# Effects of Aroclor 1254 on LH and 17,20β-P secretion in female Prussian carp (*Carassius gibelio* Bloch) in the spawning season

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**ABSTRACT**: The effects of Aroclor 1254 on the secretion of luteinizing hormone (LH) and 17,20β-dihydroxy-4-pregnen-3-one (17,20β-P) in female Prussian carp (*Carassius gibelio* Bloch) at the time of their natural spawning were determined. Aroclor 1254 at doses of 0.01, 0.1, and 1 mg/kg body weight was dissolved in 500 ml of oil and was administered three times intraperitoneally or rectally to female Prussian carp every 24 h for three days. Blood samples were collected after 3 days of Aroclor 1254 administration to determine the LH and 17,20β-P concentrations using ELISA. At 6, 12, and 24 h after injection of gonadotropin-releasing hormone analogue (GnRH-A) blood samples were collected for stimulated LH secretion determination. Aroclor 1254 administered intraperitoneally (0.1 and 1 mg/kg) and rectally (0.01 and 1 mg/kg) significantly increased spontaneous LH secretion. In the case of GnRH-A-stimulated LH release, Aroclor 1254 (administered intraperitoneally only) at concentrations of 0.1 and 1 mg/kg significantly decreased gonadotropin release. The intraperitoneal injections of the lowest tested concentration of Aroclor 1254 also significantly decreased 17,20β-P secretion. The results show that Aroclor 1254 can affect the reproductive system of Prussian carp by changing the secretion of two very important hormones, LH and 17,20β-P, at the time of natural spawning.

**Keywords**: PCBs; cyprinids; gonadotropins; LH, steroids; reproduction

Polychlorinated biphenyls (PCBs) are lipophilic industrial chemicals that accumulate in living organisms (Okumura et al., 2003; Van Geest et al., 2011) and have negative physiological effects, mostly on the reproductive system of vertebrates, including fish (Khan and Thomas, 1997, 2001, 2006; Khan et al., 2001; Gore et al., 2002; Coimbra et al., 2005; Gregoraszczuk et al., 2005; Coimbra and Reis-Henriques, 2007; Daouk et al., 2011). The impairment of reproduction in teleosts was found at a central level, at the hypothalamo-pituitary-gonadal axis, after the exposure to different PCB mixtures. Decreases in the basal and GnRH-A-induced LH secretion, together with gonadosomatic index reductions in Atlantic croaker (*Micropogonias undulatus)* and Nile tilapia (*Oreochromis niloticus*), have been observed (Khan and Thomas, 1997, 2001, 2006; Coimbra and Reis-Henriques, 2007). The disruption of LH secretion is associated with changes in the content of important brain neurotransmitters (GnRH, DA, and serotonin (5-HT)), which regulate the synthesis and release of gonadotropins in teleosts. In Atlantic croaker, PCBs influenced the synthesis of GnRH at the level of the hypothalamus (preoptic anterior hypothalamic area) and significantly lowered the number of pituitary GnRH receptors, which, in turn, affected LH secretion (Khan and Thomas, 1997, 2001).

PCBs have been noted to influence testicular and ovarian steroidogenesis in pigs, cows, and rats (Wójtowicz et al., 2000, 2005; Andric et al., 2006; Kotwica et al., 2006). There is also evidence confirming changes in steroid hormone production (testosterone, progesterone, and estradiol) due to the direct action of PCBs on the activity of specific steroidogenic enzymes, such as 17β-HSD and P450 aromatase (Wójtowicz et al., 2005; Andric et al.,

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2006). Benninghoff and Thomas (2005) suggest the existence of a novel mechanism of endocrine disruption by PCBs that involves the alteration of calcium-dependent signalling pathways regulating steroidogenesis in fish.

