Toxicity and Effects of the Herbicide Glufosinate-Ammonium (Basta) on the Marine Medaka *Oryzias dancena*

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Abstract

Glufosinate-ammonium, a component of the herbicide Basta, is one of the most extensively used pesticides worldwide. In this study, we assessed subchronic and chronic toxicities of Basta and its histopathological effects on the marine medaka *Oryzias dancena*. Marine medaka were exposed to 0, 2, 4, or 8 mg/L of Basta for 28 or 42 days. The lethal concentration (LC₅₀) of Basta for 96 h is 8.76 mg/L. Histological changes in the gills and liver were evaluated with histopathological indices, allowing quantification of the damage to fish exposed to Basta. Blood congestion, lamellar fusion, and epithelial lifting were observed in the gills, and hydropic degeneration, fibrosis, lipid degeneration, leukocyte infiltration, and necrosis were found in the liver. These responses could be useful indicators of Basta toxicity in this species.

Key words: Basta, Glufosinate-ammonium, Histopathology, Marine medaka, *Oryzias dancena*

Introduction

Glufosinate-ammonium (Basta; Bayer CropScience, Monheim am Rhein, Germany), a nonselective (broad-spectrum) herbicide, is extensively used to control a wide range of weeds in agricultural and industrial areas (Cox, 1996; Orme and Kegley, 2006). It is used as a preharvest desiccant for a variety of crops, including potatoes, peas, beans, and cereals (PAN UK, 1998), and it also being increasingly used as a selective herbicide on transgenic crops that have been genetically modified to tolerate it (Bayer CropScience, 2007; Duke and Cerdeira, 2010). Glufosinate-ammonium acts by blocking the activity of an enzyme involved in the synthesis of the amino acid glutamine (Hoerlein, 1994; Cox, 1996), and its inhibition of glutamate synthetase has been reported not only in plants but also in animals and humans (Cox, 1996; HSD, 2003). The World Health Organization (WHO) classifies glufosinate as an acute hazard (class III), and the United States Environmental Protection Agency (U.S. EPA) classifies it as “persistent” and “mobile.” However, it is registered for use in over 80 countries (Bayer CropScience, 2005) and is sold under several trade names, including Basta, Rely, Finale, Ignite, Challenge, and Liberty (Jewell and Buffin, 2001).

The exposure of nontarget aquatic organisms to glufosinate formulations by pollution of rivers and marine environments through runoff is a concern because the compound is highly water-soluble and extensively used (Cox, 1996; Jewell and Buffin, 2001). Glufosinate has been detected in soils (EFSA, 2005), groundwater (HSD, 2003; EFSA, 2005), and surface waters and sediments (KEMI, 2005). Tests of the effects of an herbicide containing glufosinate-ammonium on aquatic organisms (sheepshead minnow *Cyprinodon variegatus*, rainbow trout *Oncorhynchus mykiss*, and water flea *Daphnia magna*) showed that it was more toxic than glufosinate alone (U.S. EPA, 1990a). Several acute toxicity studies have investigated glufosinate herbicides. The U.S. EPA (1986a) re-
ported that the median lethal concentration (LC₅₀) in 96 h (96 h-LC₅₀) is 65 mg/L for bluegill sunfish Lepomis macrochirus (U.S. EPA, 1990b), 13.5 mg/L for juvenile sheepshead minnow (U.S. EPA, 1990b), and 26.7 mg/L in rainbow trout (U.S. EPA, 1986a).

Histopathological biomarkers can be used in fish species to assess the biological effects of contaminants such as agrochemicals (Van der Oost et al., 2003). The utility of histological lesions as sensitive and reliable indicators of fish health has been reported in previous studies (Stentiford et al., 2003; Bernet et al., 2004; Ramírez-Duarte et al., 2008). Numerous studies of glyphosate-based herbicides (e.g., Roundup) have demonstrated their toxic effects on fish (the Nile tilapia Oreochromis niloticus and Jenynsia multidentata), and reported that Roundup can cause histological changes in the liver, gills, and kidneys after acute and chronic exposure to sublethal concentrations (Jiraungkoorskul et al., 2002, 2003; Ayoola, 2008; Langiano Vdo and Martinez, 2008; Hued et al., 2012). However, no previous study has investigated the aquatic toxicity of glufosinate-based herbicides according to histopathological analyses.

