Chemosphere 165 (2016) 152–162

Contents lists available at ScienceDirect

Chemosphere

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

Effects of methomyl on steroidogenic gene transcription of the hypothalamic-pituitary-gonad-liver axis in male tilapia

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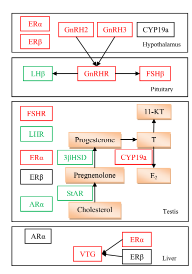
HIGHLIGHTS

- Effect of methomyl on gene transcription of HPGL in male tilapia were investigated.
- Gene mRNA in HPGL of tilapia exposed to higher than 20 μg/L changed significantly.
- Methomyl disrupted multiple sites in HPGL axis.
- 2 μg/L can be considered as no observed adverse endocrine disruption effect level.
- 200 μg/L was the threshold for methomyl-induced irreversible endocrine disruption.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 19 June 2016
Received in revised form
2 September 2016
Accepted 6 September 2016
Available online 17 September 2016

Handling Editor: David Volz

Keywords:
Methomyl
Hypothalamic-pituitary-gonad-liver axis
Male tilapia
Steroidogenic gene transcription
Recovery pattern

ABSTRACT

Male tilapia were exposed to sub-lethal methomyl concentrations of 0, 0.2, 2, 20 or 200 μg/L for 30 d, and were subsequently cultured in methomyl-free water for 18 d. Relative transcript abundance of steroidogenic genes involved in the HPGL axis of male tilapia was examined at 30 d in the exposure test and at 18 d in the recovery test. The results revealed that low concentrations of methomyl (0.2 and 2 μg/L) did not cause significant changes in gene mRNA levels in the HPGL axis of male tilapia; thus, we considered 2 μg/L concentrations as the level that showed no apparent adverse endocrine disruption effects. However, higher concentrations of methomyl (20 and 200 μg/L) disrupted the endocrine system and caused significant increase in the levels of GnRH2, GnRH3, ERα, and ERβ genes in the hypothalamus, GnRHR and FSHβ genes in the pituitary, CYP19a, FSHR, and ERα genes in the testis, and VTG and ERα genes in the liver, and significantly decreased the levels of LHR, StAR, 3β-HSD, and ARα genes in the testis and LHβ gene in the pituitary, leading to changes in sex steroid hormone and vitellogenin levels in the serum and ultimately resulting in reproductive dysfunction in male tilapia. The recovery tests showed that the toxicity effect caused by 20 μg/L methomyl was reversible; however, the toxicity effect at 200 μg/

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http://dx.doi.org/10.1016/j.chemosphere.2016.09.024

L of methomyl was irreversible after 18 d. Therefore, we concluded that 200 μg/L was the threshold concentration for methomyl-induced irreversible endocrine disruption in male tilapia.

1. Introduction

Anthropogenic activities and life-style of modern society often lead to intentional or unintentional discharge of thousands of chemicals (Scognamiglio et al., 2016). At certain doses, some of these are known to interfere with the endocrine systems of humans and animals in several ways, imitating the function of hormones and eventually affecting an organism's health, growth and reproductive system, which is why they are defined as Endocrine Disrupting Chemicals (EDCs) (Papaevangelou et al., 2016). Methomyl ($C_5H_{10}N_2O_2S$) is globally utilized and has been considered an environmental estrogen by the World Wide Fund for Nature in 1997 (Guo, 2006). Because of its broad biological activity, high efficiency against insects and relatively rapid disappearance, methomyl has been used worldwide for crop protection and soil and plant treatment (WHO, 1996). The half-life in soil is short, ranging from 3 to 50 d depending on the soil characteristics (Leistra et al., 1984; Van-Scoy et al., 2013). Furthermore, its absorbability in soil is very low (Leistra et al., 1984); therefore, the residue in soil is not high (Meng et al., 2013). However, methomyl exhibits high water solubility (57.9 g/L at 25 °C), long half-life in water (ranging from 20 to 54 weeks according to the water characteristics) (Yang et al., 2005) and weak adsorption to soils (Leistra et al., 1984), posing a potential contamination risk to surface and groundwater, especially when applied in agricultural fields where it can infiltrate into the groundwater and threaten the safety of the drinking water resource (Strathmann and Stone, 2001). Methomyl residues in environmental water have been reported to range from a minimum of no residues present to a maximum of 55.3 μg/L (Van-Scoy et al., 2013).