In addition to changes in the secretion of very important hormones regulating the reproduction, the PCBs present in water could affect the final phase of reproduction in male fish, as these compounds decrease the quality of semen, affecting sperm motility in common carp (*Cyprinus carpio* L.) and Atlantic croaker (Thomas and Doughty, 2004; Socha et al., 2008). In females (Atlantic croaker, Nile tilapia, and zebrafish (*Danio rerio*)) exposed to PCBs, the observations include decreased ovary growth, the absence of maturing oocytes, and an increased number of atretic follicles (Khan and Thomas 2001; Coimbra and Reis-Henriques, 2007; Daouk et al., 2011).

PCBs are dangerous contaminants present in the environment, mostly because of their high liposolubility, long biological half-life, and potential to bioaccumulate along the food chain. The action of PCBs might be estrogenic and/or antiestrogenic and is highly dependent on the number and position of chlorine atoms on the biphenyl rings (Kester et. al., 2000; Gregoraszczuk et al., 2005; Plíšková et al., 2005). In our study a common PCB mixture – Aroclor 1254 with 54% of chlorine and with the composition of about 71.44% hepta and 21.97% of hexa chlorobiphenyls (Taniyasu et al., 2003) was used. The aim of the present study was to evaluate the influence of Aroclor 1254 on the secretion of LH and 17,20β-dihydroxy-4-pregnen-3-one (17,20β-P), which regulate the final stage of oocyte maturation in Prussian carp (*Carassius gibelio* B.). Because it is known that the alimentary tract is one of the most important contamination routes of PCBs in organisms (Daouk et al., 2011), we tested rectal administrations of PCBs (in addition to intraperitoneal injections) resulting in the high absorption of xenobiotics (de Boer et al., 1982).

## MATERIAL AND METHODS

The experiments were conducted at the end of May 2009 on 79 sexually mature (mean gonadosomatic index (GSI) of 12.6%) female Prussian carp (*Carassius gibelio* Bloch) raised in the ponds of the Department of Ichthyobiology and Fisheries of the Agricultural University in Krakow. The experiments were conducted according to the research protocols approved by the Local Animal Ethics Committee in Krakow, Poland. Three days before the administration of PCBs, the fish were captured from the outdoor ponds and transferred to aquaria (volume of 250 l, water temperature of approximately 20 ± 0.5°C). The fish were exposed to a simulated natural photoperiod. All of the females were weighed individually and tagged after a light anaesthesia with Propiscin™ (3 ml/10 l of water). The mean body weight of fish was 72.8 ± 11.6 g.

A stock solution of Aroclor 1254 (20 mg/ml) was prepared by dissolving Aroclor 1254 in dimethyl sulphoxide (DMSO), and the injection solution was prepared by diluting the appropriate amount of the stock solution in the vehicle (sunflower oil). Aroclor 1254 was purchased from LGC Promochem (Lomianki, Poland), Propiscin™ from IRS (Żabieniec, Poland), and the Des-Gly$^{10}$ D-Ala$^{6}$ LHRH analogue and DMSO from Sigma Chemicals (Balcatta, USA). GnRH-A was injected intraperitoneally (10 μg/kg) as a solution in 0.6% saline (0.5 ml/kg vol.) into all the females at time 0 h.

In the first experiment, 39 females were divided into 4 groups and injected intraperitoneally with 0 (control), 0.01, 0.1 or 1 mg/kg of Aroclor 1254 in the volume of 0.5 ml/kg. In the second experiment, the same doses of Aroclor 1254 were administered rectally to 40 females. In both experiments, the treatment with Aroclor 1254 was repeated every 24 h for 3 days. Just before GnRH-A injection, blood samples (150–200 μl) were collected by puncturing the caudal vein with a 1-ml heparinized syringe after 3 days of PCB administration (0 h) for the estimation of the LH and 17,20β-P levels. Next, blood samples from all of the females were collected at 6, 12, and 24 h for LH measurements. After centrifugation of the blood, the plasma samples were frozen at –20°C until the levels of LH and 17,20β-P were analyzed using ELISA. The LH analysis was performed according to the method of Kah et al. (1989). The plasma samples for 17,20β-P were extracted with dichloromethane prior to the measurement of this steroid and subjected to 17,20β-P determination according to the method described by Szczerbik et al. (2008).

