Ecotoxicology and Environmental Safety 161 (2018) 342–349

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Acute and Subacute toxicity study of Olaquindox by feeding to common carp (*Cyprinus carpio* L.)

Qian Yang[a,b,1], Jie He[a,b,1], Sheng-yu He[a,b], Kai-yu Wang[a,b,*], Yi Geng[a,b], De-fang Chen[b,c], Xiao-li Huang[b,c], Ping Ou-yang[a,b]

[a] Department of Basic Veterinary, College of Veterinary Medicine, Sichuan Agricultural University, Wenjiang District, Huimin Road No. 211, Chengdu 611130, Sichuan, PR China

[b] Key Laboratory of Animal Disease and Human Health of Sichuan Province, Sichuan Agricultural University, Wenjiang District, Huimin Road No.211, Chengdu 611130, Sichuan, PR China

[c] Department of Aquaculture, College of Animal Science & Technology, Sichuan Agricultural University, Wenjiang District, Huimin Road No.211, Chengdu 611130, Sichuan, PR China

ARTICLE INFO

Keywords:
Olaquindox
Toxicity
Cyprinus carpio L.
Accumulation
Histopathology

ABSTRACT

Olaquindox, is a growth-promoting feed additive for food-producing animals. As the banned medicinal feed additive, olaquindox in animal feed and water must be concerned as an important hazard index. To improve studies of the toxicity of olaquindox, we provide a toxicological effects of olaquindox on a common freshwater fish, *Cyprinus carpio* L. The results of acute toxicity tests showed that the 7d-LD_{50} of olaquindox administered by feeding for common carp was determined to be 3746.3 mg/kg. We also found that the accumulation coefficient of olaquindox in carp was 1.45–1.9. Based on the studied hematological and blood biochemical parameters (RBCs count, hemoglobin content, ALT, AST and SOD activity), we found that olaquindox induced significant alterations in all studied parameters. Regarding bioaccumulation, the results showed that olaquindox had more efficiency to internalize fish tissues (liver, kidneys and muscle). The histopathological investigation of tissues from poisoning fish revealed various alterations that varied between adaptation responses and permanent tissue damage. Our results indicate that olaquindox are toxic to common carp and have obvious accumulation, and all the data from acute and subacute toxicity experiments in common carp may provide a useful tool for assessing the toxicity of olaquindox to aquatic organisms.

1. Introduction

Olaquindox, derived from quinoxaline-N, N-dioxides, has been widely used as a growth-promoting feed additive to improve feed conversion efficiency. In addition, olaquindox presents broad antibacterial spectrum and strong inhibition of bacterial growth, it has been used as treatment of bacterial disease etc (Liu et al., 2010). Therefore, olaquindox also has been used in fish formula feed in 1980s. However, olaquindox has a severe side effect, and there are reports about the accumulative toxicity, mutagenicity, genotoxicity, carcinogenicity, and phototoxicity of olaquindox (Belhadjali et al., 2002; Ihsan et al., 2013; Neumann et al., 2005; Wang et al., 2015; Zou et al., 2011). In 1999, the application of olaquindox has been banned by Commission of the European Community as the raising concerns over the food safety (Song et al., 2011). The Agricultural Minister of China prohibited its usage in livestock breeding except young swine feeding (≤ 35 kg) (Zhang et al., 2013).

Nevertheless, the immense and imprudent use of olaquindox is still affecting domestic animals' health directly. It is clear that the feed applied to aquaculture with a high concentration of olaquindox or olaquindox contaminated water sources can eventually lead to its residues in fish, which can damage the fish health. The animal food and water risk assessment of olaquindox has been conducted in previous literatures (Wang et al., 2012). But few researches have been done to evaluate the ecotoxicity of olaquindox in aquatic systems. Therefore, the potential toxicity of olaquindox should not be ignored.

Studying different hematological parameters and calculated blood indices in fish play an important role in understanding either normal or pathological processes, they are important health indicators (Saravanan et al., 2011). Hematological and biochemical parameters are closely

* Corresponding author at: Department of Basic Veterinary, College of Veterinary Medicine, Sichuan Agricultural University, Wenjiang District, Huimin Road No.211, Chengdu 611130, Sichuan, PR China.
E-mail address: kywang1955@126.com (K.-y. Wang).
[1] The first two authors contributed equally to this work.

https://doi.org/10.1016/j.ecoenv.2018.05.079
Received 24 January 2018; Received in revised form 25 April 2018; Accepted 24 May 2018

related to the response of the animal to the environment, an indication that the environment where fish lives could exert some influence on the blood characteristics (Fernandes et al., 2003). Moreover, the histopathology reflects the true health state of the affected organism during the assessment of aquatic contaminations, it has been recommended as a highly relevant methodology (Velma and Tchounwou, 2010). Actually, the toxic deposition in internal tissue is the major route through which a certain pollutant can transfer across the food chain and finally assimilated by human consumers resulting in health risks (Abdel-Khalek, 2015). Therefore, determination of accumulated olaquindox in fish is really significant from toxicological point of view. The present study using common carp as an animal model aimed to declare the olaquindox accumulation levels and toxic effects. In addition, this study also presented several hematological indicators, blood biochemical parameters and histopathological pictures to give a complete understanding about the health state of the exposed fish.

2. Materials and methods

2.1. Fish

Healthy common carp (60 ± 5 g) were purchased from a fishery in Ya'an, Sichuan Provence. Common carp were feed in aquarium with 24 h continuous aeration where they maintained for 14 days. The water temperature was kept at 25–28 °C, while pH and dissolved oxygen were 6.8–7.2 and 8–12 mg/L, respectively.

