Effects of 4-*tert*-octylphenol on *Xenopus tropicalis* in a long term exposure☆

Karen L. Porter [a],[*], Allen W. Olmstead [b], David M. Kumsher [a], William E. Dennis [a], Robert L. Sprando [c], Gary W. Holcombe [b], Joseph J. Korte [b], Annelie Lindberg-Livingston [b], Sigmund J. Degitz [b]

[a] *U.S. Army Center for Environmental Health Research, Fort Detrick, MD, United States*
[b] *U.S. EPA National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, Duluth, MN, United States*
[c] *U.S. FDA Center for Food Safety and Applied Nutrition, Division of Toxicology, Laurel, MD, United States*

ARTICLE INFO

ABSTRACT

Endocrine disrupting chemicals that activate the estrogen receptor are routinely detected in the environment and are a concern for the health of both exposed humans and indigenous wildlife. We exposed the western clawed frog (*Xenopus tropicalis*) to the weak estrogen octylphenol from Nieuwkoop–Faber (NF) stage 46 tadpoles through adulthood in order to document the effects of a weak estrogen on the life history of an amphibian species. Frogs were exposed to 1, 3.3, 11 and 36 μg/L octylphenol in a continuous flow-through water system. Just prior to completion of metamorphosis (NF 65), a random subsample of froglets was collected and assessed, while the remaining frogs received continued exposure through 31 weeks of exposure when the remaining animals were sampled. Significant induction of the female egg yolk protein precursor vitellogenin was observed in the high treatment at the larval subsampling for both males and females, but not at the final sampling for either sex. No significant deviation from the control sex ratio was observed for either sampling period, suggesting minimal to no effect of octylphenol exposure on gonad differentiation. No effects in the adult frogs were observed for mortality, body mass and size, liver somatic index, estradiol and testosterone serum levels, sperm counts, or oocyte counts. The development and growth of oviducts, a female-specific secondary sex characteristic, was observed in males exposed to octylphenol. These results indicate that octylphenol exposure can induce vitellogenin in immature froglets and the development of oviducts in male adult frogs. The lack of effect observed on the developing gonads suggests that in amphibians, secondary sex characteristics are more susceptible to impact from estrogenic compounds than the developing gonads.

Published by Elsevier B.V.

1. Introduction

Endocrine disrupting chemicals (EDCs) elicit their effects by altering the normal physiological functions of various components of the endocrine system and have been shown to adversely interact with the endocrine system of a variety of organisms (Kloas et al., 2009). EDCs have been detected in various and diverse environmental media including soil, ground water, and surface water (Barrek et al., 2009; Duran-Alvarez et al., 2009; Lei et al., 2009; Oehlmann et al., 2008; Teuten et al., 2009) and have been reported to negatively impact wildlife populations (Bernanke and Kohler, 2009; Taylor and Harrison, 1999). For example, male alligators in Lake Apopka, which is contaminated with DDT breakdown products and the reproductive toxicant 1,2-dibromo-3-chloropropane (DBCP), were shown to suffer from defects in testis development (Semenza et al., 1997). Female gastropods exposed to the androgenic EDC, tributyltin, developed an imposex condition that prevented proper reproduction in these animals (Bailey and Davies, 1988; Oehlmann et al., 1996). Male fish downstream from sewage treatment plants discharging estrogenic EDCs have been reported to express elevated levels of the female-specific egg yolk protein, vitellogenin (Diniz et al., 2005).

The effects of EDC exposure have also been reported in amphibian populations, especially in anurans. Several studies have associated agricultural land use and exposure to various endocrine

disrupting herbicides and pesticides with abnormalities in male gonads (McCoy et al., 2008; McDaniel et al., 2008; Mosconi et al., 2005). In addition, numerous studies have demonstrated the susceptibility of the anuran endocrine system to alterations due to toxicant exposure (Hogan et al., 2008; Storrs and Semlitsch, 2008; Mann et al., 2009). Most of these studies focus on larval development, with few studies examining the effects of EDC exposure on juvenile development or the adult frog.

This research aims to increase our understanding of the ability of EDC's and specifically weak estrogens to interfere with normal endocrine physiology in anurans through reproductive maturity of these animals. Additional research in amphibian environmental toxicology is important for several reasons. First, amphibian populations are in decline globally with roughly one third of all species considered endangered (International Union for the Conservation of Nature, 2009; Stuart et al., 2004). While habitat degradation and disease are considered larger factors in this decline, investigations into the role of environmental contaminants in negatively impacting amphibian populations would increase our ability to protect these animals (International Union for the Conservation of Nature, 2009). Amphibians have a unique life cycle patterns that differ from those organisms traditionally used in environmental toxicology studies. For example, larval development of most amphibians occurs in an aquatic environment, while juveniles and adults inhabit a diverse array of environments including aquatic, terrestrial, arboreal, and subterranean habitats. The U.S. Environmental Protection Agency in developing the Endocrine Disruptor Screening Program acknowledges the importance of an amphibian model by proposing two anuran assays: one focusing on thyroid effects during metamorphosis and the other as a life cycle assay designed to detect impacts on a suite of endocrine-related endpoints (U.S. Environmental Protection Agency, 1999, 2007).

