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Tilapia Larvae Aroclor 1254 Exposure: Effects on Gonads and Circulating Thyroid Hormones During Adulthood

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Abstract Aroclor 1254 a polychlorinated biphenyls (PCBs) mixture, when administrated through the diet, was previously found to inhibit adult tilapia (*Oreochromis niloticus*) reproduction. Since fish larvae can be more sensitive to contaminants, the objectives of this study were to evaluate in adults the impact in gonad development and in thyroid function of Aroclor 1254 administrated at larvae stages. Aroclor 1254 exposed tilapia presented both ovary and testicular alterations and a decline in T4 plasma concentration while T3 remained unaltered. This work shows exposure to Aroclor 1254 during tilapia early life stages causes a disruption of the reproductive axis that enables reproduction.

Keywords Tilapia · Aroclor 1254 · PCBs · Reproduction · Ovary · Testis · Thyroid hormones · Larvae

Anthropogenic chemicals in the environment are a major problem to human and wildlife. Many of these chemicals used in industry and/or agriculture have as final destiny the aquatic environment (Hwang et al. 2006); also some of these chemicals are known to interfere with endocrine functions, leading to reproductive and developmental anomalies in wildlife populations (Cooper and Kavlock 1997), which may cause malformations on sexual characters and disturb the reproductive function in fish (Harrison et al. 1997; Tyler et al. 1998; Mills and Chichester 2005; Goksoyr 2006) and other vertebrates (Colborn et al. 1993; Guillette and Gunderson 2001; Halldin 2005; Ottinger et al. 2005).

Polychlorinated biphenyls (PCBs) are usually present in the environment as complex mixtures of several congeners; one of them is Aroclor 1254, which is a commercial PCB mixture with a 54 wt% of chlorine. Exposure to these compounds is associated with both biological and toxicological effects such as endocrine disruption and mortality in vertebrates (Brouwer et al. 1998; Vos et al. 2000). Recently, Coimbra and Reis-Henriques (2005) demonstrated that adult tilapia exposed during 20 days through the diet to 4.5 μg g^{-1} Aroclor 1254 presented an inhibition of reproduction.

Fish early life stages are thought to be more sensitive to environmental chemicals than adult fish (Nguyen and Janssen 2002). This is particularly evident in xenoestrogens exposures (Mills and Chichester 2005). Thyroid hormones importance in fish early development, growth and reproduction is well known, as their presence in the oocytes resulting from maternal transference (Leatherland 1994; Power et al. 2001). Thyroid hormones actions are mediated by the binding of T3, which formed after T4 deiodination, to nuclear receptors (Brown et al. 2004). In fish, the origin of both hormones can be endogenous and/or exogenous (dietary) (Eales 1997). Furthermore, PCBs are known to accumulate in the fish ovary and therefore might be present in descendents of contaminated fish (Hellou et al. 1993; Monosson et al. 2003).

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Previously, we studied the effects of Aroclor 1254 in adult tilapia and observed several reproductive alterations; also PCBs are known to be very persistent in fish tissues (Antunes et al. 2007). Therefore, the objectives of our study were to evaluate the impact of Aroclor 1254 in gonad development and thyroid function of tilapias exposed to Aroclor 1254 during larval stages, after the vitelline sac absorption.

Materials and Methods

Tilapia (*Oreochromis niloticus*) larvae were removed from the mouth of their progenitor, randomly divided in two groups, control and Aroclor 1254, and placed in 70 L tanks with individual mechanical and biological filtration. Aroclor 1254 contaminated diets were prepared by immersion of a commercial fish diet, previously powdered, on an Aroclor 1254–ethanol solution and then allowed to dry. Control tilapias were fed with the same commercial fish diet, which was only immersed in ethanol. Supplemental aeration was provided to maintain dissolved oxygen near saturation. The water source was the public water supply, which was de-chlorinated before entering the tanks; an approximate 3 L renewal occurs per hour in each system. Water temperature (20°C) and photoperiod (12 h-light:12 h-dark) were maintained constant during the assay period.

