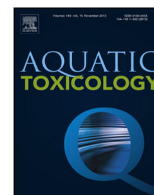
Aquatic Toxicology 160 (2015) 57–68

Contents lists available at ScienceDirect

Aquatic Toxicology

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



Occurrence and origin of sensitivity toward difenoconazole in zebrafish (*Danio reio*) during different life stages

Xiyan Mu, Tingting Chai, Kai Wang, Jie Zhang, Lizhen Zhu, Xuefeng Li*, Chengju Wang*

College of Sciences, China Agricultural University, Beijing 100913, People's Republic of China

ARTICLE INFO

Article history:
Received 12 September 2014
Received in revised form 2 January 2015
Accepted 3 January 2015
Available online 6 January 2015

Keywords:
Difenoconazole
Zebrafish
Stage-based sensitivity
Embryos
Adult fish

ABSTRACT

We report here an investigation of the mechanisms contributing to the divergent sensitivity toward the triazole fungicide difenoconazole of zebrafish (*Danio reio*) during different life stages. Adult and embryonic zebrafish were exposed to three different concentrations of difenoconazole (0.01, 0.5 and 1.0 mg/L). The death rate, bioaccumulation of difenoconazole, oxidative stress parameters and transcription of related genes were tested at 4 and 8 days post-exposure (dpe). The death rate for adult zebrafish was much higher than that of the embryos at an exposure concentration of 1.0 mg/L at both 4 and 8 dpe. The concentrations of difenoconazole in both the embryos and adult fish were similar, except for the group exposed to 0.01 mg/L difenoconazole. A decrease in antioxidant enzyme activities was observed in both the embryos and the livers of adult fish after exposure to difenoconazole. Significant lipid peroxidation was found in the livers of adult fish in all exposure groups at 8 dpe, but was not observed in the treated embryos. The gene transcription response of the embryos toward difenoconazole was different from that in the livers of adult fish at 4 dpe. At 8 dpe, the modification in the transcription of the tested genes in the embryos and adult fish was similar, except for the genes related to the synthesis of sterols.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

The replacement of the traditional toxicity test for fish by the fish embryo test has become an issue of much concern in the assessment of the ecological safety of toxic substances. The fish embryo test provides an intuitive understanding of the effects of the investigated chemical on the morphology of embryos during development and is considered to be more ethical and economical than experiments on adult fish (Russell and Burch, 1959; Strähle et al., 2011). In Germany, the use of zebrafish embryos as an alternative to animal testing in regulatory whole effluent tests has already become mandatory (Busch et al., 2011). The embryonic stage of teleosts was previously considered to be the most sensitive period in the whole life cycle of these fish (Westernhagen, 1988; Lele and Krone, 1996) and many published studies have shown that aquatic embryos are more vulnerable to chemicals than adult fish (Abe et al., 2001; Şişman, 2011; Ton et al., 2012). However, it has since been proposed that fish embryos may not be sufficiently sensitive to replace adult fish in toxicity testing. This may be because the chorion can act as a barrier to exposure for some chemicals, which may result in a reduction in toxicity (Embry et al., 2010). For example, Domingues et al. (2010) demonstrated that adult zebrafish were less tolerant to chromium (VI) exposure than embryos. We obtained similar results in a previous study of the toxicity of difenoconazole (Mu et al., 2013). These studies show that a single life stage biotoxicity assay may not be sufficient in ecological evaluation work.

Identifying the most sensitive period in the life stages of an organism is important in protecting the species from external toxic substances (Newman and Unger, 2002). It is therefore important to determine the most vulnerable life stage by carrying out multiple toxicity tests at different life stages before developing a scientific method for protection. Systematic multi-stage assays to obtain the sensitivity of species to potentially toxic chemicals based on the different life stages of the organism have been adopted in a number of studies (Brion et al., 2004; Mu et al., 2013; Wang et al., 2013). However, although there is an increasing focus on the investigation of the ecological impact of chemicals via multi-stage assays of aquatic organisms, the mechanisms leading to different sensitivities at different life stages is still poorly understood.

