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Nile Tilapia, *Oreochromis niloticus* L., Reproduction Inhibition by Dietary Exposure to Aroclor 1254

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Polychlorinated biphenyls (PCBs) belong to a family of environmental contaminants that persist for a long period in ecosystems. Their lipophilic properties are responsible for bioaccumulation in food chains. PCBs are associated with a large variety of biologic and toxicological effects (Dragnev et al. 1994), being capable of reproductive and endocrine disruption in all classes of vertebrates (Alston et al. 2003). Usually, PCBs are present as complex mixtures of several PCB congeners, like Aroclor 1254 which is a commercial mixture of PCBs congeners with 54% chlorine in weight. Reports on PCBs exposure, both in wild and laboratory fish populations, describe a wide variety of alterations connected with reproduction, such as decreased hatching success, low survival rates, impaired development of eggs and embryos, reduced and delayed spawning, inhibition of spermatogenesis, testicular abnormalities and disruption of the reproductive endocrine function (review in Örn et al. 1998).

Tilapia belong to one of the most commercially important groups of freshwater fish in world aquaculture (Coward and Bromage 2000). These factors affecting reproduction may cause severe ecological effects, as well as economical ones.

In order to determine if dietary exposure to PCBs interferes with tilapia reproduction, tilapia were exposed to Aroclor 1254 through the diet. We tested the effects of two doses of this compound at two exposure periods. In this study, progesterone and testosterone plasma levels were measured to evaluate PCBs impact on gonadal steroidogenesis. Gonadal histology was performed.

MATERIALS AND METHODS

Aroclor 1254 was purchased from NSI Solutions, Inc. (Raleigh, USA) as a pure standard.

One week before the beginning of the assay, 8 adult tilapia, <1 year old, (average size and weight: 19.9±0.4cm and 170.3±10.8g) were randomly allocated, in five 70L tanks (Control and Aroclor 1254 two doses-two exposure times), with an individual mechanical and biological filtration system to each dose group.

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Supplemental aeration was provided to maintain dissolved oxygen near saturation and water temperature was kept at 17°C, since at this temperature tilapia gonads remain immature. The water source was the public water supply. Before use water was de-chlorinated and an approximate 3L renovation occurs per hour in each system.

Throughout the assay, specimens were exposed through the diet to two different Aroclor 1254 doses (0.5 and 4.5μg/g - Aroclor 1254/g food). Animals were fed with approximately 2% of their body weight once a day with the contaminated diet. During the assay they were kept under ideal reproduction conditions: 27°C water temperature and a 12-hour light, 12-hour dark photoperiod. Control tilapia experienced the same conditions and were fed with a commercial fish diet.

Five tilapia from each group were sacrificed at the end of 10 and 20 days of exposure (control was sacrificed at the end of 20 days). Simultaneously, the specimens were anaesthetised, blood was collected from the caudal vein, centrifuged and plasma was stored, until further analysis, at -80°C. All the organs collected were frozen and stored at -80°C. Whenever possible, a part of the gonad was fixed in Bouin Liquor (Panreac) and after dehydration included in paraffin, sectioned at 5μm, mounted on glass slides and stained with haematoxylin & eosin.

Testosterone and progesterone plasma levels were measured by solid-phase radio immuno assay (RIA) using ^{125}I Coat-a-Count kits (DPC, Los Angeles,USA), with antibody precoated tubes as was described for tilapia hormones (Rocha and Reis-Henriques 1996, Fontaínhas-Fernandes et al. 2000). The sensitivities of the standard curve were 0.02ng/ml for progesterone and 0.004ng/ml for testosterone. The antibodies are highly specific for both hormones, showing a 100% binding to progesterone, 50% binding for testosterone and a low cross-reactivity with other naturally occurring plasma steroids, such 3.4% to 17α-hydroxyprogesterone and 0.1% to pregnenolone and testosterone with the progesterone kit and 16% to 11-ketotestosterone and 0.02% to estradiol with the testosterone kit. The intra-assay coefficients were 6.7% for progesterone and 19.1% for testosterone. Inter-assay variation was avoided by measuring all samples in the same assay. All samples were analysed in duplicate.

All results are expressed as mean±standard error of the mean (SEM). Statistical analysis between control and exposed tilapia data was performed by analysis of variance (ANOVA) using the LSD test ($p<0.05$). Correlation coefficients were calculated using the Pearson correlation method ($p<0.05$).

RESULTS AND DISCUSSION

Although ideal reproduction conditions were maintained during the assay, reproduction did not occur, in either the control or in Aroclor 1254 exposed tilapia.