In a first experiment, it was administrated through the diet 1.5 μg g^{-1} of Aroclor 1254 (NSI Solutions, Inc.; Raleigh, USA) to 10 days post-hatch larvae (n = 80), right after the absorption of the vitelline sac, which resulted in a high mortality (57% at the end of 15 days) that led us to end this experiment. A second experiment using a lower Aroclor 1254 concentration (0.05 μg g^{-1}) was performed (n = 130 larvae per group), where Aroclor 1254 had no effect on mortality ($p > 0.05$). Larvae were fed with the contaminated diet for 40 days and from this period on tilapia from the exposed group were also fed with the control diet. At day 80 the number of tilapia was reduced, randomly, to 15 per group and the animals were changed for 250 L tanks, this tilapia survived to adulthood and 18 months after the beginning of the exposure were sacrificed.

Testis slides were prepared for optic microscopy, stained with Hemathoxilin-Eosin and qualitative slide analysis was undertaken.

Plasma concentrations of total T3 and total T4 were determined using commercial available RIA kits (Kit Coat-a-Count, DPC) according to the manufactures instructions. Both RIA kits use antibodies with high specificity (100% of reactivity) to the correspondent hormone. Cross-reactivity of each kit is very low; the T3 kit presents a reactivity of 0.05% for T4, while the T4 kit presents a 2% reactivity for T3. Inter assay variation was avoided by analysis of all samples in the same assay. All samples were analyzed in duplicate.

All results are presented as mean ± standard error (SE). Thyroid hormones concentration and gonad somatic index statistical analysis was performed using the non-parametric Mann–Whitney test and lesions frequency was tested using the chi-square test (STATISTICA 6.0).

Results and Discussion

Eighteen months after 40 days of dietary exposure to Aroclor 1254, specimens presented ovarian and testicular abnormalities. During sampling it was observed that seven in nine of the exposed females presented ovary lesions ($p < 0.01$), characterized by an abnormal weight and the presence of atretic oocytes within the ovaries, while control females presented no ovary lesions. Three of the exposed females presented a 60% content of atretic oocytes, a value superior to the 40% described as the maximum reached by Nile tilapia (Coward and Bromage 2000); the average gonad somatic index (as % of total body weight) was of 6.4 ± 1.11 in exposed females and of 4.11 ± 0.55 in control females ($p > 0.05$). Also, two in six males ($p > 0.05$) showed a full urinary bladder (Fig. 1), while this alteration was not observed in control tilapia. In addition, testis histology showed the occurrence of Leydig cells hyperplasia and a decrease of the germinal epithelium layer of Aroclor 1254 exposed tilapia (Fig. 2).

Gonad macroscopic changes observed in the present study were similar to the ones described for adult tilapia exposed to Aroclor 1254 (Coimbra and Reis-Henriques 2005), although, in the present study, rupture of ovaries walls was not seen. Females presented the ovaries full of oocytes, including a high number of atretic oocytes; a dose dependent increase in atretic oocytes was also observed in zebrafish exposed to doses from 40 to 270 ng g^{-1} of food of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) contaminated diets (Heiden et al. 2005).

Zebrafish TCDD exposure did not arrest spawning, but females presented ovarian necrosis and egg production reduction, being these effects caused by the accumulated TCDD, in concentrations as little as 0.6 ng g^{-1} of fish (Heiden et al. 2005, 2006). Due to the severe damage observed in tilapia ovaries, it was impossible to perform a histological evaluation of this organ.

Male tilapia exposed to Aroclor 1254 during the larvae stage exhibited full urinary bladders, as was also observed when exposed as adults (Coimbra and Reis-Henriques 2005). Therefore, as we previously suggested, Aroclor 1254 seems to interfere with the urogenital pore elasticity,