The data were analyzed using a nonparametric Mann-Whitney *U*-test (two-tailed). The results are presented as the means ± SEM and are considered significant at $P < 0.05$ and highly significant at $P < 0.01$.

## RESULTS

### Effect of intraperitoneal or rectal administration of Aroclor 1254 on spontaneous LH secretion in Prussian carp females

Three days after the intraperitoneal injections of Aroclor 1254 (0.1 and 1 mg/kg), a significant ($P < 0.05$) dose-dependent increase in spontaneous LH secretion (2.19 and 4.26 ng/ml, respectively) compared with the control group was observed. Furthermore, the rectal administration of 0.01 mg/kg and 1 mg/kg Arcolor 1254 caused significant and highly significant increases, respectively, of spontaneous LH secretion compared to the control group. In the case of the smallest tested dose of Aroclor, the rectal administration caused a highly significant elevation of LH (4.64 ng/ml) in comparison to the same dose administered intraperitoneally for which the level of LH was only 1.12 ng/ml (Figure 1).

### Effect of intraperitoneal or rectal administration of Aroclor 1254 on GnRH-A-stimulated LH secretion in Prussian carp females

6 h after GnRH-A (10 μg/kg) injection, a significant decrease in LH release was observed in the groups intraperitoneally injected with Aroclor 1254 (0.1 and 1 mg/kg) in comparison with the control group. However, 6 h after analogue injection, in the females receiving Aroclor 1254 rectally there were no significant differences in the GnRH-A-stimulated gonadotropin release compared to the control group (Figure 2). At 12 and 24 h, the GnRH-A-stimulated LH levels in the females of both (intraperitoneal and rectal) experiments were not significantly different in comparison with the corresponding controls (data not shown).

### Effect of intraperitoneal or rectal administration of Aroclor 1254 on 17,20β-P secretion in Prussian carp females during the spawning season

In all females receiving Aroclor 1254 intraperitoneally, the level of 17,20β-P was lower than in the control group, but the secretion of this steroid was significantly ($P < 0.05$) decreased in comparison with the control only in the group receiving the lowest dose of PCB (0.01 mg/kg) (Figure 3A). The level of 17,20β-P in this group was also significantly decreased in comparison to the same dose (0.01 mg/kg) of Aroclor 1254 administered rectally (Figure 3). The rectally administered Aroclor 1254 caused no significant changes in 17,20β-P secretion compared with the control group (Figure 3B).

## DISCUSSION

Our results demonstrate the effect of Aroclor 1254 on the secretion of two very important regulators,

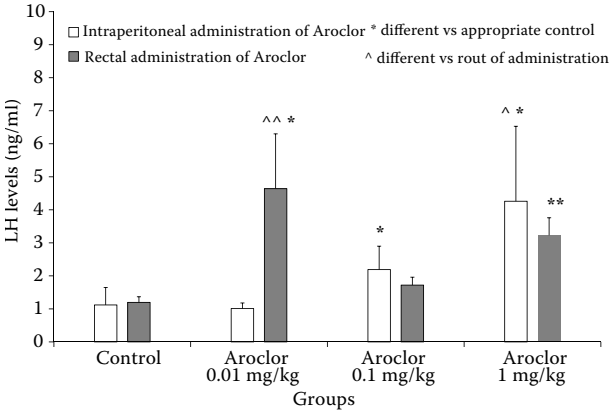


Figure 1. Effect of the intraperitoneal or rectal administration of Aroclor 1254 on spontaneous LH secretion in female *Carassius gibelio* in the spawning season

blood samples collected 3 days after treatment with Aroclor 1254 (0 h)

