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Effect of bisphenol A on maturation and quality of semen and eggs in the brown trout, *Salmo trutta f. fario*

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Abstract

In the present study male and female brown trout (*Salmo trutta f. fario*) were exposed to environmentally relevant concentrations of bisphenol A (1.75, 2.40, 5.00 $\mu g\,l^{-1}$) during the late prespawning and spawning period and the effect of this contaminant on maturation, quantity and quality of semen and eggs was investigated.

In males exposed to estimated BPA concentrations of 1.75 and 2.40 $\mu g\,l^{-1}$ semen quality was lower than in the control in the beginning of spawning (reduced sperm density, motility rate, and swimming velocity) and in the middle of spawning (reduced swimming velocity, at 2.40 $\mu g\,l^{-1}$ BPA also reduced sperm motility rate). Therefore, production of high quality semen was restricted to the end of the spawning season and delayed for approximately 4 weeks in comparison to the control. At BPA exposure levels of 5.00 $\mu g\,l^{-1}$ only one of eight males gave semen of low quality (reduced semen mass, motility rate, and swimming velocity).

The percentage of ovulated females was similar for the control group and the groups exposed to estimated BPA concentrations of 1.75 and 2.40 $\mu g\,l^{-1}$, whereas at 5.00 $\mu g\,l^{-1}$ BPA females did not ovulate during the investigation. While brown trout of the control group ovulated between the 28 October and 12 November, brown trout exposed to estimated BPA concentrations of 1.75 $\mu g\,l^{-1}$ BPA ovulated approximately 2 weeks later and brown trout exposed to 2.40 $\mu g\,l^{-1}$ BPA approximately 3 weeks later. Therefore, the tested BPA concentrations affected the percentage of ovulated females and the time point of ovulation. No effect was observed on the quality of eggs (egg mass, percentile mass increase during hardening, egg fertility).

Keywords: Bisphenol A; Brown trout; *Salmo trutta f. fario*; Spermatozoa; Eggs; Motility; Fertility

1. Introduction

Bisphenol-A (BPA) is a synthetic chemical used in the production of epoxy resins and polycarbonate plastics. Sources of environmental releases are epoxy man-

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ufacturing facilities. Recent research showed that BPA has estrogenic potency and is therefore an endocrine disrupter (Toppari et al., 1995). In fish, it changes the levels of sex hormones (*Chasmichthys dolichognathus*, Baek et al., 2003), increases the levels of plasma vitellogenin (*Rhynchocypris oxycephalus*, Park et al., 2003a; *Phoxinus oxycephalus*, Park et al., 2003b; *Fundulus heteroclitus*, Pait and Nelson, 2003) and of zona radiata proteins (*Oryzias latipes*, Lee et al., 2002), alters the fecundity of fish (*O. latipes*, Kang et al., 2002; *Pimephales promelas*, Sohoni et al., 2001), changes the testis structure (*Poecilia reticulata*, Kinnberg and Toft, 2003) and affects the egg and larval development (*Salmo salar m. Sebago*, Honkanen et al., 2001; *O. latipes*, Na et al., 2000; *O. latipes*, Yokota et al., 2000; *O. latipes*, Pastva et al., 2001).

Effects of endocrine disrupting chemicals on fish are evaluated by gross gonad morphology and histology (development of ovotestes), and induction of vitellogenin and choriogenin production in males (Kime et aal., 1999; Jobling et al., 2003). Based on the BPA levels which increased vitellogenin levels in male rainbow trout (70 $\mu g\,l^{-1}$ BPA, Lindholst et al., 2000) a predicted non-effect concentration (PNEC) of 64 $\mu g\,l^{-1}$ was defined (Staples et al., 2000). The described risk assessment is quick, reliable and easily comparable between different labs. However, changes in gonad morphology, histology and vitellogenin levels indicate biological endpoints where sex reversion already occurs. At lower pollutant concentrations alterations in reproduction could occur which are not detectable with the described methods. The maturation of spermatozoa and eggs could be disturbed resulting in reduced gamete quantity or quality, or in desynchronization of reproduction. Such changes could drastically reduce the reproductive potential of wild populations. However, data evaluating these points are very limited. Only Haubruge et al. (2000) described that sperm densities in guppies (*Poecilia reticulata*) are decreased after exposure to low levels of BPA.

Therefore, in the present study brown trout (*S. trutta f. fario*) were exposed to environmentally relevant concentrations of BPA (1.75, 2.40, 5.00 $\mu g\,l^{-1}$) during the late prespawning and spawning season to determine the effect on the final maturation processes of gametes (time point of spawning, quality and quantity of gametes). The tested BPA concentrations were selected on the basis of their occurrence in Austrian water systems and on the PNEC defined for Austria (1.6 $\mu g\,l^{-1}$) (Paumann and Vetter, 2003). Brown trout were used as a model as only very few data are available on the species and as they represent recreationally important fish populations in many parts of the world. The criteria for assessment of semen quality were the mass of produced semen, the sperm density, the sperm motility as assessed by computer assisted cell motility analysis, and the sperm fertility. The criteria for assessment of egg quality were the number of produced eggs, the egg mass, the mass increase during hardening and the egg fertility.

2. Materials and methods

2.1. Experimental design

All experiments were conducted in the hatchery of Kreuzstein in Sankt Gilgen, Upper Austria, with brown trout (*S. trutta f. fario*) and in compliance with the Austrian Federal law for animal care (GZ 68.210/58-Br GT/2003). Fish derived from a wild population in a mountain area of Salzburg (Blühnbach) with water system containing no endocrine disrupting substances (unpublished data). Fish were caught in the end of August by electroshocking and then transported to the fish farm Kreuzstein. There they were acclimated for 2 weeks before they were used for the experiments.

In order to expose fish to BPA, a flow through system was used which has been described previously (Lahnsteiner et al., 2005) (Fig. 1). Briefly, the system consisted of four 0.5 m^3 tanks. The tanks were supplied with well water of 6 °C and an oxygen content of >90% saturation. BPA was added by means of an injection pump. Consequently, the BPA concentrations were adjusted by changing the injection rates. In comparison to the previously described system (Lahnsteiner et al., 2005) the flow through system used in the present experiments was modified to increase its accuracy. Well water was supplied via a storage reservoir in a height of 1.5 m above the tanks where after the water flow was regulated by reduction pieces (diameter reduction from 30 to 6 mm) (Fig. 1). This set up reduced variations in well water flow rates to <2.0%. The injection pumps were precise, having variations of <0.5%. Final BPA concentrations were calculated based on the flow rate