Difenoconazole [*cis-trans*-3-chloro-4-(4-methyl-2-(1H-1,2,4-triazol-yl methyl)-1,3-dioxolan-2-yl) phenyl 4-chlorophenyl ether] is a typical fungicide with a substituted triazole moiety

Abbreviations: dpe, days post-exposure (count from the exposure beginning); hpf, hours post-fertilization.

* Corresponding authors. Tel.: +86 10 62733924; fax: +86 1062734294.
E-mail addresses: xiyanmu@aliyun.com (X. Mu), gedac@126.com (T. Chai), wangkai12@cau.edu.cn (K. Wang), 136426167@qq.com (J. Zhang), 542341542@qq.com (L. Zhu), Lxf1966@263.net (X. Li), Wangchengju@cau.edu.cn (C. Wang).

http://dx.doi.org/10.1016/j.aquatox.2015.01.001
0166-445X/© 2015 Elsevier B.V. All rights reserved.

that binds to the heme portion of the fungal cytochrome P450 (cyp) 51 (Vanden et al., 1990). It inhibits fungal lanosterol-14α-demethylase activity and blocks the biosynthesis of ergosterol. This, in turn, blocks the synthesis of cell wall chitin in the fungus and results in the overspill of cytoplasm (Ragsdale, 1977; Buchenauer, 1995; Hamada et al., 2011). Difenoconazole has been used in China for many years as the main pesticide to combat fungal diseases in rice crops (Wang and Zhang, 2012). This has led to more opportunities for the contamination of the aquatic environment by difenoconazole. Many studies have been published on the occurrence of difenoconazole in the environment (Table 1). Difenoconazole is reported to have a higher acute toxicity than other triazole fungicides to a wide range of aquatic organisms (Dong et al., 2013). The European Food Safety Authority considers difenoconazole to be very toxic to aquatic organisms based on its high toxicity to *Daphnia magna* (chronic NOEC = 0.0056 mg active substance/L) (EFSA, 2011). It is therefore important to carry out further environmental toxicological studies on difenoconazole to determine the risk it poses to aquatic organisms.

Our previous study showed that exposure to difenoconazole may result in a series of negative effects on both zebrafish embryos and adult zebrafish and that, according to the lethal results, adult zebrafish are more sensitive to difenoconazole than the embryos (Mu et al., 2013). The study reported here investigated the factors that contributed to this divergent sensitivity to difenoconazole between zebrafish embryos and adults by determination of the bioaccumulation of the fungicide, the responses of biomarkers and the regulation of gene transcription caused by this fungicide. An additional goal was to use this approach to better understand the impact of difenoconazole on zebrafish during different life stages.

2. Materials and methods

2.1. Zebrafish maintenance

Wild-type zebrafish were purchased from a local fish shop. All the adult zebrafish were maintained in flow-through feeding aquariums (Esen Corp.) at 26 °C with a photoperiod of 14/10 h (light/dark) and were fed daily with live brine shrimp. The preparation and collection of the zebrafish embryos followed a previously published procedure (Mu et al., 2013).

2.2. Chemicals and reagents

Standard water was prepared in our laboratory following ISO-7346-3 and contained 2 mM Ca^{2+}, 0.5 mM Mg^{2+}, 0.75 mM Na^{+} and 0.074 mM K^{+} (ISO, 1996). Difenoconazole (96%) (CAS 119446-68-3) was obtained from the Chinese Ministry of Agriculture and the stock solution used for exposure was prepared using acetone AR. All other chemicals used were of analytical-reagent grade.

