Chemosphere 128 (2015) 293–298

Contents lists available at ScienceDirect

Chemosphere

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

Alteration in the cytokine levels and histopathological damage in common carp induced by glyphosate

Junguo Ma, Xiaoyu Li *

College of Life Science, Henan Normal University, Xinxiang, Henan 453007, China

HIGHLIGHTS

- Glyphosate has low toxicity on common carp.
- Glyphosate-exposure alters the contents of cytokines.
- Glyphosate caused histopathological damage to common carp.
- Glyphosate has immunotoxic effects on common carp.

ARTICLE INFO

Article history:
Received 13 March 2014
Received in revised form 7 February 2015
Accepted 10 February 2015
Available online 3 March 2015

Handling Editor: David Volz

Keywords:
Glyphosate
Common carp
Cytokine
Immunotoxicity
Histopathology

ABSTRACT

Glyphosate is one of the most frequently used herbicides, and it has been demonstrated to generate a series of toxicological problems in animals and humans. However, relatively little is known about the effects of glyphosate on the immune system of fish. In the present study, the acute toxicity of glyphosate on common carp was first determined; then, the contents of interferon-γ (IFN-γ), interleukin-1β (IL-1β), and tumor necrosis factor -α (TNF-α) and histopathological alterations in the liver, kidneys, and spleen of common carp exposed to 52.08 or 104.15 mg L^{-1} of glyphosate for 168 h were also determined and evaluated. The results of the acute toxicity tests showed that the 96 h LC_{50} of glyphosate for common carp was 520.77 mg L^{-1}. Moreover, sub-acute exposure of glyphosate altered the contents of IFN-γ, IL-1β, and TNF-α in fish immune organs. For example, there was a remarkable increase in the IFN-γ content in the kidneys, while there was a decrease in the liver and spleen. The IL-1β content increased in liver and kidneys, but it decreased in the spleen, and TNF-α mainly increased in the fish liver, kidneys, and spleen. In addition, glyphosate-exposure also caused remarkable histopathological damage in the fish liver, kidneys, and spleen. These results suggest that glyphosate-caused cytokine alterations may result in an immune suppression or excessive activation in the treated common carp as well as may cause immune dysfunction or reduced immunity. In conclusion, glyphosate has immunotoxic effects on common carp.

1. Introduction

Glyphosate (N-[phosphonomethyl] glycine, $C_3H_8NO_5P$) is a broad-spectrum, nonselective, and nonsystemic herbicide that is frequently used in agricultural and non-agricultural systems (Baylis, 2000). Glyphosate and its putative metabolite aminomethylphosphonic acid (AMPA) have been found in urban streams, and the half-life of glyphosate in aquatic environments is normally in the range of 7–14 days (Gholami-Seyedkolaei et al., 2013). In recent years, the adverse effect of glyphosate on fish has received substantial attention (Glusczak et al., 2007; Lushchak et al., 2009) although it has been considered to have relatively low toxicity for aquatic organisms, including fish (Solomon and Thompson, 2003). There have been many few reports about the toxicity of glyphosate in fish, such as liver histological alterations induced by sublethal glyphosate exposures in common carp (Neskovic et al., 1996), Nile tilapia (Jiraungkoorskul et al., 2002), and curimbatá (Langiano and Martinez, 2008); biochemical toxicity on common carp (Gholami-Seyedkolaei et al., 2013); increased level of cortisol, plasma glucose, and catalase activity in silver catfish (Cericato et al., 2008) and curimbatá (Langiano and Martinez, 2008); and a significant reduction in superoxide dismutase, glutathione S-transferase, and glutathione reductase in goldfish (Lushchak et al., 2009). Meanwhile, the low concentrations of glyphosate in rice or soybean fields might cause alterations in the metabolic and enzymatic parameters of silver catfish (Glusczak et al., 2007) and other fish species (Glusczak et al., 2006; Lushchak et al., 2009).

* Corresponding author. Tel./fax: +86 373 3329390.
E-mail address: lixiaoyu65@263.net (X. Li).

http://dx.doi.org/10.1016/j.chemosphere.2015.02.017
0045-6535/

Due to the extensive use of glyphosate in agriculture, it may enter the surface water via spray drift, leaching from the soil and water or running off from agriculture and exerting adverse effects on fish (WHO, 2005; Gholami-Seyedkolaei et al., 2013). Glyphosate is highly soluble in water, and its half-life can vary from a few days to 60 days, depending on the conditions in the environment (Vereecken, 2005). Mörtl et al. (2013) reported that the glyphosate concentrations in the surface and ground water were nearly 1 mg L^{-1} within some samples from Békés County in Hungary. Moreover, Sobrero et al. (2007) found that the concentration of glyphosate in the Argentine water bodies covered a range of 0.1–80 mg L^{-1}, while Peruzzo et al. (2008) reported that the levels of glyphosate were 0.1–0.7 mg L^{-1} in water bodies located near the agricultural plantations of the Pampa Region (Buenos Aires, Argentina), which are higher than the drinking water limits recommended by the US Environmental Protection Agency (EPA) (0.7 mg L^{-1}) or European standard (0.1 μg L^{-1}) (WHO, 2005; CCME, 2012). Therefore, glyphosate residue in a body of water may threaten the survival of fish.