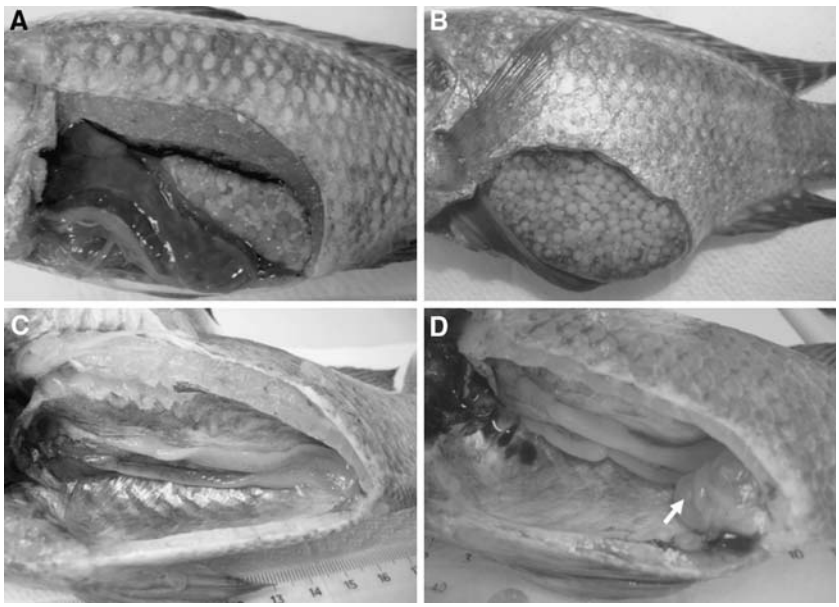

Fig. 1 **a** Control female. **b** Aroclor 1254 exposed female presenting an abnormal ovary size and atretic oocytes. **c** Control male. **d** Aroclor 1254 exposed male presenting a full urinary bladder (*arrow*)

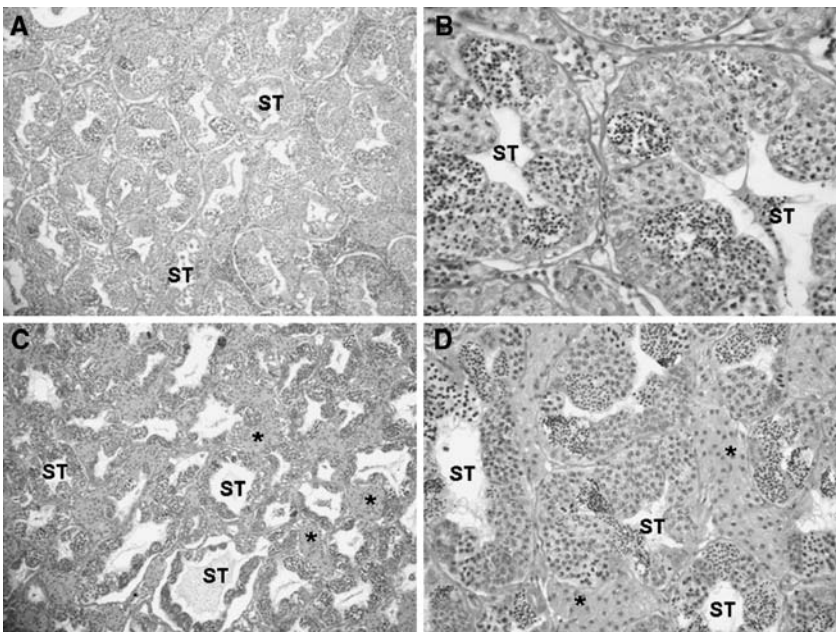

Fig. 2 Histological images of tilapia testis. **a** ×100 control tilapia; **b** ×400 control tilapia; **c** ×100 Aroclor 1254 exposed tilapia; **d** ×400 Aroclor 1254 exposed tilapia. *Asterisks* Leydig cells hyperplasia; *ST* seminiferous tubules lumen

blocking female spawning and interfering with male release of urine and possibly of spermatozoa. Uro-genital morphological alterations were also described for LE Hooded and Holtzman rats (Gray and Ostby 1995) and Syrian hamsters (Wolf et al. 1999) exposed to TCDD in utero. Gray and Ostby (1995) observed in rats dosed by gavage with 1 μg kg^{-1} of TCDD, at gestational day 15, a delay of the vaginal opening and in some females a partial vaginal occlusion by a persistent membrane or thread.

The testicular tissue lesions observed in tilapia exposed as larvae are in some degree different from lesions described for adults. Both in adult tilapia (Coimbra and Reis-Henriques 2005) and in this study it was observed a decrease of the germinal epithelium layer (Fig. 2). Identical results were observed in cod (Sangalang et al. 1981) and in other vertebrates exposed to PCBs mixtures (Ahmad et al. 2003; Zhang and Qiao 2004); however, while tilapias exposed when adults, presented an increase in the seminiferous tubules walls thickness, due to connective tissue proliferation (Coimbra and Reis-Henriques 2005), also observed in cod (Sangalang et al. 1981); tilapia exposed during the larvae stage presented a hyperplasia of Leydig cells. Increased Leydig cell number was observed in Sprague Dawley rats exposed during lactation to Aroclor

1242 (Kim et al. 2001). This Leydig cell hyperplasia may be a response to maintain androgens levels, which were shown to be depressed when adults were exposed to this pollutant (Coimbra and Reis-Henriques 2005); Aroclor 1248 also inhibit in vivo and in vitro rat testicular stereoidogenesis (Andric et al. 2000).