2.2. Acute toxicity test

To determine the LD50, olaquindox were fed with commercial pellets in 6 different doses (1400 mg/kg, 1960 mg/kg, 2744 mg/kg, 3842 mg/kg, 5378 mg/kg and 7350 mg/kg). Here, 210 fish were randomly divided into seven groups (one control group and six treatment groups), each group contained 30 fish. In each treatment, fish were exposed to these doses for 7d. Test water was renewed once daily. Mortality was recorded every 12 h. The dead fish were removed from the aquaria immediately. Finally, the LD_{50} was determined by the Karber's method.

2.3. Accumulation coefficient analysis

The accumulative effect was detected by degrees' dye-poison method. 60 fish were randomly divided into 3 groups (2 test groups and 1 control group), each group contained 20 fish. The fish of test groups was given olaquindox by esophageal feeding with the dose of 0.1 LD50 daily in the first 4 days and 1.5 fold dose in the next 4 days, until the test fish occurred half of the death. Accumulative coefficient (K) was calculated afterwards.

2.4. Subacute toxicity test

2.4.1. Exposure test

Experimental fish were randomly divided into four groups: three olaquindox treatment groups, named group I (1 g/kg), group II (2 g/kg) and group III (3 g/kg), and one control group. Each group was 40 fish and 2 replicates. The fish were exposed under olaquindox by feeding with commercial pellets for 6 weeks.

2.4.2. Hematological and blood biochemical analysis

Blood samples were collected from the caudal vein of the studied fish at 2, 3, 4, 5 and 6 weeks. Heparin was used as anticoagulant. Blood samples were mixed and diluted with physiological saline solution (0.9% NaCl). The red blood cells (RBCs) were counted using improved hemocytometer. Hemoglobin (Hb) concentration was determined by Drabkin (1964) method (Drabkin, 1946), and cyanomethemoglobin was measured spectrophotomerically at 540 nm wavelength. The serum

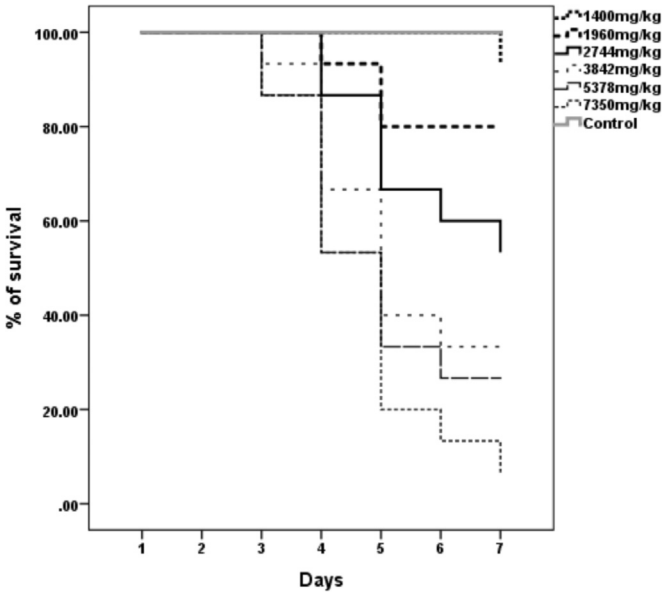

Fig. 1. Survival (%) of common carp during the acute experiment. Fish were exposed to 6 different doses (1400 mg/kg, 1960 mg/kg, 2744 mg/kg, 3842 mg/kg, 5378 mg/kg and 7350 mg/kg) for 7d.

aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity was determined according to IFCC (International Federation of Clinical Chemistry) recommended method without pyridoxal phosphate. The pyrogallol autoxidation method was used to determinate the superoxide dismutase (SOD) activity in erythrocytes. And SOD extract of erythrocytes was examined by means of a polyacrylamide gel electrophoresis to investigate the isoenzymic changes.

2.4.3. Histopathological examination

The clinical symptoms were daily observed during the experimental period. The liver, kidney, muscle, spleen and gill were collected from fish to observe the pathological changes. Histopathology was performed according to the traditional method. Briefly, the tissues of the carp were removed and fixed in 10% buffered formalin and allowed to fix for 24 h. The fixed tissues were routinely processed for histopathology in paraffin embedding, and the 5 μm thickness tissue sections were stained with haematoxylin and eosin (H&E). After staining, the sections were observed with Nikon microscope (Japan). Ultrathin sections were double-stained with uranium acetate and lead citrate and photographed by a transmission electron microscope (Jiang Guang H-600).

2.4.4. Determination of the accumulated olaquindox in the studied tissues

The concentration of olaquindox in tissues of common carps was determined by high performance liquid chromatography (HPLC). At the end of the experiment, the muscle, liver and kidney samples were collected from four randomly selected fish from each group. The samples of tissues were extracted and purified. Briefly, about 1 g thawed and homogenized sample was weighed into 1.5 ml polypropylene tube. 1 ml saturated ammonium sulfate solution were added. The mixture was shaken 10 min and then centrifuged for 15 min (4000 r/min). After centrifugation, the supernatant was collected, then 1 ml potassium dihydrogen phosphate (1 mol/L) and 5 ml of extract solution (acetonitrile: ethyl acetate = 3:2) were added into the supernatant. The mixture was shaken 20 min and then centrifuged for 10 min (4000 r/min). The organic phase was collected and the residue was extracted once. The twice organic phases were combined, and 1 ml of 0.2 mol/L NaOH was added. The mixture was shaken for 10 min and was placed stably for 10 min. The organic phase was collected and dried under nitrogen at 60–80 °C. Finally, the extract was stored at-20 °C until assayed. The olaquindox in the extraction liquid was separated and determined by