The fish immunologic system is the first defense system against pathogens or environmental chemicals (Burns-Naas et al., 2001). It is also very sensitive to internal or environmental pressure and responds rapidly to pathogen infection or toxins from the aquatic environment by creating changes in the activities or contents of the immunologic parameters, such as interleukins, interferons, and tumor necrosis factor, which may be early indicators of environmental toxicant stress (Colosio et al., 2005).

Even though adverse effects of commercial glyphosate on fish physiological and biochemical parameters have been reported, little is known about its effect on the immune system of fish. For example, El-Gendy et al. (1998) found a decrease in the total protein content of serum that was concomitant with a change in the serum antibody titers in glyphosate-treated bolti fish. Kreutz et al. (2011) also reported an altered phagocytic index, serum bacteria agglutination, and lysozyme activity in silver catfish following glyphosate-exposure. In addition, Ma et al. (2015) found that glyphosate-exposure suppressed the expression levels of IgM, C3, and LYZ in the kidneys of common carp. The present study aimed to evaluate the acute toxicity, immunological and histopathological effects of glyphosate on common carp, *Cyprinus carpio* L.

2. Materials and methods

2.1. Glyphosate, kits, and chemicals

Glyphosate was purchased from Anyang Anlin Agrochemical Co., Ltd., China as a commercial formulation (50% soluble powder). Glyphosate was first dissolved in distilled water to generate a stock solution and then diluted to obtain the experimental concentrations with dechlorinated tap water.

The kits for assaying interferon-γ (IFN-γ) (Catalogue No. DRE96157), interleukin-1β (IL-1β) (Catalogue No. DRE96017), and tumor necrosis factor-α (TNF-α) (Catalogue No. DRE96155) were purchased from the Wuhan ColorfulGene Biological Technology Co., LTD, China. The other reagents used in this study were purchased from Sigma (St. Louis, USA) and were analytical grade.

2.2. Fish

Common carp (8.14 ± 1.37 g of mean body weight) were originally obtained from a local fish farm (Feilong aquarium fishery, Xinxiang, China). The fish were subjected to a prophylactic treatment by bathing twice in 0.05% potassium permanganate for 2 min before they were raised in a 200-L tank under laboratory conditions for at least two weeks before the test. The characteristics of the water were as follows: dissolved oxygen of 7.0 mg L^{-1}, total hardness of 340 mg L^{-1}, pH of 7.6, turbidity of 1.5 NTU, and total dissolved solid content of 660 mg L^{-1}. Fish were maintained at 25 ± 2 °C and exposed to a 16-h light/8-h dark photoperiod. The water in the tank was partially changed every day with aerated tap water. During the acclimatization, the fish were fed with commercial food from the Feilong aquarium fishery at a day-rate of 1–1.5% of fish body weight. The fish were handled according to the guidelines in the China Law for Animal Health Protection and Instructions for Granting Permits for Animal Experimentation for Scientific Purposes (Ethics approval No. SCXK (YU) 2005–0001).

2.3. LC_{50} determination, sub-acute exposure of glyphosate, and sampling

A total of 160 healthy fish were used in the acute toxicity bioassays to determine the 96 h LC_{50} of glyphosate. The toxicity test design and exposure concentrations were based on the Spearman–Kärber method (Kärber, 1931) with modification (Zhang and Liu, 1997). Preliminary acute toxicity tests were conducted to determine the concentration range that causes 0–10% and 90–100% mortality in fish. Then, the acute toxicity testing was performed with 96 h of glyphosate-exposure in a 30 L glass jar at seven concentrations (771.70, 714.52, 661.58, 612.55, 567.22, 525.18, and 486.26 mg L^{-1}) of glyphosate, and one control group was treated with aerated tap water (0 mg L^{-1} of glyphosate). The various concentrations of glyphosate for acute exposure were designed according to the results of our primary acute toxicity test and the method of Spearman–Kärber (Kärber, 1931). A total of 80 fish were randomly divided into 8 groups (7 treatment groups and one control group with 10 fish in each group), and they were exposed to glyphosate solution under semi-static conditions for 96 h. During the treatment, no food was provided, but saturated oxygen was maintained in the solution for every group, and the water for all groups was completely changed every day. Each test was performed in duplicate. During the period of testing, the fish behavior was observed and dead fish were counted and removed from the aquarium. After 96 h of glyphosate-exposure, the LC_{50} was calculated with SPSS13.0 software according to the recorded number of dead fish in every group.

For the sub-acute exposure of glyphosate, 54 fish with the same body weight as above were randomly divided into three groups; two groups were the glyphosate-treatment groups (1/10 of 96 h LC_{50} and 1/5 of 96 h LC_{50}) and one was the control with aerated tap water. There were 18 fish in each group. The fish in the treatment group were exposed to glyphosate solution at concentrations of 1/10 of 96 h LC_{50} or 1/5 of 96 h LC_{50} under semi-static conditions for 168 h. During the treatment, no food was provided to avoid interference or an adverse effect on the following biochemical assay from differences in ingestion and digestion between the treatment and control groups, but saturated oxygen was maintained for the fish. The water in the three groups was completely changed daily. No fish died during the testing period. Each test was performed in duplicate.

After 24, 72, and 168 h of glyphosate-exposure, respectively, 6 fish from every group were taken each time. The carp were anaesthetized with 100 mg L^{-1} MS-222 (Tricaine) and dissected; then, the tissues (liver, kidneys, and spleen) were rapidly isolated and washed with cold PBS. One part of the tissue was stored at −80 °C until it was biochemically assayed and the other was placed in the solution of 10% neutral-buffered formalin for histopathological analysis.