The results obtained in our study demonstrate that the exposure during the larvae stage to a low and environmental relevant concentration of a PCBs mixture originates effects in the adult stage, that may led to reproductive impairment. The dose used, 0.05 μg g^{-1}, is in the range of total PCBs observed in a food web of the Kalamazoo River (Kay et al. 2005) and lower than concentrations found in adult *O. mossambicus* from the Salton Sea where a total PCBs concentrations ranging from 10.9 to 32.8 ng g^{-1} wet weight (ww) in muscle, 16.4–114.5 ng g^{-1} ww in liver and 14.4–98.4 ng g^{-1} ww in gonads were observed (Sapozhnikova et al. 2004). Thus, we believe that further studies should be performed exposing larvae to even lower Aroclor 1254 concentrations to evaluate their impact in adulthood.

The effects observed in our study reflect either a direct action of Aroclor 1254 in gonad development or a indirect impairment by acting in the hypothalamic–pituitary–gonadal axis, since this compound has the capacity to cause alterations at central level; Aroclor 1254 was found to interfere with reproduction of *Micropogonias undulates*, after a 1 mg kg^{-1} exposure for 30 days, by decreasing the secretion and release of the luteinizing hormone (LH), through inhibition of serotonin synthesis and disruption of the serotonin–gonadotropin releasing hormone pathway that controls LH secretion and resulting in gonad growth inhibition (Khan and Thomas 2001). Thus, alterations observed in ovaries could reflect an action of Aroclor 1254 at central level enabling tilapia maturation and/or ovulation.

Adult tilapia, which had been exposed to 0.05 μg g^{-1} Aroclor 1254, showed a significant decrease in T4 plasma concentrations, corresponding to a 59% reduction in this hormone levels. T3 plasma concentration was also depressed with Aroclor 1254, although differences between groups were not significant ($p > 0.05$) (Table 1).

Aroclor 1254 exposure of tilapia larvae seem to have originated a deficient thyroid hormone status, revealed by plasma T4 depletion in adults. As observed in adult tilapia, plasma T3 concentration in exposed tilapia revealed similar values presented by control, since Aroclor 1254 exposure does not interfere with the deiodinase activities responsible for the maintenance of this hormone plasmatic concentration (Coimbra et al. 2005). T4 levels decline might result from the competition of PCBs and/or of their metabolites for the binding to thyroid hormones transport proteins present in the blood (Lans et al. 1994; Cheek et al. 1999; Chauhan et al. 2000).

Table 1 Thyroid hormones, T4 and T3, plasma concentration (nM) in control and Aroclor 1254 exposed tilapia

	T4	T3
Control (n = 7)	13.6 ± 1.15	7.5 ± 1.58
Aroclor 1254 (n = 13)	8.0 ± 0.77**	5.8 ± 1.07

** $p < 0.01$

In rats, hypothyroidism has been previously associated with an increased of Leydig cell number as a direct increase of Leydig cells precursors (Hardy et al. 1993; Mendishandagama and Sharma 1994). Moreover, Coimbra et al. (2005) showed that adult tilapia fed with a diet contaminated with 0.5 μg g^{-1} of Aroclor 1254 for 35 days presented an increase in testicular activity of the deiodinase responsible for the conversion of T4 in T3, suggesting the occurrence of an increase in testicular metabolism, probably due to an effort of the cells to compensate on the steroidogenesis disruption.

Studies with other fish species showed that PCBs exposure may lead to population decline due to decreased ovary growth (Khan and Thomas 2001), induction of testicular lesions (Sangalang et al. 1981), reduction in the number of spawned eggs (Orn et al. 1998; Heiden et al. 2005), or even increase in embryo and larvae abnormalities and mortality (Palstra et al. 2006).

In conclusion, Aroclor 1254 when administrated to tilapia larvae disrupted the reproductive axis enabling tilapia reproduction during adulthood, since the injuries caused by Aroclor 1254 in tilapia gonads seem to be irreversible. The current study shows that gonads are sensitive target organs for disruption by PCBs. Exposure effects to low levels of PCBs, even for short periods of time, during early life stages, may have serious repercussions in the gonads development and thus affecting the reproductive capability of adults. Since these compounds are still widely distributed in the environment, our results indicate that even low levels may have a considerable impact in fish populations as PCBs exposure may led to a decrease of larvae recruitment and to a reproduction impairment.

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