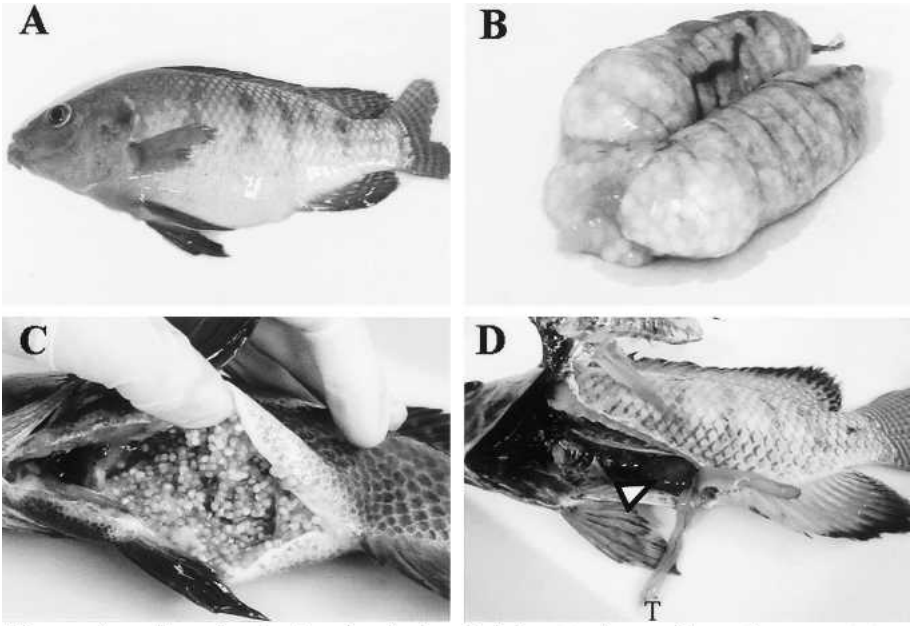

Figure 1. A-Female tilapia after being fed for 20 days with a diet containing 4.5μg/g of Aroclor 1254 presenting an increase in abdomen volume. B-Ovary of a tilapia fed for 20 days with a diet containing 4.5μg/g of Aroclor 1254, in which can be observed a high percentage of atretic oocytes. C-Ovary of a tilapia fed for 20 days with a diet containing 4.5μg/g of Aroclor 1254; in this case the ovary membrane had been damaged and a mass of atretic oocytes was found scattered in the abdominal cavity. D- Male tilapia fed for 20 days with a diet containing 4.5μg/g of Aroclor 1254. The arrowhead indicates the bladder full of urine, T-testis.

At the end of 20 days of exposure to Aroclor 1254, some tilapia from the group fed with 4.5μg/g of Aroclor 1254 presented an increased abdomen volume (Fig. 1A); during sampling it was observed that these tilapia were all females. The increase was due to an abnormally high number of oocytes in ovaries; also, the percentage of atretic oocytes was found to be greater than 40% (Fig. 1B), a value described as the maximum reached by Nile tilapia (review in Coward and Bromage 2000). Some females presented due to destruction of the ovary membrane, oocytes dispersed inside the abdominal cavity (Fig. 1C). These results indicate that while Aroclor 1254 does not interfere with tilapia oogenesis, spawning seems to be inhibited, resulting in accumulation of oocytes in the interior and rupture of ovary walls.

In male tilapia, the urinary bladder was visible and full of urine (Fig. 1D). This observation contrasts with that observed by Rocha and Reis-Henriques (1996) which described this organ as being compressed and empty between the gonads and the abdominal cavity wall upon manipulation.

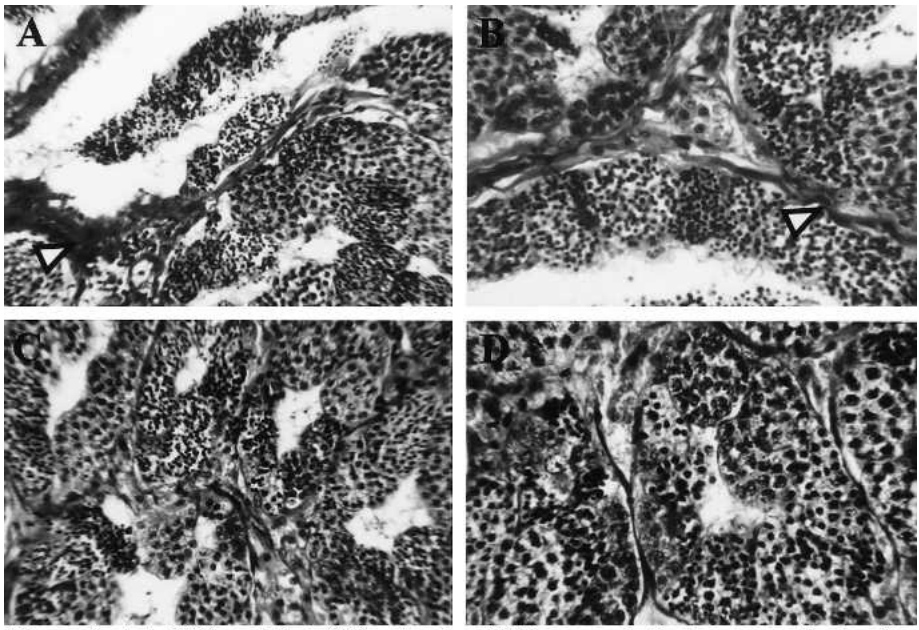

Figure 2. A (x250) and B (x400) - Aroclor 1254 exposed tilapia testes. Notice the spermatozoa in the lumen of the seminiferous tubules, the thickness of connective tissue of the walls (arrowheads) and the decline of the germinal epithelium. C (x250) and D (x400) - Control tilapia testes.