2.3. Exposure and sample collection

The experiments were performed in accordance with current Chinese legislation and were approved by the independent animal ethics committee at the China Agricultural University. Test solutions with a difenoconazole concentration of 0 (control), 0.01, 0.5 and 1.0 mg/L were prepared using standard water on the basis of pre-experimental data. Both blank control and solvent control was set up. The exposure solutions for solvent control and all treatment groups contained the same concentration of acetone (0.05 mL/L). Embryos were randomly transferred into test solutions in 1-L beakers at 2 h post-fertilization (hpf). Each beaker contained 500 mL of exposure solution and about 120 embryos, and there were eight beakers in each treatment group. The embryos were exposed to difenoconazole for 8 days and the embryos (or larvae) were transferred to freshly dosed beakers every 24 h. Three beakers were used to calculate the mortality and were not sampled until 8 dpe. The external conditions during exposure, including the temperature, humidity and light cycle, were the same as in the culture environment. The number of dead individuals and the stage of embryonic development were determined daily. For embryos, death was judged using the lethal toxicological endpoints proposed by Nagel, (2002). Larvae that had no heartbeat under micro-observation were considered to be dead. Dead individuals were removed in a timely manner. The hatching and malformation of embryos were checked daily. Morphological development was observed using a microscope. Abnormalities were recorded and the body lengths were measured using an Aigo GE-5 digital microscope (Aigo, Beijing, China). At 4 and 8 dpe, 120 embryos were collected from one beaker as one replicate sample. Three replicates were obtained for all the difenoconazole treatment groups and the control group. For each replicate sample, the embryos were divided into three groups: 50 embryos for difenoconazole analysis, 20 embryos for RNA extraction and 50 embryos for biomarker tests (for specific information, see sampling process in the Supplementary materials). The embryos were washed and the wet weight of all the samples was measured using an electronic balance (Sartorius, Gottingen, Germany). The embryos for difenoconazole analysis were stored at −20 °C and the remaining embryos were stored at −80 °C until analysis.

Adult fish were exposed to difenoconazole in aquaria and the test concentration and control groups were similar to the embryonic tests. Six-month-old zebrafish were randomly assigned to tanks (25 L volume). Eight tanks were used for each treatment and each tank contained 20 L of exposure solution and 40 fish. Three tanks were used for the calculation of mortality and were not sampled until 8 dpe. The exposure solution was renewed every 24 h to maintain the appropriate concentration of the fungicide and to maintain the water quality. The external conditions during exposure, including the temperature, humidity and light cycle, were the same as in the culture environment. The adult fish were fed daily during the test with dry food (equivalent to 2% of body weight) except for the 24 h prior to sacrifice. At 4 and 8 dpe, 13 zebrafish were randomly selected from one tank as one replicate; three replicates were used for all the difenoconazole treatment groups and the control group. For each replicate the fish were divided into three samples: two fish for difenoconazole analysis; six fish for the biomarker test; and five fish for RNA extraction (for specific information, see sampling process in Supplementary materials). All

Table 1
The reported environmental difenoconazole dosage in water area.

Area	Data source	Reported value	References
China (Changsha, Changchun, Hangzhou,)	Paddy Water (spraying day)	1.98–2.91 mg/L	Zhang et al., 2011 (20)
Thailand (Salakru, Nong Sua)	PEC[a] (agricultural water area)	0.028 mg/L	Satapornvanit et al., 2004 (21)
Malaysia (Kedah)	Surface water (river near field)	0.30 mg/L	Latiff et al., 2010 (22)
China (Fujian)	Surface water (river)	0.0039–0.0061 μg/L	You, 2008 (23)
Italy (River Meolo)	PEC (river)	0.0095 μg/L	Verro et al., 2009 (24)
Australia (Victoria)	Surface water (river and stream)	0.15 μg/L	Schäfer et al., 2011 (25)

[a] PEC = Predicted Environmental Concentration.

Download English Version:

https://daneshyari.com/en/article/6382237

Download Persian Version:

https://daneshyari.com/article/6382237

Daneshyari.com