In most cases, the concentrations of EDCs found in the aquatic environment are much lower than that expected to cause direct lethality to non-targeted species (Xu et al., 2016). However, concern has arisen about harmful effects to human or animal reproductive systems from chronic exposure to some EDCs (Gillesby and Zacharewski, 1998). Even if the binding affinity of most EDCs to the estrogen or androgen receptors is low compared with the potential of endogenous steroid hormones, several studies have found that exposure to even low concentrations of xenoestrogens or xenoandrogens can disrupt normal developmental and reproductive processes (Hachfi et al., 2012). A well-known example is ethinylestradiol (EE_2), a bioactive estrogen that is commonly used in contraceptive pills and which may be eventually discharged into ground or surface waters (Hachfi et al., 2012). Although EE_2 is generally found in lower ng/L levels in aquatic environments, this can be enough to cause sustainable impacts on reproduction and development in fish populations (Hachfi et al., 2012).

The aquatic ecosystem is one of the most exposed environments to contaminants since it represents the ultimate destination for most anthropogenic pollutants: whether initially released on land, in the atmosphere or directly into waterways, many will eventually arrive in rivers or oceans (Hachfi et al., 2012). Consequently, fish are considered as one of the primary risk organisms for EDCs (Kime, 1999). Not only are they directly exposed to a wide variety of EDCs, but also sex determination in fish is known to be very labile and consequently can be disturbed or even reversed by exogenous hormone exposure at critical developmental stages (Francis, 1992).

Many studies on the acute toxicity of methomyl against aquatic organisms have been conducted previously (Farré et al., 2002; Li et al., 2008; Pereira and Goncalves, 2007); however, studies on reproduction damage and endocrine disruption of methomyl in aquatic organisms are still limited (Gaete et al., 2013). Furthermore, there is no available information yet on the effects of the chemical on fish, except the papers we have published (Meng et al., 2014, 2015). The present study was carried out to determine the effects of chronic exposure of methomyl on steroidogenic gene transcription in tilapia (*Oreochromis niloticus*).

Environmental estrogens can induce disruption of the sex steroid hormone system by either acting as steroid hormone receptor agonists or antagonists, by affecting synthesis, transport or metabolism of steroid hormones; or by affecting the synthesis or function of the steroid hormone receptors (Goksøyr, 2006; Rotchell and Ostrander, 2003). The hypothalamus–pituitary–gonadal–liver (HPGL) axis regulates fish reproduction closely (Hachfi et al., 2012). Moreover, the fish HPGL axis as a novel graphical model is reported to have been used in ecotoxicogenomics research on endocrine disrupting materials (Hachfi et al., 2012). Therefore, in the present study, transcriptional levels of some important genes in the HPGL axis of tilapia exposed to methomyl were measured. The results of the research will help to understand the reproduction-related endocrine disruption effect of methomyl on tilapia and the associated mechanisms of neuroendocrine alteration.

2. Materials and methods

2.1. Fish and chemicals

The Nile tilapia *Oreochromis niloticus* was chosen for this study as tilapia is not only an important economic fish, but also considered as a common test species in toxicology studies because of its strong adaptability, wide distribution, high fecundity, convenient breeding in laboratory and easy access. Male tilapia with an average weight of 150.7 ± 9.7 g and total length of 19.0 ± 1.4 cm were obtained from the fish farm of the Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, in Wuxi, China. Before the experiments, fish were acclimated under laboratory conditions for 4 weeks, at a population density of 30 specimens per 200 L, in glass aquaria supplied with dechlorinated tap water. The physicochemical characteristics of the water in the aquaria were analyzed according to methods for the examination of water and wastewater published by the State Environmental Protection Agency of China (2002). The water had a pH of 7.3 ± 0.3 and a temperature of 25 ± 0.5 °C; water hardness was 107 mg/L (as concentration $CaCO_3$), and dissolved oxygen concentration was 6.5–7.0 mg/L. The farmed fish stock and experimental fish were fed 2% body mass daily with commercial fish feed (Ningbo Tech-Bank Co., Ltd., China), and kept on a 12-h light and 12-h dark photoperiod. The experiments commenced when no mortality was observed in the acclimated population. The methomyl (97% w/w) used was produced by Shanghai Focus Biological Technology Co., Ltd., China. All other chemicals used were of analytical grade and obtained from Sigma-Aldrich (St. Louis, MO, USA) or Sangon Biotech (Shanghai, China).