Environmental Research 151 (2016) 313–320

Contents lists available at ScienceDirect

Environmental Research

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

A comparison of biomarker responses in juvenile diploid and triploid African catfish, *Clarias gariepinus*, exposed to the pesticide butachlor

Ali Karami [a,*], Dzolkhifli Omar [b], James M. Lazorchak [c], Chee Kong Yap [d], Zailina Hashim [a], Simon C. Courtenay [e]

[a] Laboratory of Aquatic Toxicology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Selangor, Malaysia
[b] Laboratory of Toxicology, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Selangor, Malaysia
[c] Office of Research and Development, National Exposure Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH, United States
[d] Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 Selangor, Malaysia
[e] School of Environment, Resources and Sustainability, Canadian Water Network, Canadian Rivers Institute, University of Waterloo, Canada

ARTICLE INFO

Article history:
Received 29 May 2016
Received in revised form
5 August 2016
Accepted 7 August 2016

Keywords:
Polyploid
Gene
Biochemical biomarkers
Steroid
Morphometric parameters

ABSTRACT

Influence of waterborne butachlor (BUC), a commonly used pesticide, on morphometric, biochemical, and molecular biomarkers was evaluated in juvenile, full sibling, diploid and triploid African catfish (*Clarias gariepinus*). Fish were exposed for 21 days to one of three concentrations of BUC [mean measured μg/L: 22, 44 or 60]. Unexposed (control) triploids were heavier and longer and had higher visceral-somatic index (VSI) than diploids. Also, they had lighter liver weight (HSI) and showed lower transcript levels of brain gonadotropin-releasing hormone (*GnRH*), aromatase (*cyp191b)* and *fushi* tarazu-factor (*ftz-f1*), and plasma testosterone levels than diploids. Butachlor treatments had no effects, in either diploid or triploid fish, on VSI, HSI, weight or length changes, condition factor (CF), levels of plasma testosterone, 17-β estradiol (E2), cortisol, cholesterol, or mRNA levels of brain tryptophan hydroxylase (*tph2*), forkhead box L2 (*foxl2*), and 11 *β*-hydroxysteroid dehydrogenase type 2 (*11β-hsd2*). Expressions of *cyp191b* and *ftz-f1* in triploids were upregulated by the two highest concentrations of BUC. In diploid fish, however, exposures to all BUC concentrations decreased *GnRH* transcription and the medium BUC concentration decreased *ftz-f1* transcription. Substantial differences between ploidies in basal biomarker responses are consistent with the reported impaired reproductive axis in triploid *C. gariepinus*. Furthermore, the present study showed the low impact of short term exposure to BUC on reproductive axis in *C. gariepinus*.

1. Introduction

It has been over 70 years since the first efforts to manipulate ploidy in fish (i.e. Makino and Ozima, 1943). Since then, artificial triploidy has been successfully induced in a number of fish species. Due to sterility and/or high growth rate, triploid fish have been utilized by different industrial and environmental sectors. For example, triploid grass carp (*Ctenopharyngodon idella*) have been employed in Florida waters to control nuisance aquatic vegetation (Hanlon et al., 2000).

The broad usage of pesticides in response to the need for increasing crop yields has resulted in their ubiquitous presence in water bodies. Butachlor (BUC) is an organochlorine (OC) herbicide, used as an active ingredient of many chloroacetanilide herbicides. It is widely used in South America and Asia (Naylor, 1996; Lo et al., 2008) as a pre-emergence herbicide to control weeds in rice paddy fields (Chen et al., 2009). African catfish (*Clarias gariepinus*) was chosen for this study because it is commonly cultivated in paddy fields (Marimuthu et al., 2013) and would therefore be expected to encounter exposure to a wide range of pesticides including BUC. Also, this species is a major aquaculture species in tropical and sub-tropical regions due to its high meat quality, fast growth rate, and high resistance to disease and poor water quality (Henk et al., 1996).