*$P < 0.05$, **$P < 0.01$ vs control

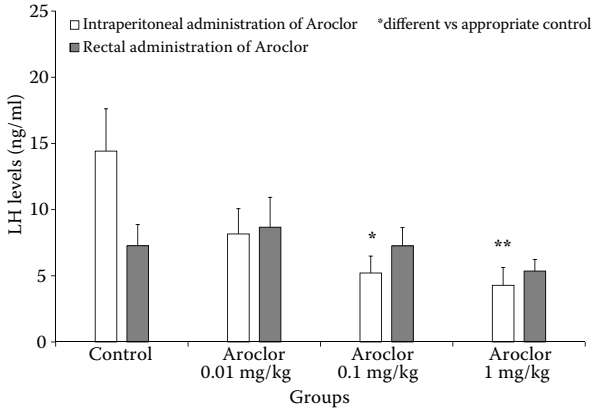


Figure 2. Effect of the intraperitoneal or rectal administration of Aroclor 1254 on GnRH-A-stimulated LH secretion in female *Carassius gibelio* in the spawning season

blood samples collected 3 days after treatment with Aroclor 1254 plus 6 h after GnRH-A injection

*$P < 0.05$ vs control, **$P < 0.01$ vs control

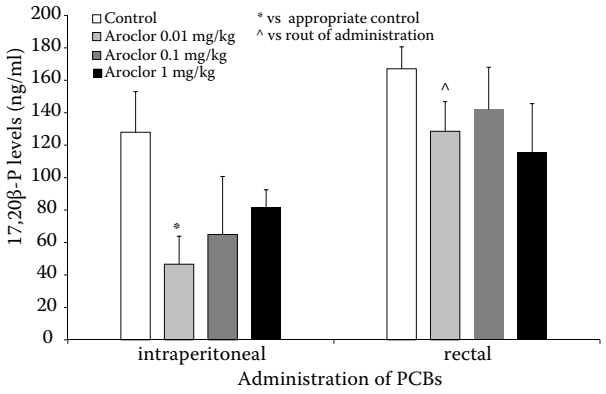


Figure 3. Effect of the intraperitoneal or rectal administration of Aroclor 1254 on 17,20β-P secretion in female *Carassius gibelio* in the spawning season

blood samples collected 3 days after treatment with Aroclor 1254 (0 h)

*$P < 0.05$ vs control

LH and 17,20β-P, which are responsible for the successful final phase of gonadal maturation in fish. Both routes (intraperitoneal or rectal) of a short (3-day) administration of Aroclor 1254 increased spontaneous LH secretion in female Prussian carp at the time of their natural spawning. The highest dose of Aroclor 1254 (1 mg/kg) in both experiments elevated the gonadotropin levels (Figure 1). It is worth noting that the lowest dose of PCBs (0.01 mg/kg) given rectally resulted in a significant elevation of LH secretion in comparison to the control group and to the same dose of Aroclor 1254 given intraperitoneally. This observation indicates that the rectal administration of low doses of Aroclor might have a stronger affect on LH secretion in Prussian carp, most likely caused by the rapid absorption of the xenobiotic by this route. The stimulation effect of Aroclor 1254 on spontaneous LH secretion in Prussian carp is in contrast with the data presented for *Micropogonias undulatus* exposed to Aroclor 1254 (1 mg/kg body weight) in their diet for 30 days (Khan and Thomas, 2001) in which the basal LH level was significantly lower than in the control group after PCB treatment. The difference in results may be due to the longer duration (10-fold) of exposure to a rather high dose of this PCB mixture for Atlantic croaker. It is worth noticing that in fish, the biotransformation of PCBs, leading to hydroxylated PCBs (OH-PCBs) formation, is possible (Buckman et al., 2006). There is also evidence that metabolites of polychlorinated biphenyls such as OH-PCBs may have stronger disrupting (estrogenic/antiestrogenic) potency (Gerpe et al., 2000; Carlson and Williams, 2001; Gregoraszczuk and Ptak, 2008). That is why in the case of longer-lasting exposure to PCBs the observed effects (changes in hormone secretion) may be the additive action of both hydroxylated PCBs as well as their parent compounds.

