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Aquatic Toxicology 71 (2005) 357–369

AQUATIC TOXICOLOGY

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Morphological abnormalities during early-life development of the estuarine mummichog, *Fundulus heteroclitus*, as an indicator of androgenic and anti-androgenic endocrine disruption

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Received 18 June 2004; received in revised form 20 October 2004; accepted 11 December 2004

Abstract

We tested the hypothesis that gross morphological abnormalities are a sensitive indicator of exposure to waterborne androgenic and anti-androgenic compounds during embryonic, larval and juvenile stages of development in the common estuarine killifish, the mummichog (*Fundulus heteroclitus*; Pisces: Cyprinodontidae). Static exposures with daily renewal were carried out with 10–100,000 ng/L of the androgen agonist, 17α-methyltestosterone (MT), or the androgen antagonist, cyproterone acetate (CA), for 60 days post-fertilization (PF) in duplicate exposures. Measured concentrations were 78.4–155.8% of nominal concentrations for MT and 13.5–168.1% for CA. No dose-related or consistent effects of MT or CA were observed before hatch. In 60 days PF juveniles, incidence of skeletal abnormalities (scoliosis, lordosis, head, facial and fin), soft tissue abnormality (anal swelling) and hemorrhaging were significantly increased by MT but only at high concentrations (≥1000 ng/L). The 10,000 and 100,000 ng/L concentrations of MT produced a wider range of abnormalities than 1000 ng/L. Over 90% of fish exposed to 10,000 or 100,000 ng/L were abnormal with an average of over 3.5 abnormalities per fish. CA did not increase the incidence of any type of abnormality. Survival of juveniles to the end of the exposure was reduced by MT at concentrations of 1000 ng/L and greater in the first experiment and at concentrations of 10,000 ng/L and greater in the second experiment. Juvenile length was reduced by high concentrations of MT (≥10,000 ng/L) in the first experiment and by most concentrations in the second experiment. We conclude that morphological abnormalities in early-life stages of mummichogs are not a sensitive indicator of exposure to androgenic or anti-androgenic waterborne EDSs at environmentally relevant concentrations.

Keywords: Mummichog; Killifish; Endocrine disruption; Methyltestosterone; Cyproterone acetate; Developmental abnormalities

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0166-445X/$ – see front matter
doi:10.1016/j.aquatox.2004.12.005

1. Introduction

Certain anthropogenic and natural substances in the environment are able to disrupt endocrine homeostasis by their ability to mimic or block the action of endogenous hormones, as highlighted by Colborn et al. (1993). In natural populations and laboratory studies, exposure to endocrine disrupting substances (EDSs) has resulted in various responses, such as vitellogenin synthesis by males (Purdom et al., 1994), altered sex steroid levels (MacLatchy et al., 2003) and induction of testis–ova (Zillioux et al., 2001). Most known EDSs are xenoestrogens, hence studies have mainly tested the effects of such compounds (Patyna et al., 1999; Länge et al., 2001; Zillioux et al., 2001; MacLatchy et al., 2003). However, environmental contaminants may also interfere with androgen dependent processes. For example, within the complex mixture of pulp and paper mill effluent are unknown substances that act as androgen receptor agonists (Durhan et al., 2002), some of which appear to be bioavailable (Hewitt et al., 2000) and also with the potential to bioaccumulate (Hewitt et al., 2003). Exposure to these effluents has been linked to masculinization of females in fish populations (Howell et al., 1980). Anti-androgens are also found in the environment as demonstrated by laboratory exposures in which pesticides and their metabolites, vinclozolin and *p*,*p*′-1,1-dichloro-2,2-bis (*p*-chlorophenyl) ethylene (*p*,*p*′-DDE) [metabolite of 1,1,1-trichloro-2,2-bis (*p*-chlorophenyl) ethane (DDT)], are able to demasculinize mature male fish (Baatrup and Junge, 2001).

To date, most bioassays developed to assess effects of EDSs on fish have focused on freshwater species (Kime and Nash, 1999; Patyna et al., 1999; Örn et al., 2000; Andersen et al., 2001; Ankley et al., 2001; Länge et al., 2001) with few studies testing impacts on marine and estuarine species (Zillioux et al., 2001; Urushitani et al., 2002; MacLatchy et al., 2003). Estuaries receive both industrial (Durhan et al., 2002) and municipal (Desbrow et al., 1998) effluents, containing compounds capable of disrupting endocrine homeostasis. Endocrine disruption could be particularly harmful in estuaries because these areas are important breeding and rearing grounds for many fish species (Locke and Courtenay, 1995). Early-life stages are known to be among the most sensitive life stages to chemical insult (McKim, 1977; von Westernhagen, 1988; Weis and Weis, 1989). Irreversible morphological abnormalities in embryos, larvae and juveniles often occur after contaminant exposure (von Westernhagen, 1988; Weis and Weis, 1989). Laboratory exposures to anthropogenic compounds (Holdway and Dixon, 1986; Kelly and Di Giulio, 2000), and also to synthetic (Kime and Nash, 1999; Barón et al., 2000) and natural (Urushitani et al., 2002) hormones, have demonstrated that early morphological development can be affected by EDSs. Consequently, endocrine disrupters could affect early-life development of estuarine fish species. Therefore, an estuarine fish bioassay should be developed to determine possible environmental impacts of EDSs during early-life development.

The mummichog (*Fundulus heteroclitus*) is a small estuarine teleost found in salt marshes and creeks along the East Coast of North America from Florida (USA) to Newfoundland (Canada). It is easily obtained in large numbers and easily kept in the laboratory where gametes of Northern populations can be made available for early-life testing throughout the year, by temperature and photoperiod manipulation (MacLatchy et al., 2003). The biology of this fish is well known from over 100 years of studies including toxicological studies (Atz, 1986). A clear chorion permits observation of the developing embryo and normal stages of early-life development have been well documented (Armstrong and Child, 1965; Vandersea et al., 1998). For these reasons, the mummichog is a good candidate for a marine–estuarine fish lifecycle bioassay.

The purpose of the present study was to test the effects of androgenic and anti-androgenic compounds with a mummichog early-life bioassay, which was previously utilized to test estrogenic and anti-estrogenic compounds (Boudreau et al., 2004). The compounds chosen for the present study were the androgen agonist 17α-methyltestosterone (MT) and the mammalian androgen antagonist cyproterone acetate (CA). MT was recommended by the OECD (OECD, 1999) as a reference chemical for EDS testing. CA, a clinical anti-androgen which can be employed as a male contraceptive (Amory and Bremner, 2000), was selected because of its high affinity for fish androgen receptors (Wells and Van Der Kraak, 2000), although it has not yet been demonstrated to be an androgen receptor antagonist in fish. A wide range of concentrations (10–100,000 ng/L), including both environmentally relevant and higher, pharmaceutical levels, were utilized to evaluate effects of these compounds on