate-ammonium rather than D-glufosinate-ammonium during the bioaccumulation phase. These findings are consistent with previous research on the chiral bioaccumulation behavior of tebuconazole in zebrafish (Liu, Dong, Xu, Liu, & Zheng, 2016), and they reveal that the enantiomers of tebuconazole in zebrafish have much better enantioselectivity when compared to high-dose treatment. But, a study by Lv (Lv, Liu, Li, Gao, Wang, Li, & Guo, 2014) found that the stereoselectivity of flucycloxazole bioaccumulation in whitefly larvae was not significant at low doses when compared to high doses. So, a variety of factors influenced the enantioselectivity of chiral pesticides in organisms, including the pesticides’ physicochemical properties, the organisms’ absorption, and metabolism, and the enzyme system in the body.

Table 1: Recoveries and RSDs (Intraday and Inter-day) for glufosinate-ammonium, NAG, MPP, and MPA in zebrafish.

| Matrix   | Spiked level (mg/kg) | Compound | Average recovery (%), intraday RSD (%), n = 5 | Interday RSD (%), n = 5 |
|----------|---------------------|----------|---------------------------------------------|------------------------|
|          |                     |          | Day 1       | Day2      | Day 3       |                      |
| Zebrafish| 0.05                | D-glufosinate | 83.67 | 101.0 | 8.77 | 101.0 | 8.77 | 9.95 |
|          |                     | L-glufosinate | 96.95 | 90.70 | 9.16 | 93.00 | 6.85 | 9.74 |
|          |                     | NAG       | 88.83 | 77.00 | 6.04 | 78.65 | 7.57 | 9.72 |
|          |                     | MPP       | 80.07 | 96.4 | 8.86 | 87.21 | 8.37 | 10.03 |
|          |                     | MPA       | 85.87 | 81.29 | 8.78 | 83.03 | 9.07 | 6.30 |
|          | 0.1                 | D-glufosinate | 98.18 | 95.00 | 7.28 | 99.00 | 1.51 | 5.01 |
|          |                     | L-glufosinate | 100.9 | 85.26 | 9.68 | 98.03 | 6.68 | 5.13 |
|          |                     | NAG       | 99.53 | 83.01 | 5.15 | 85.25 | 9.39 | 7.50 |
|          |                     | MPP       | 84.61 | 82.02 | 5.27 | 85.76 | 3.84 | 2.11 |
|          |                     | MPA       | 92.77 | 83.77 | 9.45 | 79.01 | 6.45 | 6.21 |
|          | 1                   | D-glufosinate | 100.4 | 88.01 | 8.51 | 90.03 | 3.40 | 3.67 |
|          |                     | L-glufosinate | 99.89 | 102.6 | 8.72 | 100.0 | 3.21 | 3.78 |
|          |                     | NAG       | 98.65 | 83.77 | 9.45 | 83.03 | 9.37 | 6.49 |
|          |                     | MPP       | 89.54 | 81.29 | 8.78 | 83.03 | 9.37 | 6.49 |
|          |                     | MPA       | 96.11 | 85.76 | 8.86 | 87.21 | 8.37 | 10.03 |

Fig. 2. Bioaccumulation and purification of rac-glufosinate-ammonium in zebrafish (A) at low concentration (2 µg/mL) (B) at high concentration (10 µg/mL).
The concentration of glufosinate-ammonium in zebrafish during the elimination phase was displayed in Fig. 2 from 14 to 21 days. Table 2 shows that the degradation half-lives of low-concentration D/L-glufosinate-ammonium were 2.20 and 1.63 days, respectively, with elimination rates of 92.05 and 96.13% at 7 days of the elimination phase. Similarly, the half-lives of high-concentration D/L-glufosinate-ammonium were 2.39 and 1.54 days, with 92.85 and 96.02% elimination rates at 7 days, respectively. D/L-glufosinate-ammonium is quickly eliminated during the elimination phase. The elimination speed of L-glufosinate-ammonium is faster than D-glufosinate-ammonium in terms of half-life.

Bioaccumulation and purification of glufosinate-P in zebrafish

The bio-concentration and elimination behavior of glufosinate-ammonium-P was investigated, as shown in Fig. 3. L-glufosinate-ammonium increased from 0.03 mg/kg on day 0 to 0.56 mg/kg on day 14 in the low-concentration treatment group, while it increased from 0.15 mg/kg on day 0 to 2.02 mg/kg on the 14th day in the high-concentration treatment group. The BCF values of the low and high concentration treatment groups (as shown in Fig. 3) were 0.28 and 0.21, respectively, at the end of the bio-concentration (14 days). The elimination equations for glufosinate were \( C_t = 1.4904e^{-0.304t} \) and \( C_t = 0.4679e^{-0.304t} \), with \( R^2 \) values of 0.8953 and 0.9331 for the high and low dose groups, respectively. The high and low dose groups had the same glufosinate elimination rates, and the zebrafish had a half-life of less than 2.3 days.