One of the principal mechanisms by which some EDC's elicit their effects is activation of the estrogen receptor. These chemicals are capable of mimicking endogenous estrogens and causing alterations in estrogen-responsive tissues such as the liver and gonads. Some of these chemicals are quite potent, such as 17α-ethynylestradiol, the active ingredient in some birth control formulations, which has been shown to activate estrogen receptors at lower concentrations than endogenous estrogens in some cell-based assays (Mitsui et al., 2007). Most estrogenic EDC's, however, are weak estrogens, and activate estrogen receptors at concentrations orders of magnitude higher than that of endogenous hormones (Mitsui et al., 2007). While these chemicals have been demonstrated to weakly activate the estrogen receptor in vitro, they nevertheless can elicit endocrine toxicity in vivo in a diverse group of organisms, including fish (Ortiz-Zarragoitia and Cajaraville, 2005; Rasmussen et al., 2005; Seki et al., 2003), mammals (Laws et al., 2000), reptiles (Semenza et al., 1997), amphibians (Kloas et al., 1999), and invertebrates (Andrew et al., 2008).

The weak estrogen, 4-tert-octylphenol (OP), (Bonefeld-Jorgensen et al., 2007), is a degradation product of octylphenol polyethoxylate used as non-ionic surfactants in industrial and household settings (Ying et al., 2002). OP has been measured in surface waters of the Danube near Budapest at 1.6–178 ng/L (Nagy et al., 2005), the Haihe River in China at 18–20 ng/L (Jin et al., 2004), the Mai Po Marshes Nature Reserve in Hong Kong at 11–348 ng/L (Li et al., 2007), and in Great Lakes sediments in the U.S. and Canada at 0.002–23.7 ng/g (Bennett and Metcalfe, 1998). Kloas et al. (1999) reported that exposure of larval X. laevis to OP at concentrations of 2.1 and 21 μg/L resulted in a significant shift in sex ratio towards females (Kloas et al., 1999).

In this study, we hypothesized that exposure of the western clawed frog, Xenopus tropicalis, to the weak estrogen, 4-tert-octylphenol, during larval and juvenile development would result in endocrine toxicities that would manifest itself at the end of lar-

val development as well as in the adults. These toxic effects would be similar to those seen in fish exposed to estrogenic compounds including alterations in sex ratio, induced vitellogenin in males, and abnormal testicular development. The experimental setup was designed to evaluate and compare effects at two different life stages in this organism. Although endpoints measured in this study were primarily centered on reproductive parameters (e.g., gonad development); endpoints related to the overall development and health of the organisms were also measured.

2. Materials and methods

2.1. Animals

Animals used were the western clawed frog, Xenopus tropicalis (Nigerian strain), originally obtained from Xenopus One (Dexter, MI) and bred for five generations in our in-house colony. Breeders were housed in partially filled 120 L tanks with a flow through rate of 5.4 turnovers per day. The aquaculture water used for housing and exposure of the frogs was prepared by mixing treated tap water with ground water at approximately a 50:50 ratio. This dilution was done to reduce the natural hardness of the ground water used for the study. The aquaculture water was then aerated, 10 μM filtered, UV sterilized and heated to 25 °C before use. Adult frogs were fed Nasco pellets (Fort Atkinson, WI) and tropical fish food flakes (Aquatic Eco-Systems Inc, Apopka, FL) once daily. A 12:12-h light:dark cycle was maintained.

Embryos were obtained as previously described (Knechtges et al., 2006). Briefly, ten breeding pairs were induced to mate using one injection of 200 IU human Chorionic Gonadotropin (Chorulon, Provet, Kansas City, MO) and kept overnight in the dark in 4 L breeding chambers in a 25 ± 2 °C incubator. The next morning, the most viable spawn was rinsed in 2% L-cysteine at pH 8 in order to remove the gelatinous coatings in order to increase ease of handling and sorting. Non-developing or abnormally developing embryos were removed daily. On the third day when most embryos were at Nieuwkoop and Faber (NF) stage 46 (Hubrecht-Laboratorium et al., 1967), the embryos were randomly transferred into the exposure tanks.

Tadpoles were fed 10 mL of a 50:50 mixture of Sera Micron (Pondside Herp Supply, Indian Harbor Beach, FL) and Nasco frog brittle powder (Nasco, Fort Atkinson, WI) suspended in water (0.4 g per 10 mL water) one time on the first day of exposure, then four times per day through day 50 of exposure. Half a gram of advanced tadpole diet (ATD: 2 L finely ground fish flakes, 1 L 45% protein Nasco fish food powder and 34 g Sera Micron) was added once on day 25, then four times daily and continued through metamorphosis. Half a gram of Nasco pellets were added once on day 35, then four times daily until metamorphosis. Once 95% of the animals in a tank reached stage 66 of metamorphosis, at about week 7 of exposure for control tanks, Sera Micron, Nasco frog brittle powder, and ATD feeding was discontinued.

Once post-metamorphic frogs were observed in the tanks, 0.5 g Nasco pellets were added from day 35 to 51, 1.0 g Nasco pellets from day 52 to 61, 2.0 g from day 62 through the end of the exposure. Starting on day 51, 0.5 g fish flake food (Aquatic Eco-Systems, Inc., Apopka, FL) was given four times daily, and then increased to 1.0 g on days 52–61, 2.0 g from day 62 through the end of the exposure. Feeding frequency of Nasco pellets and fish flake food was decreased to three times daily on day 91, to twice daily on day 121, and finally to once daily at day 151 until the end of the exposure.

Frogs being culled and moribund frogs were euthanized by immersion for 10 min in 0.2% Tricaine methanesulfonate (MS-222, Sigma, St. Louis, MO) adjusted to pH 7.0 with sodium bicarbon-