Science of the Total Environment 542 (2016) 876–885

Contents lists available at ScienceDirect

Science of the Total Environment

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

Acute exposure to synthetic pyrethroids causes bioconcentration and disruption of the hypothalamus–pituitary–thyroid axis in zebrafish embryos

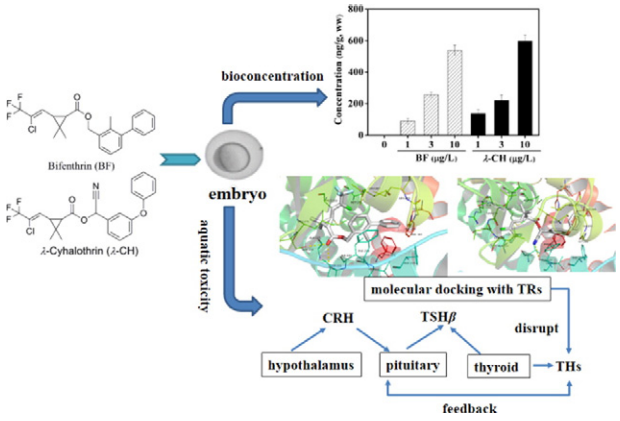
Wenqing Tu [a,b], Chao Xu [b,*], Bin Lu [b], Chunmian Lin [b], Yongming Wu [a], Weiping Liu [c]

[a] Research Institute of Poyang Lake, Jiangxi Academy of Sciences, Nanchang 330029, China
[b] Institute of Environmental Science, College of Biological and Environmental Engineering, Zhejiang University of Technology, Hangzhou 310032, China
[c] Institute of Environmental Science, College of Environmental and Resource Sciences, Zhejiang University, Hangzhou 310058, China

HIGHLIGHTS

- Following respective exposure of embryos to BF and λ-CH, thyroid endocrine disruption was investigated in zebrafish embryos.
- Thyroid hormones (T3 and T4 levels) were significantly altered after being exposed to BF and λ-CH.
- Gene transcription modulation in the HPT axis was examined.
- BF and λ-CH bioconcentration in zebrafish larvae were evident.
- BF binds to thyroid hormone receptor (TRα) protein more potently than λ-CH.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 20 July 2015
Received in revised form 18 October 2015
Accepted 26 October 2015
Available online 8 November 2015

Editor: D. Barcelo

Keywords:
Pyrethroid insecticides
Bioconcentration
HTP axis
Zebrafish embryos

ABSTRACT

Synthetic pyrethroids (SPs) have the potential to disrupt the thyroid endocrine system in mammals; however, little is known of the effects of SPs and underlying mechanisms in fish. In the current study, embryonic zebrafish were exposed to various concentrations (1, 3 and 10 μg/L) of bifenthrin (BF) or λ-cyhalothrin (λ-CH) until 72 h post fertilization, and body condition, bioaccumulation, thyroid hormone levels and transcription of related genes along the hypothalamus–pituitary–thyroid (HPT) axis examined. Body weight was significantly decreased in the λ-CH exposure groups, but not the BF exposure groups. BF and λ-CH markedly accumulated in the larvae, with concentrations ranging from 90.7 to 596.8 ng/g. In both exposure groups, alterations were observed in thyroxine (T_4) and triiodothyronine (T_3) levels. In addition, the majority of the HPT axis-related genes examined, including CRH, TSHβ, TTR, UGT1ab, Pax8, Dio2 and TRα, were significantly upregulated in the presence of BF. Compared to BF, λ-CH induced different transcriptional regulation patterns of the tested genes, in particular, significant stimulation of TTR, Pax8, Dio2 and TRα levels along with concomitant downregulation of Dio1. Molecular docking analyses revealed that at the atomic level, BF binds to thyroid hormone receptor (TRα) protein more

Abbreviations: SPs, synthetic pyrethroids; BF, bifenthrin; λ-CH, λ-cyhalothrin; CRH, corticotropin-releasing hormone; TSH, thyroid stimulating hormone; TTR, transthyretin; UGT1ab, uridine diphosphate glucuronosyltransferase; TPO, thyroid peroxidase; TG, thyroglobulin; NKX2.1 (also known as TTF1), thyroid transcription factor-1; Pax8, paired box gen 8; Dio, deiodinase; TR, thyroid hormone receptors; HPT axis, hypothalamic–pituitary–thyroid axis; TH, thyroid hormones.