The marine medaka Oryzias dancena (Beloniformes) is distributed primarily in Asia, including India, Bangladesh, and Myanmar (Roberts, 1998). This fish species can adapt to a wide range of salinities, and its viability, development, growth, and reproduction are consistent (Cho et al., 2010). It has also been successfully developed as an estrogen-responsive transgenic model (Cho et al., 2013). No aquatic toxicity study has investigated the effects of any herbicide at different levels of salinity, and marine medaka could be used as an excellent model in such studies at salinities ranging from freshwater to seawater.

As a first step in examining the toxic effects of glufosinate-containing herbicides (Basta) in aquatic environments at various salinity levels, we evaluated histological lesions in the liver and gills of marine medaka in freshwater in response to subchronic and chronic exposure to Basta for 28 and 42 days, respectively.

Materials and Methods

Fish

The marine medaka specimens used in this study were from a laboratory stock maintained at the Institute of Marine Living Modified Organisms, Pukyong National University, Busan, Korea. Their mean total length was 10.1 ± 0.6 mm and their mean weight was 10.2 ± 2.0 mg.

Short-term toxicity (LC₅₀) testing

A short-term static toxicity test was performed to estimate the median lethal concentration of Basta on marine medaka exposed for 96 h (96 h-LC₅₀). The Basta used in the present study contained 18.02% glufosinate-ammonium as the active ingredient and 9.91% surfactants (Bayer CropScience, 2013). The fish were exposed to the following nominal concentrations of Basta: 0.0, 7.5, 8.0, 8.5, 9.0, 9.5, or 10.0 mg/L. The control and each experimental concentration were tested in duplicate with 12 individuals per group in 2-L aquariums. The individuals were starved for 24 h before the experiment and were not fed during the experiment. Fish showing no respiratory movement and no response to tactile stimuli were considered dead and were removed immediately. The 96 h-LC₅₀ was computed using probit and logit analyses.

Subchronic and chronic toxicity testing

For the subchronic and chronic toxicity tests, the fish were exposed to different concentrations of Basta (2, 4, or 8 mg/L) for 28 and 42 days, respectively. The experimental groups and control groups, each containing 65 fishes, were assigned to an aquarium and the tests were performed in duplicate. The fish were maintained in 10-L aerated glass aquariums containing dechlorinated tap water at a temperature of 25 ± 1°C, with a 12 h:12 h photoperiod. They were fed twice daily with a commercial diet (Ewha Oil Co., Busan, Korea), and one-fourth of the water in each aquarium was replaced daily throughout the experiments. All the procedures in this study were performed in accordance with the Guide for the Care and Use of Laboratory Animals (NRC, 2011). The number of dead fish was recorded and they were promptly removed. After exposure, the fish were anesthetized with ice-cold water and fixed in 10% formaldehyde for over 24 h. The fish were dehydrated through a graded series of ethanol and cleared in xylene. They were embedded in paraffin, sectioned to a thickness of 5 µm, and stained with hematoxylin and eosin.

Tissue lesions were examined under a light microscope (E400; Nikon Co., Tokyo, Japan) and photographed with a digital camera (Moticam Pro 205A; Motic Co., Hong Kong, China).

Histopathological analysis

For the histological analysis of the gills, five filaments were examined per individual fish. To calculate the percentage of filaments affected, the number of secondary lamellae displaying a particular change was recorded and divided by the total number of gill secondary lamellae examined. To evaluate histological changes in the liver, the middlemost section of the liver was used because the type of lesion was the same throughout all livers. The type of change and its extent were recorded for each area and divided by the total number of areas examined, and the values for the affected liver areas were converted into percentages.

The histopathological lesions were assessed according to the standardized assessment tool used in the protocol de-