In female tilapia, the genital pore is separated from the urinary pore, while in males they form a common orifice. In this study, we observed no release of female gametes, while in males there was urine accumulation in the urinary bladder. These results suggest that exposure to Aroclor 1254 might cause obstruction of the genital pores. We speculate that the connective tissue surrounding the pore may have increased, thereby diminishing pore elasticity. Further studies on the morphology of tilapia genital pores, after Aroclor 1254 exposure are necessary to confirm this hypothesis.

Due to the severe damage of the ovaries, it was impossible to perform a histological evaluation of this organ. However, testes histology (Fig. 2) showed that Aroclor fed males presented a thicker seminiferous tubule wall compared to equivalently mature control males. Also we detected a decrease in germinal epithelial layers. Similar results were observed in mammals and birds, where the same testicular damages were described after Aroclor 1242 (Ahmad et al. 2003) and Aroclor 1254 exposure (Alston et al. 2003; Zhang and Qiao 2004).

Aroclor 1254 exposure significantly depressed progesterone levels in male tilapia (Table 1). Significant testosterone level depression was observed for males exposed to the lower dose for 20 days and to the higher dose for 10 days.

Progesterone and testosterone plasma concentrations were found to be positively

Table 1. Progesterone and testosterone (ng/ml) plasma levels of tilapia exposed to Aroclor 1254 and control. Values presented as Mean±SEM. (n) number of animals. Statistical differences between treatment and control:*p<0.05; **p<0.01; ***p<0.001.

Aroclor 1254 (µg/g)	Day	Male		Female	
		Progesterone	Testosterone	Progesterone	Testosterone
Control		2.34±0.71 (2)	51.17±5.23 (2)	0.75±0.06 (2)	37.47±1.91 (2)
0.5	10	0.56±0.36** (2)	23.26±15.68 (2)	0.17±0.07 (2)	9.63±2.88 (2)
0.5	20	0.49±0.24** (3)	17.11±6.57* (3)	0.44 (1)	19.46 (1)
4.5	10	0.25±0.14*** (3)	9.27±3.40** (3)	0.56 (1)	44.4 (1)
4.5	20	0.64±0.26** (2)	22.78±12.52 (2)	0.14±0.02 (2)	5.1±4.87 (2)

correlated (r=0.93; p<0.0001). In this study, control male tilapia presented plasma concentrations of 2.34±0.71 and 51.17±5.23ng/ml of progesterone and testosterone, respectively. In exposed tilapia (4.5µg/g for 10 days) a significant decrease of progesterone (0.25±0.14ng/ml) and testosterone (9.27±3.40ng/ml) was observed.

In male tilapia, no differences were found in testosterone and progesterone levels between 10 and 20 days of exposure to the lower Aroclor 1254 dose. Tilapia exposed 10 days to the higher dose presented significant (p<0.05) lower levels when compared to tilapia exposed 20 days. The observation that in the male both hormone levels at the end of 20 days were similar to those measured after exposure to 0.5µg/g, suggests a slight recovery of testes.

The decrease in progesterone plasma levels observed in male exposed tilapia, may explain the decline in testosterone levels, as progesterone is the precursor for testosterone production and a strong positive correlation was observed. Aroclor 1254 exposure inhibited tilapia testicular steroidogenesis. This was also described in rats where both *in vivo* and *in vitro* Aroclor 1248 exposure inhibited testicular steroidogenesis (Andric et al. 2000).

Due to the number of female specimens analyzed and the high variability among the hormone concentrations, it was not possible to observe significant differences. However, progesterone levels for females exposed to Aroclor 1254 were always lower than those of the control. The data on plasma hormone levels and ovarian morphology observations, suggest that ovarian steroidogenesis was also inhibited. The destruction of the ovary tissue, as demonstrated by the high number of atretic oocytes, is the likely cause.

In conclusion, a short exposure period to a low dose of Aroclor 1254 through the diet interrupts tilapia reproduction. The inhibiting mechanisms seem to result in alterations of sex steroid synthesis due to morphological alterations of the testicular and ovarian germinative tissues. Globally, our results do not discard the hypothesis of Aroclor 1254 being capable of producing effects at other levels of the reproductive axis.

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