Biomarkers are routinely employed to evaluate the effects of pollutants in fish. Type and concentration of contaminant, route and duration of exposure, gender and species of fish are among the well-understood factors that affect biomarker responses in fish (Ward et al., 2006; Sanchez et al., 2008). Ploidy, however, is a factor that has received very little attention in the literature on biomarker responses in fish. Our recent studies have shown substantial differences between diploid and triploid fish in biomarker

Abbreviations: BUC, Butachlor; VSI, visceral-somatic index; HSI, Hepatosomatic index; CF, Condition factor; *GnRH*, Gonadotropin-releasing hormone; *tph2*, Tryptophan hydroxylase2; *cyp191b*, Brain aromatase; *foxl2*, Forkhead box L2; *ftz-f1*, Fushi tarazu-factor 1; *11β-hsd2*, 11 *β*-hydroxysteroid dehydrogenase type 2; E2, 17-β estradiol

* Corresponding author.
E-mail address: alikaramiv@gmail.com (A. Karami).

http://dx.doi.org/10.1016/j.envres.2016.08.006
0013-9351/

responses to contaminants (Karami et al., 2015b, Karami et al., 2016c, Karami et al., 2016a).

Several behavioural factors such as courtship and spawning are required for the successful reproduction in fish (Potts, 1984). Contaminants have been shown capable of disrupting these reproductive-associated behaviours (see review by Scott and Sloman, 2004). Like other animals, reproduction in fish is controlled by a complicated series of factors at different organizational levels ranging from molecular, biochemical, organ, and whole organism. Several studies investigated the impact of BUC on different biomarker responses in fish. For example, 1000, 2000, or 2500 μg/L BUC induced oxidative stress in different tissues of *C. gariepinus* (Farombi et al., 2008). In another study, BUC caused developmental toxicity, endocrine disruption and immune toxicity in the zebrafish (*Danio rerio*) embryos (Tu et al., 2013). In contrast, our recent study showed lack of impact of BUC on skin gelatin yield and amino acid composition in diploid and triploid *C. gariepinus* (Karami et al., 2016b). However, information on the reproductive toxicity of BUC is limited. Butachlor has been shown to disrupt the reproductive axis in *D. rerio* (Chang et al., 2013) and rare minnow (*Gobiocypris rarus*) (Zhu et al., 2014). To evaluate the potential for impacts on reproductive success of *C. gariepinus* living in the areas contaminated with BUC, we investigated a suite of biomarkers related to the reproductive axis.

Morphometric parameters like CF, total length change, total weight change, visceral-somatic index (VSI), and hepatosomatic index (HSI) are frequently reported as indices of the bioenergetic and health status of fish (Adams and Ham, 2011). Teleost brains exhibit 100–1000 times higher aromatase activity than the brains of adult mammals (Pasmanik and Callard, 1985). Ovarian cytochrome P450 aromatase (cyp191a) and the brain aromatase (cyp191b) are involved in the conversion of androgens to estrogens in teleosts (Coumailleau et al., 2015). As opposed to the well-documented vitellogenin (VTG) induction in juvenile fish following exposure to estrogenic contaminants (e.g., de Vlaming et al., 2007), little attention has been given to changes in steroidal levels. The role of *fushi* tarazu-factor (*ftz-f1*) in the regulation of *cyp191b* expression has been shown in many fish species including *C. gariepinus* (Sridevi et al., 2013). Similarly, involvement of forkhead box L2 (*foxl2*) in the transcriptional regulation of *cyp191a* and *cyp191b* has been reported in *C. gariepinus* (Sridevi et al., 2013). Tryptophan hydroxylase 2 (*tph2*) is a key enzyme regulating 5-hydroxytryptamine (5-HT) biosynthesis, which in turn stimulates the release of gonadotropin-releasing hormone (GnRH) and gonadotropins (GTHs) through acting at the level of hypothalamo–hypophyseal axis (Nakamura and Hasegawa, 2007).

Cortisol oversecretion has been implicated in risk of cardiovascular diseases, homeostatic impairment, immune system suppression and tissue degeneration (Whitworth et al., 2005; Barry et al., 2010). 11-hydroxysteroid dehydrogenases are responsible for the interconversion of cortisol and cortisone which may protect mineralocorticoid-targeted tissues against cortisol excess (Zhou et al., 2012). To better understand the impacts of BUC on the mechanisms involved in cortisol inactivation, we measured 11 *β*-hydroxysteroid dehydrogenase type 2 (*11β-hsd2*) gene transcription and its impacts on circulating plasma cortisol levels in diploid and triploid *C. gariepinus*.