* Corresponding author.
E-mail address: chaoxu@zjut.edu.cn (C. Xu).

http://dx.doi.org/10.1016/j.scitotenv.2015.10.131

potently than λ-CH, consequently affecting HPT axis signal transduction. In vitro and in silico experiments disclosed that during the early stages of zebrafish development, BF and λ-CH have the potential to disrupt thyroid endocrine system.

1. Introduction

Due to their high effectiveness as insecticides and low mammalian toxicity, synthetic pyrethroids (SPs) are one of the most commonly used residential and agricultural insecticides (Alonso et al., 2012). As the application of organophosphate pesticides is restricted, the use of SPs has steadily increased, currently comprising 25% of the total global insecticide market (Kuivila et al., 2012; Yavuz et al., 2010). Consequently, SPs are ubiquitous in environments, especially fresh water (Ensminger et al., 2011; Ge et al., 2010; Hladik and Kuivila, 2009). For example, Ge et al. (2010) found the concentrations up to 0.632 μg/L for deltamethrin, 0.969 μg/L for cypermethrin and 1.246 μg/L for fenvalerate in surface water, Beijing. SPs are extremely toxic to fish and other aquatic organisms (Brander et al., 2012; Corcellas et al., 2015; Jin et al., 2009). For example, the 144-h LC50 values of zebrafish embryos to BF and λ-CH are reported as 190 μg/L and 110 μg/L, respectively (DeMicco et al., 2010). Previous studies have disclosed induction of hepatic oxidative stress, DNA damage, apoptosis and reproductive toxicity by SPs in zebrafish (Jin et al., 2012; Jin et al., 2011b). In caged wild fish collected from surface water, high SP concentrations were detected (Corcellas et al., 2015; Rawn et al., 2010), with bioconcentration factors (BCF) of 500 to 6000 in bluegill sunfish and carp (Laskowski, 2002). Our group studies showed that after short-term exposure, SPs accumulated in zebrafish larvae, with recorded maximum concentrations of permethrin (PM), BF and λ-CH of 6074, 4201 and 2415 ng/g, respectively (Tu et al., 2014). In particular, accumulated BF in zebrafish embryos has been shown to induce developmental toxicity, with 96 h EC50 of 256 μg/L for pericardial edema and 109 μg/L for curved body axis (Jin et al., 2009). Exposure to SPs, even at low concentrations, can lead to accumulation in aquatic organisms, such as fish, which is potentially associated with negative biological effects.

Thyroid hormones (TH) play a crucial role in the development, growth, metabolism and reproduction of vertebrates (Jugan et al., 2010). Mammalian studies have demonstrated a correlation between SP exposure and disruption of the thyroid hormone status (Kaul et al., 1996; Maiti and Kar, 1998). For instance, Wang et al. (2002) showed that oral administration of 400 mg/kg permethrin induces a significant decrease in TH levels, and exposure to 12.5 and 25 mg/kg deltamethrin leads to markedly reduced T_4 levels in rat serum after 15 days. Similarly, administration of 100 and 200 mg/kg fenvalerate was shown to trigger significant elevation of T_3 and T_4 in rats (Kambe et al., 1996; Kaul et al., 1996). To date, the majority of studies regarding SP interference with thyroid function have focused on mammals. However, little is known about the effects of SPs in fish and the underlying mechanisms.