Differences in the bio-concentration and elimination behavior of racemic glufosinate and glufosinate-P

Racemic glufosinate-ammonium (containing D-glufosinate-ammonium and L-glufosinate-ammonium) and glufosinate-P (pure L-glufosinate-ammonium) bio-concentration and the elimination characteristics were examined. Rac-glufosinate-ammonium and glufosinate-P have the same total concentration, with a low concentration of 2 \( \mu \)g/mL and a high concentration of 10 \( \mu \)g/mL. Total glufosinate-ammonium levels increased from 0.02 mg/kg and 0.22 mg/kg on day 0 to 0.38 mg/kg and 1.6 mg/kg on day 14, respectively, in the low- and high-dose racemic glufosinate-ammonium treatment groups. The BCF values of rac-glufosinate-ammonium at 14 days were 0.19 and 0.16, respectively, in the low-dose and high-dose treatment groups. When the 14 days BCF of glufosinate-ammonium was compared to that of racemic glufosinate-ammonium at the two doses of 0.28 and 0.21, the accumulation of glufosinate-P in zebrafish was greater.

The elimination half-lives of rac-glufosinate-ammonium were 1.84 and 1.93 days for low-dose and high-dose treatments, respectively, in the elimination phase (see Table 5, in Supporting Information). In low- and high-dose treatment, the elimination half-life of glufosinate-P is 2.28 and 2.24 days, respectively (see Table 5, in Supporting Information). The results reveal that low- and high-dose treatments for rac-glufosinate-ammonium or glufosinate-P had the same elimination half-life. Zebrafish eliminate rapidly rac-glufosinate-ammonium and glufosinate-P, with an elimination half-life of less than 2.3 days.

| Concentration (\( \mu \)g/mL) | Stereosomer | Regressive function | \( R^2 \) | \( T_{1/2} \) (d) |
|-----------------------------|-------------|---------------------|---------|-------------|
| 2                           | D-glufosinate | \( C_t = 0.12e^{-0.313t}\) | 0.8904 | 2.20        |
|                             | L-glufosinate | \( C_t = 0.1374e^{-0.292t}\) | 0.8537 | 1.63        |
| 10                          | D-glufosinate | \( C_t = 0.5264e^{-0.290t}\) | 0.7212 | 2.39        |
|                             | L-glufosinate | \( C_t = 0.697e^{-0.440t}\) | 0.9005 | 1.54        |

Formation of metabolites in zebrafish

During the bioaccumulation phase, the metabolites of glufosinate-ammonium, MPP, NAG, and MPA were examined, as shown in Fig. 4. MPP was not detected until 3 days into the bioaccumulation period, NAG was not detected until 5 days, and MPA was lower than LOD throughout the bioaccumulation period. During the bioaccumulation phase, the metabolites MPP and NAG rose with time and peaked at 14 days. MPP increased from 0.005 mg/kg to 0.031 mg/kg on the 14th day in the low-dose treatment group, and from 0.012 mg/kg to 0.051 mg/kg in the high-dose treatment group during the rac-glufosinate-ammonium accumulation period. In the low-dose treatment group, MPP production increased from 0.01 to 0.039 mg/kg, while in the high-dose treatment group, MPP production increased from 0.019 to 0.071 mg/kg during the glufosinate-P accumulation period. The high and low-dose NAG quantities in the rac-glufosinate-ammonium exposure solution were 0.021 mg/kg and 0.014 mg/kg, respectively, while the high and low-dose NAG amounts in the glufosinate-P exposure solution on the 14 days were 0.027 mg/kg and 0.014 mg/kg, respectively. Glufosinate-P always creates more metabolites (NAG and MPP) than rac-glufosinate-ammonium when the total concentration of glufosinate is the same. We believe that zebrafish have a very low metabolite content. MPP arrived first and had a higher concentration than NAG. The metabolites MPP and NAG are rapidly removed 2 h after elimination and are below the LOD during the elimination phase.

Conclusion

We developed an effective approach for detecting glufosinate-ammonium and metabolite residues in fish in this investigation. The differences between rac-glufosinate-ammonium and glufosinate-P were compared, as well as the selective bioaccumulation and elimination behaviors of glufosinate-ammonium in zebrafish. The findings reveal that L-glufosinate-ammonium has a clear preference for bioconcentration, with selectivity being greater at low concentrations than at high concentrations. The accumulation of glufosinate-P in zebrafish was larger than that of racemic glufosinate-ammonium when the total quantity of glufosinate-ammonium was the same. Also, at equilibrium, the BCF value of glufosinate is less than 0.3, showing that glufosinate is a low bioaccumulation pesticide in zebrafish. D/L-glufosinate-ammonium was quickly eliminated in the elimination phase, with an elimination half-life of less than 2.3 days. Both metabolites created in zebrafish indicated NAG was less abundant than MPP, and the greatest metabolite MPP produced was very tiny, accounting for less than 4% of the parent culture concentration. This is the first study that we are aware of that shows glufosinate bioaccumulation depression in fish under controlled laboratory conditions. Low bioaccumulation and fast elimination imply the food contamination of fish by rac-glufosinate-ammonium and glufosinate-P in environment is low.

CRediT authorship contribution statement

Fei Wang: Conceptualization, Methodology, Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision. Qiao Lin: Conceptualization, Validation, Formal analysis, Data curation, Writing – review & editing, Supervision. Xueqin Shi: Validation, Formal analysis. Yunfang Li: Methodology, Validation, Formal analysis. Pengyu Deng: Methodology, Validation. Yuping Zhang: Conceptualization, Methodology, Validation, Formal analysis, Resources, Data curation, Writing – review & editing, Supervision, Funding acquisition. Deyu Hu: Writing – review & editing, Visualization, Supervision, Funding acquisition.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2022.100383.

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