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Tissue-specific bioconcentration of the synthetic steroid hormone medroxyprogesterone acetate in the common carp (*Cyprinus carpio*)

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ARTICLE INFO

Article history:
Received 2 July 2013
Received in revised form
16 September 2013
Accepted 20 September 2013
Available online 5 October 2013

Keywords:
Progestins
Bioconcentration
Common carp
Pharmaceuticals

ABSTRACT

The steroid hormone medroxyprogesterone acetate (MPA), commonly used in oral and injectable contraceptives, has been detected in surface and wastewaters near urban and agricultural areas in several rivers of the world. The objectives of this study were to examine the accumulative potential and tissue distribution of MPA in fish. A freshwater species, the common carp (*Cyprinus carpio*), was exposed to 100 μg/L of MPA for a 7-day period followed by a depuration phase in which fish were maintained in dechlorinated tap water for an additional 7 days. Tissues (muscle, brain, plasma, and liver) were sampled during the uptake (days 1, 3, and 7) and depuration (day 14) phases of the experiment. Tissue-specific bioconcentration factors (BCF) ranged from 4.3 to 37.8 and uptake was greatest in the liver > brain > plasma and lowest in the muscle. From a regulatory standpoint, MPA shows little tendency to bioaccumulate in fish.

1. Introduction

Synthetic steroid hormones, commonly used in oral contraceptives, hormone replacement therapy, and livestock production, are present in the aquatic environment and can act as potent endocrine disruptors when exposed to aquatic organisms (Cripe et al., 2010; Durhan et al., 2006; Peterson et al., unpublished data; Vajda et al., 2008; Viglino et al., 2008). Surface waters receive synthetic hormones from a number of sources, including discharge from waste water treatment plants (WWTPs), sewage runoff from livestock farms, and excrement from fish hatcheries (Chang et al., 2008; Kolodziej et al., 2004; Ternes et al., 1999). Several steroid hormones have been shown to reduce fecundity or cause intersex in fish at concentrations (1–50 ng/L) relative to those detected in WWTP effluent, runoff from beef cattle feeding operations, and even streams (Ankley et al., 2003; Durhan et al., 2006; Fernandez et al., 2007; Jobling et al., 2006; Kolodziej et al., 2003; Santos et al., 2007; Zeilinger et al., 2009). 17-α-Ethinylestradiol (EE_2), a synthetic estrogen, has been detected in WWTP effluent at concentrations up to 7 ng/L (Desbrow et al., 1998) and has induced vitellogenin, an egg yolk precursor protein, in male rainbow trout at a 100 pg/L exposure level (Purdom et al., 1994). The presence of synthetic steroid hormones in wastewaters of some streams and rivers is associated with sexual abnormalities in fish (Allen et al., 1999; Kirby et al., 2004). Jobling et al. (2002), report that all male fish sampled from waterways

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http://dx.doi.org/10.1016/j.etap.2013.09.013

receiving wastewater effluent in the U.K. contained both male and female reproductive tissues. EE2 and other estrogenic chemicals are linked to the feminization of fish in waste water effluent dominated waters (Sumpter and Johnson, 2008).

Whereas much attention of eco-toxicological studies focused on synthetic steroid hormones has been directed toward estrogens, the efficacy of combination estrogen/progestin oral contraceptives in humans is apparently derived from progestins (Erkkola and Landgren, 2005). Additionally, synthetic progestins are likely to exist in the environment at higher concentrations than estrogens because birth control medications usually contain 3–100 times more progestin than estrogen (Zeilinger et al., 2009).

In mammals, synthetic progestins prevent pregnancy through several different mechanisms within various target tissues (Flores-Herrera et al., 2008). These mechanisms include the prevention of follicle stimulating hormone (FSH) and luteinizing hormone (LH) surges that stimulate ovulation (Letterie, 1998; Richter et al., 2002), the alteration of cervical mucus cell content and molecular structure (McCann and Potter, 1994), and the reduction in the number of cilia, and the frequency and intensity of cilia action on the tubal epithelium, thereby inhibiting the transport of the fertilized egg from the oviduct to the uterus (Flores-Herrera et al., 2008; McCann and Potter, 1994). In female fish, natural progestins play a critical role in oogenesis (Miura et al., 2007), regulation of oocyte maturation (Nagahama and Yamashita, 2008), and, in some species, ovulation (Pinter and Thomas, 1999). Progestins are associated with sperm motility (Tubbs and Thomas, 2008) and initiation of spermiation (Ueda et al., 1985) in males. Because synthetic progestins have the ability to mimic natural progestins in fish, and thus disrupt reproductive and developmental processes, these compounds pose a risk to fish communities. Previous research indicates that synthetic progestins are capable of inhibiting reproduction in fathead minnows at concentrations as little as 0.8 ng/L (Zeilinger et al., 2009) and completely halting reproduction at concentrations ranging from 85 ng/L to 100 ng/L (Paulos et al., 2010; Runnalls et al., 2013).