Similar to numerous vertebrates, thyroid homeostasis in fish is subject to regulation of the hypothalamus–pituitary–thyroid (HPT) axis by which the thyroid endocrine system is controlled. The corticotropin-releasing hormone (CRH) secreted by the hypothalamus coordinates HPT axis function by controlling the release of thyroid stimulating hormone (TSH) from the pituitary, which regulates TH synthesis and release (Shi et al., 2009). T_4 is converted to biologically active T_3 via the action of deiodinase enzymes (such as Dio1 and Dio2). T_3 binds to TR receptors and mediates actions in the target organs (Jin et al., 2011a). Zebrafish are an ideal model for investigating thyroid-disrupting chemicals owing to their small size, ease of culture, high embryo yield and morphological and physiological similarities to mammals (Chen et al., 2012; Tu et al., 2013; Wang et al., 2013). Previous studies have reported a number of chemicals that affect the HPT axis of zebrafish and induce developmental toxicity. For instance, exposure to 400 μg/L perfluorooctane sulfonate (PFOS) led to a significant reduction in body length and weight, increased T_3 levels, and altered expression of several genes in the HPT axis (Shi et al., 2009). Treatment with 300 and 600 μg/L Tris (1,3-dichloro-2-propyl) phosphate (TDCPP) caused developmental toxicity, including decreased body weight and increased malformation, increased T_3 and T_4 levels, and upregulated transcription of genes involved in the HPT axis (Wang et al., 2013). In the current study, zebrafish embryos were employed to investigate the effects of BF and λ-CH on the HPT axis. Furthermore, transcriptional expression of target genes involved in the HPT axis and TH levels were examined and bioconcentrations of the two SPs in larvae determined. Our findings facilitate understanding of the potential impact of SPs on the fish thyroid endocrine system.

2. Materials and methods

2.1. Chemicals

BF (CAS No. 82657-04-3; 99.5% purity) and λ-CH (CAS No. 91465-08-6; 98.5% purity) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Stock solutions were prepared in acetone at concentrations of 1–10 mg/L and stored in darkness at 4 °C. The internal standard, deltamethrin (99% purity), was purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade and purchased from Tedia Company Inc. (Fairfield, OH, USA).

2.2. Zebrafish maintenance and embryo exposure

Adult male and female zebrafish were purchased from National Zebrafish Resources of China (Shanghai, China) and raised in a flow-through system (dechlorinated tap water, pH 7.2–7.6, hardness 44.0–61.0 mg $CaCO_3$/L) at 28.5 °C under a 14 h:10 h (light:dark) photoperiod. Fish were fed with live brine shrimp twice daily. With the aim of alleviating suffering, all animals were humanely maintained in accordance with current Chinese legislation. The study was approved by the independent animal ethics committee at Jiangxi Academy of Sciences.

Male and female adult fish (male/female ratio of 2/1) were separated using isolation boards in spawning boxes overnight. The following morning, isolation boards were removed and spawning triggered once the light was turned on. Embryos were siphoned from the spawning boxes and washed three times with zebrafish system water. Embryos that developed normally and reached the blastula stage (2 h post fertilization [hpf]) were exposed to BF or λ-CH (0, 1, 3, and 10 μg/L) in a 1 L beaker until 72 hpf. Each beaker contained 500 mL of exposure solution and 500 embryos. The concentrations selected for exposure were based on our previous findings, and did not induce statistically significant malformation or mortality (Tu et al., 2014). SP-free water and water containing acetone (0.1%) were used as blank control and solvent control, respectively. All test concentrations and controls were replicated three times. Throughout the experiment, all embryos were raised in an incubator at 28 ± 1 °C under a photoperiod of 14 h light:10 h dark. The exposure solution was renewed daily. After exposure, 430 larvae from each replicate were collected and divided into three groups: 30 for RNA extraction, 100 for SP analysis, and 300 for the TH assay. Larvae for SP analysis were stored at −20 °C and those of the other groups at −80 °C until analysis.

2.3. BF and λ-CH determination in exposure solutions

Prior to the first water replacement, exposure solutions were collected from each replicate beaker for all the treatments at the