The next finding in our study was the weaker response of LH secretion to gonadoliberin stimulation in Aroclor 1254-treated female Prussian carp. In our first experiment at 6 h after GnRH-A injection (10 μg/kg), the level of LH was significantly lower in the experimental groups (Aroclor 1254 at 0.1 and 1 mg/kg) compared to the control. However, there were no significant changes in analogue-stimulated LH secretion in the case of the rectal administration of PCBs for the same time point (6 h) (Figure 2). At the next time points (12 and 24 h) after GnRH-A injection, there were no significant differences in LH secretion for either route of Aroclor 1254 administration. The weak response of LH release to GnRH-A stimulation in female Prussian carp after Aroclor 1254 treatment was similar to the observations in Atlantic croaker (Khan and Thomas, 2001; Khan et al. 2001): a decreased level of GnRH-A-stimulated LH secretion was observed 1 h after analogue injection in croaker exposed to Aroclor 1254. The authors explained that the lack of response to GnRH-A stimulation by LH secretion might be due to a decreased number of GnRH receptors in the pituitary of the fish after PCB exposure. Khan et al. (2001) found a decreased concentration of GnRH receptors in the pituitary of Atlantic croaker after Aroclor 1254 treatment in addition to a lower GnRH content in the preoptic anterior hypothalamic area (POAH), indicating that PCBs could impair the synthesis and/or enhance the degradation of GnRH in this species. Taking into consideration these findings and our data, it seems possible that the observed changes in LH secretion in Prussian carp, even after a short-term exposure to PCBs, might be due to disturbances at the hypothalamic and/or pituitary level caused by these synthetic compounds.

The results of the present study also demonstrate the changes in 17,20β-P secretion in female Prussian carp after the administration of Aroclor 1254. In both experiments, the levels of 17α,20β-P in the females treated with the PCBs were lower than in the control group (Figure 3), but a significant decrease was observed only after the intraperitoneal injections of the lowest dose of Aroclor 1254 (Figure 3A). These results show that a short-term administration of Aroclor 1254 significantly decreased the level of 17,20β-P, a very important endogenous factor

that regulates successful reproduction. In teleosts, 17,20β-dihydroxy-4-pregnen-3-one functions in the induction of oocyte final maturation and spermiation (Scott et al., 2010). A low level of this steroid might be due to decreased activity of 20β-HSD, the enzyme responsible for the conversion of 17α-hydroxyprogesterone to 17α,20β-P (Nagahama et al., 1993). There is evidence showing that PCBs as well as other endocrine disrupting chemicals (EDCs) impair steroidogenesis through an influence on the activity of enzymes involved in the steroid biosynthesis cascade in vertebrates (Benninghoff and Thomas, 2005; Wójtowicz et al., 2005; Andric et al., 2006; Hatef et al., 2012). In our study, it is possible that Aroclor 1254 was able to influence gonadal steroidogenesis by acting directly on the gonads. In common carp Aroclor 1254 was found to inhibit the final stage of oocyte maturation and cause a significant, dose-dependent, decrease of *in vitro* 17,20β-P secretion (Socha et al., unpublished data). Low plasma steroid concentrations could also be the result of elevated hepatic deactivation by pollutants (Kime, 1999). In experiments on carp after intraperitoneal injections of Aroclor 1254, Yano and Matsuyama (1986) reported that the decreased level of gonadal steroids (progesterone, testosterone, and estradiol) was a consequence of increased steroid catabolism. However, *in vitro* experiments with PCBs are needed to explain the site and mechanism of their action in the Prussian carp reproductive system. In conclusion, the results of the present study demonstrate that the short-term exposure to Aroclor 1254 in the spawning season might impair reproduction in female *Carassius gibelio* by affecting the endocrine system by altering the secretion of LH and 17,20β-P.

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