Cholesterol is the precursor for major classes of steroid hormones including androgens and estrogens (Bolté et al. 1974). Changes in the circulating plasma cholesterol levels could be an indicator of cellular damage, organ dysfunction and/or impaired chemical toxicokinetics (Smitz et al., 1989; Gad and Saad, 2008). In turn, any alterations in cholesterol levels could result in hormonal imbalance with potential implications for maintenance of the population (Miller et al., 2007).

In the shallow aquifers beneath irrigated and rainfed rice fields of Laguna, Philippines, BUC had the highest concentration (max 1.14–1.26 μg/L) among other pesticides (Bhuiyan and Castaneda, 1995). The recommended application of BUC in a paddy field (1–3 kg/ha) with about 3 cm water depth would result in the concentration of around 11–32 μM (Chen et al., 2007). Also, illegal or out of control usages of pesticides by farmers (Wilson and Tisdell, 2001; Williamson and others, 2003), their gradual leaching from sediments into the water (Vryzas et al., 2009), and the runoff of BUC from paddy fields (Iwakuma et al., 1993), could increase the concentrations BUC in the surface waters to the critical levels. Therefore, this study covered the biological impacts of high BUC concentrations. Furthermore, the selected BUC levels in this study (nominal concentrations: 50, 100, and 150 μg/L) are within (Chang et al., 2013) or below (Farombi et al., 2008) the range used by the earlier studies.

The first objective of this study was to compare basal morphometric (VSI, HSI, total weight change, total length change, and CF), biochemical [plasma testosterone, 17-βestradiol (E2), cortisol, and cholesterol levels], and molecular (*tph2*, *GnRH, cyp191b*, *foxl2*, *ftz-f1,* and *11β-hsd2* expressions) biomarker levels in control diploid and triploid *C. gariepinus*. We then compared changes in these biomarkers, particularly molecular responses as early signals of further physiological changes, within BUC-treated diploid and triploid African catfish following a short exposure to BUC.

2. Materials and methods

2.1. Chemicals and other materials

Artemia (Utah strain) was purchased from Bio-marine Inc. (USA); commercial powder (crude protein: 38–40%) and pellets (crude protein: Min 45%) were purchased from Cargill and Star Feed, respectively; phosphate buffer saline (PBS), propidium iodide (PI), and RNase from Sigma (USA); BUC (Pestanal® analytical standard grade) from Sigma–Aldrich (USA). HPLC-grade ethanol, acetone, and dichloromethane (DCM), NaCl and Na_2SO_4 were supplied by Fisher Scientific; Vacutainer tubes treated with ethylenediaminetetraacetic acid (EDTA) from Becton Dickinson (Franklin Lakes, NJ); enzyme immunoassays (EIAs) kits obtained from Cayman Chemicals (Ann Arbor, Michigan USA; testosterone #582701, E2 #582251, cortisol #500360), RNAeasy Mini kit, RNase free DNAse set, Quantitect Reverse Transcription Kit, and QuantiNova SYBR Green PCR Kit from Qiagen (Valencia, CA), and oligonucleotide primers First Base (Singapore).

2.2. Production of diploid and triploid fish

Mature *C. gariepinus* were obtained from local farmers in Selangor, Malaysia and raised for 12 months before breeding. Full-sibling diploid and triploid *C. gariepinus* were produced according to established protocols (Richter et al., 1986; Karami et al., 2010). Briefly, sexually mature male and female fish were injected with Ovaprim® 10 h before breeding. Eggs and milt were obtained, gently mixed, and divided into two batches. The first batch (triploids) was cold-shocked 3 min after fertilization at 5 °C for 40 min. The second batch did not receive any treatment and was considered as diploid. Each batch of eggs was transferred to separate fiberglass tanks filled with 500 UV-sterilized dechlorinated tap water. Ploidy status of 20 larvae was examined according to the method of Karami et al. (2015a). If the initial ploidy induction rate was higher than 95%, then diploid and triploid fish were raised separately in 2000 L fiberglass tanks. Larvae were fed *ad libitum* with freshly hatched *Artemia* nauplii for 7 days. Fingerlings and juvenile fish were fed with commercial powder and pellets at the rate of 5–10% of body weight per day for 15 weeks. Throughout the study, fish were maintained on a 12:12 light: dark cycle.