The synthetic progestin, medroxyprogesterone acetate (MPA), is widely used as an injectable and oral contraceptive and as a therapy for breast cancer and hormone replacement. MPA has been detected in wastewater effluent at concentrations up to 18 ng/L (Chang et al., 2009) and in surface water up to 1 ng/L (Kolodziej et al., 2004). In mammals, MPA has been shown to interact with receptors for progesterone (Winneker et al., 2003), androgen (Bentel et al., 1999; Hackenberg et al., 1993), and estrogen (Di Carlo et al., 1983). Like many other steroid hormones, MPA is relatively hydrophobic (Table 1) giving it the ability to partition into the lipid portion of organisms and bioaccumulate (Lindenmaier et al., 2005). Xenobiotics that bioaccumulate may trigger certain toxicological responses, such as reduced fecundity, as a result of increased tissue burden over an extended period of time (Nallani et al., 2012). From a regulatory perspective, bioaccumulative potential is an important aspect in determining the risk a compound poses to aquatic organisms. For these reasons, the objectives of the current study were to determine the tissue specific and plasma bioconcentration factor (BCF) of MPA in fish.

2. Materials and methods

2.1. Chemicals and reagents

The test chemical, Medroxyprogesterone acetate (MPA, 17α-acetoxy-6α-methylprogesterone, CAS# 71-58-9), was purchased from Sigma–Aldrich (St. Louis, MO). Medroxyprogesterone-d3 (MP-d3, CAS# 162462-69-3), also acquired from Sigma–Aldrich, was used as an internal standard. HPLC grade methanol, dichloromethane, and dimethylformamide were obtained from Fisher Scientific (Houston, TX). Milli-Q water was obtained from the Milli-Q Water System (Millipore, Billerica, MA) within the laboratory.

2.2. Fish exposure and study design

Juvenile common carp (*Cyprinus carpio*) were cultured at the University of North Texas aquatic toxicology facility. The carp were maintained in a 16:8-h light/dark cycle and fed flake food once a day. Fish exposures were accomplished using a continuous flow-through system that incorporated two 20 L tanks. Each tank received the test chemical from a mixing chamber that was dosed with MPA by direct infusion from a syringe pump, and both tanks drained via overflow into a common drain pan. Complete tank turn over with dechlorinated tap water occurred approximately 9 times per 24 h. Carp ($n = 28$) were randomly distributed between the two tanks and exposed to 100 μg/L MPA (in dimethyl formamide, DMF < 0.003%) for 7 days followed by a depuration phase where the fish were held in clean water for an additional 7 days. Because other fish BCF studies on pharmaceuticals have indicated little accumulation difference among varying exposure concentrations (Garcia et al., 2012; Nallani et al., 2012), this study design involved one exposure concentration. To account for any possible effects of the carrier solvent (DMF), the experiment included a solvent control ($n = 14$) that introduced the fish to DMF by the same exposure method as that of the MPA exposed carp. Although a total of 12 fish were sampled from each tank during the study, two extra fish were maintained in each exposure chamber to help ensure sampling objectives were accomplished in case fish fatalities occurred.

2.3. Tissue and water sample collection

On days 1,3,7, and 14, three fish from each tank were sampled for plasma and tissues (muscle, liver, and brain). Fish were anesthetized with tricaine methanesulfonate (MS-222) prior to removal of any blood or tissue samples. To ensure there was enough plasma to analyze, blood samples were combined from the three fish sampled from each tank on each sampling day. Blood was taken from the caudal vein using a heparinized capillary tube and subsequently placed in a microfuge tube with heparin. Following centrifugation at 2500 × *g*, plasma was taken from the sample and deposited in another heparinized microfuge tube. Plasma and tissues were stored at −80 °C for further processing. To determine the realized exposure concentrations, 5 water samples were collected from each of the 20 L exposure tanks on days 1 and 7 of the 7-day uptake phase.