Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Marine Environmental Research 61 (2006) 352–362
MARINE ENVIRONMENTAL RESEARCH
www.elsevier.com/locate/marenvrev

Oral single pulse exposure of flounder *Platichthys flesus* to 4-*tert*-octylphenol: Relations between tissue levels and estrogenic effects

Louise L. Madsen *, Bodil Korsgaard, Poul Bjerregaard

Institute of Biology, University of Southern Denmark, Odense, Campusvej 55, DK-5230 Odense M, Denmark

Received 1 September 2005; received in revised form 15 November 2005; accepted 17 November 2005

Abstract

The accumulation of 4-*tert*-octylphenol and the associated estrogenic effects were studied after a single pulse exposure to flounder *Platichthys flesus*. 4-*tert*-octylphenol was administered orally in a single dose of 50 mg kg^{-1} and tissue (liver, muscle and testis) and plasma concentrations of 4-*tert*-octylphenol as well as plasma vitellogenin were measured 3, 6, 12, 18, 24, 48, 72, 144 and 216 h after administration of the dose. Concentrations of 4-*tert*-octylphenol in plasma and tissues were determined by Liquid Chromatography Mass Spectrometry (LC–MS).

4-*tert*-octylphenol was detectable in liver, testis, muscle and plasma 3 h post administration and an accumulation was observed in liver, muscle and plasma up to 12 h and in testis 18 h post administration, respectively. The maximum concentrations of 4-*tert*-octylphenol in liver, muscle and testis were 67, 3.2 and 6.8 μg g^{-1}, respectively.

An increase in plasma vitellogenin levels was seen 48 h post administration and the vitellogenin level continued to increase (from <100 ng ml^{-1} to 1.4 mg ml^{-1}) until the end of the experiment 9 days after the administration of 4-*tert*-octylphenol.

Keywords: *Platichthys flesus*; 4-*tert*-octylphenol; Vitellogenin; Disposition; Pulse exposure; Xenoestrogens; Biomarker; Fish; Oestrogen mimics

* Corresponding author. Tel.: +45 65502760; fax: +45 65930457.
E-mail address: louisem@biology.sdu.dk (L.L. Madsen).

0141-1136/$ - see front matter
doi:10.1016/j.marenvres.2005.11.002

1. Introduction

In nature, fish are likely to be exposed to xenoestrogens in pulses released from e.g. wastewater treatments plants. However, most of the present knowledge on the effects of xenoestrogens is from water exposure experiments with a constant water regime.

Alkylphenols (e.g. octylphenol and nonylphenol) are the final breakdown products of the alkylphenol polyethoxylates. Alkylphenol polyethoxylates are a large group of non-ionic surfactants, which are used both as detergents and in many formulated products such as paints, pesticides and plastics. Alkylphenol polyethoxylates enters the aquatic environment either in untreated sewage or in effluent from sewage treatment plants. In sewage treatment systems alkylphenol polyethoxylates are biodegraded under aerobic and anaerobic conditions to more toxic and estrogenic metabolites such as the alkylphenols that are not readily biodegradable (Bennie, 1999). 4-*tert*-octylphenol used in the present study is found in the environment at concentration one order of magnitude lower than those of 4-nonylphenol (Bennie, 1999).

One of the most well established specific and sensitive biomarker of estrogenic effects in both laboratory and field studies is the induction of the yolk-precursor protein vitellogenin in juvenile and male fish (Harries et al., 1997; Sumpter & Jobling, 1995). Normally, estrogen stimulates the liver of female fish to produce vitellogenin, which is incorporated into the yolk of developing oocytes. However, the liver of male (and immature male and female) fish is also able to produce vitellogenin when stimulated by estrogens. Thus, accumulation of xenoestrogens in male liver may induce vitellogenin production.

4-*tert*-octylphenol is the most potent xenoestrogen of the alkylphenols, with 4-*tert*-octylphenol being 1000 times less potent compared to 17β-estradiol when measuring vitellogenin gene expression in trout hepatocytes. The alkylphenols compete with estradiol for binding with the estrogen receptor (White, Jobling, Hoare, Sumpter, & Parker, 1994). 4-*tert*-octylphenol induces vitellogenin after water exposure (21 days) in medaka *Oryzias latipes* (Gronen et al., 1999), rainbow trout *Oncorhynchus mykiss* (Jobling, Sheahan, Osborne, Matthiessen, & Sumpter, 1996) and roach *Rutilus rutilus* (Routledge et al., 1998). Orally administered 4-*tert*-octylphenol induces vitellogenin in both rainbow trout *O. mykiss* (LOED: 30 $mg\,kg^{-1}$) (Pedersen, Pedersen, Pedersen, Korsgaard, & Bjerregaard, 2003) and flounder *P. flesus* (LOED: 5 $mg\,kg^{-1}$) (Madsen, Korsgaard, & Bjerregaard, 2002, 2003) after dosage given every second day for 10 days.

Alkylphenols are metabolized in fish but residues including the parent compound may persist in various tissues. In flounder *P. flesus* orally exposed to 4-*tert*-octylphenol, a significant accumulation of 4-*tert*-octylphenol is found at a dose of 2.5 $mg\,kg^{-1}$ BW in liver, and an accumulation was also observed at a dose of 5 $mg\,kg^{-1}$ BW in muscle and testis (Madsen, Korsgaard, & Bjerregaard, 2003). Similarly, 4-*tert*-octylphenol accumulated as the major residue in muscle and testis after water exposure in rudd *Scardinius erythrophthalmus* (Pedersen & Hill, 2002). In rainbow trout *O. mykiss* exposed to 4 $\mu g\,l^{-1}$ of [^{14}C] *t*-OP in water, the major tissues accumulating 4-*tert*-octylphenol were muscle (13%), bone (7%) and skin (5%) (% of total OP residues in the body) (Ferreira-Leach & Hill, 2001).

So far very little is known as to the effects of pulse exposure to xenoestrogens. Therefore the aim of the present study was to investigate the accumulation of 4-*tert*-octylphenol and the associated estrogenic effects after a single pulse exposure to flounder *Platichthys flesus*. We investigated the induction of vitellogenin and the concentrations of 4-*tert*-octylphenol in plasma and tissue (liver, muscle and testis) throughout the experiment.